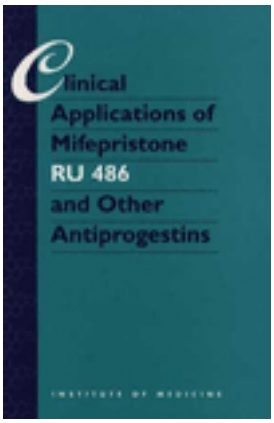

Clinical Applications of Mifepristone (RU486) and Other Antiprogestins: Assessing the Science and Recommending a Research Agenda

Molla S. Donaldson, Laneta Dorflinger, Sarah S. Brown, and Leslie Z. Benet, Editors; Committee on Antiprogestins: Assessing the Science, Institute of Medicine

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Assessing the Science and Recommending a Research Agenda

Committee on Antiprogestins: Assessing the Science
Division of Health Promotion and Disease Prevention
INSTITUTE OF MEDICINE

Molla S. Donaldson, Laneta Dorflinger, Sarah S. Brown, and Leslie Z. Benet, Editors

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The image adopted as a logotype by the Institute of Medicine is based on a relief carving from ancient Greece, now held by the Staatlichemuseen in Berlin.

COMMITTEE ON ANTIPROGESTINS: ASSESSING THE SCIENCE

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Preface

The first clinically available antiprogestin, mifepristone (RU 486), has generated great interest in the research community since its discovery in France in the early 1980s. Seldom has a research discovery (in this case, a compound exhibiting a high affinity for progesterone receptors, but with little or no progesterone-like activity) been so rapidly applied in clinical medicine. Today it is recognized that mifepristone and other antiprogestins have a significant potential role in human health and disease, with likely applicability to a variety of pregnancy-related conditions (e.g., management of labor), contraception, endometriosis, and cancer, as well as several other diseases and conditions.

Mifepristone has been most widely studied and used as a means of nonsurgical abortion in early pregnancy and has been approved for marketing for that purpose in France, the United Kingdom, and Sweden. Although great interest in mifepristone and other antiprogestins has been shown by both the medical and the lay communities in the United States, the political issues surrounding abortion have thus far prevented the introduction of the drug into the U.S. market, and research on this compound in America has been limited. However, pressure is mounting to accelerate the pace of approval and use of mifepristone. An Executive Order signed by President Clinton in January, 1993, signalled a change in climate and called for research on the benefits and risks of the antiprogestins as therapeutic agents. Such research is a necessary first step before their wider availability in the United States. In late April, 1993, it was announced that Roussel-Uclaf had agreed to grant a license for distribution of mifepristone to the non-profit organization the Population Council. The Population Coun

cil has agreed to sponsor an application to the U.S. Food and Drug Administration (FDA) and manage a clinical trial in the United States. Roussel-Uclaf has also agreed to provide the FDA with its toxicologic and chemical data on mifepristone.

To provide an unbiased evaluation of the science and potential clinical uses of antiprogestins for numerous diseases and conditions in addition to inducing abortion, the Institute of Medicine (IOM) of the National Academy of Sciences, with funding from the Henry J. Kaiser Family Foundation, convened an expert committee to conduct an evaluation and to develop recommendations concerning future research on the potential clinical use of antiprogestins in the United States. The seven member committee included individuals with expertise in cell biology, pharmacology, epidemiology, reproductive endocrinology, care of women with hormone-dependent clinical conditions, population demography, and oncology. The committee held a two-day invitational workshop in Washington, D.C., on April 13 and 14, 1993, to review the status of scientific and clinical investigations regarding antiprogestins. Leading researchers throughout the world with expertise in antiprogestin science accepted the IOM's invitation to prepare papers and to participate in this workshop. Their papers are included in this volume as Appendix B to the committee's report. Although these manuscripts have not been peer-reviewed, they summarize the extensive published literature of those invited to make presentations, as well as many recent and often, as yet, unpublished studies related to the science and clinical uses of the antiprogestins. Besides the invited speakers and committee members, 54 outside observers attended the workshop and provided thoughtful commentaries as well as supplementary information and discussion during the two-day workshop. The workshop agenda and a listing of the observers are included in Appendixes A and C, respectively. The committee and speakers had the opportunity to hear from Margaret Cately-Carlson, president of the Population Council, during an evening presentation. Immediately following the workshop, the committee met to review and discuss an initial set of conclusions and recommendations concerning the scientific issues presented at the workshop. Each of the committee members then prepared a draft section of the committee's report, including initial recommendations related to that section. During and after another meeting in early May, the committee reached consensus on its report and recommendations. The committee's report is a summary of what is known; it reviews the data that served as the bases for approval of mifepristone in other countries. The report includes 20 recommendations about various scientific issues that are important to the evaluation of mifepristone and other antiprogestins. The report has been subject to external review by a specially appointed expert panel according to National Research Council procedures.

The committee's report begins with an overview of research and three cross-cutting recommendations that should lead to a better understanding of the mechanisms of action of the antiprogestins and the potential for developing additional compounds that are directed exclusively toward progesterone receptors. The next nine recommendations are ordered to follow the time sequence of potential pregnancy-related uses of antiprogestins (e.g., pre-coital contraception, post-coital contraception, missed menses, pregnancy termination, term and post-term labor induction). The final eight recommendations relate to other possible therapeutic uses of antiprogestins for the treatment of endometriosis, uterine leiomyomas, breast cancer, meningiomas, and antiglucocorticoid-dependent conditions.

These 20 recommendations reveal how uneven current scientific knowledge is regarding the many actual and potential uses of antiprogestins in clinical practice. In some areas, such as treatment for endometriosis, much research is needed to understand whether and how to use antiprogestins to treat this particular condition; in other areas, such as the use of antiprogestins to induce early abortion or to serve as post-coital contraception, clinical data gathered in Europe appear complete enough for their prompt review by U.S. regulatory authorities.

I am extremely grateful to my fellow committee members for the dedication and industry that they exhibited in preparing this evaluation within such a short time frame, without sacrificing scientific integrity. With the rapid acceleration of interest in the possible introduction of mifepristone and other antiprogestins into the U.S. market, this IOM report provides a timely independent review and assessment of current knowledge that should be useful to researchers and clinicians, federal officials in the Department of Health and Human Services (the National Institutes of Health and the Food and Drug Administration, especially), the U.S. Congress, the report's sponsors as well as other foundations, and the more knowledgeable public.

The committee and I are extremely grateful to the IOM staff who tirelessly and with unselfish dedication helped to prepare this report in a timely manner. We are particularly appreciative of the efforts of the Study Director, Molla S. Donaldson; the Senior Program Officer, Sarah S. Brown; and the Director of the Division of Health Promotion and Disease Prevention, Michael Stoto. We are cognizant of the excellent judgment that they exhibited in assisting the committee in evaluating many of the controversial and technical issues related to this study. The committee also acknowledges and appreciates the work of Senior Project Assistant, Helen Rogers, and Project Assistant, Terri Barba, who were unflagging in their responses to committee needs.

The committee had the good fortune to have the services of Laneta Dorflinger, Director of Regulatory Affairs and Quality Assurance at

Family Health International. Dr. Dorflinger provided extensive expertise in the issues addressed by the committee, and served as an excellent and knowledgeable science editor for the committee's draft report and recommendations. She also helped to edit the papers presented at the workshop and published in Appendix B.

The committee and the Institute of Medicine are particularly grateful for the support provided by the Henry J. Kaiser Family Foundation, which requested that the IOM conduct this study, and thank the Foundation's project officer, Sarah Samuels, for her assistance.

LESLIE Z. BENET, Ph.D.

Chair

Contents

Clinical Applications of Mifepristone (RU 486) and Other Antiprogestins

Summary

Progesterone is a steroid hormone that plays a pivotal role in establishing and maintaining pregnancy. It exerts well-documented actions on a number of target tissues within the reproductive system (e.g., uterus, cervix, breast, pituitary-hypothalamic unit), and less well-defined actions on tissues outside the reproductive system (e.g., brain, vascular endothelial cells) and on lipid metabolism. Given the broad role progesterone plays in normal physiology and in certain pathophysiologies, logic suggested that compounds to inhibit its action (termed antiprogestins) might be useful in the pharmacological regulation of a variety of conditions and diseases.

The first antiprogestin was discovered fortuitously by scientists searching for an antiglucocorticoid, a compound that would interfere with the action of a class of adrenal gland hormones called glucocorticoids that are involved in the physiologic regulation of virtually all tissues in the body. Now, more than a decade after the report of this first antiprogestin, mifepristone (RU 486), numerous antiprogestins have been synthesized and studied. Although all of the antiprogestins also have some degree of antiglucocorticoid activity, a property that causes unwanted side effects under many conditions of use, the potential role of these compounds in human health and disease is great. They have applicability to a variety of pregnancy-related conditions—management of labor, contraception, and infertility; endometriosis; the treatment of certain types of tumors; and as a result of their antiglucocorticoid activity, the treatment of Cushing's syndrome (a disease resulting from excess glucocorticoid production by the adrenal glands) and several other conditions.

Although many antiprogestins are under investigation by pharmaceutical companies for various therapeutic purposes, mifepristone is the only antiprogestin that has been extensively studied in human beings. It has been most widely studied as a means of nonsurgical abortion in early pregnancy, and has been licensed for that purpose in France, the United Kingdom, and Sweden. Because of the perceived promise of this class of compounds for a variety of therapeutic uses, the Institute of Medicine (IOM) established a committee of seven individuals to assess the state of scientific knowledge about antiprogestins, to clarify what is and is not known about these compounds, and to make recommendations for future research that might lead to improvements over currently available therapies for a variety of conditions.

Political controversy has focused public attention on the use of antiprogestins to induce miscarriage; this committee, however, was specifically charged with considering the full spectrum of clinical applications of antiprogestins, not just their use for medical abortion.

Using information provided in the literature, in commissioned papers, and presented during a workshop held at the IOM in April 1993, the committee reviewed and assessed the basic and clinical research available on the use of antiprogestins for post-coital contraception, continuous-use contraception, induction of missed menses, first-trimester termination of pregnancy, second-trimester termination of pregnancy, as well as induction of labor, and for the treatment of endometriosis, uterine leiomyomas, meningioma, breast cancer, and Cushing's syndrome. The committee made a series of general recommendations that included several cross-cutting issues for investigation, as well as specific recommendations for research on each of the topics discussed at the workshop. Those recommendations appear below. The committee's full report includes a more complete explanation of its conclusions and presents additional materials supporting its recommendations. The background papers prepared for the IOM workshop are included as Appendix B of this report.

CROSS-CUTTING RECOMMENDATIONS

Recommendation No. 1. Research is needed to define the basic mechanisms and modes of action of mifepristone (RU 486) and other antiprogestins in order to understand the effects that have already been demonstrated and to develop compounds with more specific antiprogestin activity.

Recommendation No. 2. Because antiprogestins as a class have clear potential for preventive and therapeutic applica

tions in human health beyond those that have already been documented, the committee strongly recommends additional clinical testing of mifepristone and of newer antiprogestins as they are developed.

Recommendation No. 3. Because some uses of antiprogestins may require long-term administration, the committee recommends studies to evaluate the potential toxicity, maintenance of efficacy, and development of drug resistance for antiprogestins used over longer periods of time.

USES OF ANTIPROGESTINS: THE REPRODUCTIVE CYCLE

Contraception

Progesterone is produced by the ovary during the latter half of a normal menstrual cycle, and by the ovary and placenta during pregnancy. Because of the complex hormonal interplay that characterizes the female reproductive cycle and the role that progesterone plays in initiating and maintaining pregnancy, it is clear that antiprogestins could alter many of these functions. As a pharmacologic class, the antiprogestins appear to have great promise as regulators of reproductive potential.

Data in humans, however, are limited with respect to most of the possible contraceptive applications, and the data that exist are confined largely, if not exclusively, to mifepristone. The committee reviewed a number of studies on the potential contraceptive effectiveness of mifepristone when given during the follicular, periovulatory, and luteal phases of the cycle. In these studies, administration of the antiprogestin varied: in some cases the drug was given continuously for various periods of time; in other cases it was given intermittently (once weekly or monthly for several days); and in some studies it was given in combination with other agents such as progestins or prostaglandins.

In general, single-dose or short-term administration on an intermittent, as opposed to a continuous, basis appears to have limitations. Administration during the follicular phase merely delays follicular growth and ovulation. Early luteal phase administration delays the development of a secretory endometrium but does not affect the corpus luteum. Timing of such early luteal phase administration must be linked to ovulation, which is not easy to achieve. Late luteal phase administration will suppress the corpus luteum and cause bleeding, but it appears to require prostaglandins as well as the antiprogestin for uniform effectiveness.

There are no data on the contraceptive efficacy or safety of continuous

long-term administration of low-dose antiprogestins. However, data derived from a number of therapeutic studies of mifepristone to treat endometriosis, leiomyomas, meningiomas, and Cushing's syndrome suggest that long-term use of this compound would inhibit ovulation and induce consistent amenorrhea (lack of menstrual bleeding). Toxicity experienced by women in these various studies was minimal except at the highest doses. Furthermore, the antiglucocorticoid activities of the compound were not manifest at low doses (less than 25 mg/day), even though doses as low as 2 mg/day were effective at inhibiting ovulation.

Recommendation No. 4. Clinical research should be undertaken promptly to evaluate the efficacy and safety of mifepristone and other antiprogestins as low-dose contraceptives. Such research should address, among other issues,

- **a. mechanisms of contraceptive action**
- **b. the effectiveness of various regimens in preventing pregnancy (e.g., continuous versus cyclic administration, possibly with the addition of other hormones)**
- **c. the lowest effective dose to prevent pregnancy for each regimen**
- **d. potential short- and long-term toxicities affecting bone (osteoporosis), lipids (alteration in profile), endometrium (histologic changes), ovary (cyst formation), and brain (mood); and**
- **e. benefits and risks, both contraceptive and noncontraceptive, of antiprogestin contraception relative to other hormonal contraception.**

Post-Coital Contraception

A post-coital contraceptive is a pill or other method that can be used to reduce the chance of undesired pregnancy after unprotected intercourse around the probable time of ovulation (midcycle). In the United States, the most common post-coital approach (termed the "morning-after pill") involves a combination oral contraceptive containing both a synthetic estrogen and a progestin. Although the regimen is not 100 percent efficacious, currently available oral contraceptives reduce the risk of pregnancy by at least 75 percent after unprotected midcycle sexual intercourse. Unfortunately, the relatively high amount of estrogen used results in side effects such as nausea and vomiting, and a 25 percent failure rate is highly undesirable.

Because of the critical role progesterone plays in early transformation of the uterus, and its possible roles in ovulation and tubal transport, it

was reasoned that mifepristone might also be an effective post-coital method. Two available studies (from England and Scotland) on the use of mifepristone for post-coital contraception were reviewed by the committee. These studies compared mifepristone with other post-coital methods and have shown that it is, indeed, effective in this context. Furthermore, it is potentially preferable to the post-coital oral contraceptive regimen in that it appears to have higher efficacy and a lower frequency of side effects.

Recommendation No. 5. The committee recommends expeditious submission to the U.S. Food and Drug Administration of existing clinical trial data on the use of mifepristone as a post-coital contraceptive to determine whether these data meet current U.S. regulatory requirements.

Menses Induction

Studies have evaluated the use of mifepristone to induce menses after an undesired mid-cycle exposure when administered within one week following the day of the expected onset of a menstrual period. Once-a-month administration at the end of the luteal phase of each menstrual cycle has also been studied. The major problem with both of these approaches has been that mifepristone alone does not consistently induce menstruation, and administering prostaglandin two days after mifepristone is required for consistently successful results. The need for a second agent makes this regimen less convenient from the patient's point of view, possibly reducing compliance. Given that a more specific antiprogestin compound would be expected to induce menses without any additional treatment, research that resulted in the development of such a compound would be particularly useful for menses induction.

Recommendation No. 6. Research is needed to develop the best means for delivering combinations of antiprogestins and prostaglandins for menses induction and regulation, ranging from sequential oral doses of each component, to new ways of providing both drugs simultaneously with delayed release of the prostaglandin. Studies of compliance with these various approaches are needed, especially for sequential drug delivery.

Recommendation No. 7. The committee recommends the development and evaluation of new antiprogestins that may in themselves induce menses without the need to add prostaglandin.

Pregnancy Termination During the First Trimester

Ample progesterone must be produced by the ovarian corpus luteum to maintain early pregnancy. In fact, low progesterone secretion in the luteal phase has been implicated in habitual abortion. It was, therefore, logical to hypothesize that antiprogestins given during early pregnancy might act as abortifacients, and thus provide a medical alternative to current techniques of surgical abortion. Almost a decade of research is now available on the use of one of the antiprogestins, mifepristone, for first-trimester abortion. Drug regulatory officials in France, Sweden, and the United Kingdom have concluded that mifepristone, when given in combination with prostaglandin, is safe and efficacious medical treatment for early pregnancy termination.

Recommendation No. 8. The committee recommends expeditious submission to the U.S. Food and Drug Administration of all existing preclinical and clinical trial data on mifepristone and prostaglandin for early pregnancy termination to determine whether these data meet current U.S. regulatory requirements.

Recommendation No. 9. The committee recommends that in considering the use of mifepristone and prostaglandin for early pregnancy termination in women who smoke more than 10 cigarettes per day or are over 35 (two groups of women who were excluded from European studies), the documented risks must be compared with the risks of continuing pregnancy or of surgical termination of pregnancy. Furthermore, such assessment should attempt to distinguish between the risks attributable to mifepristone and those attributable to prostaglandin.

Recommendation No. 10. With respect to using mifepristone for first-trimester termination of pregnancy, health services research should be conducted in the United States to determine which approaches (e.g., required number of visits, type of health care provider administering the drugs, site of service delivery) are most suitable from the standpoint of safety, efficacy, accessibility, and acceptability.

The committee does not recommend that consideration of a New Drug Application by the Food and Drug Administration for the use of mifepristone for pregnancy termination be delayed until the research outlined in Recommendations Nos. 9 and 10 has been completed.

Recommendation No. 9 addresses a group of potential patients excluded from previous trials. Recommendation No. 10 relates to the appropriate setting for use of the drug combination, reflecting the committee's concern that the imposed criteria of four medical visits, as set by protocols from the innovator-manufacturer, may not be necessary or acceptable to patients in the United States.

Pregnancy Termination During the Second Trimester

Currently, second-trimester pregnancy termination is available in the United States by dilation and evacuation (D&E), by intra-amniotic saline injection, by intra-amniotic prostaglandin $F_{2\alpha}$, or by prostaglandin E_2 suppositories. A series of clinical studies has suggested that treatment with mifepristone before the administration of prostaglandin significantly decreases the level of prostaglandin needed to complete an abortion and shortens the time interval from administration to abortion. Overall, in clinical studies to date, the use of antiprogestins for this purpose during the second trimester has been well tolerated with minimal side effects.

Given the multiday admission required for a second-trimester prostaglandin abortion, as well as the high level of discomfort for the woman, the ability to shorten by one day the duration of hospitalization using antiprogestin would be significant.

Recommendation No. 11. With regard to second-trimester abortion, the committee recommends conducting clinical trials in the United States to compare the established surgical procedure of dilation and evacuation (D&E) both to antiprogestins in combination with prostaglandins and to prostaglandins used alone. Such trials should clarify the optimal dose of antiprogestin and prostaglandin for this use, and should assess relative pain, interval to fetal expulsion, blood loss, and frequency of infection, uterine perforation, and incomplete expulsion requiring surgical intervention.

Cervical Ripening

The application of mifepristone for cervical ripening has been tested and shows promise. This property may help to manage clinical situations such as (1) preparation for second-trimester abortion, (2) preparation for labor induction at term, and (3) preparation of the cervix when labor must be induced because of intrauterine fetal demise. Because of the discomfort associated with either elective termination of second-trimester pregnancy or the termination of the genetically abnormal or

dead fetus, the ability of antiprogestins to shorten the therapeutic process is attractive. The use of antiprogestins given 36 to 48 hours before either a surgical procedure or prostaglandin installation appears to be well tolerated without the addition of significant clinical complications.

Labor Induction in Late Pregnancy

Studies in ewes and monkeys have indicated that, at term, antiprogestins induce uterine contractions and enhance the myometrial sensitivity to oxytocin, a drug used to induce labor. An initial study in humans with promising results compared mifepristone to a placebo for labor induction in term or post-term pregnancies. In this study, a significantly higher percentage of women receiving mifepristone experienced a spontaneous onset of labor, and the time to onset of labor was about 24 hours earlier than in the placebo control group. Mifepristone also reduced the amount of oxytocin required to induce labor in the patients who did not have a spontaneous onset of labor.

Research should include physiologic studies to assess the effects of antiprogestins on maternal lactation and on primate neonates to evaluate the pulmonary, cardiac and adrenal status of neonates as well as their later development and fertility potential.

Recommendation No. 12. The committee recommends studies to determine the minimal dose of antiprogestins necessary to induce labor. Studies in animal models (most likely the primates) should assess possible adverse outcomes on infants. Research is also needed to determine the effect of antiprogestins on maternal lactation.

OTHER POTENTIAL THERAPEUTIC USES OF ANTIPROGESTINS

Endometriosis

Endometriosis is a disease caused by the presence of endometrial tissue in ectopic (outside the endometrium of the uterus) locations, most commonly within the pelvic cavity. During the menstrual cycle this tissue undergoes changes similar to those in the endometrium. Endometriosis is a common disease (some have estimated that 5 to 15 percent of reproductive-age women and 30 to 40 percent of infertile women have this disorder), and it can be painful.

The etiology of endometriosis is uncertain, but there is little question about the hormonal responsiveness of the ectopic endometrial tissue. Current therapeutic approaches are designed to interrupt cyclic hor

monally induced changes in the ectopic tissue. Common medical therapies include danazol and gonadotropin-releasing hormone (GnRH) agonists, neither of which is uniformly successful. Surgical therapy is sometimes uses as well, particularly when endometriosis is associated with infertility. The side effects of the currently available therapies for treating endometriosis are sufficient to warrant continued research on alternative treatments. Further, drug and surgical treatments are only palliative, and it would be an important advance to have a curative treatment.

Human studies using antiprogestins to treat endometriosis are limited. Only a single antiprogestin (mifepristone) has been evaluated, and treatment periods have been short (three and six months). In these limited studies, mifepristone at several different doses produced uniform amenorrhea and reduced pain in all subjects. However, significant disease regression was observed only with longer treatment (six months). Daily administration of mifepristone in this context seemed to be well tolerated, with limited side effects, particularly at the lowest doses studied (<25 mg/day). A study using 5 mg/day of mifepristone for six months is ongoing and will establish whether dosages this low are effective in treating endometriosis.

The main promise offered by antiprogestins for treating endometriosis is preservation of the follicular phase levels of estradiol. This would protect women from the consequences of very low estrogen levels encountered with other forms of therapy (e.g., GnRH agonist). A goal of therapeutic studies should be to develop an antiprogestin regimen that is devoid of antiglucocorticoid side effects such as fatigue, nausea, and vomiting.

Uterine Leiomyomas (Fibroids)

Uterine leiomyomas, also known as fibroids, are non-malignant tumors of smooth muscle cell origin. Leiomyomas are the most common pelvic tumor; some have estimated that up to 20 percent of women over 30 years of age may have these benign tumors. They represent one of the most frequent reasons for surgery (including hysterectomies) in women of reproductive age.

These tumors are clearly hormonally dependent. Medical therapies such as high-dose progestin therapy and gonadotropin-releasing hormone agonists decrease overall uterine volume markedly, usually over a three-month treatment period. However, the effect of medical therapy is temporary, and no therapy has thus far been successful on a long-term basis. In the face of persistent symptoms, surgical therapy is usually applied in advanced disease following the failure of medical therapy and, ideally, when no pregnancies are desired.

There are very limited data from a few small studies on the use of antiprogestins in the treatment of uterine leiomyomas. In these studies, mifepristone at doses ranging from 5 to 50 mg/day for three months produced a marked decrease in leiomyoma volume. Side effects were limited and promptly resolved after discontinuation of the drug. Importantly, estrogen levels were maintained in a range sufficient to prevent bone loss as measured in the spine and hips. At the highest dose studied (50 mg/day), however, antiglucocorticoid effects were observed.

These early studies on the use of antiprogestins in the treatment of both endometriosis and uterine leiomyomas appear promising; however, larger studies than those currently available are required to establish the long-term efficacy and safety of these drugs for such purposes. In particular, efforts should be made to determine whether antiprogestins improve fertility as compared to other available regimens. As with many areas of antiprogestin research, substantial additional studies are also needed to elucidate the molecular mechanism of action of antiprogestins in the treatment of these diseases. Understanding the mechanism of action of these compounds is critical and might provide leads for future therapeutic uses. In particular, the noncompetitive "antiestrogenic" properties reported in some studies should be characterized, especially as they relate to defining potential long-term side effects of these therapies.

Recommendation No. 13. The committee recommends further studies to determine the minimal effective dose of mifepristone and other antiprogestins for the treatment of endometriosis and uterine leiomyomas. Measures of outcome should not be limited to pain relief alone, but should also address the likelihood of improving fertility. Once such studies are completed, randomized clinical trials should be undertaken to compare the safety and efficacy of mifepristone and other antiprogestins with current therapies for the treatment of endometriosis and uterine leiomyomas.

Recommendation No. 14. The committee recommends additional research to elucidate the antiestrogenic property of mifepristone and other antiprogestins. Research models should include endometrial cultures, explants, and in vivo systems. It is also important to clarify the molecular events involved and, in all such investigations, to characterize whenever possible the steroid-receptor status of the endometriotic and fibroid tissues under study.

Breast Cancer

Interest in the study of antiprogestins in breast cancer is understandable, given the known prognostic importance of the progesterone receptor in this disease and the current use of diverse endocrine interventions in its treatment. Approximately 30 percent all metastatic breast cancer patients, and 50 to 80 percent of the subset of patients whose tumors have estrogen or progesterone receptors, respond to a variety of endocrine interventions (e.g., drugs such as tamoxifen or progestins, or surgical removal of the ovaries for premenopausal women). Although such patients may improve in response to these treatments, the treatment results are relatively short-lived, lasting about 8 to 12 months. The antiprogestins have potential as growth inhibitory compounds against breast cancers. Whether this antitumor activity will be unique among the many other available endocrine therapies for breast cancer remains to be seen. In reviewing the literature, the committee concluded that too few clinical data are currently available to assess adequately the clinical potential of antiprogestins in the treatment of metastatic breast cancer, much less to assess their potential applications for adjuvant therapy (postoperative therapy undertaken when no detectable tumor is present to reduce the risk of recurrence) or chemoprevention (treatment of well women to lower the risk of initial development of cancer).

The exact mechanism by which antiprogestins exert their antitumor effect is unclear at present. However, data suggest that more than one mechanism exists. Although animal models can provide hypotheses, the biologic complexity and heterogeneity of breast cancer, and the limitations of these models, will require that many questions be addressed in basic research and in human clinical trials.

Recommendation No. 15. The committee recommends research to clarify the activity of antiprogestins in women with advanced (metastatic) breast cancer. Trials should be conducted by using more homogeneous groups of patients. Potential sources of heterogeneity should be reduced by including patients with only minimal prior therapy for their breast cancer and by performing pharmacokinetic evaluations to ensure consistent drug exposure and to facilitate concentration-response correlations. In these studies, tumor progesterone-receptor and estrogen-receptor status should be measured routinely.

Recommendation No. 16. Clinical trials of antiprogestins for treatment of breast cancer should include ancillary investigations to clarify mechanisms underlying the activity of antiprogestins. Examples of such studies include

a. histologic evaluation of tumor tissue before and after treatment

b. assessment of cell-cycle distribution before and after therapy by use of flow cytometry

c. assays of transforming growth factor (TGF_{β})induction and other potential indicators of cell differentiation

d. characterization of the progesterone receptor, including quantitation not only of total receptor content but also of A and B receptors and receptor mutants (Western blot technology currently exists for this analysis); and

e. assays for the expression of other growth factors such as TGF_{α} epidermal growth factor (EGF), and insulin-like growth factor (IGF), which may be modulated by antiprogestins.

Recommendation No. 17. The committee recommends that studies establish the maximum tolerated dose of antiprogestins for treatment of breast cancer. Further exploration of potential toxicity at various doses should be undertaken in additional Phase 1 clinical trials.

Recommendation No. 18. The committee recommends additional preclinical exploration of the molecular mechanisms of action of antiprogestins for treatment of breast cancer. Such studies should include

a. further characterization of progesterone receptors, including the natural A and B forms, as well as possible genetic mutants, and their distribution in normal and malignant tissue; this will be important for the rational use of antiprogestins in future clinical trials.

b. investigation of the use of antiprogestins as differentiating agents that produce programmed cell death (apoptosis)—a possible novel mechanism of action; and

c. exploration of the mechanism of tumor resistance to antiprogestins and other effects of long-term administration.

Meningioma

Meningiomas are tumors arising from the membranes surrounding the brain. Although they are not usually malignant in the sense of bringing about a patient's death through metastasis, the enlarging mass can be life threatening. The vast majority of meningiomas have progesterone receptors, though there is some controversy as to whether the progesterone receptors are functional in meningiomas.

The committee reviewed results of a small initial clinical trial using an antiprogestin, mifepristone, in patients with meningiomas. About 25 percent (6 of 24 patients) had some improvement in their disease. Toxicities associated with daily chronic administration of the drug appeared tolerable. Although mifepristone appears to have some activity in recurrent or unresectable (inoperable) meningiomas, its clinical importance for treating a disease with a highly variable natural history remains to be defined. However, the lack of good alternative treatments for this group of patients and the mechanistic rationale make this an attractive treatment for further study.

Recommendation No. 19. A randomized Phase 3 trial will be required to define the role of antiprogestins in the management of patients with unresectable meningioma. Such a trial is ongoing in the Southwest Oncology Group. The committee recommends that these data be reviewed carefully to define directions for further research on this disease.

ANTIGLUCOCORTICOID EFFECTS OF ANTIPROGESTINS

Glucocorticoids are steroid hormones that are produced by the adrenal glands and that have biological effects in virtually every system of the body. Like other steroids (e.g., estrogens, progestins), glucocorticoids exert their actions through specific receptors in target cells. All antiprogestins identified to date can bind to glucocorticoid receptors and exert some glucocorticoid antagonist activity (antiglucocorticoid effects). In the context of most uses of antiprogestins, these antiglucocorticoid effects are undesirable. There are, however, diseases that involve excess adrenal production of glucocorticoid hormones (generically termed Cushing's syndrome) for which drugs that have antiglucocorticoid activity would be highly desirable. Indeed, the committee reviewed the literature on the use of mifepristone in the treatment of Cushing's syndrome, and found the results promising. For most of the documented uses of the drugs as antiprogestins, their antiglucocorticoid actions were clearly viewed as undesired side effects. For obvious medical reasons, it would be preferable to have separate classes of pure antiprogestins and of pure antiglucocorticoids that do not display any other endocrine effects.

Recommendation No. 20. Antiglucocorticoid effects are an unwanted property of existing antiprogestins. Therefore, the committee recommends expanded efforts to produce pure antiprogestins that would not display any other endocrine effects at therapeutic doses.

1

Introduction

BACKGROUND AND HISTORICAL PERSPECTIVES

The human ovarian cycle is controlled by the gonadotropic hormones (the luteinizing hormone—LH—and the follicle-stimulating hormone—FSH) secreted by the pituitary gland, which are, in turn, under the control of a neuropeptide (gonadotropin-releasing hormone, GnRH) produced by the brain. The gonadotropic hormones direct follicular development, ovulation, the formation of the corpus luteum, and the secretion of estrogen and progesterone (the female sex hormones). These ovarian steroid hormones, in turn, inhibit the production of the gonadotropic hormones (negative feedback). If estrogen and progesterone are given in sufficient quantities they can completely block gonadotropin secretion, thereby arresting gonadal function. Historically, this observation is the physiological basis for the development of oral contraceptives that contain varying proportions of analogues of these ovarian steroids. Although it is now known that the estrogenic and progestational constituents of oral contraceptives may control fertility in many ways other than the inhibition of follicular development and ovulation, the fact remains that many details of their molecular actions are not yet clear. The desirability of finding precisely defined targets for the interruption of the reproductive process, with unambiguously understood consequences, has remained an elusive goal that continues to occupy the attention of many in the scientific and medical communities, and to a certain extent in the pharmaceutical industry (IOM, 1990).

The discovery that hormones bind to specific receptors before acting on their target cells provided new opportunities for circumscribed

contraceptive strategies. The notion that competition with the active hormone for its receptor by an inert or less active compound was anticipated by Segal and Thompson (1956) some 40 years ago when they demonstrated that the weak estrogen estriol inhibited the stimulatory action of estradiol-17β on the uterus of ovariectomized rats. The subsequent demonstration of the existence of estrogen receptors by Jensen and Jacobson (1962) in the late 1950s and the subsequent elucidation of the mechanisms of estrogen action led to the development of antiestrogenic compounds that block the effects of estradiol to varying degrees. Most of these, however, were not "pure" antiestrogens in that they possessed estrogenic properties as well. One such compound is currently used to stimulate ovulation in women, and there is still some debate about whether it acts as an estrogen or as an antiestrogen in this context.

The discovery that in the absence of progesterone, pregnancy cannot be initiated or maintained provided the basis for a massive effort designed to interfere with the normal functioning of the corpus luteum, the source of progesterone, during the luteal phase of the menstrual cycle and during early pregnancy. Because in sheep and a number of other mammals the normal regression of the corpus luteum is caused by uterine prostaglandin production, a variety of these compounds were used without success in attempts to induce luteolysis in primates. The physiological demise of the corpus luteum in humans is occasioned by quite different mechanisms that remain to be fully elucidated.

This experience underlines the belated recognition that seemingly fundamental reproductive processes in different mammalian species such as ovulation, the recognition and maintenance of pregnancy, and the initiation of labor, are governed by a variety of different control systems and that findings in one species cannot be extrapolated to others and particularly cannot be extrapolated to humans without rigorous verification.

Finding an Antiprogestin

Although attempts to interfere with the production of progesterone in humans have been without success, the recognition that progesterone, like all other steroids, binds to specific receptors as the first step in its action provided a more precise target for blocking the action of progesterone and thus preventing pregnancy. Some compounds that bind to receptors stimulate activity (agonists); other compounds bind to the receptor and inhibit the action of the hormone (antagonists). Compounds that are antagonists of progesterone—antiprogestins—formed the central theme of the Institute of Medicine (IOM) project presented in this report. Although most of the data reviewed by the committee were

related to one particular antiprogestin, mifepristone (RU 486, manufactured by Roussel-Uclaf, a French pharmaceutical company), there is published information on other antiprogestins as well. More than 400 compounds with possible antiprogestin activity have been synthesized. The main antiprogestins that have been studied to date, however, are listed in Table 1.1.

TABLE 1.1 Selected Antiprogestins: Name and in Vivo Testing

Generic Name	Company	Company Code Name	In Vivo Data Available
Mifepristone	Roussel-Uclaf	RU 486	Human, monkey, sheep, rabbit, dog, rat, mouse
Onapristone	Schering AG	ZK 98 299	Human, monkey, guinea pig, rabbit, rat, mouse
Lilopristone	Schering AG	ZK 98 734	Human, monkey, guinea pig, rabbit, rat, mouse
None	Schering AG	ZK 112 993	Monkey, guinea pig, rabbit, rat, mouse
None	Organon	ORG 31710, 31806	Monkey, rabbit, rat
None	Research Triangle Institute	HRP 2000	Rabbit

None of the antiprogestins are "pure" antiprogestins, having marked antiglucocorticoid properties as well, especially at higher doses. In addition, mifepristone and onapristone, and perhaps other antiprogestins, have as yet poorly understood antiestrogenic activity and, in some circumstances, progesterone-like actions. Mifepristone also appears to antagonize gonadotropin secretion directly at the level of the pituitary gland, an effect that, curiously, is reversed by the addition of progesterone. Furthermore, mifepristone has been reported to block ovulation and progesterone production directly at the ovarian level. While these non-antiprogestational actions of mifepristone may not have particular relevance to its current application as an abortifacient or post-coital contraceptive, they do emphasize the hazard of using mifepristone, or other antiprogestins with mixed activity, as probes in attempts to study the mode and mechanisms of action of progesterone.

Less studied than mifepristone are other antiprogestational compounds, produced primarily by Schering AG and Organon. Some of these are claimed to have greatly diminished antiglucocorticoid activity relative to mifepristone and to differ from mifepristone in not blocking ovulation in monkeys, while preventing pregnancy (Baulieu, Appendix B1; Van Look and von Hertzen, Appendix B12). It is not clear at present how extensively, if at all, these compounds will be subjected to clinical trials. For the future, a large number of new antiprogestins are known to be under development by Roussel-Uclaf, Schering, Organon, Research

Triangle Institute, and others, and these efforts may yield compounds with greater specificities as antiprogestins, with the goal of eventually obtaining a "pure" antiprogestin. The mechanisms of action and the structure/function relationships of antiprogestational and antiglucocorticoid compounds are discussed at length in papers included in Appendix B (Baulieu, Appendix B1; Weigel, Appendix B2).

THE IOM REPORT

During spring, 1993, the IOM convened an expert committee to assess current knowledge about the clinical uses of antiprogestins and to develop recommendations for research on antiprogestins in the United States. Political controversy has focused public attention on the use of antiprogestins to induce miscarriage; this committee, however, was specifically charged with considering the full spectrum of clinical applications of antiprogestins, not just their use for medical abortion.

The seven-member committee held a two-day invitational workshop in Washington, D.C., to hear from leading researchers in this field. Speakers were asked to prepare papers (1) highlighting the current state of the science, focusing on clinical and animal studies including their own research and cell studies as they are pertinent, and (2) identifying important and promising areas for future research. These papers are included in Appendix B. The speakers presented summaries of their papers during the workshop. After reading each paper and participating in the workshop (which included lively discussion among speakers, committee, and observers), the committee developed its conclusions and recommendations that appear in this report.

In this report the committee considers current information and research needed to advance knowledge about the following clinical applications of antiprogestins: intermittent or continuous use to alter the reproductive cycle (contraceptive use); use for post-coital contraception; inducing missed menses; pregnancy termination during the first trimester; pregnancy termination and cervical ripening during the second trimester; labor induction in late pregnancy; use as a therapy for endometriosis and uterine leiomyomas; and uses in breast cancer and meningioma therapy. In addition, uses of these compounds that take advantage of the antiglucocorticoid properties of the antiprogestins were considered.

CROSS-CUTTING RECOMMENDATIONS

Each chapter contains specific recommendations pertinent to that issue. The committee also had three recommendations that seemed to be cross-cutting issues for all the applications considered:

Recommendation No. 1. Research is needed to define the basic mechanisms and modes of action of mifepristone (RU 486) and other antiprogestins in order to understand the effects that have already been demonstrated and to develop compounds with more specific antiprogestin activity.

Recommendation No. 2. Because antiprogestins as a class have clear potential for preventive and therapeutic applications in human health beyond those that have already been documented, the committee strongly recommends additional clinical testing of mifepristone and of newer antiprogestins as they are developed.

Recommendation No. 3. Because some uses of antiprogestins may require long-term administration, the committee recommends studies to evaluate the potential toxicity, maintenance of efficacy, and development of drug resistance for antiprogestins used over longer periods of time.

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2

Uses of Antiprogestins: The Reproductive Cycle (Part I)

Progesterone is a steroid hormone produced by the ovary during the latter half of a normal menstrual cycle, and by the ovary and placenta during pregnancy. During the first half of a menstrual cycle (the follicular phase), estrogen is produced by a developing follicle. After ovulation (luteal phase), the follicle transforms to a corpus luteum, which produces both estrogen and progesterone. Each month, the cyclic fluctuations of estrogen and progesterone induce sequential, well-characterized changes in the lining of the uterus (endometrium).

If conception does not occur, the corpus luteum undergoes a process of regression. This regression is accompanied by declining levels of estrogen and progesterone, which in turn precipitate shedding of the endometrium; this shedding is experienced as a woman's monthly menstrual bleeding, or menses. If conception occurs, the corpus luteum continues to produce estrogen and progesterone under the influence of a hormone from the developing embryo (human chorionic gonadotropin), and endometrial shedding is prevented.

Because of the complex hormonal interplay that characterizes the female reproductive cycle and the role that progesterone plays in initiating and maintaining pregnancy, it is clear that antiprogestins could alter many of these functions. As a pharmacologic class, the antiprogestins appear to have great promise as regulators of reproductive potential. Data in humans, however, are limited with respect to most of the possible contraceptive applications, and the data that exist are largely, if not exclusively, confined to mifepristone (RU 486). Therefore, many of the committee recommendations for research on

clinical potential and mechanisms of action must be based on results from limited human studies that use one specific antiprogestin.

CONTRACEPTION

Existing oral contraceptives (synthetic estrogen-progestin combinations, as well as progestin alone) are available and efficacious. Nonetheless, contraceptives based on new approaches (hormonal, antihormonal, or nonhormonal) would be welcome, because there remain some populations for whom use of oral contraceptives is contraindicated, and because not all women comply fully with the various regimens of pill-taking. New methods that are easier to use consistently and properly, and that preserve the beneficial effects of estrogen for women's health while minimizing any possible side effects would be of enormous social value. With regard to possible side effects, for example, currently used estrogen-progestin combination oral contraceptives are undergoing continuing epidemiologic study because of their possible role in increasing the risk of early-onset breast cancer (IOM, 1991). Other possible side effects for which questions persist include alteration in lipid profiles and, for older women who smoke, cardiovascular effects as well as possible thromboembolic events. Even the concern about such effects, whether or not they exist, means that new contraceptive formulations that are free of these worries would be very favorably received.

Antiprogestins present a new pharmacologic approach to contraception. Any such application of mifepristone or other antiprogestins would, of course, require testing in large-scale clinical trials to evaluate formally their efficacy and safety for use as low-dose contraceptives.

The committee reviewed a number of studies on the potential contraceptive effectiveness of mifepristone when given during the follicular, periovulatory, and luteal phases of the cycle (see Baird, Appendix B4; Baulieu, Appendix B1; Van Look and von Hertzen, Appendix B12; and Spitz and Bardin, in press). In these studies, administration of the antiprogestin varied; in some cases the drug was given continuously for various periods of time; in other cases it was given intermittently (once weekly or monthly for several days); and in some studies it was given in combination with other agents such as progestins or prostaglandins.

In general, single-dose or short-term administration on an intermittent, as opposed to a continuous basis, has limitations. Administration during the follicular phase merely delays follicular growth and ovulation. Early luteal phase administration delays the development of a secretory endometrium but does not affect the corpus luteum. Timing of such early luteal phase administration must be linked to ovulation,

which is not easy to achieve. Late luteal phase administration will suppress the corpus luteum and cause bleeding, but it appears to require prostaglandins as well as the antiprogestin for high effectiveness.

There are no data on the contraceptive efficacy or safety of continuous long-term administration of low-dose antiprogestins. Of interest, however, are data derived from a number of studies using mifepristone to treat endometriosis, leiomyomas, meningiomas, and Cushing's syndrome. These studies suggest that long-term use of this compound would inhibit ovulation and induce consistent amenorrhea (Yen, Appendix B8; Nieman, Appendix B10). In one study reported at the IOM workshop, with perhaps the longest continuous use of mifepristone, 15 women with meningiomas (3 of whom were premenopausal and 12 postmenopausal) were treated for meningiomas with 200 mg daily for six months to five years (Grunberg et al., 1991a, b). During the study, the three premenopausal women became amenorrheic. Toxicity experienced by these women was minimal; reported side effects included fatigue, hot flashes, breast tenderness, thinning of hair, and rash. At low doses (less than 25 mg/day) used for up to six months to treat subjects with uterine leiomyomas, the antiglucocorticoid activities of the compound were not manifest (Yen, Appendix B8), even though doses as low as 5 mg/day were effective at inhibiting ovulation. These data substantiate the fact that mifepristone is more potent as an antiprogestin than as an antiglucocorticoid; however, pure antiprogestins without any associated antiglucocorticoid activity would remain preferable for contraceptive purposes.

In studies to evaluate potential contraceptive efficacy, experience with continuous low-dose mifepristone appears to be limited to only 30 days of treatment and doses of 2 to 10 mg/day of mifepristone (see Baird, Appendix B4; Ledger et al., 1992; Croxatto et al., 1993). The 2-mg/day dose consistently suppressed ovulation; a 1-mg dose did not. A possible advantage of continuous, low-dose mifepristone administration is that it appears to "spare" estrogen despite the inhibition of ovulation. Serum estrogen levels similar to those seen in late follicular phase were noted in these studies (see Baird, Appendix B4, and Croxatto et al., 1993, referenced therein), suggesting that such estrogen-affected factors as bone density, lipids, and sense of well-being should be well maintained. However, the few samples of endometrial tissue that have been obtained from women using mifepristone do not show proliferative effects, nor do they show a progestin-induced secretory effect. If changes in the endometrium caused by mifepristone are sufficient to prevent implantation, then a dose lower than the 2 mg daily required to suppress ovulation may be adequate. Endometrial changes associated with mifepristone require further study. Evaluation of possible ovarian follicular cysts, which have been reported to occur, and the recognized antiglucocorticoid effects will also require study.

Recommendation No. 4. Clinical research should be undertaken promptly to evaluate the efficacy and safety of mifepristone and other antiprogestins as low-dose contraceptives. Such research should address, among other issues,

- **a. mechanisms of contraceptive action**
- **b. the effectiveness of various regimens in preventing pregnancy (e.g., continuous versus cyclic administration, possibly with the addition of other hormones)**
- **c. the lowest effective dose to prevent pregnancy for each regimen**
- **d. potential short- and long-term toxicities affecting bone (osteoporosis), lipids (alteration in profile), endometrium (histologic changes), ovary (cyst formation), and brain (mood); and**
- **e. benefits and risks, both contraceptive and noncontraceptive, of antiprogestin contraception relative to other hormonal contraception.**

POST-COITAL CONTRACEPTION

A post-coital contraceptive is a pill or other method that can be used to reduce the chance of undesired pregnancy after unprotected intercourse around the probable time of ovulation (midcycle). Post-coital contraception is frequently desired after unplanned or unwanted sexual intercourse, or after a contraceptive failure such as a skipped pill, slipped diaphragm, or a loose or broken condom. In the United States, the most common post-coital approach (commonly called the "morning-after pill") involves using an oral contraceptive containing both a synthetic estrogen and a progestin. Although this regimen is not 100 percent efficacious, oral contraceptives reduce the risk of pregnancy by at least 75 percent after unprotected midcycle sexual intercourse (Trussell and Stewart, 1992). The treatment schedule is one dose (100 µg of ethinyl estradiol and 1.0 mg of *dl*-norgestrel) as soon as possible (beginning no more than 72 hours after unprotected intercourse), and a second dose 12 hours after the first. The total regimen is therefore 200 µg of ethinyl estradiol and 2.0 mg of *dl*-norgestrel. Because of the relatively high dose of estrogen, this method has a high frequency of estrogen-related side effects such as nausea and vomiting, and a 25 percent failure rate is undesirable.

A more recently studied experimental post-coital therapy consists of three 200-mg tablets of danazol taken within 72 hours of unprotected intercourse and repeated 12 hours later. Danazol, a synthetic steroid used in the treatment of endometriosis, lowers the estrogen level and so produces a lower incidence of nausea, vomiting, and breast tenderness

when compared to oral contraceptives in post-coital administration (Rowlands et al., 1983; Zuliani et al., 1990; Webb et al., 1992). However, the efficacy of danazol is not uniform, and it has a variety of undesired side effects with sustained use.

Two studies have been reported on the use of mifepristone as a post-coital contraceptive (Glasier et al., 1992; Webb et al., 1992). In these studies, mifepristone was better than combined oral contraceptives or danazol. In a British randomized trial involving all three regimens, women assigned to mifepristone and danazol experienced much lower incidences of nausea and vomiting; none of 195 women assigned to mifepristone became pregnant, compared with 2.6 percent and 4.7 percent of those receiving combined oral contraceptives and danazol, respectively (Webb et al., 1992). In a larger Scottish trial, 800 women were randomly assigned to use either mifepristone or the combined oral contraceptives regimen. Although 23 pregnancies were expected in each group had no interference been made, there were only 4 pregnancies in the group receiving combined oral contraceptives, and there were no pregnancies in the group receiving mifepristone alone. Rates of nausea, vomiting, headache, and breast tenderness were significantly lower in the group receiving mifepristone (Glasier et al., 1992). In both trials, a significantly greater proportion of women receiving mifepristone experienced a delay in the onset of the following menses.

The results of these studies indicate that the mifepristone regimen is potentially preferable to existing therapies in that it has higher efficacy and fewer side effects (nausea and vomiting). A significant advantage of mifepristone is that only a single 600-mg dose is required within 72 hours of unprotected intercourse; compliance in taking a second dose is therefore not an issue. Delay of menses by a few days following post-coital mifepristone should not be a serious disadvantage if women are informed that this delay may occur.

Recommendation No. 5. The committee recommends expeditious submission to the U.S. Food and Drug Administration of existing clinical trial data on the use of mifepristone as a post-coital contraceptive to determine whether these data meet current U.S. regulatory requirements.

MENSES INDUCTION

Studies have evaluated the use of mifepristone to induce menses (e.g., after an undesired mid-cycle exposure) when administered within one week following the day of the expected onset of a menstrual period. Once-a-month administration at the end of the luteal phase of each menstrual cycle has also been studied (Baulieu, Appendix B1; Van Look

and von Hertzen, Appendix B12; Baird, Appendix B4). The major problem with both of these approaches has been that mifepristone alone does not consistently induce menstruation, and administering prostaglandin two days after the mifepristone is required for consistently successful results. In the few small studies reported, pregnancy rates on the order of 20 percent were seen either when mifepristone was used before the day of expected menses or when mifepristone alone was used to induce abortion within 10 days of missed menses (Baird, Appendix B4, and references therein). These studies suggest the need for prostaglandin to increase efficacy. This need for a second agent makes the regimen less convenient from the patient's point of view, possibly reducing compliance. Research on new methods to deliver antiprogestins and prostaglandins is warranted. These methods of delivery might include vaginal suppositories, intramuscular administration, cutaneous patches, or sustained-release capsules. Given that a more specific antiprogestin compound would be expected to induce menses without any additional treatment, research that results in the development of such a compound would be particularly useful for menses induction.

Recommendation No. 6. Research is needed to develop the best means for delivering combinations of antiprogestins and prostaglandins for menses induction and regulation, ranging from sequential oral doses of each component, to new ways of providing both drugs simultaneously with delayed release of the prostaglandin. Studies of compliance with these various approaches are needed, especially for sequential drug delivery.

Recommendation No. 7. The committee recommends the development and evaluation of new antiprogestins that may in themselves induce menses without the need to add prostaglandin.

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3

Uses of Antiprogestins: The Reproductive Cycle (Part II)

PREGNANCY TERMINATION DURING THE FIRST TRIMESTER

The need for ample progesterone production by the ovarian corpus luteum to maintain early pregnancy is well established. In fact, low progesterone secretion in the luteal phase has been implicated in habitual abortion (Giudice et al., 1989). It was, therefore, logical to hypothesize that antiprogestins given during early pregnancy might act as abortifacients, and thus provide a medical alternative to current techniques of surgical abortion. On the basis of successful animal experiments (Baulieu, Appendix B1), mifepristone (RU 486) was initially tested as an efficient medical means of early pregnancy termination. The first studies in humans were successfully performed by Herrmann et al. (1982) in Geneva, documenting the ability of mifepristone alone to interrupt early pregnancy. These results were confirmed in a dose-finding study conducted under the auspices of the World Health Organization (Bygdeman, Appendix B5). Two conclusions could be drawn from these early studies. First, the frequency of successful complete abortion using mifepristone decreased with advancing age of the fetus, with approximately a 60 to 70 percent incidence of complete abortion up to eight weeks. Second, there appeared to be no relationship between the success rate and the treatment regime employed for women at the same stage of gestation.

In an attempt to interrupt early gestation more effectively, several groups have used different prostaglandin preparations in combination with mifepristone. In the first study with combined therapy, 25 mg of

mifepristone were given twice daily for three to six days accompanied by 0.25 mg sulprostone, an injectable prostaglandin (PGE_2) analogue (Schering AG, Berlin), on the last day. With this combined regime, the overall frequency of complete abortion was 94 percent (Bygdeman and Swahn, 1985).

Subsequent studies (Bygdeman, Appendix B5) reported successful abortion rates of between 95 and 100 percent when mifepristone was combined with vaginal administration of 0.5 to 1 mg gemeprost, a vaginal pessary prostaglandin (PGE_1) analogue. These high success rates for complete abortion led to the French approval in September 1988 of mifepristone used in conjunction with prostaglandin administration for pregnancies up to 49 days of amenorrhea as calculated from the last menstrual period.

In Great Britain, successful trials were conducted using gemeprost up to 63 days of amenorrhea. This protocol for medical interruption of pregnancy was approved in Great Britain in July 1991, and subsequently in Sweden in 1992, using the same vaginal prostaglandin protocol. The French have reported the largest experience, treating more than 2,000 women with up to 49 days of amenorrhea with a single 600-mg dose of mifepristone followed 36 to 48 hours later by the administration of either gemeprost (1 mg by vaginal suppository) or sulprostone (0.25 to 0.5 mg by intramuscular injection). In a study reported by Silvestre et al. (1990), the overall efficacy rate was 96 percent, with 1 percent continuing pregnancies, 2.1 percent incomplete expulsions, and 0.9 percent required dilation and curettage (D&C). Only one woman required blood transfusion.

The procedure is not only highly efficient, but it is generally acceptable to women. The clinical events of the mifepristone-gemeprost protocol are quite similar to those of a spontaneous abortion with bleeding and increased uterine contractility. About 50 percent of patients have begun to bleed at the time of prostaglandin treatment, and virtually all bleed within four hours after the administration of prostaglandin, with a mean duration of bleeding of eight days (Bygdeman, Appendix B5). Uterine pain is common, especially in the first few hours following prostaglandin treatment, with approximately 30 percent of women requiring an analgesic and another 30 percent requiring a narcotic.

In a large study in France, serious cardiovascular side effects following prostaglandin administration (sulprostone injection) were reported in 4 out of 16,000 women treated after sulprostone injection (Ulmann et al., 1992). These included one acute myocardial infarction and three cases of severe hypertension. By now, over 60,000 women have used RU 486 for abortions. Two more myocardial infarctions have occurred, one of which was fatal. The overall frequency of severe cardiac complications

after sulprostone is approximately one in 20,000 cases. So far, myocardial infarction has not been reported as a complication of vaginal gemeprost use, but given the French experience with sulprostone, Roussel-Uclaf recommends that mifepristone plus any prostaglandin not be used in women who smoke more than 10 cigarettes per day, who are older than 35, or who have any other cardiovascular risks (Bygdeman, Appendix B5).

In France, patients must agree to a surgical termination of pregnancy if the medical therapy is not successful. The six children born after unsuccessful therapy with mifepristone are reported to be normal (Ulmann, IOM workshop). However, there is conflicting evidence about the teratogenicity of the mifepristone-prostaglandin regimen (Spitz and Bardin, in press), resulting in the current recommendation of surgical termination in cases of a failed medical procedure.

The ideal combination of an antiprogestin and prostaglandin remains to be established. Certainly, oral administration of prostaglandin may be advantageous for patient convenience. An oral preparation, misoprostol (G.D. Searle & Co., Chicago), is licensed for sale in many countries as a treatment for gastric and duodenal ulcers. Although one patient death was reported during the first trial of mifepristone plus misoprostol (Aubeny and Baulieu, 1991), a much larger study has documented the safety of this protocol. In the latter, 600 mg of mifepristone, plus 400 µg (two tablets) of misoprostol administered 48 hours later, were given to 895 women (Peyron et al., 1993). Approximately 70 percent of women had completely expelled the conceptus four hours after misoprostol ingestion. If expulsion did not occur, a third misoprostol tablet was given. This increased the efficacy to greater than 98 percent. No adverse treatment events were recorded. In addition, uterine cramping or discomfort was reported to be far less than that experienced with injectable or vaginal prostaglandins, with only 16 percent of patients requiring analgesia and 0.1 percent requiring narcotics.

A recent dose-finding study using 200, 400, and 600 mg of mifepristone suggests that doses as low as 200 mg are equally efficacious in inducing first-trimester abortion (Van Look et al., in press). The lowest effective dose of mifepristone in conjunction with prostaglandin is as yet unknown; however, the committee believes that a dose lower than 600 mg is effective.

The cardiovascular side effects in healthy nonsmoking women under the age of 35 appear to be minimal. The additional oral prostaglandin apparently results in a less painful but equally efficacious procedure compared to other prostaglandin preparations, with minimal bleeding and a failure rate of less than 2 percent.

Drug regulatory officials in France, Sweden, and the United Kingdom have judged that the use of antiprogestins in combination with prostaglandin is a safe and efficacious medical treatment for early pregnancy

termination. There is now a vast world literature with more than 60,000 patients treated in France. Repetition of large Phase 3 trials in the United States (to demonstrate efficacy and document side effects) does not appear necessary.

Recommendation No. 8. The committee recommends expeditious submission to the U.S. Food and Drug Administration of all existing clinical trial data on mifepristone and prostaglandin for early pregnancy termination to determine whether these data meet current U.S. regulatory requirements.

Recommendation No. 9. The committee recommends that in considering the use of mifepristone and prostaglandin for early pregnancy termination in women who smoke more than 10 cigarettes per day or are over 35 (two groups of women who were excluded from European studies), the documented risks must be compared with the risks of continuing pregnancy or of surgical termination of pregnancy. Furthermore, such assessment should attempt to distinguish between the risks attributable to mifepristone and those attributable to prostaglandin.

Health Services Research

At this point, the major research need in using antiprogestin for pregnancy termination is not in efficacy, but rather in health services research. The medical regimens appear to be safe and efficacious, as demonstrated by experience in France, Great Britain, and Sweden. However, the United States has a very different health care system in which access to reproductive health care is a major problem. Research can help clarify how to increase access to a medical program for terminating pregnancy, as well as provide benefit-cost analyses comparing the medical procedure to surgical termination of pregnancy. Another area of research includes the number of visits required to oversee this regimen safely. In France, pregnancies are terminated only in legally authorized centers and under a Roussel-Uclaf protocol requiring that medical termination occur before 49 days of amenorrhea. The protocol is administered during four required visits to the center. An initial visit documents pregnancy and educates the patient about the procedure; at a second visit, 600 mg of mifepristone is given. Thirty-six to forty-eight hours later, the woman returns to the clinic, and prostaglandin is administered either vaginally or orally. Patients are monitored as inpatients for at least four hours on the day of prostaglandin administration. They return for a fourth, follow-up visit 8 to 12 days

after mifepristone administration (Bygdeman, Appendix B5). The apparent safety and efficacy of orally administered misoprostol (which does not require either parenteral administration or refrigeration) raise the possibility of fewer visits for this medical procedure (see also Grimes, Appendix B7). Advantages of this medical procedure are decreased cost (in Sweden, providers are reimbursed $600 for a surgical abortion and $300 for a medical abortion; Bygdeman, IOM workshop), as well as an increased sense of patient privacy and autonomy during the procedure. The safety of decreasing the number of patient visits to the physician must be evaluated.

In the United States, patient acceptability and access are major issues. Although the medical antiprogestin regimen (as distinct from surgical protocols) has found widespread patient acceptability in Europe (David, 1992; Winikoff et al., 1992), the committee suggests additional studies to determine appropriate protocols for this country, which may vary from those used elsewhere. Such research should help to define the optimal number of visits, appropriate doses and delivery route, and patient acceptability and access, including how best to ensure equal access for U.S. women of all economic and social classes.

Recommendation No. 10. With respect to using mifepristone for first-trimester termination of pregnancy, health services research should be conducted in the United States to determine which approaches (e.g., required number of visits, type of health care provider administering the drugs, site of service delivery) are most suitable from the standpoint of safety, efficacy, accessibility, and acceptability.

The committee does not recommend that consideration of a New Drug Application by the Food and Drug Administration for the use of mifepristone for pregnancy termination be delayed until the research outlined in Recommendations Nos. 9 and 10 has been completed. Recommendation No. 9 addresses a group of potential patients excluded from previous trials. Recommendation No. 10 relates to the appropriate setting for use of the drug combination, reflecting the committee's concern that the imposed criteria of four medical visits, as set by protocols from the innovator-manufacturer, may not be necessary or acceptable to patients in the United States.

PREGNANCY TERMINATION DURING THE SECOND TRIMESTER

Although the specific mechanisms of human labor and delivery are not fully understood, several methods have been examined for inducing

labor during the second and third trimesters of pregnancy. A variety of prostaglandins have been shown to induce uterine contractions in both second- and third-trimester pregnancies. The experience with mifepristone in first-trimester pregnancy termination suggested that mifepristone treatment sensitizes the myometrium to the action of prostaglandins, thereby reducing the amount of prostaglandin necessary to induce expulsion. This led to the hypothesis that antiprogestins might be useful in termination of pregnancies during the second and third trimesters as well.

Currently, second-trimester pregnancy termination is available in the United States by dilation and evacuation (D&E), by intra-amniotic saline injection, intra-amniotic prostaglandin $F_{2\alpha}$, or prostaglandin E_2 vaginal suppositories. These procedures are used for elective pregnancy termination in the second trimester, including termination of pregnancies where fetal genetic or structural abnormalities have been documented. Prostaglandin termination of second-trimester pregnancies requires two to three days of inpatient hospital stay with significant discomfort. The alternative surgical procedure of D&E, although widely used, requires highly skilled technical ability, which may not be available in all clinical settings. A series of clinical trials has been performed to study the efficacy of mifepristone treatment before prostaglandin administration for second-trimester pregnancy termination to determine whether smaller doses of prostaglandin following mifepristone are as effective as larger doses of prostaglandin alone. All of these studies (Ulmann and Silvestre, Appendix B6) have suggested that treatment with mifepristone before prostaglandin, whether gemeprost, sulprostone, or PGE_2, decreases significantly the level of prostaglandin needed to complete an abortion and shortens the time interval from administration to abortion. Given the multiday hospital admission required for a second-trimester prostaglandin abortion, as well as the high level of discomfort for the woman, the ability of antiprogestins to shorten the duration of hospitalization by one day would be significant. Other studies comparing the effects of antiprogestins with those of laminaria tents (Dilapan®) in gemeprost-induced second-trimester abortion documented that the use of mifepristone resulted in a significantly shorter induction-to-abortion interval than did the Dilapan (Thong and Baird, 1992).

Recommendation No. 11. With regard to second-trimester abortion, the committee recommends conducting clinical trials in the United States to compare the established surgical procedure of dilation and evacuation (D&E) both to antiprogestins in combination with prostaglandins and to prostaglandins used alone. Such trials should clarify the optimal dose of antiprogestin and prostaglandin for this use,

and should assess relative pain, interval to fetal expulsion, blood loss, and frequency of infection, uterine perforation, and incomplete expulsion requiring surgical intervention.

CERVICAL RIPENING

It appears that antiprogestins not only sensitize the myometrium to subsequent prostaglandin installation but also help to ripen the cervix. This property may help to manage clinical situations such as (1) preparation for second-trimester abortion, (2) preparation for labor induction at term or post-term, and (3) preparation of the cervix when labor must be induced because of intrauterine fetal demise.

Animal studies have demonstrated that mifepristone matures and ripens the cervix, as measured by an increase in cervical diameter and decrease in cervical resistance to mechanical dilation (Ulmann and Silvestre, Appendix B6). This effect occurs through the molecular mechanisms that characterize the normal cervical-ripening process, including increase in water and hyaluronic acid content as well as collagenase activation. Several studies documenting the ability of antiprogestins to induce cervical maturation and ripening before vacuum aspiration have shown a significant effect 24 to 48 hours after mifepristone administration (Ulmann and Silvestre, Appendix B6). A dose-finding Canadian study using placebo or 100, 200, 400, or 600 mg of mifepristone as a single dose, administered 24 or 48 hours before cervical calibration, showed a significant increase in cervical diameter that was linearly related to dose up to 400 mg (Lefebvre et al., 1990). Patients treated with mifepristone had either an equivalent or a lower blood loss after vacuum aspiration than those treated with a placebo (Ulmann and Silvestre, Appendix B6). Thus, mifepristone in doses up to 600 mg appears to be efficacious in ripening and dilating the second-trimester cervix before D&E, as well as in decreasing treatment to expulsion time in nonsurgical protocols when used in conjunction with prostaglandin.

It should be noted that prostaglandin alone in an intravaginal formulation is approved by the Food and Drug Administration for the indication of cervical ripening. Studies will be needed to determine the relative efficacy of mifepristone.

Because of the discomfort associated with either elective termination of second-trimester pregnancy or the termination of the genetically abnormal or dead fetus, the ability of antiprogestins to shorten the therapeutic process is attractive. Overall, the antiprogestins in the second trimester are well tolerated, with the most frequently reported side effects being abdominal pain, nausea, and vomiting. All second-trimester pregnancy terminations carry the risk of bleeding and the

potential need for surgical intervention. The use of antiprogestins given 36 to 48 hours before either a surgical procedure or prostaglandin installation appears to be well tolerated without the addition of significant clinical complications.

Recent advances in molecular biology and genetic, karyotypic diagnosis, as well as our increased ability to diagnose early structural abnormalities with ultrasonography, will inevitably increase the number of abnormal second-trimester gestations identified. The emotional stress of terminating such a pregnancy is great, as is the case of fetal demise, and therapeutic modalities to reduce both the time necessary for such a procedure and the discomfort involved merit further investigation.

LABOR INDUCTION IN LATE PREGNANCY

In addition to its possible use in cervical ripening in preparation for labor induction, mifepristone may also be useful in inducing labor, for instance in cases of intrauterine fetal demise and during late pregnancy when the medical complications require prompt delivery.

Fetal Demise

Fetal demise is an unfortunate occurrence in both second- and third-trimester pregnancies, and requires evacuation of the uterus. Studies have evaluated mifepristone's ability to initiate labor in cases of fetal demise. In the study of Cabrol et al. (1990), 600 mg of mifepristone, given on two consecutive days after diagnosis of fetal death, caused the initiation of labor within 72 hours in 63 percent of women, compared to spontaneous initiation of labor in 17 percent of the placebo-treated women. Thus, it appears that mifepristone alone is able to induce labor in patients with intrauterine fetal demise and that expulsion takes place significantly earlier than in placebo-treated patients.

Studies in ewes and monkeys have indicated that, at term, antiprogestins induce uterine contractions and enhance the myometrial sensitivity to oxytocin. Newborn monkeys of mothers treated with antiprogestins were normal and, in fact, grew more rapidly than did newborns from untreated mothers (Wolff et al., 1989). This is thought to be an effect of increased milk output in primate mothers treated with antiprogestins and is believed to be secondary to abrogation of the suppressive effect of progesterone on prolactin secretion. An initial study in humans by Frydman et al. (1992) evaluated the efficacy and safety of mifepristone for induction of labor in post-term pregnancies or in term pregnancies when other medical indications for labor induction were present. This placebo-controlled study used 200 mg of mifepristone daily for two consecutive days and showed a marked increase in the number of

women with spontaneous onset of labor. Of those women receiving mifepristone, 54 percent had spontaneous onset of labor, compared with only 18.2 percent of those given a placebo. The interval between day 1 of treatment and the onset of labor for patients given mifepristone was 51.7 hours; for those on placebo it was 74.5 hours. In addition, the total dose of oxytocin required in mifepristone-treated patients was significantly lower than in those treated with placebo, although the cesarean section rate was equivalent. The evaluation of newborns by Apgar scores and measurement of umbilical vein pH levels showed no difference in outcome between mifepristone and placebo-treated patients. The authors concluded that mifepristone appeared to be a safe, efficient, and suitable induction agent for initiation of labor in women at term (Frydman et al., 1992).

It would be valuable in clinical practice to initiate labor more easily in post-term patients, as well as in women with medical conditions requiring relatively immediate (within two to four days) delivery. As mifepristone has been, and presumably will continue to be, given to women in late gestation, the opportunity to study any effects should be used. Prudence and caution dictate follow-up of infants born when labor is induced using antiprogestins. Assessments should include pulmonary, cardiac, and adrenal status of neonates as well as their later development and fertility potential. In addition, the early initiation of abundant lactation reported in the primate model suggests the need for studies in women to determine the impact of antiprogestins on lactation. The committee encourages dose-finding studies to determine the minimal dosage of antiprogestin necessary to induce labor.

Recommendation No. 12. The committee recommends studies to determine the minimal dose of antiprogestins necessary to induce labor. Studies in animal models (most likely the primates) should assess possible adverse outcomes on infants. Research is also needed to determine the effect of antiprogestins on maternal lactation.

SUMMARY

Mifepristone is currently used with government approval in France, the United Kingdom, and Sweden. There are a variety of applications during pregnancy ranging from early first-trimester pregnancy termination to termination during second-trimester and even third-trimester labor induction (e.g., preeclampsia, post-term pregnancy, fetal demise). The application of mifepristone for cervical ripening at term, and for labor induction in late pregnancy when medical complications require it, has been tested and shows promise. Mifepristone appears to be effica

cious and safe and to add valuable alternatives to the obstetrical armamentarium. Studies to determine optimal doses, administration regimens, and the incidence of complications or side effects, as well as benefit-cost analyses, are warranted. The therapeutic options offered by antiprogestins have the potential to enhance clinical care during pregnancy.

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4

Other Therapeutic Uses of Antiprogestins

ENDOMETRIOSIS

Endometriosis is a non-malignant, albeit progressive, disease attributable to the presence of endometrial glands and stroma (supporting connective tissue) in ectopic locations (outside the endometrium of the uterus) most commonly within the pelvic cavity. Although multiple pathophysiologic mechanisms underlying the genesis of endometriosis have been proposed, the precise etiology of the disease remains unknown.

Hard numbers on the actual prevalence of endometriosis are not available, and because diagnosis is frequently difficult and not regularly reported, the estimates of prevalence constitute approximations at best. Some afflicted patients are diagnosed incidentally during laparoscopy or exploratory laparotomy for a variety of other indications. Some investigators have reported that endometriosis may be detected in 5 to 15 percent of abdominal laparotomies performed on reproductive-age females. That incidence may increase to 30 to 40 percent in the infertile population.

The most typical symptomatic complaints associated with endometriosis include chronic pelvic pain, dysmenorrhea (painful menses), dyspareunia (painful intercourse), and infertility. However, not all patients with endometriosis are symptomatic. Moreover, correlation between the severity of this disease and the manifestation of symptomatology is far from absolute. Pelvic examination may reveal evidence of active endometriosis, provided the disease has extended to the lower pelvis, most importantly to the uterosacral ligaments. Alternatively, the presence of

large ovarian cysts, also known as endometriomas, may be detected. Sonographic assessment of the latter may enhance the clinician's diagnostic suspicion. In the final analysis, however, the diagnosis of endometriosis requires surgery and is a histologic diagnosis complemented by direct observation of the typical lesion through laparoscopy or laparotomy.

Unanswered questions about the precise etiology of endometriosis notwithstanding, there is little doubt about the hormonal responsiveness of ectopic endometrial tissue. In general, estrogen deprivation, progestin excess, and androgen excess appear to have a favorable, albeit reversible, impact on the disease. Although regression of the lesions in many, but not all, patients can be documented when such regimens are used, the effect is always palliative, not curative. By using these findings therapeutic strategies over the last 30 years have made use of the progestational effect of high-dose combination oral contraceptives to produce pseudopregnancy, the apparent antiendometrial effect of testosterone, the progestational-androgenic effect of danazol, and the hypoestrogenic impact of gonadotropin-releasing hormone (GnRH) agonists to produce pseudomenopause. Surgical therapy has always constituted a component of the treatment regimen, particularly when endometriosis is associated with infertility. Here, too, only palliation can be anticipated. Moreover, significant concerns currently exist as to the very utility of this approach in light of the iatrogenic insult acquired in the process. Clearly then, long-term prevention may well be the best strategy for individuals with known early disease.

Utility of Mifepristone (RU 486) for Treatment of Endometriosis

The first suggestion about the utility of mifepristone to treat endometriosis can be traced to an article by van Uem et al. (1989), who observed that the administration of mifepristone to female monkeys treated with human menopausal gonadotropin and human chorionic gonadotropin resulted in the genesis of an atrophic to weakly proliferative endometrium on menstrual cycle day 25, despite serum estradiol concentrations in excess of 300 pg/ml. These effects were observed whether mifepristone was begun on cycle day 3 or 7. Thus, when administered daily in early or midfollicular phase at a dose of 10 mg/kg, mifepristone elicited a persistent retardation of early proliferative endometrium. Apparently it antagonized the mitogenic effects of estrogens. This observation prompted the investigators to suggest that mifepristone might exhibit noncompetitive antiestrogenic activity.

To elaborate on this action of mifepristone, Wolf and associates (1989) examined its action in estradiol-treated monkeys. Use of mifepristone

alone at a dose of 1 mg/kg was associated with the induction of a secretory endometrium, but higher doses (5 mg/kg) inhibited both proliferation and secretory activity. Thus, in the absence of progesterone, the actions of mifepristone were biphasic in nature. This mifepristone action appears to be associated with an increase in the estrogen-receptor content of the relevant endometrium, a dose-dependent phenomenon (Neulen et al., 1990). However, the latter observation may be affected to some degree by the choice of the denominator—that is, protein versus cellular DNA content.

More recently, the effects of mifepristone were examined using rats in which endometriosis had been experimentally induced (Tjaden et al., 1993). Animals were treated daily with mifepristone (10 mg/kg body weight subcutaneously) for two, four, six, or eight weeks. No regression of endometriosis was observed. The precise reasons for this apparent species/model specificity remain unknown.

Stimulated by the preceding observations, Kettel and associates (1991) reported the first relevant (though preliminary) human study on the endocrine response to long-term administration of mifepristone to patients with endometriosis. Six women who had normal menstrual cycles and who had endometriosis were recruited for the study, and received mifepristone at a dose of 100 mg/day for three months. Importantly, this regimen produced uniform amenorrhea. The mean circulating levels of luteinizing hormone (LH) and the LH pulse amplitude (but not frequency) were increased. An antiglucocorticoid effect was apparent as assessed by an increase in the circulating levels of cortisol and adrenocorticotropic hormone (ACTH). Pelvic pain was reportedly lessened in all subjects, but there was no significant change in the extent of the disease as evaluated by follow-up laparoscopy.

Unpublished observations reported by Yen at the Institute of Medicine workshop addressed the administration of mifepristone for a period of six months. Nine women with symptomatic endometriosis, who had no improvement with other forms of medical therapy, were recruited and treated with 50 mg/day mifepristone. Again, amenorrhea was uniformly induced. Likewise, all subjects reported a significant decrease in pelvic pain and dysmenorrhea. American Fertility Society (AFS) scores, a measure of the extent of disease, improved significantly in eight of nine subjects. Bone mineral density measurements of the lumbar spine and femur revealed no adverse effect. An initial increase in the circulating levels of LH and testosterone was noted in the first month of treatment. The levels of estradiol remained in midfollicular phase range. No antiglucocorticoid effect was noted. Side effects included transient, mild increases in liver transaminase (20 percent of patients), which returned to normal after one month. A follow-up study using a lower dose of 5 mg/day of mifepristone administered for six

months is ongoing. Early results in this study appear promising. It is possible, then, that doses of mifepristone as low as 5 mg/day may prove effective in treating the symptoms and extent of disease in endometriosis.

Summary

The very limited body of data currently available to assess the use of mifepristone for the treatment of endometriosis is nevertheless encouraging enough to warrant additional investigation. The main promise offered by mifepristone therapy for treating endometriosis is preservation of follicular phase levels of estradiol. This would protect women from the consequences of very low estrogen (hypoestrogen), a side effect of other effective forms of therapy (e.g., GnRH agonists). These side effects include transient decrements in bone density and hot flashes. It is important to note, however, that although GnRH may well induce a hypoestrogenic state, such an effect may be indispensable to therapeutic efficacy. With regard to other side effects, it is quite conceivable that the dose of mifepristone could be reduced to a level at which its antiglucocorticoid side effects would be negligible. Other antiprogestins with a higher level of specificity for the progesterone receptor (lower antiglucocorticoid activity) could also be tested.

It appears that much of the therapeutic benefit derived from antiprogestins in general or from mifepristone in particular may have to do with an "antiestrogenic" effect exerted directly at the level of the endometrium. By extrapolation, similar effects would be exerted at the level of the ectopic endometrial implants. Although the precise cellular mechanism involved remains unknown, empirical observations in the monkey appear to bear out this effect of mifepristone. For the most part, the women in the available studies reported pain relief and, at least in one study, a real decrease in the overall extent of disease as assessed by the AFS scores. Clearly, additional studies would be required to further evaluate these initial promising observations.

UTERINE LEIOMYOMAS (FIBROIDS)

Uterine leiomyomas, also known as fibroids, are benign tumors of smooth muscle cell origin and varying amounts of fibrous tissue (thus the term "fibroid"). Leiomyomas, which may be single or multiple and highly variable in size, are the most common pelvic tumor recognized, occurring with the highest frequency during the fifth decade of a woman's life. Although the precise cellular mechanism underlying the pathogenesis of leiomyomas remains unknown, there is little question about the hormonal dependence of this condition.

Indeed, it would appear that estrogen deficiency and perhaps pro

gestin excess exert a beneficial, though temporary, effect on leiomyoma growth. The symptoms related to leiomyomas are primarily a result of pressure from an enlarging pelvic mass. Dysmenorrhea and abnormal menstrual bleeding are also relatively common symptoms. The diagnosis of uterine leiomyoma can be made by pelvic examination and confirmed by sonographic or magnetic resonance imaging.

Treatment, medical as well as surgical, is contingent upon the age of the individual, the severity of the symptoms, and the attendant medical complications. High-dose progestin therapy as well as GnRH agonists have been shown to decrease overall uterine volume by as much as 50 percent, usually in the course of a three-month treatment period. Clearly, however, the effect of medical therapy is temporary, and no therapy has thus far been used on a long-term basis. In the face of persistent symptoms, surgical therapy is usually applied in advanced disease following the failure of medical therapy and, ideally, when no pregnancies are desired.

Utility of Mifepristone for Treatment of Uterine Leiomyomas

The only published study using antiprogestins to treat uterine leiomyomas was conducted by Murphy et al. (1993). The investigators examined the effects of daily administration of mifepristone at a dose of 50 mg for three months on 10 patients with uterine leiomyomas and regular menstrual cycles. Invariably, amenorrhea was induced. The volume of the leiomyomas decreased by 49 percent after 12 weeks of therapy. The circulating levels of LH (but not follicle-stimulating hormone) virtually doubled during the first three weeks of treatment, in association with a significant increase in the circulating levels of androstenedione and testosterone. However, these hormonal alterations were limited to the first month of therapy and returned to normal thereafter. Similarly, a significant increase was noted in the circulating levels of dehydroepiandrosterone sulfate and cortisol at 12 weeks, which suggests an antiglucocorticoid effect. Importantly, however, the circulating levels of estradiol and estrone were unchanged relative to early follicular phase values. Myomectomy or hysterectomy was performed in 6 of 10 patients at the end of treatment. Reportedly, progesterone-receptor (but not estrogen-receptor) immunoreactivity was significantly reduced in both leiomyomas and myometrium after therapy, compared to untreated controls. Side effects included mild atypical hot flashes and a transient elevation in serum transaminase accompanied by joint pain. These were experienced at the end of treatment in one patient who had prompt resolution of the side effects after discontinuing the drug. Bone mineral density at the level of the spine and hips remained unaffected after three months of therapy.

In a follow-up dose-response study discussed at the workshop, Yen

(Appendix B8) reported on the use of 25- and 5-mg daily doses of mifepristone. Specifically, 17 patients were given 25 mg daily; seven patients were given 5 mg daily for a total of three months. Again, all patients became amenorrheic. Two patients given a 25-mg dose had mild elevations of liver transaminase, which resolved within one month of discontinuing the medication. After three months of treatment, Yen found a 68.4 percent decrease in leiomyoma volume in patients given 25 mg/day. The corresponding decrease for the 5-mg/day dose was 29.2 percent. No antiglucocorticoid effect was noted at the 25- and 5-mg/day dose levels. The 25-mg/day dose resulted in a substantial reduction of uterine blood flow as assessed by Doppler sonography; but long-term efficacy has yet to be established.

Recommendation No. 13. The committee recommends further studies to determine the minimal effective dose of mifepristone and other antiprogestins for the treatment of endometriosis and uterine leiomyomas. Measures of outcome should not be limited to pain relief alone, but should also address the likelihood of improving fertility. Once such studies are completed, randomized clinical trials should be undertaken to compare the safety and efficacy of mifepristone and other antiprogestins with current therapies for the treatment of endometriosis and uterine leiomyomas.

Recommendation No. 14. The committee recommends additional research to elucidate the antiestrogenic property of mifepristone and other antiprogestins. Research models should include endometrial cultures, explants, and in vivo systems. It is also important to clarify the molecular events involved and, in all such investigations, to characterize whenever possible the steroid-receptor status of the endometriotic and fibroid tissues under study.

BREAST CANCER

Interest in the study of antiprogestins in breast cancer is understandable, given the well-known prognostic importance of the progesterone receptor in this disease and the activity of a variety of diverse endocrine manipulations for its treatment (Hamm and Allegra, 1991). Approximately 30 percent of unselected patients and 50 to 80 percent of estrogenand progesterone-receptor-positive, metastatic breast cancer patients will have some degree of benefit from a variety of endocrine interventions. Although these patients may improve temporarily in response to these treatments, they are not cured.

Paradoxically, both estrogen and progesterone in low doses stimulate breast cancer growth, whereas high dosages of either are growth inhibitory and are used clinically in the treatment of breast cancer. In addition, the antiestrogen tamoxifen is generally the mainstay of first-line therapy for advanced estrogen-receptor-positive tumors because of its efficacy, safety, and convenience (Hamm and Allegra, 1991). In this context, it is not surprising that another endocrine manipulation with antiprogestins appears to have activity in breast cancer.

Although research on the potential cancer applications of antiprogestin therapy has lagged behind endocrine and reproductive research, a number of issues that merit further study were identified at the committee's workshop, and some of these issues are discussed below. However, given the many complexities of the progesterone receptor and of the effects of binding with agonists and antagonists in different tissues described at the workshop, there are undoubtedly many reasonable research questions to be addressed, and the issues raised in the following discussion are meant to be illustrative rather than exhaustive.

Mechanism of Action

The mechanism by which antiprogestins exert their growth-inhibitory effects is complex and not fully understood. Even among the antiprogestins there are structural and functional variations suggesting that there may be important differences among this class of compounds in their mechanisms of action. Weigel (Appendix B2) points out that the three-dimensional structures of the two clinically available antiprogestins, mifepristone and onapristone, are quite different and that they appear to inhibit progesterone-receptor function by different mechanisms.

Horwitz (Appendix B9) describes the tissue-specific differences in progesterone effects and the implications of these differences for breast cancer treatment. In contrast to its actions in the uterus, progesterone at low dosage approximating physiologic ranges is growth stimulatory in the normal breast; most mitoses occur in the late luteal phase of the menstrual cycle coincident with the rise in progesterone. Blockade of this mitogenic effect was described as a potential strategy for breast cancer prevention. On a molecular basis, there are a number of differences among progesterone receptors when bound to various antiprogestins (some antiprogestin-receptor complexes bind to DNA, and others do not). These differences govern agonist versus antagonist activities. For example, as Horwitz described at the IOM workshop (see also Horwitz, 1992), several progesterone antagonists are "transcriptionally silent" when occupying smaller type A progesterone receptors (PR-A) but stimulate strong transcription when occupying the larger type B receptors (PR-B) (Weigel, Appendix B2). The tissue distribution

of these naturally occurring progesterone receptors has not been fully elucidated but has obvious biologic and toxicity implications, and this represents an important area for future research.

The complex effects of PR-A and PR-B are further complicated by the fact that some PR-B effects may not appear to require DNA binding. In other studies of these receptors it appears that PR-A dominates PR-B; equimolar amounts of A abolish the agonist activity of B. The agonist activity of an antiprogestin bound to the PR-B represents a potential mechanism of clinical resistance to antiprogestin. The fact that PR-B agonist activity may be mediated by binding, not to DNA but to other regulatory proteins that might be distributed differently than the progesterone receptors, may also help explain tissue-specific differences in response to progesterone and antiprogestins.

Intriguing data by Michna et al. (1992) and Henderson at the IOM workshop have suggested a novel mechanism of action for the antiprogestins onapristone (ZK 98 299) and Schering's ZK 112 993. Specifically, morphologic and cell-cycle distribution data suggest that they induce terminal differentiation and produce cell death through apoptosis (programmed cell death)[1] rather than necrosis. Data on down-regulation of tenascin, an extracellular matrix glycoprotein of tumor stroma, in rat mammary tumors provide additional support for the induction of terminal differentiation as an underlying mechanism of growth inhibition by onapristone. Interestingly, although ovariectomy and antiestrogen therapy were associated with growth inhibition in this model, neither was associated with decreased expression of tenascin (Volleyer et al., 1992). These observations may be important in attempting to define a unique clinical role for antiprogestins in the endocrine armamentarium and certainly deserve additional study.

Data from other studies that demonstrate enhanced antitumor activity with combination endocrine therapy may also have important implications for the future clinical development of antiprogestins (Baker et al., 1989). Improved antitumor activity was observed in rat mammary tumor models with combination endocrine therapies incorporating antiprogestins and antiestrogens or HL-releasing hormone (LHRH) agonists. Significant down-regulation of estrogen- and progesterone-receptor content was noted. Data on endocrine combinations are also presented by Horwitz (Appendix B9) in a rat dimethylbenzanthracene (DBA) (carcinogen-induced) breast cancer model with established tumors. These data demonstrated that the combination of an

[1] In the normal course of events cells of the body die and are replaced by new cells; the programming and mode of this metabolic "suicide" have been given the name apoptosis to distinguish them from the type of death called necrosis. This latter implies that some harmful agent, foreign to the cell's own metabolic programs, has caused the cell to die.

antiestrogen (tamoxifen) and antiprogestin produced tumor regressions comparable to ovariectomy, whereas either agent alone only produced tumor stasis. In the DMBA model, tamoxifen down-regulated the estrogen receptor; however, it also exerted agonist activities and thereby up-regulated the progesterone receptor. Mifepristone down-regulated both the estrogen receptor and the progesterone receptor. In other DMBA experiments with established tumors, progesterone was able to overcome the growth inhibition produced by tamoxifen. This finding suggests that tamoxifen could not inhibit the progestin-mediated growth-stimulatory effects. Addition of mifepristone with progesterone effectively reestablished tamoxifen growth inhibition.

These results provide a rationale for future study of combination endocrine therapy. Such study would be further enhanced by the availability of compounds with pure antiprogestin properties and, therefore, presumably more specificity and less toxicity.

Clinical Issues

To date, the clinical activity of antiprogestins in breast cancer patients is sparse and clinically unimpressive. Data on 33 patients treated with mifepristone have been published. No other clinical trials have been completed, although onapristone recently entered its first clinical trial in cancer patients in Europe, and larger trials of mifepristone are ongoing in Europe and Canada.

In the largest experience (Horwitz, Appendix B9, and references in Maudelonde et al., 1987, and Romieu et al., 1987, therein), 22 postmenopausal or oophorectomized patients with advanced breast cancer were treated with mifepristone (200 mg daily). All patients' tumors had progressed while they were on tamoxifen, and all had received at least one other prior therapy including chemotherapy, radiation, or another endocrine treatment. Conventional response criteria were not used in this trial, so it is difficult to determine whether any of the patients had an objective partial response.[2] Twelve patients were judged to have had partial regression or stabilization of disease. In only four patients was the effect persistent for three months. An analgesic effect on bone pain was also noted. Estrogen- and progesterone-receptor status were not available for many of these patients.

In a second study, 11 postmenopausal patients received mifepristone doses of 200 to 400 mg daily as second-line treatment following tamoxifen (Michna et al., 1989; Bakker et al., 1990; Horwitz, Appendix B9).

[2] Typical criteria for partial response require a 50 percent reduction in the sum of the products of the perpendicular diameters of the measured lesion.

One partial response in a patient with lymph node metastases was observed, but the response lasted for only five months. Disease did not progress in six patients for periods of three to eight months. The appearance of toxicities was delayed; these included weight loss in ten patients, fatigue, anorexia, nausea, malaise, somnolence, and one grand mal seizure. A rise in serum creatinine and an increase in eosinophil counts were also observed. Of interest is the fact that 3 of the 11 patients responded to third-line treatment with the progestin megestrol acetate. This included the one patient who had also responded to mifepristone.

A number of endocrine levels were evaluated in this study. Increases were noted in estradiol, ACTH, cortisol, and androstenedione, but sex hormone-binding globulin levels decreased with treatment. The authors attributed increases in estradiol in these postmenopausal women to increased adrenal stimulation secondary to antiglucocorticoid effects of mifepristone and subsequent peripheral conversion of adrenal androgens to estrogens in these postmenopausal women. This increase in estrogens, which could be potentially deleterious to the treatment of breast cancer, hypothetically could be overcome by combining the antiprogestin with an antiestrogen or aromatase inhibitor. Preclinical data supporting the utility of such an approach are mentioned above (Kian et al., 1989; Bakker et al., 1990).

Adjuvant Therapy and Chemoprevention

Potential uses for antiprogestins in adjuvant therapy (postoperative therapy undertaken when no detectable tumor is present to reduce the risk of recurrence) or chemoprevention (treatment of well women to lower the risk of initial development of cancer) arose at the committee's workshop during the discussion of Horwitz's presentation, undoubtedly stimulated in part by the experience with the use of the antiestrogen, tamoxifen, for these indications. However, given the current status of the clinical development of antiprogestins, specifically mifepristone, such applications to chemoprevention are purely speculative at present. Although a number of interesting theoretical applications have been identified, clinical experience with these compounds is too limited at present. In addition, the potential for agonist as well as antagonist activity (discussed in several papers in Appendix B) and the preclinical observation of tumor stimulation (Bowden et al., 1989) suggest that cautious observation will be required prior to clinical trials in early breast cancer or prevention. Substantial clinical activity and acceptable toxicity in advanced disease patients would be required before one could seriously consider using antiprogestins as an adjuvant treatment for breast cancer and certainly prior to their introduction for the chemoprevention of breast cancer. In addition, antiprogestin therapy would need

to distinguish itself from other available endocrine therapies, most notably tamoxifen, for this latter indication. Long-term toxicity experience will also be an important issue in assessing the therapeutic potential of antiprogestins in the treatment of curable early breast cancer and/or chemoprevention.

Resistance

Eventually all advanced breast cancers become hormone independent and increasingly resistant to any subsequent therapy. It is this development of resistance that limits the potential utility of antiprogestins and other endocrine therapies for the treatment of advanced disease. Further understanding of the mechanisms of resistance will be essential for future attempts to improve the efficacy of endocrine treatment.

Dose Issues

The issue of optimal dose remains unsettled. In some studies the antiproliferative effects of antiprogestins on breast cancer cells in vitro have been shown to be dependent on dose and progesterone-receptor content (Bardon et al., 1985). The use of a biologic end point (induction of menses with onapristone) to define an adequate dose makes certain assumptions regarding the relative sensitivities of tumor and endometrium to antiprogestins. It also assumes that the antihormonal mechanism underlies the antiproliferative actions of antiprogestins and that optimal doses for antiproliferative effects, differentiation, and other important actions such as growth factor induction will be similar. In that regard, it is interesting to note that tamoxifen, the best studied of the antihormonal agents used in breast cancer, is active in some patients with estrogen-receptor-negative tumors, suggesting that nonhormonal antitumor effects may be important. Tamoxifen exhibits other potential antitumor mechanisms, including induction of a number of polypeptide hormones, and inhibition of activation of protein kinase C and calmodulin (O'Brian et al., 1985; Horgan et al., 1986; Musgrove et al., 1989; Sunderland and Osborne, 1991). However, increased doses have not proved beneficial in tamoxifen therapy (Stewart et al., 1982; Rose et al., 1992). Nonetheless, given the uncertainties regarding the mechanism of action of antiprogestins, further exploration of dose, toxicity, and activity relationships seems reasonable.

Conclusions

The committee reached the following conclusions about the current state of the science for the use of antiprogestins to treat breast cancer:

1. The antiprogestins have potential as growth-inhibitory compounds against breast cancers. Whether this antitumor activity will be unique among the many other available endocrine therapies for breast cancer remains to be seen. There are at present too few clinical data to assess adequately the clinical potential of antiprogestins in the treatment of metastatic breast cancer, much less to assess their potential applications for adjuvant therapy or chemoprevention.
2. The exact mechanism by which antiprogestins exert their antitumor effect is unclear at present. However, data suggest that more than one mechanism exists because the receptor-antiprogestin complexes of at least two of the clinically available antiprogestins appear to interact with DNA differently (the mifepristone-receptor complex binds DNA, but the onapristone-receptor complex does not).
3. Although animal models can provide hypotheses, the biologic complexity and heterogeneity of breast cancer and the limitations of these models will require that many questions be addressed in human clinical trials.
4. Even if the clinical experience with antiprogestins demonstrates substantial activity with an acceptable toxicity profile, it will still be important to define a unique mechanism or role for the use of antiprogestins as compared to other available endocrine therapies. Further elucidation of the antiproliferative mechanism of action, especially the differentiating effects, and of potential synergistic combination hormonal therapies will be useful in this regard.

Recommendation No. 15. The committee recommends research to clarify the activity of antiprogestins in women with advanced (metastatic) breast cancer. Trials should be conducted by using more homogeneous groups of patients. Potential sources of heterogeneity should be reduced by including patients with only minimal prior therapy for their breast cancer and by performing pharmacokinetic evaluations to ensure consistent drug exposure and to facilitate concentration-response correlations. In these studies, tumor progesterone-receptor and estrogen-receptor status should be measured routinely.

Recommendation No. 16. Clinical trials of antiprogestins for treatment of breast cancer should include ancillary investigations to clarify mechanisms underlying the activity of antiprogestins. Examples of such studies include

a. histologic evaluation of tumor tissue before and after treatment

b. assessment of cell-cycle distribution before and after therapy by use of flow cytometry

c. assays of transforming growth factor (TGF_{β}) induction and other potential indicators of cell differentiation

d. characterization of the progesterone receptor, including quantitation not only of total receptor content but also of A and B receptors and receptor mutants (Western blot technology currently exists for this analysis); and

e. assays for the expression of other growth factors such as TGF_{α}, epidermal growth factor (EGF), and insulin-like growth factor (IGF), which may be modulated by antiprogestins.

Recommendation No. 17. The committee recommends that studies establish the maximum tolerated dose of antiprogestins for treatment of breast cancer. Further exploration of potential toxicity at various doses should be undertaken in additional Phase 1 clinical trials.

Recommendation No. 18. The committee recommends additional preclinical exploration of the molecular mechanisms of action of antiprogestins for treatment of breast cancer. Such studies should include

a. further characterization of progesterone receptors, including the natural A and B forms, as well as possible genetic mutants, and their distribution in normal and malignant tissue; this will be important for the rational use of antiprogestins in future clinical trials

b. investigation of the use of antiprogestins as differentiating agents that produce programmed cell death (apoptosis)—a possible novel mechanism of action; and

c. exploration of the mechanism of tumor resistance to antiprogestins and other effects of long-term administration.

MENINGIOMAS

Meningiomas are tumors arising from the membranes surrounding the brain. Meningiomas are common tumors, generally nonmalignant and slow-growing, which can, however, threaten brain function or even life if they are not surgically removed. More frequent in women, meningioma growth is accelerated during pregnancy. Several papers in Appendix B allude to a rationale for the treatment of meningiomas with antiprogestins. The vast majority of meningiomas express progesterone

receptors, although there is some controversy as to whether the progesterone receptor is functional in meningioma. Recent data from 33 meningiomas evaluated for progesterone-receptor mRNA by Northern blot analysis (Carroll et al., 1993) revealed that 64 percent of specimens were positive (indicating positive receptor status). Of note was a statistically significant difference in expression between women and men. For women, 81 percent of specimens—and for men, 19 percent of specimens—were positive. Additional evidence of functional receptors has been reported by using immunohistochemistry to identify progesterone-receptor protein product. Six of eleven samples demonstrated intense nuclear staining at this location, which was compatible with a functional receptor (Carroll et al., 1993).

The results of an initial clinical trial using an antiprogestin in patients with recurrent meningiomas following surgery and radiotherapy have been published and updated (Grunberg et al., 1991a,b). Twenty-four patients with recurrent or unresectable meningiomas have been treated with mifepristone (200 mg daily) for considerable periods—some for more than two years. Thirteen patients were treated for more than 12 months; 20 patients were treated for more than 6 months. (At the IOM workshop, Grunberg described results extending the published data for 28 patients treated for a median of 27 months. Maximum duration of therapy is 62 months. Twenty patients have been treated for periods of one year or longer. All five of the premenopausal women in this study had cessation of menses for the duration of therapy, and two who ceased therapy (one after eight months and one after two years) had a return of normal menses.)

Response criteria in the 1991 reports were less stringent than those conventionally employed in cancer clinical trials. However, six patients had some improvement manifest by a minor decrease in tumor measurement by computed tomography or magnetic resonance imaging, or some improvement in visual field examination associated with amelioration of visual symptoms or headache. Progesterone-receptor status could not be obtained on most patients. Toxicities associated with daily chronic administration appeared tolerable and included fatigue, hot flashes, breast enlargement and tenderness, thinning of hair, and rash. Amenorrhea occurred in the three premenopausal patients; however, menses resumed in two patients following cessation of therapy.

Conclusions

The committee reached the following conclusions about the current state of the science for the use of antiprogestins to treat meningioma:

1. Mifepristone appears to have some activity in recurrent or unresectable meningiomas, though the clinical importance of this observa

tion in patients having a disease with a highly variable natural history remains to be defined. The lack of good alternative treatments for this group of patients and the mechanistic rationale make this an attractive treatment for further study.

2. Daily chronic administration of mifepristone appears tolerable in this group of patients.

Recommendation No. 19. A randomized Phase 3 trial will be required to define the role of antiprogestins in the management of patients with unresectable meningioma. Such a trial is ongoing in the Southwest Oncology Group. The committee recommends that these data be reviewed carefully to define directions for further research on this disease.

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5

Antiglucocorticoid Effects of Antiprogestins

All antiprogestins studied to date have been found to have some antiglucocorticoid effects as well as antiprogestational effects (Baulieu, Appendix B1; Nieman, Appendix B10; Spitz and Bardin, in press). Although antiprogestins can be used as probes to study glucocorticoid function, their antiglucocorticoid effects are undesirable, and it would be preferable to have classes of pure antiprogestins and of pure antiglucocorticoids that would not display any other endocrine effects. Present knowledge is insufficient to set a rational strategy that would assure the synthesis of such pharmaceutical agents; therefore, the search now proceeds using empirical tactics and the outcome of this research is, as always in research, unpredictable.

GLUCOCORTICOID FUNCTION

Glucocorticoids are steroid hormones produced by the adrenal glands in response to stimulation by the pituitary hormone adrenocorticotropin (ACTH), which is in turn regulated by a hypothalamic hormone, corticotropin-releasing hormone. The control of glucocorticoid secretion is summarized by Nieman (Appendix B10). Briefly, production of cortisol, the main glucocorticoid, is stimulated by ACTH. ACTH secretion, in turn, is inhibited by increasing levels of cortisol. This process is termed negative feedback regulation. The system is fine-tuned, therefore, through a feedback control mechanism that is similar to those of many other hormone systems, including estrogen and gonadotropins in the reproductive system.

Glucocorticoids, like other steroid hormones, exert their biologic effects by binding to specific intracellular receptors. There are two

receptors that bind with high affinity to glucocorticoids; these have been dubbed the type I and type II receptors. The receptors and their involvement in glucocorticoid action are described in detail by Nieman (Appendix B10).

Several factors influence glucocorticoid actions on a given tissue. These include the type and amount of steroid available, and the number of glucocorticoid receptors. The number of glucocorticoid receptors in a cell is usually inversely regulated by glucocorticoid exposure, and glucocorticoid administration may decrease the number of receptors by as much as 50 percent (down-regulation). Moreover, the response to a specific dose of glucocorticoid may vary widely across tissues, so that a dose of steroid that elicits a maximal response in one tissue may elicit only a small response in another (Nieman, Appendix B10).

The importance and function of glucocorticoids are made clear by examining information from states of deficiency and excess. Cushing's syndrome and adrenal insufficiency are diseases of too much and too little glucocorticoid, respectively. These diseases involve nearly all tissues and many physiologic processes, including fuel consumption and economy, structural catabolism (especially of bone, collagen, and muscle), the immune system, and inflammation (Nieman, Appendix B10).

THERAPEUTIC USE OF MIFEPRISTONE (RU 486) AS AN ANTIGLUCOCORTICOID[1]

Nieman et al. (1985) were the first to use the antiglucocorticoid properties of mifepristone to treat Cushing's syndrome, a condition caused by excess glucocorticoid levels and characterized by depression, hypertension, carbohydrate intolerance, and gonadal and thyroid hormone suppression. Cushing's syndrome is a generic term describing chronic excess glucocorticoid production that may be secondary to ACTH secretion by a tissue other than the pituitary (ectopic secretion), or elevated ACTH due to a defective feedback mechanism in which the homeostatic regulatory mechanism does not function correctly (changed "set point"). Ectopic secretion of ACTH is not responsive to the normally operative negative feedback by glucocorticoids. In contrast,

[1] During the committee's work, Irving Spitz and C. Wayne Bardin of the Population Council shared with the committee an exhaustive review, "Clinical Pharmacology of RU 486: An Antiprogestin and Antiglucocorticoid," which the authors have submitted to *Contraception*. A shorter version of the manuscript is in press, in the *New England Journal of Medicine* . The following discussion borrows extensively from their manuscript.

ACTH secretion in the subset of patients with a defective feedback mechanism (Cushing's disease) will respond to changing concentrations of glucocorticoids.

In patients with an ectopic ACTH-secreting tumor, it was possible to alleviate the deleterious effects of the high levels of cortisol in 7 of 11 subjects by using mifepristone at doses of 5 to 22 mg/kg per day for periods ranging from four weeks to one year (Chrousos et al., 1989). This study showed that mifepristone may have a role to play in the preoperative preparation for surgery of a patient with Cushing's syndrome caused by fixed cortisol secretion. By contrast, in Cushing's disease where cortisol secretion is not fixed but the system set point is altered, mifepristone was not effective in alleviating symptoms. In this case, the antiglucocorticoid activity of mifepristone actually enhances ACTH and cortisol secretion (Spitz and Bardin, in press).

There are also potential therapeutic applications of mifepristone as a local antiglucocorticoid. Studies in rabbits have demonstrated that local application of eye drops containing mifepristone can lower intraocular pressure (Philips et al., 1984). Moreover, it is unlikely that enough mifepristone would be administered by this route to increase adrenal cortisol secretion. There have been no studies to date to evaluate the applicability of these data to humans.

In addition to its use in the treatment of Cushing's syndrome, Spitz and Bardin (in press) discuss the use of mifepristone to counteract (antagonize) large doses of exogenous glucocorticoid given for therapeutic purposes. For example, Konagaya et al. (1986) used mifepristone to block dexamethasone-induced loss of muscle and body weight in rats. Mifepristone may have potential use in humans in the treatment of steroid-induced myopathy, such as that produced by dexamethasone when it is given as an anti-inflammatory agent; however, Spitz and Bardin (in press) point out that use of antiglucocorticoids for therapy would require selective antagonistic effects on muscle without reducing the beneficial effects for which the glucocorticoid was given. In other words, selective inhibition of the catabolic, but not the antiinflammatory, properties of glucocorticoids has, to date, eluded chemists trying to synthesize antiglucocorticoid agents.

Use of mifepristone to antagonize endogenous cortisol might also be of benefit in a wide variety of disorders. Spitz and Bardin (in press) reviewed some of these potential uses such as attenuation of muscle atrophy associated with androgen withdrawal, denervation, and muscular dystrophy. In addition, Regelson et al. (1990; reviewed in Spitz and Bardin, in press) suggest that antagonism of the action of endogenous cortisol could prevent progression of certain viral diseases. However, systematic administration of antiglucocorticoids is likely to cause a compensatory increase in cortisol, which might blunt the desired effect.

Whether dose amounts and schedules can be developed that allow selective beneficial actions of glucocorticoid antagonists in the above settings will have to be established by clinical trials for each specific condition (Spitz and Bardin, in press). Glucocorticoid-induced animal or tissue culture models of hypertension, wound healing, cataracts, inflammation, and arthritis have suggested a potential role for antiglucocorticoids in these states. Whether these results will pertain in humans is largely unexplored (Nieman, Appendix B10).

ADVERSE ANTIGLUCOCORTICOID EFFECTS OF ANTIPROGESTINS

Mifepristone has been used at doses of up to 10 mg/kg per day for as long as seven days with few adverse effects, including adverse antiglucocorticoid effects. Daily doses of more than 3 mg/kg given for more than seven days have been associated with fatigue in the majority of subjects (Grunberg et al., 1991); anorexia and nausea may also occur (Klijn et al., 1989; Bakker et al., 1990; Grunberg et al., 1991; Lamberts et al., 1991). These effects are consistent with relative adrenal insufficiency, and improve with administration of dexamethasone or other glucocorticoids; however, it is not completely clear that they represent adrenal insufficiency (Nieman, Appendix B10; Spitz and Bardin, in press).

Clearly, for long-term antiprogestin therapy applied to conditions other than Cushing's syndrome, the antiglucocorticoid actions of these compounds are an undesirable side effect. For obvious medical reasons, it would be preferable to have separate classes of pure antiprogestins and of pure antiglucocorticoids that do not display any other endocrine effects.

Recommendation No. 20. Antiglucocorticoid effects are an unwanted property of existing antiprogestins. Therefore, the committee recommends expanded efforts to produce pure antiprogestins that would not display any other endocrine effects at therapeutic doses.

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6

Animals as Models for Studying Antiprogestins

Newly synthesized compounds are generally screened by using assays that assess their in vitro binding affinities for the progesterone receptor. Usually, these compounds are also screened for binding to other steroid hormone receptors. In general, compounds that have high binding affinity for the progesterone receptor are assessed further for their agonist or antagonist activity by using animal models (Van Look and von Hertzen, Appendix B12). Papers presented in Appendix B of this report provide a history of the development of antiprogestins including the use of animal models (Baulieu, Appendix B1); provide an overview and perspective of the use of animal models in general to predict the pharmacologic effects in humans (Van Look and von Hertzen, Appendix B12); address the applicability of the primate model to humans (Hodgen, Appendix B11); address animal and cell culture models for their relevance to evaluating the treatment potential of these compounds for breast cancer and mammary tumors (Henderson, IOM workshop; Horwitz, Appendix B9); describe species specificity of receptor binding and the effect of amino acid substitutions within the progestin-receptor-binding site (Weigel, Appendix B2); and discuss research on antiprogestin effects on estrogen-receptor levels and induction of labor in primates versus humans (Baird, Appendix B4; Ulmann and Silvestre, Appendix B6). The committee has concluded from these discussions that results in most animal models appear to have been fairly predictive of the effects of antiprogestins in humans despite the fact that there are major differences between humans and other species, including nonhuman primates, in terms of the pharmacokinetics of the antiprogestins and the type of placentation (Van Look and von Hertzen,

Appendix B12). Some of the similarities and differences are discussed below.

Antiprogestins bind to progesterone-receptor preparations from a variety of species, including rat, rabbit, calf, marmoset, bonnet monkey, and human, but they do not bind to chicken or hamster progesterone receptors (Baulieu, Appendix B1; Weigel, Appendix B2; Van Look and von Hertzen, Appendix B12). In the latter cases, the lack of mifepristone (RU 486) binding to the progesterone receptor has been attributed to a single amino acid change in the hormone-binding domain—the replacement of a glycine by a cysteine at positions 575 and 722 for the chicken and hamster, respectively. All receptors that bind mifepristone, including glucocorticoid and androgen receptors, have a glycine residue at a corresponding position in the hormone-binding domain (Van Look and von Hertzen, Appendix B12). Both the chicken and the hamster progesterone receptors can be modified by a substitution of glycine (but not methionine or leucine) for this key cysteine, and the mutated receptor will bind mifepristone. Conversely, replacing the key glycine in the human progesterone receptor with cysteine renders the receptor incapable of binding mifepristone (Baulieu, Appendix B1). This exquisite sensitivity of progestin-receptor binding to a single amino acid substitution suggests that studies of the molecular actions of antagonists that have potential clinical applications should be conducted by using human steroid receptors, since receptors from other species may respond somewhat differently (Weigel, Appendix B2).

When using animal models, it is important to recognize that there are marked species differences in plasma proteins that can bind to the steroid hormones and to the antihormones. For example, no animal species appears to have the high-affinity binding protein α_1-acid glycoprotein, which is found in the human (Baulieu, Appendix B1; Van Look and von Hertzen, Appendix B12). This plasma glycoprotein binds to some, but not all, antiprogestins, and appears to affect clearance rates of these compounds. For example, α_1-acid glycoprotein strongly binds to mifepristone and probably lilopristone, but not to onapristone. Research suggests that the low clearance of mifepristone is exacerbated by this tight protein binding and is reflected in the long half-life of mifepristone (20 to 24 hours) versus that for onapristone (2 to 4 hours) (Van Look and von Hertzen, Appendix B12).

The pregnant guinea pig model has been used extensively for the study of abortifacient potency and the mechanism of action of antiprogestins. Studies using this model led to the development and testing of the sequential treatment regimen of mifepristone followed by prostaglandin. However, studies in the guinea pig model have not always correlated with those in humans. For example, in the pregnant guinea pig model, a marked synergism was demonstrated between antipro

gestins and the antiestrogen, tamoxifen, in inducing abortion. This was not seen in one study in women (Van Look and von Hertzen, Appendix B12). However, the study in women may not have been expected to demonstrate synergy due to the drug regimens tested. The concentration of mifepristone used in the human study was a maximal, or near maximal, dose. By definition, synergism could not be demonstrated under these circumstances, and lower (submaximal) doses would have to be utilized to evaluate the potential synergistic action properly.

Cook of Research Triangle Institute (RTI) presented information at the Institute of Medicine workshop on a new RTI compound that has a basic structure similar to other antiprogestins, but exhibits agonist rather than antagonist effects in the rabbit. Chwalisz of Schering AG pointed out that the rabbit progesterone receptor was anomalous and many antiprogestins that exhibit antagonist effects in other animals often exhibit agonist effects in rabbits. Therefore, the rabbit does not appear to be a good model for evaluating antiprogestins for potential application in humans.

Most of the control systems that govern reproductive function in the higher primates are fundamentally different from those in other mammals. This holds true for the control of ovulation, the recognition and maintenance of pregnancy, and the initiation of labor. The rhesus monkey has been a good model for the human in the context of neuroendocrine control of the menstrual cycle and ovulation; however, it differs markedly in the control of pregnancy. Progesterone metabolism is totally different from that in the human. In the rhesus monkey, progesterone is not converted to pregnanediol, and its concentration during pregnancy does not rise much above luteal phase except in the last few days before parturition (Neill et al., 1969). In this regard, the rhesus monkey behaves like sheep, cow, and other ungulates.

Despite the differences described above, Hodgen (Appendix B11), in reviewing the primate model for the study of antiprogestins, suggested that data from macaques (both rhesus and cynomolgus monkeys) and humans are quite similar. His comparisons focused on the noncompetitive antiestrogenic activity of progesterone antagonists, the dose-dependent blockade by mifepristone of the proliferative action of estradiol on the endometrium, and the elevation of estrogen receptors in the endometrium induced by mifepristone (Hodgen, Appendix B11). Some differences between primates and humans, however, have been noted. For example, Spitz and coworkers (1993; and Danforth et al., 1989) found that intermittent mifepristone was more effective at inhibiting ovulation in the monkey than in women. Furthermore, Frydman and coworkers (1991) found mifepristone to be more effective at inducing labor in women at the end of the third trimester than had been previously reported in monkeys.

Ulmann and Silvestre (Appendix B6) reported on studies in ewes (Burgess, 1992) and monkeys (Wolf et al., 1989) showing that mifepristone induces uterine contractions and enhances the myometrial sensitivity to oxytocin at term. Newborn animals from mothers treated with mifepristone were normal. Very limited data in both animals and humans are available to corroborate this finding, and more studies are necessary on this use (see also Chapter 3).

Extensive discussions of the use of animal models in evaluating potential effects of new antiprogestins on breast cancer are presented in the paper by Horwitz (Appendix B9). Progesterone agonists increase the incidence of spontaneous mammary tumors in dogs and mice and, at physiologic levels, increase the growth of established tumors in some species (Horwitz, Appendix B9). Various animal models of hormone-dependent mammary cancer have been used to study the antiproliferative properties of progesterone antagonists and estrogen antagonists. These include rats that have chemically induced tumors and mice bearing transplanted tumor lines (Horwitz, Appendix B9). In these animal models, combined treatment with mifepristone and antiestrogens or gonadotropin-releasing hormone agonists produces high rates of tumor remission (Bakker et al., 1990). Other antiprogestins have been shown to have similar effects (Horwitz, Appendix B9). However, these models have limitations. For example, the nude mouse model has low progesterone-receptor levels and, despite hormone dependence, was found to be resistant to antiprogestins. Another difference is that the time of induction of breast cancer is weeks in rodent models versus years in human. This may explain why hormonal effects in cancer induction are more obvious or exaggerated in animal models than in humans. In women, only preliminary clinical studies have been reported on the potential use of antiprogestins in the treatment of advanced breast cancer, and there has been no report of the combined use of antiprogestins and antiestrogens as studied in the animal models described above (see Chapter 4). Although data from animal models are promising, long-term comparative human studies will be necessary to establish whether antiprogestins, or antiprogestins in combination with antiestrogens, might form a treatment modality for human breast cancer.

In conclusion, as for many drugs, animal models have been useful in understanding the mechanism of action and evaluating the potential of treatment modalities with antiprogestins. As would be expected, animal models are not always accurate predictors of the results in human beings. However, in the antiprogesterone data presented to and reviewed by the committee, particularly on mifepristone, animal models have been extremely useful in providing clues to the documented

beneficial effects of these compounds and as leads for potential alternate uses described elsewhere in this report.

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APPENDIXES

A

Agenda for the IOM Workshop "Antiprogestins: Assessing the Science," April 13–14, 1993

APRIL 13	Room 104 Green Building 2001 Wisconsin Avenue, N.W. Washington, D.C.
8:00–8:30	Continental Breakfast, Room 110
8:30–8:45	Welcome to Participants
8:45–9:00	Purpose of the Workshop *Leslie Z. Benet, Ph.D., Chairman* *Committee on Antiprogestins: Assessing the Science*
9:00–10:30	**SESSION I. Overview and Background: Background, Development, Mechanism of Action**
9:00–9:30	Development and Future Directions in the Development of Steroid Antagonists *Étienne-Émile Baulieu, M.D., Ph.D.* *Professor of Biochemistry* *School of Medicine* *University of Paris-Sud* *Director of Research, INSERM*
9:30–10:00	Antiprogestational/Antiglucocorticoid Compounds: Structure-Function Relationships *Nancy L. Weigel, Ph.D.* *Research Associate Professor* *Department of Cell Biology* *Baylor School of Medicine*

10:00–10:30	Discussion of Session I
10:30–10:45	BREAK
10:45–2:15	**SESSION II. State of the Science and Research Directions—Uses of Antiprogestins: The Reproductive Cycle**
10:45–11:00	Introduction *Lynnette K. Nieman, M.D.* *Senior Investigator, Deputy Clinical Director,* *Special Assistant to the Scientific Director* *National Institute of Child Health and Development* *National Institutes of Health*
11:00–11:30	Daily or Luteal Phase Administration: Mechanism of Action of the Antigestagens on the Endometrium *David T. Baird, M.D.* *MRC Clinical Research Professor of Reproductive Endocrinology* *Center for Reproductive Biology* *Department of Obstetrics and Gynecology* *The University of Edinburgh*
11:30–12:00	Uses of Antiprogestins Before 63 Days *Marc Bygdeman, M.D.* *Chairman* *Department of Obstetrics and Gynecology* *Karolinska Institute* *Stockholm*
12:00–12:15	Uses of Antiprogestins After 63 Days *André Ulmann, M.D., Ph.D.* *Direction; Domaine Thérapeutique Endocrinologie* *Roussel-Uclaf* *Romainville, France*
12:15–1:30	LUNCH
1:30–1:45	Comments on Session II *David Grimes, M.D.* *Professor and Vice Chair* *Department of Obstetrics/Gynecology and Reproductive Sciences* *University of California at San Francisco*

1:45–2:15	Discussion of Session II
2:15–2:30	BREAK
2:30–4:20	**SESSION III. State of the Science and Research Directions: Therapeutic Uses of Antiprogestins**
2:30–3:00	Use of Antiprogestin in the Management of Endometriosis and Leiomyoma *Samuel S.C. Yen, M.D.* *Professor and W.R. Persons Chair* *Department of Reproductive Medicine* *University of California at San Diego*
3:00–3:30	Use of Antiprogestin in the Treatment of Breast Cancer *Kathryn B. Horwitz, Ph.D.* *Departments of Medicine and Pathology* *Division of Endocrinology* *University of Colorado Health Sciences Center*
3:30–3:50	Animal Models and Studies on the Mode of Action of Antiprogestins in Growth Inhibition of Mammary Tumors *David Henderson, Ph.D.* *Head, Experimental Oncology* *Schering AG, Berlin*
3:50–4:20	Discussion of Session III
4:20–4:50	General Discussion
4:50 p.m.	Adjourn for the Day
APRIL 14	
8:30–9:00	CONTINENTAL BREAKFAST (Room 110)
9:00–10:15	**SESSION IV. State of the Science and Research Directions: Therapeutic Uses of Antiglucocorticoids**
9:00–9:45	*Lynnette K. Nieman, M.D.* *Senior Investigator, Deputy Clinical Director,* *Special Assistant to the Scientific Director* *National Institute of Child Health and Development* *National Institutes of Health*

9:45–10:15	Discussion of Session IV
10:15–10:30	BREAK
10:30–10:50	**SESSION V. Primate Models for the Study of Antiprogestins in Reproductive Medicine** *Gary D. Hodgen, Ph.D.* *The Howard and Georgeanna Jones Professor of Reproductive Medicine* *President, The Jones Institute Foundation* *Eastern Virginia Medical School*
10:50–11:00	Discussion of Session V
11:00–11:20	**SUMMING UP** *Paul F.A. Van Look, M.D., Ph.D.* *Acting Director Special Programme of Research, Development and Research Training in Human Reproduction* *World Health Organization, Geneva*
11:20–11:45	Late-Breaking Research Issues and Final Comments
11:45	ADJOURN WORKSHOP

B

Background Papers and Presentations (In Order of Presentation at IOM Workshop)

B1 1993: RU 486—A DECADE ON TODAY AND TOMORROW

Étienne-Émile Baulieu, M.D., Ph.D.

Unité de Recherches sur les Communications Hormonales (INSERM U 33) and Faculté de Médecine Paris-Sud, Bicêtre Cedex, France

The development of RU 486[1] (Figure B1.1), the first efficient antiprogestin, may be seen as a result both of the biomedical revolution of the last few decades and the efforts of the women's movement during the twentieth century to control their reproductive life. This conjunction was exemplified in the early 1950s when Margaret Sanger went to Gregory Pincus to discuss the possibility of developing a medical method to achieve "planned parenthood." The result of this meeting, which merged science (hormone research) and the *cause des femmes,* was the invention of the contraceptive pill (Pincus, 1965). "The pill" remains at least as important symbolically as it is useful practically. Scientifically, this development was based on the physiological concept that sex steroid hormones exert negative feedback control on ovulation. With progress in steroid chemistry, orally active compounds that mimicked the action of endogenous steroids were developed (Djerassi, 1970).

In the 1960s and 1970s, it became clear that the available contraceptive methods did not completely meet the needs of women and their families; nor would they alone have a sufficient demographic impact to

[1] Mifepristone (RU 38486): 17β-hydroxy-11β-(4-dimethylaminophenyl-1)-17α (prop-1-ynyl)-estra-4,9-dien-3-one. Many publications are already available for reference: Herrmann et al. (1982) presented the first laboratory and clinical data on RU 486; the book edited with S. Segal (Baulieu and Segal, 1985) reported on the Bellagio meeting, which grouped almost all contributors known at that time; and many other reviews have partially covered the field, which has become very large: Henderson (1987); Neef (1987); Baulieu (1989a,b, 1991a,b); Laue et al. (1989); Avrech et al. (1991); Philibert et al. (1991); Ulmann et al. (1990); Cook and Grimes (1992); Horwitz (1992); Mao et al. (1992); Brodgen et al. (1993).

limit the population explosion. During those decades, ideas for new methods of contraception emerged as biology became focused more on the cellular and molecular elements of regulation of the reproductive system. The hormone-responsive proteins of target cells in the reproductive tract (termed receptors) were discovered, while progesterone (P), designated as the hormone of gestation (*pro gestare*) by Corner (in 1932) (Corner, 1963), was now easy to quantitate by radioimmunoassays (Lieberman et al., 1959). The uterine progesterone receptor (PR) (Milgrom et al., 1970) and the synthesis and action of prostaglandins (PG) (Bergström et al., 1972) were described, while the role of progesterone in the establishment and maintenance of pregnancy in women was demonstrated (Csapo and Pulkkinen, 1977). As it became clear that progesterone is involved at all steps of the reproductive processes, antagonists of progesterone were actively sought. As early as 1975, the concept of a "midcycle" contraceptive, a method based on progesterone receptor down-regulation with an "antiprogesterone" ligand, was proposed (Baulieu, 1975). Now, in 1993, we have a number of efficient antiprogestins. Although induction of abortion has been the most immediate application of such compounds, other potential applications include delivery, contraception, and treatment of several hormone-dependent diseases.

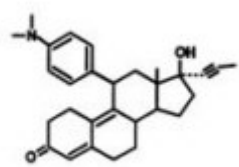

FIGURE B1.1 Mifepristone (RU 486).

When developing a procedure for the termination of pregnancy in women, it is important to be aware of both moral and physiological ideals, as well as psychological concerns. For centuries, abortion has been not only a morally difficult event for women, but also a physically painful and often dangerous procedure. A medical means for pregnancy termination should diminish this threat to women's health and, in turn, allow them to maintain their dignity. Furthermore, the distinction between abortion and contraception has lessened because the beginning of pregnancy is now understood, in physiological terms, to be a progression of steps. Hence, the term "contragestion" was proposed (Baulieu, 1985, 1989a, b) to clearly designate a method that can provoke pregnancy interruption (contra gestation) and operates as soon as possible after fertilization might have occurred, before the word abortion is appropriate (is an IUD considered an abortifacient?, see later discussion). This change in concept may be one of the most important outcomes of RU 486 development and usage.

ANTIHORMONES: THE 20 YEARS BEFORE RU 486

The aim of suppressing hormone activity is almost as old as the word hormone (wrm‘‘ein: to excite) itself. If a hormone molecule is excitatory for the target cells, then suppression of its effects can be attained by (1) abolition of its production, (2) blockade of its transport from the gland that produces it to target organs, or (3) blockade of its action at the target cell. In the case of small, lipophilic steroids such as P, which act intracellularly, the latter possibility could mean prevention of its entry into potentially responsive cells.

The first of these possibilities, suppression of biosynthesis, seems feasible in humans for some situations. For example, enzymatic inhibitors such as Epostane (4,5-epoxy-17β-hydroxy-4, 17α-dimethyl-3-oxo-5α-androstane-2-carbonitrile), which inhibits 3β-hydroxysteroid dehydrogenase, have been tested with some success in abortion (Birgerson and Odlind, 1987; Crooij and Janssens, 1988). An approach that blocks the action of hormones using specific antihormone antibodies—such as antibodies that interact with P in the blood or in target organs (Wang et al., 1989)—does not seem easily applicable to humans. However, an antihormone that operates directly at the receptor level may act more rapidly and be more specific than an inhibitor of a key enzyme involved in the synthesis of many steroids. In fact, the center of hormone action and thus the best molecular target for antihormonal action is the receptor (R) protein molecule, a mandatory element for cellular responses to hormone.[2]

The image of a receptor portrayed as a lock whose key is the hormone and whose keyhole (in fact a "binding site") can be competitively occupied and consequently put out of order by a false key (an antihormone) has been popular for decades. Because steroids are rigid molecules of well-defined conformation, as the high-affinity binding site of the receptor should be, it seemed logical to expect that a breakthrough in the hormone antagonism field would occur first in the antisteroid field. Initially, steroid receptors were detected by the binding of a traceable (radiolabeled) hormone to tissue extracts. The first of these so-called "radioreceptor" experiments was performed with tritiated estradiol (the natural estrogen) and MER 25, an antiestrogen (Segal and Nelson, 1958) that competed efficiently for radioactive hormone uptake and retention in the uterus (Jensen and Jacobson, 1962). The structure of MER 25 (Figure B1.2) is not that of a steroid. It is a triphenylethylene stilbene derivative with two phenyl rings mimicking rings A and D of

[2] RU 486 can be accommodated between partially unwound, double-stranded DNA bases by computer modeling. Yet the altered conformation of DNA cannot be correlated with the pharmacological properties of antiprogestins (Hendry and Mahesh, 1992).

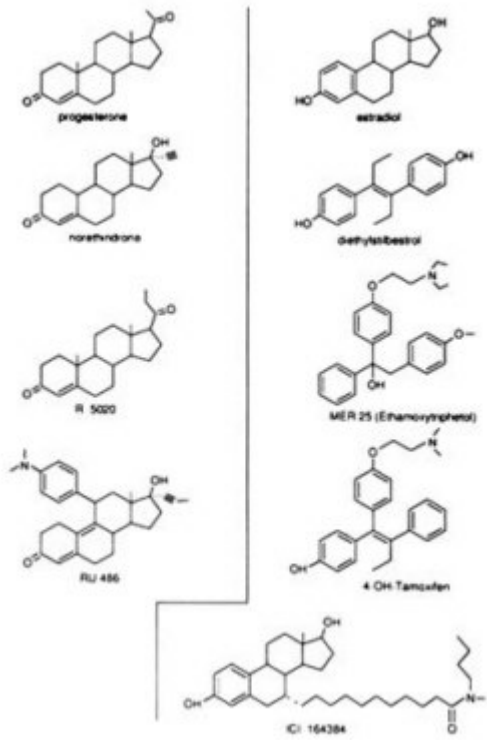

FIGURE B1.2 Structure of some progestins, estrogen agonists, and antagonists.

the steroids. X-ray crystallographic studies of the nonsteroidal estrogen diethylstilbestrol (DES) and estradiol (E) have delineated their similarity (Hospital et al., 1972). The third ring of triphenylethylene derivatives is perpendicular to the rest of the steroid-mimicking skeleton (Figures B1.2 and B1.3)—a fact that was of great importance. Given the high affinity that molecules such as E and DES show for the receptor, it was not surprising that triphenylethylene derivatives such as MER 25 and tamoxifen (Figure B1.2) had lower affinity than the agonists. However, the presence or absence of the third phenyl ring is not the critical factor for determining binding affinity since 4-hydroxytamoxifen, with an additional hydroxyl on the ring A equivalent of the tamoxifen molecule, mimicking the 3-hydroxyl group of estradiol, is a compound with high affinity for the receptor and has a resulting strong antiestrogenic effect ("pure" antagonist with no agonist activity in the chick) (Sutherland et al., 1977).

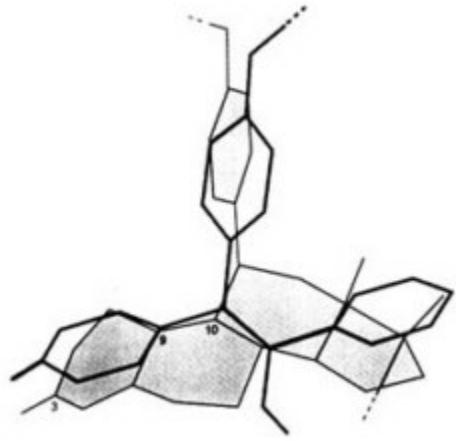

FIGURE B1.3 Superimposition of RU 486 and tamoxifen skeletons. Calculations from X-ray crystallographic data were made by Jean-Paul Mornon (Laboratoire de Crystallographie, CNRS URA 09 and Universités Paris VI and Paris VII).

I was very impressed by these last data concerning 4-hydroxytamoxifen because they contradicted the thinking of the time—all known antagonists had low affinity for their respective receptors: antiestrogens (e.g., tamoxifen), antiandrogens (e.g., cyproterone acetate, flutamid), antiglucocorticosteroids (e.g., P), and antialdosterone (e.g., spironolactone, P). Screening for antihormonal steroids tended to eliminate compounds demonstrating a high affinity for the receptor. In fact, there was no adequate theoretical reason to equate the quantitative notion of high affinity with the qualitative property of hormone antagonism. The latter was predictably due to specific conformational changes of receptor domain(s) that are involved in the transcription activation functions (TAF) of the receptor (see Figure B1.7 and later discussion), in particular in the ligand binding domain (LBD). In contrast, steroid binding affinity reflects interaction with the binding site, also located in the LBD, and is important only for kinetic quantitative aspects of the antihormone activity. I presented this scenario to Robert Bucourt who was head of chemistry at Roussel-Uclaf in the early 1970s.

Interestingly, Dr. Bucourt and his colleagues had collaborated with us to purify the estrogen receptor by affinity chromatography. Initially, this involved the screening of potential receptor ligands. Among the synthetic derivatives tested by Hélène Richard-Foy were estrogens with a long side chain grafted at the 7α-position. We selected one of them for receptor purification (Bucourt et al., 1978); however, Roussel did not test its biological activity. [About 10 years later, it was found to be an antagonist of estrogens by ICI researchers (Wakeling and Bowler, 1988).] It is important to note that the 7α-substitution on the steroid skeleton is somewhat symmetrical to an 11β-substitution, consistent with the

structures of the triphenylethylene antiestrogens and of the 11β-phenyl derivative compounds of the RU 486 series.

Also in the early 1970s, the Roussel chemists were working to improve the synthesis of new glucocorticosteroids and found a new way to produce 11β-derivatives of steroids. They discovered that 5α, 10α-epoxides obtained by metachloroperbenzoic acid treatment of 5(10), 9(11)-estradienes are prone to nucleophilic opening with Grignard reagents (Nédélec and Gasc, 1970). In addition, either copper chloride-catalyzed Grignard reagents or lithium organocuprates efficiently gave the corresponding regio- and stereospecific 11β-substituted 4,9-estradienes (Teutsch and Bélanger, 1979; Bélanger et al., 1981). Interestingly, the size of the substituent appears to largely determine agonistic or antagonistic activities.

Thus chemical research on the synthesis of glucocorticosteroids and biological studies of estrogens/antiestrogens converged when the RU 486 series of compounds was synthesized by Georges Teutsch and colleagues (Teutsch et al., 1988). The remarkable analogy of orientation of the third ring of tamoxifen and the fifth ring of RU 486 (approximately coplanar with the C-9 to C-11 bond, both perpendicular to the basic stilbene or steroid skeletons), is shown in Figure B1.3. Indeed, the 11β-phenyl-*N*-dimethyl-substituted estradiol is a strong antiestrogen (unpublished result).

The rest of the RU 486 story, which has been presented in several publications (see footnote 1), continued with the observation of the antiglucocorticosteroid activity of RU 486, and thereafter the demonstration of its antiprogesterone property. The decision to test it for human abortion was made after the endocrinological and pharmacological studies performed by Daniel Philibert and colleagues. We proposed that the compound was active and probably safe, but the idea of using RU 486 in human beings was almost "killed" by toxicologists who did not correctly interpret the signs of cortisol insufficiency when the product was given at very high doses in monkeys for several consecutive weeks. RU 486 was rescued by my insistence that it was just a beautiful (in vivo) demonstration of the antiglucocorticoid activity of the compound in primates (Baulieu, 1991c).

This compound became the subject of a political debate that is not relevant to this review. However, the scientific story is not complete, and should be pursued in order to improve and to extend the first discoveries.

CHEMISTRY: NOVEL MOLECULES

Almost all the potent antiprogestins and antiglucocorticosteroids so far described are 11β-phenyl-substituted steroids. The exception is RU 43044 (Figure B1.4), a 17β-substituted steroid: see later in the text. The

relatively long half-life of RU 486 in human beings (≈20 hours) seems to be due to its ability to bind to plasma orosomucoid (an α_1-glycoprotein) (Moguilewski and Philibert, 1985; Grimaldi et al., 1992). This binding is not found in nonhuman primates or other animals. RU 40555 (see Figure B1.4 for structure of this and other compounds discussed in this section) does not bind to the orosomucoid and has a shorter half-life, which may be of interest for kinetic assessment of the hypothalamus-pituitary-adrenal axis in clinical endocrinology (Bertagna et al., 1984; Gaillard et al., 1984). However, the binding of RU 486 and lilopristone (ZK 98734) to orosomucoid may enhance the antisteroid activity since it protects the drug against metabolic inactivation and provides a reservoir system for sustained delivery to target cells.

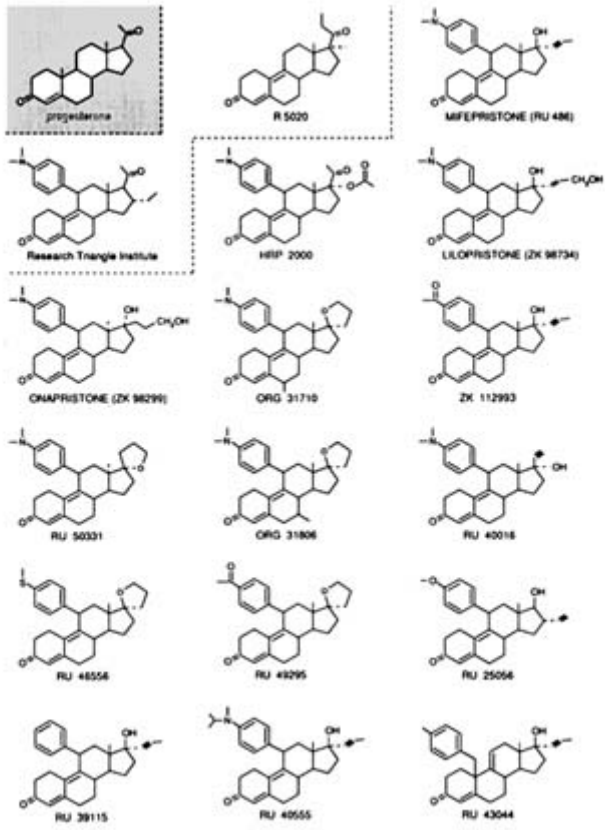

FIGURE B1.4 Structure of some currently available progestins and antiprogestins.

Since the early studies with RU 486, chemists have tried to dissociate the two main antihormonal activities of the compound and have aimed, for obvious medical reasons, to obtain "pure" antiprogestin(s) and

"pure" antiglucocorticosteroid(s) that would not display any other endocrine effects.

At present, there is no published account of a *pure antiprogestin* compound. However, it is important to note that for abortion the antiglucocorticosteroid effect is apparently neither necessary nor even useful, and that a single dose of ≤600 mg of RU 486 does not create any medical problem related to corticosteroid insufficiency. RU 486 derivatives,* such as RU 46556 and RU 49295, are strong antiprogestins with limited antiglucocorticosteroid activity. ORG 31710 (more active) and ORG 31806 have less antiglucocorticosteroid activity than RU 486 (Mizutani et al., 1992). A 17α-acetoxy derivative such as HRP2000 (Research Triangle Institute, Cook et al., 1992), with a 17β-progesterone side chain and a 11β RU 486-like substituent, is both an antiprogestin and an antiglucocorticosteroid. Curiously, 17β-acetyl, 16α-ethyl derivatives of 11β-phenyl-substituted steroids are progestin agonists (Cook et al., 1992). The Schering group has synthesized lilopristone, with a 17β side chain slightly different from that of RU 486; it has less antiglucocorticosteroid activity and higher binding to the androgen receptor. Another compound (ZK 112993) also has reduced antiglucocorticosteroid activity in the rat, due to an acetyl group on the 11β-phenyl moiety. A significant change in the RU 486 structure was obtained by making onapristone (ZK 98 299); due to photochemical epimerization at C-13, inversion of the D ring and substitutions at the C-17 position occur (Elger et al., 1986; Neef et al., 1984). Onapristone does not bind to orosomucoid (contrary to RU 486), does not bind to the chicken (c) PR (like RU 486) (Nath et al., 1991), is an antiprogesterone (but less active than RU 486), and has weak antiglucocorticosteroid activity. Its mechanism currently is controversial (see later).

A *pure antiglucocorticosteroid* may be easier to use chronically in premenstrual women. One possibility is RU 40016, an RU 486-like compound with inversion of substituents at the C-17 position. Although not very active, it has relatively more antiglucocorticoid and fewer antiprogestin effects than RU 486. RU 43044 is chemically very different, since the additional phenyl substituent is in the 10β position, and although this ring is partially superimposable spatially, with a phenyl group in 11β, there is no binding to the PR, and the activity is purely antiglucocorticosteroid (but weaker than that of RU 486). The compound, perhaps because of its metabolism, has no activity in vivo in animals; however, its activity in situ may provide some clues for the synthesis of a series of locally active thereapeutic agents.

In conclusion, the 11β-phenyl substitution is essential in determining the antagonistic properties of most antisteroids, while an 11β-aliphatic

* RU 46534 is a very active "contragestive" agent in dogs. The only structural difference with RU 486 is its allylic 17α-side chain.

chain may result in agonistic derivatives (Figure B1.5). However, most steroidal structures do not carry an absolute intrinsic property of agonism or antagonism per se, as demonstrated by steroid binding differences between the PR of different species, changes of activity when mutating the receptor LBD, and activity differences in various target cells under different physiological states.

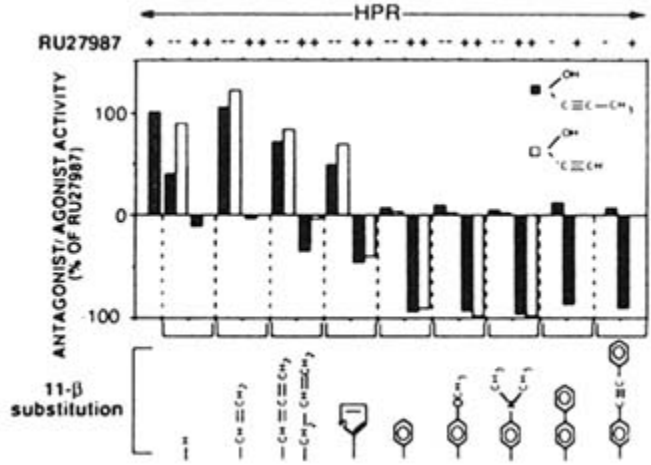

FIGURE B1.5 Agonists and antagonists of 11β-substituted steroids. Agonistic and antagonistic potential of two series of 11*β*-substituted steroids that differ from RU 486 only in their 11*β*-substitutions or their 11*β*- and 17α-substitutions. The chemical symbols in the top right corner of the upper panels illustrate the 17α-substitutions. Transcription activation was quantitated from normalized CAT assays in HeLa transiently-transfected cells with the MMTV-CAT reporter gene and the human progesterone-receptor (hPR) expression vector hPR1 in the presence of the various compounds. The agonistic potential of the hPR in the presence of 1 μM of these steroids alone (- RU 27987, at the top) is expressed as a positive value relative to the activation seen with 10 nM RU 27987 (arbitrarily assigned +100). Antagonistic potential was assayed by exposing transfected cells to 1 μM of a given steroid plus 10 nM RU 27987 (+ RU 27987 at the top) and is expressed as a negative value, with -100 indicating complete inhibition of RU 27987-induced transcription. The individual 11β-substitutions are depicted. RU 27987 is a 17α,21-dimethyl-3,20-dioxo-21-hydroxy-19-nor-pregna-4,9-diene progestin agonist. SOURCE: Garcia et al. (1992); © The Endocrine Society.

RU 486 and many corresponding compounds from Schering and Organon do not bind to cPR (Groyer et al., 1985), although they bind to the PR of humans (hPR) and most other mammals. The change of a cysteine (Cys) in the N-terminal region of the cPR LBD (Cys 575) to a glycine (Gly), as found in the hPR (cPR Cys 575 → Gly), permits the binding of RU 486 and antisteroid activity. Interestingly, RU 39115 (which is RU 486 minus *N*-dimethyl) is an antagonist of the hPR, but an agonist of the "humanized" chicken PR (cPR 575 → Gly). This indicates that the interaction of steroid and receptor is more complex than just binding

ability, and may depend on the overall structure of the LBD and consequent modification of TAF2 function (see later). Systematic experiments indicate that depending on the nature and positioning of the 11β-phenyl substituent, one may produce 11β-substituted steroids with progestin agonistic, antagonistic, or mixed agonistic and antagonistic activities (with, as expected, no relationship to binding affinity) (Benhamou, 1992; Garcia et al., 1992) (Figure B1.6).

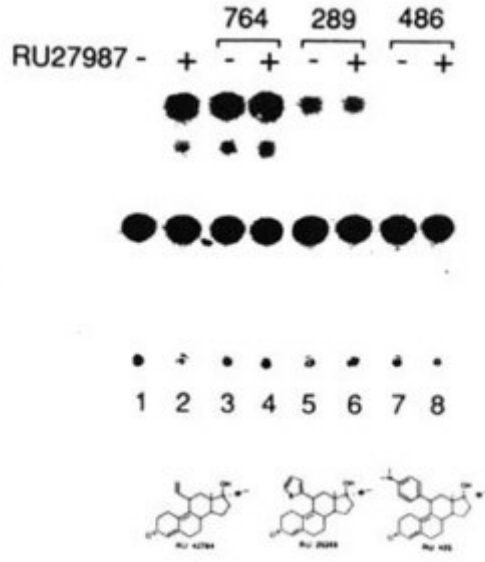

FIGURE B1.6 Agonists, antagonists, and mixed agonists-antagonists. Ligands for the hPR may generate three distinct types of TAF2-dependent transcriptional responses: they act as *agonists* with no antagonistic potential or as *antagonists* with no agonistic potential, or they may generate a *mixed* response, since they both activate and antagonize transcription activation. HeLa cells were transiently transfected with a reporter gene and a progesterone response element and exposed to the steroids in the absence or presence of RU 27987 (Figure B1.5). Steroids were used at 1 µM (- RU 27987; lanes 3, 5, 7); in cases where the antagonistic potential was analyzed, activation was achieved with 10 nM (RU 27987; lanes 4, 6, 8). Note that RU 28289 acts as both agonist and antagonist. SOURCE: Garcia et al. (1992); © The Endocrine Society.

Indeed it is logical that the structure of the steroids and of the LBD combine ultimately to direct the conformation and, thus, the function of TAF2, therefore "deciding" if a compound will act as an agonist or an

antagonist. This is potentially important for cancer treatment, since the steroid-receptor mutations that are observed in certain tumors may radically change the properties of their receptors and the effectiveness of steroidal drugs.

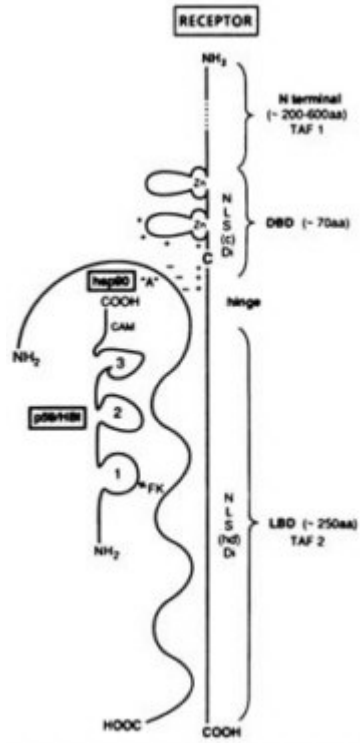

FIGURE B1.7 Schematic representation of a steroid hormone-receptor consensus structure in its "8S" heterooligomeric form. NOTE: aa = amino acid; NLS(c) = constitutive nuclear localization signal; NLS(hd) = hormone-dependent nuclear localization signal; Di = dimerization; FK = FK506; CAM = calmodulin; C = cysteine; + and - = conserved charged amino acids. Other abbreviations are as indicated in text.

CELLULAR AND MOLECULAR MECHANISMS OF ACTION OF ANTIPROGESTINS: THE RECEPTOR AT THE CENTER

The "consensus" anatomy of steroid receptors (Evans, 1988) and the concept of associated proteins (Lebeau et al., 1993) are illustrated in Figure B1.7. Shown in Table B1.1 and Figure B1.8 are several steps involved in the intracellular mechanism of steroid hormone and antihormone action.

Progesterone, cortisol, and their cognate synthetic agonists and antagonists seem to enter target cells freely and appear not to be

significantly metabolized. They bind to receptors with high affinity ($K_D \leq 10$ nM). A given ligand may bind to different receptors (for example, P and RU 486 bind to both PR and the glucocorticosteroid receptor [GR]), and a given receptor can bind multiple hormones (e.g., the GR binds both cortisol and P).

TABLE B1.1 Steps in the Intracellular Mechanisms of Steroid Hormone/ Antihormone Actiona

Entry into target cells
Intracellular metabolism (activation/inactivation)
Ligand binding to intracellular receptor(s)
Receptor transconformation
Modulation of hsp90 and other receptor-associated proteins
Nuclear localization of receptor
Dimerization of receptor
Binding of ligand-bound receptor to hormone-responsive elements (HREs) of regulated genes
(DNA dependent) receptor hyperphosphorylation
Change of chromatin structure/function
Modulation, via receptor transcription activation function(s) (TAF[s]), of the transcription factors (TFs)/transcription intermediary factors (TIFs)
Receptor down-regulation
Other gene expression interference
Overall increase or decrease of transcription and/or translation

NOTE: The most important (and/or more studied) steps are in boldface.

[a] Membrane mechanisms of action of steroids have been demonstrated for progesterone and its metabolites (Baulieu et al., 1978; Finidori-Lepicard et al., 1981; Baulieu, 1991d), but not for RU 486 and antiprogestins (no effect on ovocyte meiosis, GABAA-receptor function, sperm activation).

Upon binding of a ligand, transconformation of the receptor protein occurs. This molecular "reaction" is *central* to the mechanism of action of hormones and antihormones, and determines nuclear localization, binding to the hormone response element (HRE) of regulated genes, chromatin change, activation or inhibition of transcription, and possibly other activities.

The GR and PR, like other steroid hormone receptors, form heterooligomeric, non-DNA binding, "nontransformed" 8S complexes that include *receptor-associated* proteins (Baulieu et al., 1989; Lebeau et al., 1993). The most studied of these proteins are a heat shock protein of MW = 90,000 Da (hsp90), a "chaperone" protein (Baulieu and Catelli, 1989), and p59-HBI (a hsp90 binding immunophilin) (Callebaut et al., 1992) (Figures B1.7 and B1.8). Although their roles are not completely understood and remain controversial, certain relationships among these proteins are apparent.

The p59-HBI, a peptidyl-proline isomerase, also known as FKBP56 or 52, binds immunosuppressants such as FK506 and rapamycin. The binding of hsp90 by p59-HBI is not competitive with the binding of the immunosup

pressants, which are inhibitors of the isomerase activity. The possible effect of p59-HBI binding to hsp90 on receptor function is, at present, unknown. However, binding of p59-HBI to FK506 or rapamycin in vitro increases progestin and RU 486 binding to the rabbit uterus PR (Renoir et al., 1992) and immunosuppressants augment the response of transfected reporter gene to corticosteroids (Ning and Sanchez, 1993).

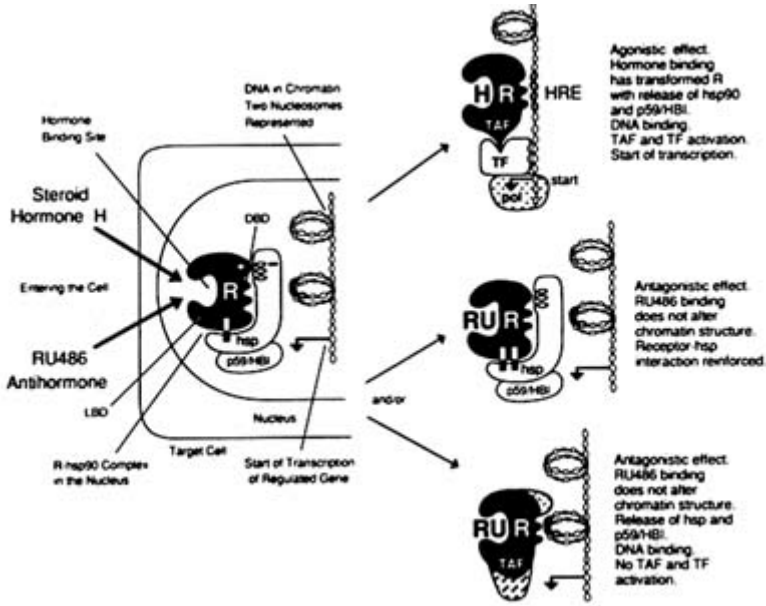

FIGURE B1.8 Cellular and molecular mechanisms of action of steroid hormone and antihormone. NOTE: hsp = heat shock protein, molecular mass ≈ 90,000 Da; pol = RNA polymerase II; TF = transcription factor. Other abbreviations are as indicated in text.

Hsp90 binds to the LBD apparently in a multipoint arrangement (Pratt et al., 1988; Cadepond et al., 1992), and also binds to a positively charged region at the C-terminal extremity of the DBD (Chambraud et al., 1990). This disposition obliterates the DNA binding to the DBD (Baulieu and Catelli, 1989), and hsp90-receptor complexes do not bind to DNA (Bourgeois et al., 1984).

In hsp90-containing 8S receptor complexes, there are two molecules of hsp90 (Radanyi et al., 1989) and one molecule of the PR, GR, or MR, or two molecules of the ER (Redeuilh et al., 1987a; Rexin et al., 1988; Rafestin-Oblin et al., 1989; Renoir et al., 1989, 1990).

Hsp90, in the role of chaperone, maintains the structure of the GR LBD in the appropriate ligand-binding conformation and seems to protect non-ligand-bound steroid receptors from chemical or enzymatic attack. It was also recently observed that hsp90 may competitively

interfere with the binding of the HRE of an estrogen-regulated gene to the ER, and thus could modulate the process of transcription (Redeuilh et al., unpublished). Agonist binding to the LBD modifies the structure of the latter in such a way that it favors the release of hsp90 from the receptor, and therefore allows binding of the receptor to the HRE and interaction with transcription factors (TFs) engaged in the transcription machinery. There may be a change of ligand affinity for the receptor even before dissociation of hsp90 (Redeuilh et al., 1987b). LBD modifications after binding of ligand are involved in TAF function, receptor dimerization, and probably chromatin changes.

Whether hsp90-receptor complexes are formed in the cytoplasm, the nucleus, or both, and whether hsp90 binding plays a role in the transfer and shuttling of the receptor between the two compartments, are not yet clear. Nuclear binding to the HRE and recycling of the receptor depend upon these movements.

The hormone-dependent homodimerization of receptors is important for their binding to the two halves of the (imperfect) palindromic HRE. The LBD and the DBD include domains for dimerization.

The progesterone response element (PRE) and the glucocorticosteroid response element (GRE) are enhancer sequences, as are other HREs for other steroid receptors. They are ligand activated following receptor binding and are most often physically situated in the 5'-promoter region of hormone-regulated genes. Schematically, HREs may be seen as allowing the receptor dimer to be placed in the appropriate position for interaction with other TFs, which bind to their specific DNA sequences and are involved in the function of RNA polymerase, the enzyme that is ultimately operational in gene transcription. There are proteins that appear functionally to link receptors and TFs, and are sometimes designated as transcription intermediary factors (TIFs). These factors are necessary when the HREs are situated far away, in molecular terms, from the transcription initiation site. Receptor transconformation following ligand binding determines the appropriate interaction of the receptor with TFs/TIFs. Receptor transconformation is also likely to be involved in inducing chromatin changes, which themselves eventually cooperate in hormone action by allowing or inhibiting TF/TIF function. The receptor domains involved in TAFs are described later.

RU 486 may interfere with several of the steps indicated in Table B1.1, which are discussed separately below.

Ligand Binding

RU 486 has high affinity for the hPR and the hGR (as 4-hydroxytamoxifen has for the hER), weak affinity for the human androgen receptor, and no affinity for the ER and the MR (thus being a useful

compound for the study of this last receptor in the presence of GR, since GR and MR share many high-affinity hormone ligands and GR is found in almost all cells). Kinetic experiments have shown differences between P and RU 486 binding to the PR (Skafar, 1991).

Receptor Transconformation

Receptor transconformation is a conformational change in the receptor that occurs after ligand binding. Transconformation may take place before dissociation of hsp90 from the 8S complexes (Allan, 1992a,b), as observed with the 8S ER (Redeuilh, 1987b), and thus precedes DNA binding and activation of the transcription function.

Selective, hormone-, or antihormone-related transconformation has been physically suggested by several observations:

1. Proteolytic enzymes do not have the same effect on the PR LBD when bound to progestin or RU 486 (Allan, 1992a).
2. An antibody such as mAb C262, raised against the last 14 C-terminal amino acids of the PR, binds to the RU 486-PR complex but not to the P-PR complex (Weigel, 1992).
3. The electrophoretic mobility of RU 486-PR bound to the PRE (gel shift experiments) is faster than that of P-PR bound to the same PRE (El-Ashri et al., 1989; Meyer et al., 1990). A difference in the receptor structure when bound to hormone versus antihormone has also been observed with antiestrogen-ER complexes (Sabbah et al., 1991).
4. In contrast to R 5020-PR (R 5020 is a synthetic progestin agonist of the 4S sedimentation coefficient), RU 486-PR forms 6S entities in salt-containing gradient centrifugation experiments (Mullick and Katzenellenbogen, 1986; Renoir et al., 1989). It is not clear whether this form is transconformed monomeric PR, homodimeric PR, or a heterodimer of one molecule of PR plus another protein, perhaps hsp70.

The lack of RU 486 binding by the cPR and the Cys 575 → Gly mutation that corrects this defect have been discussed above. Conversely, if the corresponding Gly of the hPR is transformed into cysteine, RU 486 no longer binds. In the hGR, if the corresponding Gly is transformed to Cys, the receptor does not bind dexamethasone or RU 486. Thus, exchanging cysteine and glycine may modify, in a complex manner, the overall LBD structure, particularly at the level of the pocket where it binds the 11β-substituent.

A truncated PR molecule (minus the 42 C-terminal amino acids) does not bind progestin agonists but does bind RU 486, which remarkably becomes an agonist (Vegeto et al., 1992). This, and other results mentioned above, suggest that there are both a common

binding site for progestin and antiprogestin involving the C-terminal amino acids of the LBD, and another binding site for RU 486 (and other 11β-phenyl-substituted steroids) at the N-terminal extremity of the LBD. Moreover, from these results, it has been postulated that the C-terminal region of the receptor could act as an inhibitor of TAF1 in the absence of ligand or when bound to RU 486, with the negative function being released in the presence of an agonist (Vegeto et al., 1992).

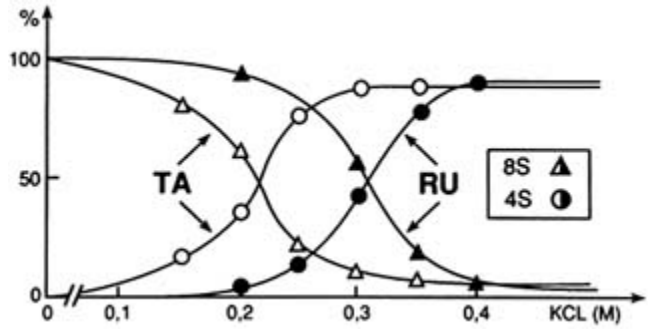

FIGURE B1.9 Glucocorticosteroid receptor (Δ0-417) 8S → 4S versus ligands. Stabilization of RU 486 by the heterooligomeric, non-DNA-binding form, of the glucocorticosteroid receptor in KCI-containing medium, as compared to the transformation effect of the agonist triamcinolone acetonide (TA). The receptor mutant Δ0-417 includes almost exclusively the DBD and the LBD. SOURCE: Segard-Maurel et al. (1992); reprinted with permission from Pergamon Press Ltd.

Heat Shock Protein Binding

Transconformation of the LBD is probably involved in the modulation of hsp90 interaction with the receptor, and the in vitro stabilization of hsp90-containing 8S complexes after RU 486 binding has been demonstrated for RU 486-bound GR (Groyer et al., 1987) and PR (Renoir et al., 1989) (several other 11β-substituted steroids do the same) (Figure B1.9). In intact cellular systems, this stabilization has also been observed by several workers (Rajpert et al., 1987; Lefebvre et al., 1988; Segnitz and Gehring, 1990). Moreover, some experiments have indicated that RU 486-GR remains more cytoplasmic than agonist-GR (Ylikomi et al., 1992).

It should be acknowledged, however, that the situation is complex; nuclear retention of GR may vary depending on the phase of the cell cycle (Hsu et al., 1992). Also on this point, a number of studies in intact cells have strongly suggested that RU 486-receptor complexes move to the nucleus and then bind to HREs (see "Binding to DNA"). The matter

is still controversial, and we cannot definitively state whether RU 486-receptor stabilization plays a significant role in antihormone action.

Receptor Dimerization

The extent of receptor dimerization frequently correlates with the specific DNA-binding activity of receptors. Indeed, RU 486 favors more dimerization of the PR than R 5020 (De Marzo, 1992). Due to the natural occurrence of distinct A and B subunits in the PR (PR-A and PR-B) and to the fact that the cPR does not bind RU 486, experiments have been done to analyze the potential heterodimerization of two PR subunits, one subunit binding the agonist R 5020 and the other subunit binding the antagonist RU 486 (Guiochon-Mantel et al., 1989; Meyer et al., 1990; De Marzo et al., 1992). In spite of different transconformations, R 5020-PR and RU 486-PR can form a heterodimer in solution, but not if the subunits bind to an artificially symmetrical palindromic PRE sequence (Meyer et al., 1990). If the PRE palindrome is imperfect, as it is naturally, formation of a heterodimer is possible (De Marzo et al., 1992). Therefore, dimerization is dependent not only on ligand-receptor interaction but also on receptor-DNA interaction. Heterodimerization including one RU 486-bound PR unit may be involved in the strong antihormonal activity of RU 486 via the negative effect of the RU 486 subunit on the heterodimer function.

Binding to DNA

RU 486, lilopristone, and ORG compounds all stimulate the binding of the PR to PREs (Bailly et al., 1986; Guiochon-Mantel et al., 1989; Turcotte et al., 1990; Mizutani et al., 1992). These results, in which the receptor is transformed and dissociated from hsp90, are consistent with the agonistic effects of RU 486 observed in some cell systems (Meyer et al., 1990) and some cell-free experiments with the GR (Schweizer-Groyer et al., 1988). The term "agonistic" implies that the receptor can bind to appropriate specific DNA. In the case of an antihormonal effect, indirect but suggestive evidence has been obtained by competition of a constitutively active (truncated) form of progesterone receptor with RU 486-PR complexes (Guiochon-Mantel et al., 1988).

When agonist-GR and RU 486-GR complexes bind to DNA, however, the results are kinetically different (Shauer et al., 1989). Since RU 486 binding to DNA is not followed by an effect on transcription, the RU 486-receptor complexes are unproductive, possibly due in part to a defect in chromatin structure and/or interaction with TF/TIF.

It has been suggested that the onaprisone-receptor complex does not have the ability to bind to HREs (Klein-Hitpass et al., 1991). This would

define a *new type of antisteroid* ("type one" for Klein-Hitpass, "type two" for Bocquel et al. [1993]). In competition experiments, onapristone inhibits the induction of DNA binding of PR by progestins and DNA-binding antiprogestins. This could be due to the lack of formation of a stable receptor dimer, but it has been recently disproved by Bocquel et al. (1993). In vitro, onapristone, in contrast to RU 486 (Schweizer-Groyer et al., 1988), may not display agonistic activity (Klein-Hitpass et al., 1991). *Hyperphosphorylation* of the receptor that is observed under agonist or RU 486 action is not observed using onapristone, again consistent with the hypothesis that a DNA-dependent protein kinase activity is involved (Bocquel et al., 1993).

However, the same proteolytic pattern of the PR was found with complexes involving so-called DNA binding and non-DNA-binding antiprogestins (Allan et al., 1992a). Moreover, these experimental differences between onapristone and other antiprogestins, particularly RU 486, may be ascribed to its low affinity for PR (Delabre et al., 1993).

Studies of Chromatin

In contrast to glucocorticosteroids, RU 486 does not modify chromatin structure, as assessed by DNase I experiments in the mouse mammary gland tumor virus (MMTV) and the liver tyrosine aminotransferase (TAT) systems (Véronique Marsaud and Hélène Richard-Foy, personal communication). The most likely explanation is that the chromatin modification induced by receptor transconformation after agonist binding does not occur after RU 486 binding. In addition, the binding of NF1, a nonspecific transcription factor, to its DNA site in the MMTV system does not take place after RU 486 binding as it does after the binding of an agonist.

Gene Transcription

Activation of gene transcription is regarded as the major mode of action of steroid hormones, mediated by two transcription activation functions of the receptor (Evans, 1988; Lees et al., 1989; Tora et al., 1989; Gronemeyer, 1991). TAF1 is situated in the N-terminal portion of the receptor molecule, is not hormone dependent, and can be regulated by cell-specific factors. TAF2 is hormone dependent, activated by agonist binding, and inhibited by antagonists, as demonstrated with tamoxifen derivatives for the ER (Webster et al., 1988) and RU 486 for the PR (Meyer et al., 1992). When TAF2 is inhibited, TAF1 may still operate differently depending on the cell type, and thus an antihormone may show some agonistic activity following binding of the receptor to DNA. This is true with RU 486 and theoretically should never be found with

non-DNA-binding antagonists (if they exist). The type of receptor may itself influence the result, with a probable role for the highly variable N-terminal domains. For instance, there are experiments in which RU 486 PR-B is agonistic and RU 486 PR-A is antagonistic toward the activation of a PRE-TK reporter gene (Meyer et al., 1990). TAF1 may also be controlled by an inactivation factor similar to that reported in yeast (McDonnell et al., 1992); if such an inactivation factor gene is suppressed, RU 486 becomes an agonist.

The number and positioning of the HREs, their varying structures in different genes stimulated (or repressed) by the same hormone, the variety of TFs/TIFs present along with receptors and HREs in different cells, and the other informational influences that reach the steroid-dependent transcription systems make the global network responding to steroids extremely diverse and difficult to interpret. An example of this is the modifying effect on antiprogestin action of another cAMP-dependent gene (Sartorius et al., 1993).

The expression of the receptor itself may change under the effect of its own ligand, as demonstrated for the *down-regulation* of the PR by progesterone (Milgrom et al., 1973). However, the effect of RU 486 on receptor down-regulation cannot be systematized, not being observed in certain cases (Sheridan et al., 1988) or being described in other instances (El-Ashry et al., 1989).

In conclusion, as would be predicted, everything depends on the complex formed by the ligand and the receptor, which is then transconformed after binding. It is important to note that genetic variation may abrogate the response to RU 486. Perhaps this is the case in the 1 percent of failures in abortion (see later), or in cancers if there are mutations such as those cited above. Hormone action involves a number of phenomena that are narrowly connected—at least temporally: release of hsp90 and other associated proteins, dimerization of the receptor, DNA binding and chromatin changes, and interaction with TFs/TIFs. The net result may be to increase or decrease gene transcription, and depends in part on specific HREs and TF(s). Antiprogestins are remarkable tools for dissecting complex cellular networks. In addition to more discoveries in cell biology, the study of these networks should lead to improved use of RU 486 and the development of novel molecules.

How lucky we were not to know of this complexity before testing RU 486, which worked so well on the basis of a "simple" hypothesis! (Herrmann et al., 1982.)

PHYSIOPHARMACOLOGICAL REPRODUCTIVE EFFECTS

The activity of RU 486 has been studied in many progesterone-responsive reproductive systems (Baulieu, 1989a; Brodgen et al., 1993) (Table B1.2). For

example, studies in the uterus, endometrium, myometrium, and cervical tissue directly relate to the use of RU 486 in fertility control.

TABLE B1.2 Targets for Antiprogestins

Reproductive System	Hormone or Process
Central nervous system	GnRH
	Glial cells (meninges)
Pituitary	LH
	FSH
Ovaries	Folliculogenesis
	Ovulation
	Oocyte maturation (?)
	Granulosa cells
Fallopian tubes	Tubal transport
Uterus	Endometrium
	Estrogen-dependent growth
	Maturation
	Decidua
	Implantation
	Immunological reaction
	Myometrial contractility
	Cervical maturation
	Maintenance of pregnancy
	Labor/delivery
Placenta	Placenta hormones
Sperm	Activation

NOTE: FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone.

In the human myometrium (Table B1.3), RU 486 reverses the quiescence effect of progesterone, which depends on (poorly defined) effects on calcium cellular distribution and metabolism leading to decreased excitability; stimulates intercellular gap junction; and decreases β_2-adrenergic receptor synthesis (Vivat et al., 1992). In addition, prostaglandin $PGF_2\alpha$ synthesis is increased and prostaglandin (PG) metabolism is decreased by RU 486. There is a decrease of prostacyclin release (Lobaccaro-Henri et al., 1992), and the myometrium sensitivity to the contractile effect of PGs of the F and E series is enhanced (Norman et al., 1991). In the rat, the increase of PG levels may not precede the stimulation of uterine contractility (Arkaravichien and Kendle, 1992).

Some hormonal components of RU 486 activity are difficult to classify. For instance, RU 486 and onapristone display an ER-independent *antiestrogenic* activity in the endometrium (Van Uem et al., 1989; Wolf et al., 1989a; Chwalisz et al., 1991; Shi et al., 1992), where they decrease the

glandular formation. This seems paradoxical since RU 486 and onapristone do not bind to the ER, and there is a PR-dependent increase of ER levels (indicated by both hormone binding and immunocytochemistry) (Haluska et al., 1990; Neulen et al., 1990).

TABLE B1.3 Interactions of Steroids/Prostaglandins/ Catecholamines in the Human Myometrium

Stimulatory Agents	Transducing Steps	Effects	Results
Progesterone	↑ Ca^{2+} ↓ cAMP β-Adrenergic receptor ↑	Relaxation	Contractility
Prostacyclin			
Estrogens	↑ Ca^{2+} α-Adrenergic receptor ↑	Contraction	
Oxytocin $PGF_{2\alpha}$			

NOTE: cAMP = adenosine cyclic 3′,5′-phosphate; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$.
SOURCE: Egarter and Husslein (1992).

In pregnancy, RU 486 inhibits the production of human chorionic gonadotropin (hCG) and prolactin in cultured syncytiotrophoblast cells and explants (Herrmann et al., 1985; Das and Catt, 1987). It decreases a marker of ER function, p29, in the placenta and the decidua (Rivera et al., 1991). RU 486 also increases the natural killer activity of lymphocytes (Hansen et al., 1992). In rats, it augments the interstitial collagenase activity of the cervix (Ikuta et al., 1991). RU 486 can inhibit the ovulation process (Loutradis et al., 1991) directly at the level of the ovaries, and may decrease gonadotropin-stimulated P production of granulosa cells (Parinaud et al., 1990).

Changes in the *hypothalamus-pituitary-gonadal* system have been reported. The PR is present in β-endorphin neurons and dopamine neurons in the arcuate nucleus, but not in gonadotropin-releasing hormone (GnRH) neurons themselves, which are indirectly under P control (Yen, 1991). Luteinizing hormone (LH) is essentially depressed by RU 486 (Schaison et al., 1985; Garzo et al., 1988), and a decrease of follicle-stimulating hormone (FSH) may be explained by an increase of inhibin (Sanchez-Criado et al., 1992). *Sexual behavior* can be modified by the antiprogestin effect of RU 486 (Brown and Blaustein, 1986; Pleim, 1990).

In breast cancer cells, RU 486 inhibits P-induced transcriptional activity, which leads to the synthesis of *fatty acid synthetase* but, surprisingly, it stabilizes the mRNA of this enzyme (Chalbos et al., 1991).

Agonistic effects of RU 486 have been observed in vivo in the uterus (e.g., Gravanis et al., 1984) and are also responsible for 3T3 differentiation of preadipocytes (Rondinone et al., 1992), consistent with the

observations made in vitro, as discussed above.

TABLE B1.4 Uses of RU 486

Contraception/Contragestion/Abortion
5–9 weeks of amenorrhea
Occasional luteal contragestion
Once-a-month menses induction
Emergency contraception
Once-a-month anti-implantation
"Endometrial" contraception
Suppression of ovulation
Medical Interruption of Pregnancy
Second trimester
Third trimester
Labor Induction
Medical Indications
(Antiprogestin)
Endometriosis
Uterine fibroid
Breast cancer
Meningioma
(Antiglucocorticosteroid)
Endocrine test
"Peripheral" hypercorticism
Glaucoma
Wound/burn healing

Thus, administration of RU 486 may produce effects in different directions, which have to be studied separately in each tissue. However, to provoke abortion or trigger labor, the effects on the decidua, endometrium, cervix, and even the trophoblast combine to make RU 486 a very efficient agent for the termination of pregnancy.

REPRODUCTIVE MEDICINE (TABLE B1.4)

Voluntary Early Pregnancy Interruption

On the basis of animal experiments, we expected RU 486 to meet the recognized need for an efficient medical means of early abortion, to be safer than a surgical technique, and to be relatively convenient and cheap (no anesthesia, no operating room). To develop a medical method of abortion was a must in terms of women's health and potentially a step toward more privacy for those having taken the difficult decision of pregnancy termination.

Early abortion offered the first opportunity to test RU 486 activity in

humans, and this was initially performed on a small group of women volunteers, under the direction of Walter Herrmann at the Hôpital Cantonal in Geneva in 1982. Since the postulated mechanism of action (Figure B1.10) suggested that a single dose should be sufficient, and since the compound was rapidly eliminated from the body, only rather short toxicological studies were necessary.

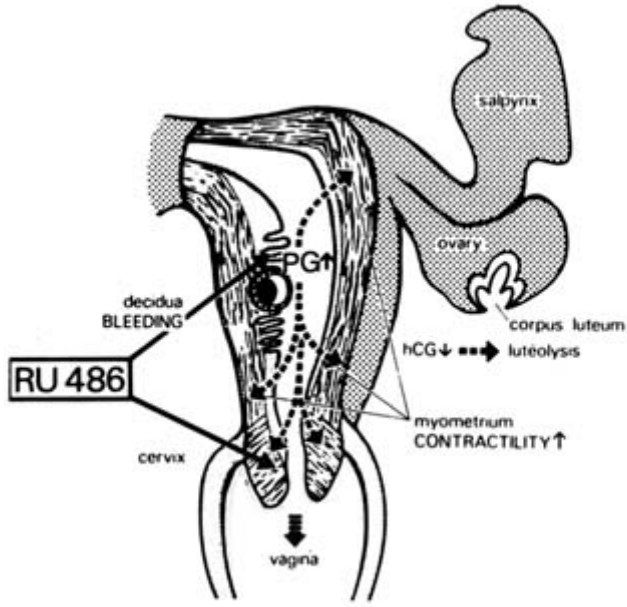

FIGURE B1.10 Physiopharmacological mechanism of action of RU 486 on the implanted blastocyst. Temporally, the antiprogesterone effect of RU 486 comes first, and then an increase in PG concentration and action, followed by a decrease in hCG-sustained corpus luteum function.

The results were so striking that RU 486 immediately got the nickname "abortion pill," despite the many other potential medical uses already predicted when the compound was announced (they progressively became reality). The first large-scale studies indicated approximately 80 percent success in pregnancies of £42 days of amenorrhea (Couzinet et al., 1986), and the compound was presented for registration to the French Ministry of Health. However, after the study by Bygdeman and Swahn (1985) (Table B1.5), the improved results obtained with the administration of a small dose of prostaglandin 48 hours after RU 486 administration (Baird et al., 1988; Dubois et al., 1988a) led to the approval (AMM: *autorisation de mise sur le marché*) in September 1988 of RU 486 plus prostaglandin for up to 49 days of amenorrhea pregnancies, in the context of the French abortion law. Sulprostone (a PGE_2 analogue,

250 µg injected intramuscularly) was the prostaglandin used most. In Great Britain, the trials with gemeprost (a PGE_1 analogue, 0.5 mg administered vaginally) were satisfactory when given up to 63 days of amenorrhea; registration (approval) was acquired there in July 1991, and in Sweden in 1992 with the same protocol. The largest study (Ulmann et al., 1992) indicated 95 percent complete efficacy, with 1 percent of ongoing pregnancies, emphasizing the obligation to evacuate the uterus instrumentally in case of failure. There were no particular bleeding problems—only approximately 1/1000 patients received a transfusion. However, three myocardial infarctions, including one fatal case (a medical mistake occurred when sulprostone was injected into a woman at great risk), were recorded after more than 60,000 cases. Sulprostone for intramuscular administration has since been withdrawn from the market in France.

TABLE B1.5 Uterine Activity During Early Pregnancy in Control and RU 486-Treated Patients (Montevideo units; mean ± SEM)

	Control	36 Hours After 50 mg of RU 486
Mean uterine activity	6 ± 4	222 ± 93
Sulprostone, 0.05 mg[a]	49 ± 24	711 ± 136

NOTE: SEM = Standard error on the mean.
[a] Administered 0.5 hour after start of recording.
SOURCE: Swahn and Bygdeman (1988).

Coincidentally, the death of a patient (having received intramuscular Sulprostone) was reported at the same time as the first trial of RU 486 plus orally active misoprostol (a PGE_1 derivative) was published (Aubeny and Baulieu, 1991). We had hoped to use a safer prostaglandin (misoprostol has a record of millions of users for the prevention and treatment of gastrointestinal ulcers in individuals often at greater cardiovascular risk than normal pregnant women). It was also obvious that an orally effective, already available, cheap, and easy to store prostaglandin had the potential to be an important improvement, since it could allow a more convenient and private method of abortion. Most results (Peyron et al., 1993) have been obtained by using 600 mg of RU 486 and 400 µg (two tablets) of misoprostol 48 hours later. Four hours after misoprostol, approximately 70 percent of women aborted; if expulsion did not occur, a third misoprostol tablet (200 µg) was proposed to the patients. Greater than 98 percent efficacy was achieved according to the current trials for women with ≤49 days amenorrhea pregnancies (Table B1.6). Besides safety, this regimen was well tolerated—uterine cramps were minimized. Details and discussion of the method are found elsewhere (Peyron et al., 1993).

TABLE B1.6 Interruption of Early Pregnancy with RU 486 and One or Two Doses of Misoprostol

Treatment	Total	Number of Successes[a] (% of total)	Number of Failures[b] (% of total)	
Mifepristone, 600 mg	385			
Abortion before the time of administration of misoprostol (400 µg)	385	21 (5.4%)		
Administration of first dose of misoprostol followed by abortion within 4 hours	364	266 (69.1%)		
Later outcome	98			
Refuse more misoprostol	27	26 (6.8%)	Ongoing pregnancy	1 (<1%)
Second dose of misoprostol (200 µg)	71	67 (17.4%)	Partial retention	2 (<1%)
			Synechiae with ongoing pregnancy	1 (<1%)
			Ectopic pregnancy	1 (<1%)
Total		380 (98.7%)		5 (1.3%)

[a] Success was defined as interruption of pregnancy and complete expulsion of the ovum.
[b] Failures were defined as indicated.
SOURCE: Peyron et al. (1993).

Currently in France, a woman suspecting an unwanted pregnancy sees a physician at a first visit, and after a delay for reflection she may return (second visit) to take the RU 486 pills. Two days later, she makes a third visit to the center to receive prostaglandin and then stays for four hours. A control (follow-up) visit (the fourth) should take place 10 to 15 days later. This method is currently not applicable to heavy smokers and women older than 35. All these precautions need to be reexamined, however, and most appear to be dispensable. In the future, it is hoped that a woman would consult her physician as early as possible in the case of missed menses and then receive, if this is her choice, RU 486 from a medically competent person who will have examined her. She will then take home the misoprostol pills for self-administration 48 hours later and return for a checkup in approximately two weeks.

Although RU 486 and misoprostol are safe drugs, pregnancy itself is a risk for women, no matter whether they wish to interrupt or continue it (e.g., ectopic pregnancy is not aborted by RU 486 and may be fatal if not treated surgically). Maintaining contact with a physician is mandatory, and there should be an appropriate permanent connection with a competent medical center in case of complications. Although it may be sufficient in the vast majority of cases for physicians (preferably gyne

cologists) to see patients privately, it has been reported that many women prefer to be treated in a group at a medical center (Thong et al., 1992). Research should be conducted to define the best ways to administer the medications under specific conditions. It is certain that requirements for skilled personnel and sterile surgical facilities will be decreased (El-Refaey et al., 1992). The mechanism of action of prostaglandin at low doses indicates that the effect takes place only when progesterone activity has been much decreased by the antisteroid —after more than 24 hours. Whether a combination of RU 486 and prostaglandin to be administered simultaneously will become available is not predictable, since no technology for delayed prostaglandin release is in sight.

Application of this method in developing countries is necessarily more difficult, and local conditions must be considered, including the availability of medical facilities and personnel, cultural traditions (bleeding for several days may be a problem), and the social context. However, women have the right to obtain medical assistance in case of suspected pregnancy, to have the choice to decide to abort, and if so, also to have the choice of either a surgical or a medical method. Whether in developing or industrialized countries, we ought to offer a complete medical choice to women. Even the RU 486 plus misoprostol method may be imperfectly applied for a period of time in certain countries, but it can only be a definite improvement of the present situation. It has also been successfully used for missed abortions and anembryonic pregnancies (El-Refaey et al., 1992). Whether RU 486 plus misoprostol may be used to compensate for the lack of access to family planning and to health facilities is another question. Generally speaking, the best solution is to make available widely accepted and very efficient methods of contraception.

Pregnancy Interruption After Nine Weeks of Amenorrhea

The effects of progesterone, essentially on the decidua (implantation), the myometrium (calming effect), and LH secretion (depressed with lack of ovulation), are observed throughout the course of pregnancy; thus, it is not surprising that an antiprogesterone is potentially useful for pregnancy interruption and labor induction.

In France, voluntary pregnancy interruption is legally permitted until 12 weeks of amenorrhea. When women have passed beyond the current legal limit for RU 486 plus misoprostol treatment (seven weeks), vacuum aspiration is performed. This can be greatly facilitated by RU 486 taken 24 to 48 hours before the procedure—preoperative cervical preparation (ripening) (Henshaw and Templeton, 1991; Urquhart and Templeton, 1990). The cervical ripening may be due not to a change of prostaglandin metabolism in the cervix (Rådestad and Bygdeman, 1992), but to a

decrease of α_2-adrenoreceptors (Kovacs and Falkay, 1993). A dose of 200–600 mg of RU 486 decreases the force required to dilate the cervix and has significantly fewer side effects than gemeprost (vaginal pessary). It also compares favorably with mechanical dilators such as Lamicel or Dilapan (Cohn and Stewart, 1991; Henshaw and Templeton, 1991; Gupta and Johnson, 1992; Thong and Baird, 1992).

In therapeutic second- and third-trimester abortions, RU 486 is most often used before the administration of prostaglandin, so that the dose of prostaglandins can be decreased, while pain and other side effects are reduced and expulsion is accelerated (Rodger and Baird, 1990). RU 486 also decreases the waiting time, and thus the pain and psychological suffering, in cases of a fetal demise (Cabrol et al., 1985).

Initiation of Labor

A decrease of progesterone activity occurs during parturition, but its precise role in successful delivery is unclear, particularly in primates (including humans) where it does not seem to be the primary event. In rats, RU 486 can synchronize delivery (Bosc et al., 1985), and in cattle (Li et al., 1991a,b) it is very efficient in facilitating parturition. In rhesus macaques near term, RU 486 provokes changes of prostaglandins and of the cervical status, but these modifications do not follow the same orderly sequence as those found during spontaneous delivery (Haluska et al., 1987; Wolf et al., 1993). It is not known if RU 486 increases gap junctions between myometrial cells in women as it does in rats (Garfield et al., 1987), but β_2-adrenoreceptor levels are unchanged in the myometrium (El Alj et al., 1989).

RU 486 has been tested in women at term who require labor induction for various medical indications such as post-term pregnancy and preeclampsia. When compared to placebo controls, the number of spontaneous deliveries is significantly increased; the amount of oxytocin, if required, is much lower; and the time to induce labor is shortened by RU 486 (Frydman et al., 1992). No undesirable incident, in mothers and newborns, was observed with the dose used (two times, 200 mg each), similar to observations made in monkey studies (Wolf, 1989b).

In summary, RU 486 appears to be safe for inducing labor when continuation of pregnancy is a risk for the fetus, the mother, or both. Systematic studies should now follow the development of babies born after RU 486 treatment, since it is known that in primates, RU 486 passes from mother to fetus during pregnancy (Wolf et al., 1988). The effects of neonatal and even embryonic (Wolf et al., 1990) exposure need to be assessed carefully (Weinstein et al., 1991). Until the absolute safety of antiprogestins is demonstrated in cases where there is a medical indication for labor induction, its use for convenience should be forbidden.

Contragestive and Contraceptive Methods Using RU 486

The previous considerations apply to pregnancy that has been demonstrated by missed menses and a positive pregnancy test, a situation clear to all women. Before this well-defined state, even if the biological steps are known, there is still confusion and ignorance as to when pregnancy begins. As a result, the vocabulary used to define the possible antihormonal interventions during the processes establishing pregnancy needs to be clarified.

If menses does not occur and a pregnancy test is positive, interruption is clearly defined as abortion. However, vacuum aspiration practiced very early, within approximately two weeks of menses delay, is called "menses regulation" (e.g., officially in Bangladesh and Turkey) or menstrual induction, and can be considered as contragestive. On the other hand, any maneuver inhibiting fertilization is called contraception, for contra conception. The word conception is generally understood as fertilization; this is wrong etymologically, since *concipire* (Latin) means to retain (originally retain sperm and mother blood in the uterus to make the child).

Contraception is, therefore, commonly understood as a method to preclude fertilization, for instance, by suppression of ovulation or preventing sperm from reaching the ovum. However, physicians also designate as contraceptives methods that are applied before implantation is completed. They argue that a fertilized ovum that is not implanted after in vitro fertilization does not define a pregnancy. In fact, the available pregnancy tests are based on the measurement of human chorionic gonadotropin, produced by the embryonic chorion, which passes into the woman's blood and occurs only after implantation has been initiated. Intrauterine devices (IUDs) can be defined as "contraceptive" tools because they work, in part, as anti-implantation agents. The word post-coital contraception is also largely accepted and applies to a possibly fertilized ovum. Note also that the process of implantation is not instantaneous and takes several days during the last week of the fertile menstrual cycle, just before the time at which menses would occur. Coincidentally, the development of the embryo is characterized by the streak (a marker indicating that an individual embryo has been formed, and there is no further risk of twins), which should occur at approximately the same time—about 15 days after fertilization. Before that time, not only may genetic abnormalities or defects of implantation stop the process leading to pregnancy, but the very definition of a single potential person cannot be rigorously applied. In short, during the period between fertilization and the time at which menses should occur, interrupting methods are contragestive, differing from both abortion and contraception as defined above, and not hiding the fact that they oppose pregnancy.

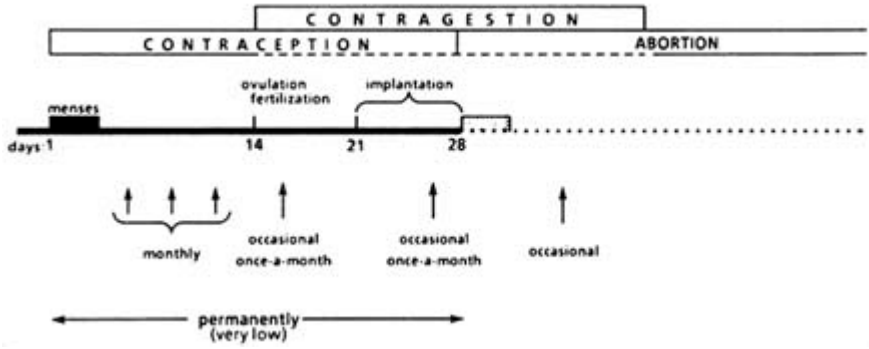

FIGURE B1.11 Fertility control with RU 486.

In summary, *contragestion* includes all treatments that operate over a period of approximately four weeks postfertilization. Such treatments include RU 486, other "morning-after" pills, early vacuum aspiration, and IUDs (as well as a potentially anti-hCG "vaccine").

According to the previous discussion, *six methods* for using RU 486 can be classified as contraceptive/contragestive terms (Figure B1.11):

Emergency Post-Coital Contraception (Contraceptive or Contragestive According to When it is Applied)

Recent studies have shown that RU 486 (single 600-mg dose) given to women after unprotected intercourse within the preceding 72 hours is highly efficient at preventing pregnancy (Glasier et al., 1992; Grimes, 1992; Webb et al., 1992), most likely acting as an anti-implantation agent. It is at least as effective as the high-dose combined estrogen and progestin preparations, and better tolerated by the women. Research needs to be pursued in order to determine the appropriate dose, and whether the administration can be repeated, how many times, and for how long, a possibility that appears rather remote because of probable changes in menstrual cyclicity.

Late Luteal Phase Administration (Occasional Use) (Contragestive)

The administration of 400 or 600 mg of RU 486, once or twice, two days before the expected menses, results in about a 20 percent failure in terms of initiating pregnancy. Since the probability of being pregnant is about 20 percent in normal couples, this leaves only 4 percent of women being pregnant (to be secondarily interrupted by other means) (Dubois et al., 1988b; Lähteenmäki et al., 1988; Grimes et al., 1992). The method is well tolerated and may be improved with the associated use of misoprostol.

Monthly Premenstrual, Late Luteal Phase Administration (Repeated Use) (Contragestive)

Giving RU 486 approximately two days before the expected day of menses over several months has not been successful (Van Santen and Haspels, 1987), the main reason being the irregularity of cycles, often due to the retardation of ovulation that is induced by the compound. In order to overcome this difficulty, a trial using a lower dose of RU 486 plus misoprostol, two days before and on the expected day of menses, has been undertaken. The prostaglandin may permit more rapid evacuation of the blastocyst and therefore a faster decline of hCG, which disturbs folliculogenesis during the next cycle (Aubény and Baulieu, work in progress).

Early Luteal Phase Administration (Once a Month) (Anti-Implantation)

Progesterone acts on the endometrium to prepare for implantation. This has been studied in detail in the ovariectomized rhesus monkey by analyzing the decidual transformation and the epithelial plaque formed in response to deciduogenic stimulus (Ghosh and Sengupta, 1992). An immunocytochemical study, assessing an endometrial secretory glycan (sialo-oligosaccharide), has shown that its production is P dependent (Graham et al., 1991). Moreover, experiments in rabbits have shown that endometrial receptivity and embryo implantation can be modified by antiprogestins (Hegele-Hartung et al., 1992), and RU 486 induces epithelium apoptosis (Rotello, 1992). Treatment with 200 mg of RU 486 was performed on women at the 2-day post-LH stage who had had unprotected intercourse at least once during the period three days before to one day after ovulation. Over 157 cycles, only one pregnancy occurred (Gemzell-Danielson et al., 1993). The main drawback of the method is the timing of the treatment. To use the method monthly, ovulation detection needs to become routine in the future. Doses of 10 mg of RU 486 administered on days 5 and 8 after the LH surge do not provoke hormonal change but do interrupt endometrial maturation with lowered PR levels (Greene et al., 1992).

"Endometrial Contraception" (Daily Delivery of Very Low Dose) (Contraceptive and Contragestive)

The provisional name "endometrial contraception" is given to the continuous exposure to a very low dose of RU 486. This may modify the genital tract in such a way that implantation and possibly fertilization do not occur, while ovulation and the pattern of estrogen and progesterone secretion are unchanged, and adrenal function is unmodified. Daily use in cycling guinea pig prevents implantation (Batista et al., 1991).

After administration of a low dose of RU 486 (12.5 mg to rhesus monkeys once per week), there was complete temporary infertility, but modifications of the cycle were very limited during three cycles of the experiment (Gary Hodgen, unpublished). In five women, 1 mg of RU 486 given daily for 30 days established a plasma concentration of ≥ 50 mg/ml; in one case, ovulation was suppressed (Croxatto et al., 1993). In a similar study, RU 486 (1 mg) given daily to nine women delayed ovulation and endometrial maturation, with a reduced peak of placental protein 14 (a glycoprotein marker for endometrium function) (Batista et al., 1992b).

Currently, studies are being organized using even lower daily doses of RU 486 given to women. An international comparative study will define the highest dose of RU 486 (actually very small) that can be administered chronically to women without perturbation of the cycle. Furthermore, this dose will be tested for contraceptive efficacy. Initially, these studies will be conducted using RU 486-containing pills administered daily. Secondarily, in case of success, we will move from pills to injectable microspheres that will allow the slow release of RU 486 for several months.

Ovulation Suppression (Daily Delivery of a Low Dose) (Contraceptive)

A number of observations demonstrate that P contributes to ovulation (Collins and Hodgen, 1986; Liu et al., 1987; Shoupe et al., 1987; Luukkainen et al., 1988; Danforth et al., 1989; Batista et al., 1992a). The administration of RU 486 during the follicular phase delays or suppresses ovulation. This may designate a new method of estrogen-free contraception. The main problem is to find an effective, well-tolerated dose. Using 5 mg of RU 486 per day may block ovulation without causing a change in adrenal function (Ledger et al., 1992; Croxatto et al., 1993). The sequential administration of RU 486 and progestin to maintain menstrual bleeding has been proposed (Croxatto and Salvatierra, 1991). We submit that this method with ovulation suppression will be more difficult to implement relative to continuous administration of a very low dose.

In conclusion, there is already hope, not to say certainty, for an occasional contraceptive/contragestive method, and "endometrial contraception" is a very appealing possibility. However, any contraceptive method should be studied carefully for a rather long period of time to delineate the possibility of side effects for the women and alteration of the embryo in case of failure (Wu, 1992).

Male Contraception

Progesterone increases calcium uptake by human sperm and favors the acrosomal reaction (Baldi et al., 1991; Blackmore et al., 1991; Parinaud et al., 1992; Uhler et al., 1992). There is likely to be a membrane

receptor mediating its action as in the progesterone-induced reinitiation of meiosis in *Xenopus laevis* oocytes (Blondeau and Baulieu, 1985). However, in sperm, as well as in oocytes, RU 486 may not act as an antiprogestin at the membrane-receptor level. A preliminary report (C.G.P. Puri, patent preview WO 9210194A1, 1992) of a contraceptive effect of RU 486 in monkeys with a decrease of sperm counts has not, to my current knowledge, yet been confirmed.

MEDICAL APPLICATIONS (TABLE B1.4)

Uterine Diseases

Endometriosis

RU 486 can suppress ovulation, and inhibit the mitotic action of estrogens in the monkey endometrium. These effects suggest that the compound may be useful for the treatment of endometriosis (Kettel et al., 1991). As expected, in women, RU 486 suppressed ovulation and menstrual cycle, and brought about an increase of LH (with augmented pulse amplitude but not frequency), adrenocorticotropic hormone (ACTH), and cortisol. Pain was decreased, but there was no decrease in the extent of the disease (corroborating observations in the rat by Tjaden et al., 1993); an effect on lipid peroxidation has also been described (Morales et al., 1992).

Fibroids

Murphy and coworkers (1993) have reported a 50 percent regression of uterine leiomyomas by daily administration of 50 mg of RU 486 for several weeks. This may be due principally to induced anovulation. Modulation of uterine growth factors and insulin-like growth factor (IGF)-binding proteins may also be involved (Murphy et al., 1993).

The treatment provokes an increase of LH, but not of FSH, and of dehydroepiandrosterone sulfate, androstenedione, testosterone, and cortisol, while estrogens, progesterone, sex steroid-binding plasma protein (SBP), thyroid-stimulating hormone (TSH), and prolactin (PRL) are unchanged, compared to original early follicular phase levels. In myometrial and leiomyomatous tissue, PR but not ER immunoreactivity is decreased. Amenorrhea and anovulation are constant. The significant decrease of PR levels may indicate a direct antiprogesterone effect; however, an alteration in ER function cannot be excluded, as discussed above.

The human papillomavirus (type 16, episomal) expression in ectocervical cells (cervical keratinocytes) is stimulated by glucocorticosteroids and progesterone (Mittal et al., 1993). Since RU 486 may inhibit this

induction in premalignant cervical lesions, do such data have application in human pathology?

Breast Cancer

Breast cancer in women is multifaceted and probably corresponds to several distinct pathophysiological and molecular processes (see recent review by Horwitz, 1992). Human carcinomas occur at different ages (before and after menopause); tumors in different animal species can be provoked by carcinogens and viruses; and various cultured cancer cells demonstrate different responses to progesterone. For example, in the classical experiments of Huggins and Yang (1962), progesterone was shown to be a promoter of DMBA-induced mammary tumors in the rat, while in contrast, a high dose of progestins is therapeutically useful in advanced cases of breast tumors in women (Pronzato et al., 1990).

RU 486 inhibits the growth of breast cancer cells in a PR-dependent manner (Bardon et al., 1985; Thomas and Monet, 1992). Interestingly, one may demonstrate (1) antiprogestin-, progesterone-, and PR-dependent effects of RU 486, lilopristone, and onapristone (Michna et al., 1992); and (2) hormone-independent, PR-dependent effects of the same "antiprogestins." In the latter cases, instead of simple degeneration and necrosis of tumor cells due to hormone deprivation, there may be terminal differentiation with a decrease in the number of cells in S-phase, an increase in cells in G0-G1, and evolution to apoptosis (Rotello et al., 1992). This has been observed in human tumor cells either cultured in vitro or transplanted into appropriate animals.

Clinically, only three studies concerning the effects of RU 486 and tumor development have been published (Maudelonde et al., 1987; Bakker et al., 1990; Klijn et al., 1990), but the percentage of objective remissions is promising enough to justify new trials. Trials are currently being performed in Canada and France. Indeed, clinical studies should concern pre- as well as postmenopausal women, and should analyze the mitotic pattern and the steroid and growth factor receptors under simultaneous or successive association of various antiprogestins with tamoxifen. Studies must also include compounds that may have less antiglucocorticosteroid activity, the association of antisteroid with antisteroidogenic drugs, and detailed molecular genetic description of PR mutations. We have seen that the latter may preclude hormone/antihormone action or even shift the response of some 11β-phenyl derivatives from antagonistic to agonistic effects.

The hormonal biology of the breast and its tumors is different from that of normal and cancerous endometrium, including, therefore, the proliferative/antiproliferative potencies of progestins and antiprogestin; this difference justifies specific bioassays (Schneider et al., 1989; Michna

et al., 1991). Breast cancer studies may unveil original PR-dependent control mechanisms of the cell cycle—in particular, hormone-independent, receptor-dependent or -independent effects (as already suggested for tamoxifen). In other words, just as for antiestrogens (Bardon et al., 1987), there may be receptor-mediated antihormonal cytostatic activity, receptor-mediated cytotoxic activity (nonantihormonal), and nonspecific cytotoxic activity of RU 486 and parent drugs.

The growth of other cancers may be decreased by RU 486 treatment. It may act via the PR as in the case of androgen-insensitive R3327HI rat prostatic carcinoma (Mobbs and Johnson, 1991).

Meningioma

Meningiomas are common tumors, generally benign and slow growing, that can threaten brain function—or even life—if they are not surgically removed. More frequent in women, meningioma growth is accelerated during pregnancy. Most meningiomas contain PR (and often little or no ER), and it has been suggested that progesterone has either a permissive or a facilitating effect on their evolution, or possibly both (Magdalenat et al., 1982; Blankestein et al., 1983; Haak et al., 1990; Grunberg et al., 1991; Lamberts, 1992a,b,c). Administration of RU 486 over several months (usually 200 mg/day) has essentially been well tolerated; even the expected side effects reproducing the features of the familial syndrome of cortisol resistance have been observed—that is, relative glucocorticosteroid insufficiency in the presence of high blood cortisol with arterial pressure is maintained because aldosterone secretion is increased.

The treatment should be restricted to unresectable meningiomas. The spontaneous variety of evolution of these tumors makes a definitive evaluation of the beneficial effects very difficult, even though definite, sometimes spectacular improvement has been observed in about one-third of available reports. Instead of RU 486, an antiprogesterone without antiglucocorticosteroid activity would be welcomed. Conversely, there are gliomas whose growth is sensitive to glucocorticosteroid that may benefit from antiglucocorticosteroid compounds (Langeveld et al., 1992; Pinski et al., 1993).

ANTIGLUCOCORTICOSTEROID EFFECTS

While demonstrating antiglucocorticosteroid activity in vitro (Philibert et al., 1981; Jung-Testas and Baulieu, 1983), RU 486 is the first powerful antiglucocorticosteroid whose activity has also been demonstrated in vivo (Bertagna et al., 1984; Gaillard et al., 1984). As one might predict, given the elimination of the negative feedback control of cortisol on ACTH, RU 486 leads to increased ACTH and cortisol secretion. This

overcomes the hypocorticosteroid effect, which explains the good tolerance of RU 486 when it is administered briefly, as in abortion or emergency contraception.

There are several potential uses of RU 486 or antiglucocorticosteroid analogues. The long-term use of RU 486—for instance, to treat tumors—may be improved if a compound blocking steroid biosynthesis is given simultaneously. In breast cancer this could be an antiaromatase because production of estrogens increases when adrenal androgen hypersecretion occurs with RU 486 treatment.

An antiglucocorticosteroid with a short half-life should be useful for the kinetic studies of the *hypothalamus-pituitary-adrenal* axis, in particular to classify different types of *depression* (Ammar et al., 1986; Kling et al., 1989; Krishnan et al., 1992). In several instances, an acute increase of glucocorticosteroids, for instance, during the stress of aggressive conditions, might be antagonized by RU 486, which could therefore protect against immune depression. Conversely, RU 486 might be detrimental in the pharmacological manipulation of septic shock (Broukaert et al., 1992).

It is not impossible that RU 486 or another antiglucocorticosteroid deprived of antiprogesterone effect might be useful in the treatment of certain cases of *psychosis* or *arterial hypertension,* since these two pathological features are remarkably cured when present in *Cushing's syndrome* treated with RU 486 administration [here RU 486 has been a lifesaving drug (see review in Chrousos et al., 1989)]. However, this will concern only a small number of patients. Whether some chronic conditions involving hypercortisolism, such as certain forms of obesity, can benefit from RU 486 is still debatable (Okada et al., 1992). The use of RU 486 (or analogues) has been suggested in premenstrual syndrome, postmenopausal flushes, Alzheimer's disease, and AIDS, but there is currently no firm scientific background to justify trials.

More generally, any long-term treatment with an antiprogestin or an antiglucocorticosteroid should be carefully followed up. Chronic antiprogesterone activity may create an unopposed estrogenic state, counteract the osteogenic effect of P, or interfere with the activity of P in the central nervous system. Signs of adrenal insufficiency may also develop.

It is probably the local use of RU 486, or its derivatives with antiglucocorticosteroid activity, that will develop rapidly. Trials are on the way for treating glaucoma, and for accelerating or amplifying the slow healing of wounds and burns, particularly in stressed or aging patients (who have increased or normal cortisol and low adrenal androgen levels).

CONCLUSIONS

Much work remains to be done (Hodgen, 1991). I single out four approaches that I believe to be most important.

Use for Voluntary Pregnancy Interruption

The RU 486-plus-misoprostol combined method is *ready* to be used at large. It works, is safe, and is close to being as convenient as a medical method of abortion may be. Given the global demographic issue, the suffering of women, and related health problems, it must soon be made available in the United States—a key to further worldwide development. Studies must rapidly discern the best conditions for its *distribution* in parts of the world where there are obstacles, including developing countries. The early use of RU 486, as soon as a woman fears a pregnancy that she does not want, will help to defuse the abortion issue.

Contraception

Research should be conducted to define convenient and safe *contraceptive methods* with RU 486 or other antiprogestins. There are serious hopes, and it is now a matter of conducting systematic studies. However, it will take several years and a great deal of money. Significant success also will contribute to decreasing the practice of abortion as we know it.

Cancer

Nonreproductive medicine should investigate the regulatory properties of RU 486 and its derivatives in several diseases. The most cruel, breast cancer, should be first on the list of trials. Again, this may take time and money, but there already are clues that cannot be neglected.

Novel Antiprogestins: Biology and Chemistry

There is enough data to suggest that RU 486 is only the first in a series of new compounds with significant differences that could be medically exploitable (Table B1.7). Therefore, basic, novel, interactive chemical and biological research should be continued forcefully.

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TABLE B1.7 Objectives of Research for Novel Antiprogestins

No binding to orosomucoid (shorter half-life)
Absolute specificity for progesterone receptor (no antiglucocorticosteroid activity)
Novel cellular/molecular mechanisms
No receptor dimerization
No DNA binding
Direct effect on cell membrane function
Direct effect on enzymes
Steroidogenesis
PG synthesis
Activity specific to central nervous system
Activity exclusive of the central nervous system

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B2 OVERVIEW AND BACKGROUND: MECHANISM OF ACTION OF ANTIPROGESTINS

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INTRODUCTION

Potent and specific progesterone antagonists have been sought for many years. Although some progress had been made in developing such compounds (Raynaud and Ojasoo, 1986), it has been only a little more than a decade since the first progesterone antagonist, RU 486, was developed by researchers at Roussel-Uclaf (Philibert et al., 1982). Other progesterone antagonists have also been described (Philibert et al., 1981; Neef et al., 1984; Raynaud and Ojasoo, 1986). To date, all of the described progesterone antagonists also show at least some degree of glucocorticoid antagonism (Raynaud and Ojasoo, 1986; Mao et al., 1992). Thus the search for the "ideal" progesterone antagonist continues.

Although the mechanism of action of the various progesterone antagonists may differ, studies suggest that most, if not all of the antagonism, is mediated by the progesterone receptor. The progesterone receptor is a member of a superfamily of nuclear receptors that includes the other steroid receptors as well as the thyroid hormone, retinoic acid, and vitamin D receptors (Evans, 1988).

In order to understand the mechanism of action of antagonists, it is first necessary to understand the structure and function of the receptors themselves. The members of this family of receptors share three regions of homology. The locations of these three regions within the human progesterone receptor are shown in Figure B2.1. The first and most highly conserved is termed C1. It encodes the DNA binding domain and contains two zinc finger structures (Evans, 1988). The other two regions of homology, C2 and C3, are small segments of the carboxyl terminus,

which are contained within a larger region important for ligand binding and for receptor dimerization. Upon activation, the receptors dimerize, bind to specific DNA sequences termed steroid response elements, and activate the transcription of target genes. Typically, the activation of receptors is studied by using artificial target genes on plasmids that contain one or more of the specific response elements placed 5' of a promoter and a cDNA for an enzyme such as chloramphenicol acetyl-transferase which can be easily assayed (Denner et al., 1990b). The activity of the receptor can then be studied by transfecting the reporter gene (and a plasmid that expresses receptor if necessary) into a target cell, treating with the desired agonist or antagonist, and measuring the resulting enzyme activity. Although there are many similarities in the mechanisms of action of the various steroid receptors, there are also significant differences. The structure and function of the human progesterone receptor are described below.

FIGURE B2.1 Location of the conserved regions in the steroid-receptor superfamily. Shown here, as an example, is the structure of the human progesterone receptor. C1 is the conserved region containing the DNA binding domain. C2 and C3 are conserved regions important for ligand binding and receptor dimerization. The numbers represent the number of the first amino acid in each of the conserved regions and the last amino acid in the receptor. SOURCE: Reproduced from Weigel et al. (1993).

STRUCTURE AND FUNCTION OF THE PROGESTERONE RECEPTOR

The human progesterone receptor is expressed as two forms, hPR-B and hPR-A. Both are derived from the same gene, but are produced from different mRNAs (Kastner et al., 1990). The PR-A is essentially a truncated version of the PR-B lacking the 164 amino terminal amino acids (Figure B2.2). These receptor forms share common hormone binding and DNA binding domains. Either form can activate transcription of a target gene in cells co-transfected with the corresponding expression vector for PR-A or PR-B, as well as a suitable reporter plasmid (Bocquel et al., 1989). However, the two forms differ in their relative activities, depending upon the target gene studied (Bocquel et al., 1989). In the cases examined thus far, both forms are expressed in cells and in tissues that contain progesterone receptor (Horwitz and Alexander, 1983; Lessey et al., 1983; Feil et al., 1988). Although it is presumed that both forms exist in the same cells, this has not been unequivocally demonstrated.

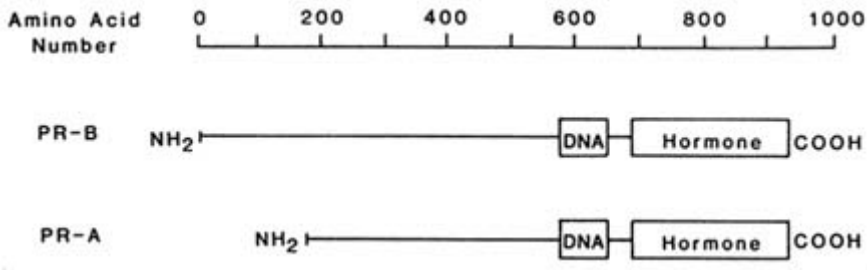

FIGURE B2.2 The structures of hPR-B, hPR-A, and the regions containing the DNA and hormone binding activities.

The Hormone Binding Domain

The ligand binding domain resides in the carboxyl terminal 30 kDa of the progesterone receptor (Figure B2.2). Despite the large size of this domain, analysis of this region using both deletions and point mutations suggests that most of this region is required to maintain the high-affinity hormone binding activity (Carson-Jurica et al., 1990; Danielson, 1991). Photoaffinity labeling studies of the glucocorticoid receptor using R 5020 show that two amino acids, which are about 150 amino acids apart in the linear sequence, are close enough in the three-dimensional structure of the receptor that they both react with the same portion of the R 5020 molecule (Carlstedt-Duke et al., 1988). This suggests that the region is extensively folded back upon itself to produce the binding pocket for the ligand. Consistent with these data is the observation that the entire hormone binding domain is relatively resistant to digestion by a number of proteases when bound with R 5020 (Allan et al., 1992).

Within this domain reside two additional functions of the receptor. The active form of the receptor is a dimer, and the region most important for receptor dimerization is found in the ligand binding domain (Fawell et al., 1990). In addition, this domain is important for transcriptional activation of steroid receptors.

The DNA Binding Domain

The DNA binding domain is a highly conserved region that is responsible for the DNA binding specificity of the receptor. The region contains two zinc finger structures (Umesono and Evans, 1989). The first zinc finger is important for the interaction with DNA, and the second is involved in dimerization with the other receptor molecule (Hard et al., 1990). Although some members of the steroid-receptor family are capable of heterodimerizing with other members of the family (Berrodin et al., 1992; Yen et al., 1992), to date only homodimers of the progesterone receptor have been observed. However, there are two forms of

the progesterone receptor, PR-A and PR-B, and these are capable of forming both homodimers and heterodimers as measured by gel retardation assays (El-Ashry-Stowers et al., 1989) and by immunoprecipitation (DeMarzo et al., 1991). Whether such heterodimerization occurs in vivo and plays a role in preferential activation of specific target genes is not yet known.

Transactivation Function

The amino terminal portion of the progesterone receptor is not required either for DNA binding or for hormone binding. However, it is important for transcriptional activation (Tasset et al., 1990). Studies have shown that there are two regions in the progesterone receptor that are important for transcriptional activation (Bocquel et al., 1989; Meyer et al., 1990). The first is the amino terminus of the protein, and the second resides in the hormone binding domain (Bocquel et al., 1989; Meyer et al., 1990). The relative importance of these two domains depends on both the target gene and the cell type in which they are examined.

Association with Other Proteins

In the absence of hormone, the progesterone receptor is found associated with several non-steroid binding proteins including two heat shock proteins, hsp90 and hsp70, as well as some less well characterized proteins (Dougherty et al., 1984; Smith et al., 1990). The role or roles of these proteins in vivo have not been elucidated. These large receptor complexes each contain a single steroid binding molecule. The receptors are not in the dimer form and do not bind to DNA, which suggests that one role of the non-steroid binding proteins is to inhibit DNA binding of the receptors in the absence of ligand. There is evidence, in the case of the glucocorticoid receptor, that the heat shock protein complex is important for maintaining the integrity of the hormone binding site in the absence of ligand (Pratt et al., 1990), but the progesterone receptor does not appear to require the heat shock proteins to maintain ligand binding activity.

Phosphorylation of the Progesterone Receptor

The steroid receptors, including the human progesterone receptor, are phosphoproteins (Dougherty et al., 1982; Housley and Pratt, 1983; Grandics et al., 1984; Denner et al., 1987). The human progesterone receptor is phosphorylated at numerous sites (Sheridan, et al., 1989), and its phosphorylation is increased in response to hormone treatment. Beck et al. (1992) have shown that the receptor in T47D breast cancer

cells undergoes a rapid (less than 10 minutes) twofold increase in phosphorylation in response to R 5020 administration, followed by a slower additional phosophorylation that results in a receptor form with decreased mobility on SDS (sodium dodecyl sulfate) polyacrylamide gels. Takimoto and coworkers (1992) have shown that the final phosphorylation that alters receptor mobility occurs only if the receptor can bind to DNA. Weigel and coworkers (1992) have shown that the chicken progesterone receptor is phosphorylated during in vitro transcription assays by a DNA-dependent kinase in HeLa nuclear extracts. This enzyme was identified as the double-stranded DNA-dependent kinase purified by Carter et al. (1990). Subsequent studies by Bagchi and coworkers (1992) have shown that the human progesterone receptor undergoes a similar DNA-dependent phosphorylation in vitro, suggesting that this enzyme may be responsible for the DNA-dependent phosphorylation observed in T47D breast cancer cells. In addition to the phosphorylations that are common to both PR-A and PR-B, PR-B has at least two additional phosphorylation sites as judged by mobility on SDS gels and by peptide mapping (Sheridan et al., 1989; Beck et al., 1992). These sites may play a role in the differential activities displayed by the PR-B and PR-A forms.

The role of phosphorylation in progesterone-receptor function has not been determined directly. However, receptor isolated from cells treated briefly with R 5020 so that the phosphorylation is enhanced shows enhanced specific DNA binding (Beck et al., 1992). Moreover, treatment of cells with activators of kinases enhances the receptor-dependent transcriptional activity (Beck et al., 1992), indicating that phosphorylation may also enhance the transcriptional activity of the receptors.

Progesterone-Receptor Function

In the absence of hormone, the progesterone receptors is found in a complex with heat shock proteins in the cytosol fraction of cell homogenates. Based on these types of assays, the unliganded receptor was originally believed to be cytoplasmic. However, immunocytochemical studies suggest that in vivo the unliganded receptor is found in the nucleus (Perrot-Applanat et al., 1992a, b). This binding is less tight than in the presence of hormone since receptor isolated from hormone-treated cells is tightly bound to the nuclear fraction and requires high-salt treatment for extraction. There is evidence that the progesterone receptor cycles continuously between the cytoplasm and the nucleus in the absence of hormone and that this recycling and nuclear retention require energy (Perrot-Applanat et al., 1992a, b). Taken together, the data suggest that in the absence of hormone, the bulk of

the receptor molecules are loosely bound in the nucleus but that they cycle through the cytoplasm with perhaps a relatively short residence time in the cytoplasm. In contrast, the distribution of the glucocorticoid receptor, which also cycles (DeFranco, 1991), appears to favor cytoplasmic localization in the absence of hormone.

A proposed model for the mechanism of progesterone-receptor activation and the possible steps at which antagonists might act to inhibit receptor function are shown in Figure B2.3. Treatment with hormone results in dissociation of the receptor-heat shock protein complex, dimerization of the receptor, and binding to specific DNA response elements termed PREs, or progesterone response elements. Since the glucocorticoid receptor recognizes the same DNA sequence, these elements are also termed GREs or PRE/GREs. Although one or two of these elements placed in the 5' flanking region of a target gene are sufficient to produce a gene whose transcription is activated in response to progesterone (Tsai et al., 1989), natural target genes have much more complicated flanking regions consisting of sequences that bind many transcription factors. In some cases, genes that are steroid inducible do not appear to contain consensus steroid response elements in their 5' flanking regions. Thus, the receptors may also act at nonconsensus sequences through interaction with other factors to induce or repress genes.

Studies in vitro demonstrate that the receptor is important for formation of stable preinitiation complexes that then allow transcription of the target gene (Klein-Hitpass et al., 1990). Characterizing the proteins with which the steroid receptors interact to activate transcription is currently an active area of research. It is likely that cell-specific and target gene-specific transcription factors play important roles in the extent and nature of the response to activation of steroid receptors.

MECHANISMS OF ACTION OF PROGESTERONE ANTAGONISTS

Shown in Figure B2.4 are the structures of progesterone, the commonly used progesterone agonist R 5020, and examples of two antagonists, RU 486 developed by Roussel-Uclaf and ZK 98 299 developed by Schering. Although the two antagonists appear to have very similar structures in this two-dimensional depiction, the C and D rings of RU 486 are fused in the *trans* position, whereas the C and D rings of ZK 98 299 are fused in a *cis* position. Thus in three dimensions the structures are quite different, and as described below, the antagonists appear to inhibit progesterone-receptor function by different mechanisms.

Evidence that the progesterone antagonists act through the progesterone receptor comes from many studies. First, antagonists compete

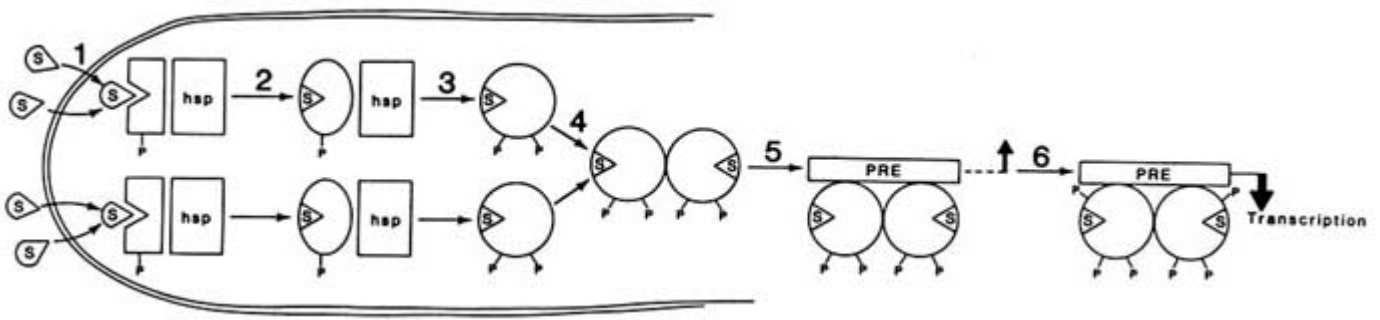

FIGURE B2.3 A model for the mechanism of action of the human progesterone receptor. In the absence of steroid, the receptor is associated with heat shock proteins and is basally phosphorylated. In this model, each P represents a class of phosphorylation sites rather than a single site. Binding of hormone (step 1) results in a conformational change in the receptor (step 2), which allows additional phosphorylation and dissociation of the receptor-heat shock complex (step 3), dimerization between two receptor molecules (step 4), binding to a steroid response element (step 5), and additional phosphorylation and interaction with the appropriate transcription factors to produce a transcriptionally active complex (step 6). NOTE: hsp = heat shock protein complex including hsp90 and hsp70; P = a class of phosphorylation sites; PRE = progesterone response element; S = steroid.

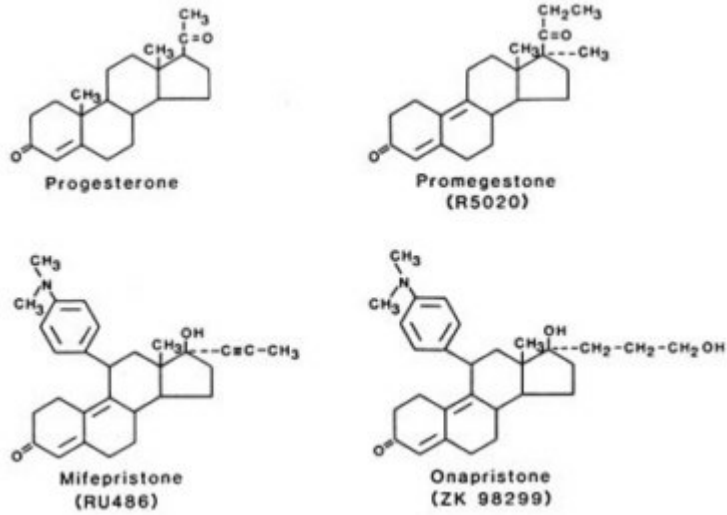

FIGURE B2.4 Structures of progesterone agonists and antagonists. Shown in the upper portion of the figure are the structures of progesterone and the progesterone agonist R 5020 (promegestone) and in the lower portion, the two antagonists RU 486 (mifepristone) and ZK 98 299 (onapristone), which are representative of the classes of antagonists that promote DNA binding and prevent DNA binding, respectively.

with progesterone itself and with progesterone agonists for binding to the progesterone receptor, and with dexamethasone and triamcinolone acetonide for binding to the glucocorticoid receptor (Philibert et al., 1981; Elger et al., 1986; Raynaud and Ojasoo, 1986; Kalimi, 1987). These studies have been done both in extracts containing endogenous receptors and in expression systems, such as the yeast expression system, where binding can be shown to be dependent upon the presence of the expressed receptor (Vegeto, 1992). Similarly, the progesterone-dependent transcriptional activation either of endogenous progesterone receptors (Beck et al., 1993) or of transiently transfected receptors (Meyer et al., 1990) can be inhibited by progesterone antagonists. The primary question is by what mechanism antagonists act to block receptor function. Transcriptional activation of the progesterone receptor is a multistep process and antagonists potentially can act at several places in the process as shown in Figure B2.3. Antagonists may (1) block the binding of progesterone to the receptor, (2) alter or block the conformational changes associated with the binding, (3) block dissociation of the heat shock protein complex, (4) alter or block receptor dimerization, (5) alter or block DNA binding, or (6) alter interaction with

other factors to produce transcriptionally active receptors. Different antagonists may act at different steps in receptor function, and a single antagonist may affect more than one stage of receptor activation.

Binding of Antagonists to Progesterone Receptors

The initial studies of RU 486 showed that the compound competed with progesterone and glucocorticoid agonists for binding to the progesterone and glucocorticoid receptors. Additional information specifically on the mechanism of action of RU 486, and on RU 486 and breast cancer, can be found in the reviews of Mao et al. (1992) and of Horwitz (1992), respectively. Other progesterone antagonists also compete for steroid binding. Surprisingly, RU 486 does not bind to either the chicken or the hamster progesterone receptor (Baulieu, 1989), and none of the other reported antagonists bind to the chicken progesterone receptor. Although there are many amino acid differences in the ligand binding domains of the progesterone receptors, a single amino acid substitution is sufficient to convert the chicken progesterone receptor from a protein that does not bind RU 486 to a protein that does bind it (Benhamou et al., 1992). This exquisite sensitivity suggests that studies of antagonists for potential clinical applications will have to be done with human steroid receptors, since receptors from other species may respond somewhat differently.

Although competition studies suggest that the antagonists are competing for the agonist binding site, detailed studies also showed that the binding of agonists and antagonists was not identical. In particular, the binding of agonists and RU 486 showed different sensitivities to sulfhydryl reagents, suggesting either a different availability of sulfhydryl groups in the receptors or differences in the interaction of the steroids with sulfhydryl groups (Moudgil et al., 1989). That the antagonists do in fact bind differently has been shown in several studies. Allan et al. (1992), using partial proteolysis of in vitro translated ^{35}S-methionine-labeled receptor as an assay for receptor conformation, have shown that all of the receptor is susceptible to proteolysis in the absence of ligand. In the presence of agonist, limited proteolysis produces a resistant fragment of just under 30 kDa. Essentially the same fragment was produced whether the receptor was in the heat shock complex or was dissociated from the heat shock proteins. Fragments of similar sizes were produced by a variety of proteolytic enzymes, suggesting that there is a protease-resistant core in the presence of agonist. When the antagonist RU 486 was used, a resistant fragment, which was approximately 3 kDa smaller than that detected with agonist, was produced, indicating that the conformation induced by RU 486 was different from that produced by the agonist. Immunoprecipitation with C262, a mono

clonal antibody that is specific for the 14 carboxyl terminal amino acids of the receptor, showed that the receptor fragment from the agonist-containing digest was immunoprecipitated, whereas the smaller fragment from the antagonist-containing digest was not recognized (Allan et al., 1992). This study demonstrated that the carboxyl terminal portion of the receptor is not protected by the antagonist although it is protected by the agonist. Vegeto et al. (1992) described a carboxyl terminal deletion mutant of the progesterone receptor that was incapable of binding the agonist R 5020, but still bound RU 486 with essentially normal affinity. These data imply that the primary determinants of RU 486 binding are different from those of R 5020 binding. It is possible that the bulky hydrophobic substitution on the RU 486 is interacting with another portion of the ligand binding domain, which allows it to bind in the absence of the carboxyl terminal amino acids. Presumably the binding sites for the agonist and antagonist are overlapping; nonetheless, it is clear that the extreme carboxyl terminal portion of the receptor is unnecessary for RU 486 binding, whereas it is critical for binding of R 5020.

Formation of Dimers and DNA Binding Complexes

Initial studies with the glucocorticoid receptor (Moguilewsky and Philibert, 1984; Formstecher et al., 1988) had suggested that RU 486 inhibited dissociation of the receptor-heat shock protein complexes and that the mechanism for antagonism might be due to failure to produce DNA binding complexes. Studies have shown that RU 486 does not block dissociation of the progesterone receptor-heat shock protein complex and that RU 486 does permit receptor dimerization and DNA binding (El-Ashry-Stowers et al., 1989). The receptor is found in the nucleus as a result of RU 486 treatment (Sheridan et al., 1988), and additional studies have shown that the RU 486-bound receptor can compete for the same DNA binding sites in vivo (Meyer et al., 1990). However, the conformations of the dimers and of the DNA complexes are different from those produced with agonist alone. The DNA complexes have typically been examined using a gel retardation assay in which the receptor is mixed with ^{32}P-labeled PRE and unlabeled nonspecific DNA, and the resulting protein-DNA complexes are detected by gel electrophoresis and autoradiography. The free DNA migrates very rapidly, and the bound receptor complexes, which are much larger, migrate more slowly. The receptor produces three dimer forms, A-A, A-B, and B-B. Because of the difference in the sizes and possibly the shapes of these complexes, the three forms are detected as three closely spaced bands in gel retardation assays (El-Ashry-Stowers et al., 1989). In the absence of hormone, the receptor does not bind to DNA and no receptor complexes are detectable. RU 486 also produces

three receptor forms, but these forms have somewhat higher mobility than the agonist-containing forms. These studies show that the conformations of these complexes are different in the presence of agonist compared to antagonist, which is consistent with the proteolytic data described above.

Early studies had suggested that PR dimers could bind either agonist or antagonist but that mixed agonist-antagonist dimers were not produced (Meyer et al., 1990). Other studies (DeMarzo et al., 1992) showed that such heterodimers can be produced. Additional data supporting the fact that heterodimers can be formed comes from the studies of Skafar (1991), who examined the cooperativity of hormone binding in receptor dimers versus monomers. Both RU 486 and R 5020 alone showed cooperative binding to dimers, suggesting that a conformational change is induced in each case that favors binding of a second similar molecule. In dilute solution containing monomers, RU 486 had no effect on the affinity of R 5020 for the receptor, whereas at high concentrations of receptor, which presumably contains predominantly dimers, the cooperativity of R 5020 binding to the receptor was abolished by RU 486. Thus the progesterone antagonists may be able to inhibit progesterone action by binding to a single site of a dimer as well as by binding to both sites.

Although RU 486 and several other progesterone antagonists do cause receptor binding to DNA (El-Ashry-Stowers et al., 1989), another antagonist, ZK 98 299, from Schering apparently does not. Initial studies by Klein-Hitpass and coworkers (1991), as well as subsequent studies (Takimoto et al., 1992; Beck et al., 1993), have shown that under the same conditions in which R 5020 and RU 486 promote DNA binding of the receptor in gel retardation assays, ZK 98 299 does not produce a DNA binding form of the receptor. Thus, this antagonist appears to act by a very different mechanism. Since DNA binding is required for activation of transcription, any antagonist that blocks DNA binding will block the activity of the progesterone receptor. Interestingly, whereas treatment of T47D breast cancer cells with RU 486 produces the receptor form with reduced mobility on SDS gels characteristic of the DNA-dependent phosphorylation, treatment with ZK 98 299 does not (Takimoto et al., 1992). Whether the ZK 98 299 prevents the dissociation of the receptor-heat shock protein complexes, blocks dimerization, or simply alters the conformation of the dimer to reduce the affinity for DNA has not been reported.

Effects of Antagonists on Transcriptional Activation

The effects of antagonists on transcriptional activation of progesterone receptors have been examined by using endogenous receptors in

T47D breast cancer cells, transfected reporters and receptors in a variety of cell lines, and in vitro transcription studies. The effects of the antagonists vary depending upon the system used for analysis of activity.

Meyer and coworkers (1990) have shown that RU 486 acts as an antagonist in HeLa cells transfected with either hPR-A or hPR-B and a reporter gene MMTV-CAT, and that RU 486 exhibits no detectable agonist activity under these conditions. However, if a simpler promoter, PRE/GRE-*tk* -CAT, is used, the PR-B form shows some agonist activity with RU 486, whereas the PR-A is still inactive. They have shown that the progesterone receptor contains two regions important for transcriptional activation by the receptor termed TAF-1 (the amino terminus of the protein) and TAF-2 (the hormone binding domain). The strength of these domains as transcriptional activators depends both on the reporter gene used and on the cell type (Bocquel et al., 1989). Studies with deletion mutants of the receptor show that RU 486 does not activate TAF-2 (Meyer et al., 1990). However, for genes that predominantly require TAF-1 for transcriptional activation, RU 486, through PR-B, will partially activate the transcription of the gene. It is interesting that PR-A is unable to stimulate transcription in the presence of RU 486. This suggests that the region unique to PR-B may contain an additional activation function not present in PR-A.

Vegeto et al. (1992) found that a mutant of hPR-B, which lacked a portion of the extreme carboxyl terminus, no longer bound R 5020 and was thus not transcriptionally active. However, this mutant did bind RU 486 with normal affinity. The receptor was transcriptionally active in response to RU 486 both when the receptor was expressed in yeast and when it was expressed in mammalian cells. These data, combined with the conformational data and the antibody data described above, suggest that the carboxyl terminal portion of the receptor may act as a repressor (Allan et al., 1992; Vegeto et al., 1992) and that one of the roles of the agonist is to change the conformation of this region and eliminate the repressor activity. In this mutant, the repressor region has been removed, which allows the RU 486 to act as an effective agonist. These studies do not address the issue of whether the deletion produces an active TAF-2 in the presence of RU 486 or whether repression has simply been relieved, allowing TAF-1 to function.

Recent studies by Denner et al. (1990b) and Power et al. (1991a,b) have shown that some of the steroid receptors can be transcriptionally activated in the absence of hormone. This ligand-independent activation occurred in response to activation of kinases or inhibition of phosphatases and was strictly dependent upon the presence of chicken progesterone receptor (Denner et al., 1990b), the orphan receptor COUP-TF (Power et al., 1991a), or other steroid receptors such as the

human estrogen receptor (Power et al., 1991b). Studies with the human progesterone receptor have failed to demonstrate ligand-independent activation of the receptor, although the same compounds, 8-Br cAMP (8-bromoadenosine cyclic 3',5'-phosphate, which stimulates protein kinase A) and okadaic acid (an inhibitor of phosphatases 1 and 2A), which were used for chicken progesterone-receptor studies, do stimulate the progesterone-dependent activity of the human progesterone receptor (Beck et al., 1992).

One major difference between the human progesterone receptor and the chicken progesterone receptor is that the human progesterone receptor absolutely requires ligand to bind to DNA (Beck et al., 1992), to be phosphorylated by the DNA-dependent kinase (Bagchi et al., 1992), and to be active in the in vitro transcription assay (Bagchi et al., 1991), whereas the chicken progesterone receptor does not (Klein-Hitpass et al., 1990; Weigel et al., 1992). However, antagonists such as RU 486 do cause binding of receptor to DNA. Beck and coworkers (1993) have shown that whereas RU 486 is incapable of activating transcription from a reporter gene stably transfected into T47D cells and can block the activity of R 5020, when RU 486 and 8-Br cAMP are given in combination, the RU 486 exhibits a substantial amount of agonist activity. Since T47D cells contain both PR-B and PR-A, it is not clear whether one or both of these receptors are involved in the mediation of transcriptional activation by RU 486. A possible concern with these results is the question of whether the RU 486 was metabolized to produce an agonist as has been observed in some cases with the estrogen antagonist tamoxifen. However, all previous studies with RU 486 suggest that it is not metabolized, and analysis of RU 486 recovered from cells treated with 8-Br cAMP showed that it had not been metabolized to new compounds (Beck et al., 1993). It is of interest to note that whereas okadaic acid and TPA (a phorbol ester that stimulates the activity of protein kinase C) both stimulated R 5020-mediated activation, neither was capable of activating transcription in the presence of RU 486. In contrast, the antagonist ZK 98 299, which blocks receptor binding to DNA, functions as an antagonist in the presence of R 5020, R 5020 + 8-Br cAMP, or RU 486 + 8-Br cAMP. These data support the conclusion that the effects of RU 486 and 8-Br cAMP occur through the classical progesterone receptor, rather than through another molecule. Thus, the same reporter gene that is completely unresponsive to RU 486 alone can be activated under some sets of conditions, provided that an appropriate signal transduction pathway is also activated.

In vitro transcription studies also show that the antagonists that stimulate DNA binding can act as agonists. In vitro transcription mediated by the human progesterone receptor requires the presence of a ligand that produces the DNA binding form of the receptor (Bagchi et

al., 1991). However, Klein-Hitpass and coworkers (1991), using a G-free cassette assay with a PRE as the response element in the 5' flanking region, have found that the antagonists that allow receptor binding to DNA act as agonists. In this assay, ZK 98 299, which does not promote DNA binding, is inactive and can antagonize the activity of both R 5020 and RU 486. Whether the antagonists will function as agonists in all in vitro transcription assays or whether this is again a function of the target gene has not been determined.

Other Effects of Antagonists on Receptors

Several studies have shown that activation of progesterone and glucocorticoid receptors is accompanied by enhanced phosphorylation (Hoeck et al., 1989; Orti et al., 1989; Sheridan et al., 1989; Denner et al., 1990a), and other reports have shown that activation of kinases will enhance activation of the receptors (Denner et al., 1990b; Beck et al., 1992). Besides inhibition of binding, another point at which the antagonists may alter receptor action is by altering receptor phosphorylation. Treatment of T47D breast cancer cells with RU 486 stimulates phosphorylation of the receptor, and the magnitude of the increase in phosphorylation is severalfold more than in the presence of R 5020 (Sheridan et al., 1989; Takimoto et al., 1992). RU 486-treated receptors also exhibit the altered mobility on SDS gels characteristic of receptors isolated from R 5020-treated cells. However, it is possible that the molecule is aberrantly phosphorylated and that this phosphorylation, perhaps in combination with an altered conformation, produces the antagonist activity of the receptors that bind to DNA. In contrast, analysis of the phosphorylation of the RU 486-treated glucocorticoid receptor suggests that RU 486 blocks the normal hormone-dependent phosphorylation of the glucocorticoid receptor (Hoeck et al., 1989; Orti et al., 1989; Mao et al., 1992). Thus, this same antagonist may be acting through different mechanisms in inhibiting the progesterone and the glucocorticoid receptors. Treatment of T47D breast cancer cells with ZK 98 299 results in some enhancement of receptor phosphorylation, but the phosphorylation that alters the mobility on SDS gels is blocked (Takimoto et al., 1992).

Although most of the interest in hormone action has been focused on the activation of receptors, receptor processing subsequent to nuclear binding and receptor recycling are integral parts of receptor function. Very little is known about these steps. Typically, activation of the progesterone receptor results in a decrease in the level of progesterone receptors in T47D cells (Sheridan et al., 1988). RU 486 does not cause this same loss in receptors, which suggests that some of the effects of RU 486 may be to interfere at this step.

SUMMARY AND FUTURE AREAS OF STUDY

Although much has been learned about the mechanism of action of progesterone receptors, there are still many unanswered questions related to receptor function and the role of antagonists in inhibition of progesterone action. This review has focused on the classical nuclear progesterone receptor that appears to mediate most of the progesterone-dependent effects for which an antagonist would be clinically useful. However, in addition to receptor-mediated effects of progesterone, there is evidence of an unrelated membrane-bound progesterone receptor, whose function has not been well characterized, that presumably acts through completely different mechanisms (Ke and Ramirez, 1990). The steroid binding specificities of this receptor may be different from those of the classical receptor. If this is, indeed, a physiologically important molecule, then care may need to be taken to design antagonists that inhibit the nuclear receptor but not the membrane-bound receptor, or vice versa. Moreover, studies with the currently available antagonists suggest that although compounds such as RU 486 bind to both the progesterone and the glucocorticoid receptors, the effect on function of these receptors differs. This characteristic, in addition to the relative binding affinities, might be used to formulate more specific antiprogestins.

Several of the studies described here suggest that those antagonists that promote DNA binding will act as agonists under some conditions. This observation raises the question of whether, for some applications, it would be better to focus on antagonists that block DNA binding and therefore completely block receptor function. On the other hand, there may be instances in which specific partial agonist activity could be beneficial.

The studies utilizing antagonists that promote DNA binding suggest that in some fashion the conformation of the receptor is inappropriate to interact productively with other transcription factors to initiate transcription. However, almost nothing is known about the detailed mechanism of transcriptional activation by steroid receptors. For example, must the receptor dissociate from the gene and then reassociate to reinitiate transcription, or once bound, does it stay bound throughout many cycles of transcription? The RU 486 data suggest that the RU 486-bound receptor may stay associated with the DNA and cause aberrant processing (Sheridan et al., 1988). If dissociation is necessary, then the RU 486 would block transcription after a single round. This might explain the apparent agonist activity in the transcription assays in vitro. Usually, the calculated efficiency of transcription in these assays is not more than one transcript per template, so that the difference between R 5020 and RU 486 might not be detected in this assay. A

detailed understanding of the mechanism of transcriptional activation and the role of antagonists in blocking this activity should be very helpful in designing more effective antagonists.

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B3 THE USE OF ANTIPROGESTINS IN THE REPRODUCTIVE CYCLE

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Progesterone is required for the development of the endometrium in preparation for pregnancy. It is also required for maintenance of pregnancy until the luteal-placental shift in steroidogenesis occurs about midway through the first trimester. During pregnancy, progesterone promotes a quiescent uterine myometrium and a closed cervical os. Because progesterone is essential to these functions, in theory, blockade of its action would result in infertility or abortion. Steroid antagonists of progesterone have been developed that block progesterone action at the receptor level. This paper introduces the use of antiprogestins to alter the reproductive cycle of women, and reviews the published literature regarding such use in the follicular phase of the menstrual cycle. Although a number of such compounds exist, only RU 486 has been studied for these properties in clinical trials. Although those data constitute the bulk of what is reviewed here, it should be kept in mind that other antiprogestins might not produce the same results.

USE OF ANTIPROGESTINS IN THE REPRODUCTIVE CYCLE

Basic Concepts and Assumptions

Apart from its effects on the endometrium, progesterone exerts important activity on the breast, cervix, lipids, pituitary-hypothalamic unit, and brain. It follows, then, that an antiprogestin would block or alter these effects. Progesterone participates in the differentiation of mammary tissue and in the preparation for lactation. It inhibits estro

gen-induced increases in a cellular, elastic cervical mucus, and induces a cellular, viscous, scanty mucus that is hostile to sperm. Although the impact of endogenous progesterone on circulating lipids is probably small, synthetic progestins, especially those with androgenic properties, antagonize the enhancement of high-density lipoprotein (HDL) cholesterol levels caused by estrogen. Progesterone is important in the feedback regulation of the gonadotropins, especially luteinizing hormone (LH). Although 1-2 mg of progesterone is secreted daily in the follicular phase, its impact is most clear during the luteal phase, when secretion rates rise to 25 mg/day, and circulating levels may reach 10 mg/ml. At this time, progesterone decreases LH pulse frequency and amplitude. Progesterone may have other central actions: at high doses in lower animals it acts as an anesthetic, and speculation regarding its role in the mood changes of pregnancy and in the premenstrual syndrome are long-standing, but unconfirmed.

Given the importance of progesterone to female reproductive competence, the availability of progesterone antagonists was hailed as providing new venues for exploration of progesterone action and for treatment of progesterone-dependent conditions. Much of this speculation was based on the assumption that progesterone antagonists would simply reverse or inhibit progesterone actions. This assumption has not always been proven to be correct. Thus, a number of assessment issues must be addressed in the design and interpretation of studies to evaluate progesterone antagonists.

Assessment Issues in Evaluating Antiprogestins

First, a dose and route of administration must be chosen to maximize the desired effect. RU 486 has glucocorticoid, androgen, and progesterone antagonist properties and little, if any, agonist activity. It does not react with the mineralocorticoid or estrogen receptor (Baulieu, 1989). Only an oral preparation is available for human administration. RU 486 has a prolonged half-life of about 20 hours when given in this way, probably because of extensive binding to plasma proteins (Kawai et al., 1987). Although antiglucocorticoid effects are seen at single doses of >3 mg/kg, antiprogestational activity is seen at smaller doses, in part allowing for the exploitation of antiprogestin properties without clinical compromise of glucocorticoid status.

Second, the physiologic status of the subject must be considered. The effects of an antiprogestin may differ dramatically in the absence or presence of progesterone or estrogen. Thus, stage of the menstrual cycle, anovulation, and pregnancy might all show differing effects. Also, end points must be chosen to measure efficacy, to evaluate potential

actions on other parts of the reproductive axis, and to assess toxicity and unexpected effects. Evaluation of cervical mucus, endometrial histology or proteins, follicular diameter, body temperature, and plasma concentrations of RU 486, estrogen, progesterone, and gonadotropins may all be useful.

Finally, evaluation of the mechanism(s) by which RU 486 affects reproduction should include evaluation of antiglucocorticoid effects. These include antagonism of cortisol action at target tissues, RU 486-induced increases in corticotropin-releasing hormone and opiates, and non-receptor-mediated drug reactions and potential toxicity.

Potential Uses of Antiprogestins

Given the known actions of progesterone on the reproductive tract, many potential uses of an antiprogesterone exist. RU 486 has been tested as a physiologic probe of progesterone action, as a contraceptive, as an abortifacient, and as an agent to promote cervical ripening and induce labor. The effects of RU 486 in pregnancy are reviewed elsewhere in this report (Bygdeman, Appendix B5; Ulmann and Silvestre, Appendix B6). Antiprogestins have shown promise in the treatment of endometriosis and leiomyomas, also reviewed elsewhere in this report (Yen, Appendix B8). These compounds may be useful in induction of lactation and may have intriguing effects on polycystic ovary syndrome, although such potential actions have not been formally evaluated. The treatment regimens used to test the potential of RU 486 as a contraceptive agent are logical extensions of the dose-response effects at various times in the menstrual cycle. RU 486 might be given during one portion of the cycle, either the follicular phase or the luteal phase; either chronically (daily), intermittently, or in a timed fashion, such as on a specific day of the cycle; or after intercourse.

Follicular Phase Administration of RU 486

The effects of RU 486 in the follicular phase depend on the dose and the progress of folliculogenesis at the time of administration. Except for a minimal decrease in serum estradiol levels, RU 486 had little effect on folliculogenesis, timing of ovulation, or duration of the menstrual cycle when given during the first three days of the cycle (Stuenkel et al., 1990). When given during the midfollicular phase at single doses of 10 or 100 mg, LH and follicle-stimulating hormone (FSH) levels decreased in proportion to the plasma estradiol levels at the time the dose was given (Permezel et al., 1989). Thus, the ability of RU 486 to reduce gonadotropins seems dependent on prior exposure to estrogen.

Mid- to late-follicular administration of RU 486 at daily doses of 100 mg or 3 mg/kg for three to seven days inhibits both folliculogenesis and ovulation as judged by estradiol production, follicular growth by ultrasound, and the absence of a midcycle LH surge. Follicular recruitment is reinitiated after discontinuation of the agent, and ovulation occurs about 14 days later (Liu et al., 1987; Shoupe et al., 1987). This effect is dose dependent: lower doses (25 mg on days 1-14 or 50 mg/day on days 7-10) allow continued follicular growth, accompanied by lower than normal daily increments in serum estrogen and a delay in the LH surge until after discontinuation of the agent (Luukkainen et al., 1988; Swahn et al., 1988). At the lowest doses tested, 1 mg/day, started when the dominant follicle was 14-16 mm, the follicular phase is prolonged by 1-11 days; the LH surge is delayed, but occurs in most women (Batista et al., 1992b). These effects on the timing of the LH surge may reflect central antagonism of progesterone action, since the small preovulatory rise in progesterone may be the ultimate trigger of ovulation. This concept is supported by the observation that follicular phase administration of RU 486 reduces LH amplitude (Permezel et al., 1989; Shoupe et al., 1990), whereas progesterone administration increases it (Permezel et al., 1989). RU 486 can inhibit LH secretion from cultured rat pituicytes (Wolf et al., 1989), which suggests a pituitary rather than a hypothalamic site of action.

The ability of RU 486, at doses of 25 mg or more, to inhibit ovulation suggests that this may be an effective contraceptive strategy. The degree of suppression of steroidogenesis is critical in this approach. Severe suppression of estrogen secretion would be undesirable because of deleterious effects on libido, bones, and lipids. Conversely, normal unopposed levels of estrogen might lead to endometrial hyperplasia. One strategy uses a dose of RU 486 that should allow adequate estrogen secretion to be maintained while inhibiting ovulation. Endometrial differentiation and shedding are induced monthly by periodic short-term administration of a progestin. This approach has been tested in women who are not at risk for pregnancy by using a combined regimen of RU 486, 25 mg/day, from days 1-14 of the menstrual cycle, followed by norethisterone, 5 mg/day, during days 15 to 24. In that study, follicular phase estradiol levels were blunted, and ovulation was inhibited in most women, but normal cyclic bleeding was maintained (Kekkonen et al., 1990). Ovulation occurred in two of eight women during norethisterone treatment. There are no data regarding additional effects of this regimen on cervical mucus or endometrial histology that might contribute to a contraceptive effect apart from inhibition of ovulation. The contraceptive efficacy in women of follicular phase administration has not been tested directly.

Other Strategies for Administration of RU 486 During the Menstrual Cycle

The effect of progesterone on endometrial tissue is most apparent in the luteal phase. Deficiency of progesterone during the luteal phase is thought to result in infertility or spontaneous abortion. Thus, it was logical that the effects of RU 486 in nonpregnant women were first investigated in the luteal phase (Herrmann et al., 1982). Administration of the agent after luteal phase day 3 at doses of 25 mg (or greater) causes endometrial shedding. At doses higher than 5 mg/kg, luteolysis occurs concurrently with menses; the timing of the cycle is reset so that the next follicular phase begins (Nieman et al., 1988). At lower doses, luteolysis is not achieved consistently with endometrial shedding, and another bleeding episode is commonly observed within two weeks when estrogen and progesterone levels fall to the follicular phase range. Presumably, this second bleeding reflects regrowth of the endometrium under the influence of continued corpus luteum function and subsequent shedding as luteolysis occurs. The differential effects of RU 486 on the endometrium and corpus luteum must be weighed when considering its use as a contraceptive in the luteal phase of the menstrual cycle.

Daily administration of an antiprogestin throughout the cycle might impede endometrial maturation without suppressing ovulation or steroidogenesis, and thus offer another contraceptive strategy (Batista et al., 1991, 1992a). Alternatively, administration of RU 486 to inhibit ovulation or prevent implantation may represent an effective strategy for post-coital contraception (Glasier et al., 1992; Webb et al., 1992). The contraceptive potential of daily, post-coital, and luteal phase administration of RU 486 is reviewed elsewhere in this report (Baird, Appendix B4).

TOXICITY

Side effects have been noted in chronic studies using 200 mg/day of RU 486 for the treatment of meningioma. One study reported fatigue and symptoms consistent with adrenal insufficiency beginning in the second week of drug administration (Lamberts et al., 1992), but another group using a similar regimen noted only fatigue without symptoms of adrenal insufficiency (Grunberg et al., 1993). At this dose, previously cycling women became amenorrheic; three of five women developed metrorrhagia, and one of these women had endometrial hyperplasia. Five patients developed a transient maculopapular rash. At a daily dose of 100 mg of RU 486, one of six women treated for endometriosis reported anorexia, nausea, and fatigue after two weeks. The symptoms resolved within two weeks without treatment (Kettel et al., 1991).

No other adverse side effects have been reported in any study using RU 486 at a daily dose of less than 10 mg/kg for more than seven days, or at single doses of up to 25 mg/kg. The range of doses used in contraceptive studies are well within these limits. Since few published studies have examined the effects of chronic administration of antiprogestins for more than one week, definitive information regarding adverse reactions of long-term administration is lacking.

QUESTIONS FOR FUTURE RESEARCH

A variety of questions remain unanswered concerning follicular phase administration of RU 486.

What is the mechanism of estradiol inhibition? Does RU 486 have direct effects on the granulosa cells, or are its actions mediated primarily through suppression of gonadotropins? Related to this issue is whether LH pulsatility is normal and whether LH and FSH bioactivities are affected by exposure to RU 486 and other antiprogestins.

How is follicular development affected by antiprogestins? Estradiol concentrations have been used to reflect follicular development. However, assessment of follicular diameter by ultrasound measurement has been used in relatively few studies. A corollary to this is the question of whether ova within developing follicles are normal. If ovulation occurs during RU 486 administration, are ova fertilizable, and would subsequent embryos develop normally?

Is follicular phase administration of RU 486 for contraception practical? A number of questions, both social and biologic, pertain. The amount of estrogen produced must be adequate to maintain bone mass, normal lipid profiles, and sexual function. However, if this estrogen level is achieved, some women are likely to develop endometrial hyperplasia because progesterone levels may not be sufficient to transform adequately the endometrium. Thus, some plan for exogenous progestin exposure would need to be built into the schedule, perhaps on a monthly basis, as has been tested. Alternatively, for women in whom long intervals without menses would be acceptable, perhaps progestin could be given at longer intervals. However, this is likely to provoke fears of pregnancy.

The short-term (one to three cycles) effects of antiprogestins may change when they are administered for longer times; thus, long-term studies are needed of any regimen. Other dose schedules might also be considered.

Finally, the bulk of data on follicular phase administration derive from studies with RU 486. Other antiprogestin compounds will need to be tested, because those compounds may have different properties.

TABLE B3.1 Effects of RU 486 on Follicular Phase Parameters

References	Amount	Days	FP	LH	FD	E_2	P4	Toxicity
Stuenkel et al.(1990)	3 mg/kg	1–3	NE	nl LHS and pulses	NE	dec	NE	1/7 HA
Permezelet al.(1990)	10 or 100 mg	6, 10	NE	LH dec	NE	NE	NE	NE
Swahn et al. (1988)	50 mg	7–10	21	NE	NE	dec	NE	None
Kekkonan et al.(1990)	25[a] mg	1–14	NE	2/6 ovulated	<10	dec	dec	NE
Luukkainen et al.(1988)	25 mg	1–14	21	NE	NE	dec	NE	None
Liu et al. (1987)	3 mg/kg	Late FP[b]	29	nl LHS and FSH levels	NE	dec	NE	None
Shoupe et al.(1987)	100 mg	10–17	28	nl LHS	NE	dec	NE	None
Batista et al.(1992)	1 mg	Late FP[c]	16	nl LHS	inc	NE	NE	None

NOTE: Days = day(s) of follicular phase on which RU 486 was given; FP = follicular phase length in days; FD = follicular diameter in mm; E_2 = estradiol levels; P4 = progesterone levels; NE = not examined; LHS = LH surge; dec = decreased; inc = increased; nl = normal; HA = headache.

[a] Norethindrone given on days 15–24.

[b] RU 486 given at FD 15–20 for 3 days.

[c] RU 486 given at FD 14–16 for 5 days.

SUMMARY

RU 486 and other progestin antagonists represent a novel and promising approach to contraception. Further work needs to be done to define optimal schedules of administration that would be convenient and effective.

Finally, large-scale contraceptive trials remain to be done to establish with certainty the utility of any of the regimens discussed.

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B4 POTENTIAL CONTRACEPTIVE EFFECTS OF ANTIGESTOGENS

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ABSTRACT

Antagonists of progesterone have the potential to be used to interrupt a wide range of reproductive processes that are dependent on this key hormone. Compounds such as mifepristone, onapristone, and lilopristone interact with progesterone receptors throughout the body and block the action of endogenous progesterone. The effect of mifepristone on the ovarian and endometrial cycles depends on the stage of the cycle. Throughout the follicular phase there is little effect on the endometrium. In the early follicular phase it has little effect on the ovarian cycle. In contrast, in midfollicular phase, gonadotropin levels are suppressed and follicular development is arrested. In the early luteal phase, the corpus luteum forms normally, but the development of secretory endometrium is retarded. In the mid- and late luteal phases, bleeding is provoked due to an effect on the endometrium, while luteal regression occurs inconsistently.

The effects of antigestogens on the endometrium and hypothalamic-pituitary system have the potential to be used as antifertility agents by inhibiting ovulation, preventing or disrupting implantation, and/or inducing luteal regression. Ovulation can be suppressed by continuous administration of mifepristone in doses as low as 2 mg/day. Given during the early luteal phase, mifepristone has been shown to be an effective post-coital agent used either in an emergency after unprotected intercourse or as a once-a-month pill. Antigestogens have the ability to alter endometrial histology in doses that have little effect on the ovarian cycle. If these changes in function are incompatible with the establishment of pregnancy, it may be possible to develop an "endometrial" contraceptive pill.

TABLE B4.1 Mifepristone and the Ovarian Cycle

	Effect		
Phase	LH/FSH	Cycle	Endometrium
Early follicular	•	None	None
Midfollicular	•	Delayed ovulation	None
Late follicular	•	± Inhibit ovulation	None
Early luteal	•	None	Retarded
Mid to late luteal	•	± Luteal regression	Low: desynchronization High: bleeding

INTRODUCTION

Progesterone is a key hormone in the regulation of many reproductive processes, including the establishment and maintenance of pregnancy. In many species it is involved in the mechanism of ovulation, and both parturition and lactation occur as a result of withdrawal of progesterone secretion. In 1980 a group of chemists at Roussel-Uclaf discovered a synthetic steroid (mifepristone or RU 486) which is a potent antagonist of progesterone and cortisol but has no antiestrogen activity (Philibert et al., 1982). Very soon, it was reported in a pilot study to induce bleeding from the uterus when it was given to women in the luteal phase of the cycle or in early pregnancy (Herrman et al., 1982). This latter property has been utilized in the development of a method of inducing abortion. Mifepristone, in combination with a suitable prostaglandin (gemeprost, sulprostone, or misoprostol), has been shown to be a highly effective alternative to vacuum aspiration to induce abortion in the first nine weeks of pregnancy and has been licensed for this use in France, Great Britain, Sweden, and China (Baird, 1993).

The political controversy surrounding the "abortion pill" has obscured the fact that antigestogens have many other potential therapeutic uses. In this paper I describe the effects of mifepristone when given to women at various stages of the menstrual cycle. Although there are several other synthetic antigestogens with slightly different pharmacological properties (e.g., onapristone and lilopristone), the data in women are virtually confined to mifepristone.

PHYSIOLOGICAL STUDIES IN THE MENSTRUAL CYCLE

The effect of mifepristone on ovarian activity is dependent on the time of the cycle and the dose used (Table B4.1). Since mifepristone binds to progesterone receptors in the hypothalamus and anterior pituitary as well as in the uterus, it is likely that it would interfere with the ovarian as well as the endometrial cycles. In the original clinical report in which mifepristone was given to three women in a dose of 50 mg/day by mouth

for four days, bleeding occurred within 48 hours of the first tablet (Herrman et al., 1982). There was a simultaneous decrease in the concentration of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and progesterone, suggesting that luteal regression had occurred prematurely as a result of inhibition of gonadotropins. This finding was surprising because it would be expected that an elevation, not a suppression, of LH would result following antagonism of progesterone.

Subsequent studies have detailed the result of administration of mifepristone at different stages of the cycle. In the early follicular phase at a dose of 3 mg/kg, there is a suppression of the concentration of estradiol, although the length of the cycle is unaffected (Stuenkel et al., 1990). However, when given late in the follicular phase, folliculogenesis is arrested and ovulation is delayed (Liu et al., 1987; Shoupe et al., 1987) (Figure B4.1). The mechanism by which follicular growth is inhibited is not entirely clear but probably involves suppression of LH. In one study there was no difference between 10 and 100 mg of RU 486, and the magnitude of suppression was greater in the late follicular phase when the concentration of estradiol was highest (Permezel et al., 1989). This suppression of LH and FSH concentration occurs apparently with little or no change in the frequency or amplitude of LH pulses. However, in the preovulatory phase, it is not possible to arrest follicular development, and an LH surge and ovulation may occur (Spitz et al., 1993). Although mifepristone has agonistic activity when given to postmenopausal women treated with estrogen, it has little effect on the histology of the endometrium in the follicular phase of the cycle (Gravanis et al., 1985; Swahn et al., 1988).

The effect of mifepristone in the luteal phase is also dependent on the dose and on the stage of the cycle. When mifepristone is given in the early luteal phase (LH + 2–3 days), the corpus luteum functions normally and menstruation occurs at the appropriate time (Swahn et al., 1990). In the mid-and late luteal phase, however, doses of >50 mg/day mifepristone suppress LH and progesterone, and result in premature luteal regression in approximately 50 percent of cases (Schaison et al., 1985; Garzo et al., 1988; Swahn et al., 1988) (Figure B4.2). In those women in whom the corpus luteum does not regress prematurely, bleeding occurs within 48 hours due to a direct effect on the endometrium and is followed by a second episode at the time of expected menses.

The bleeding that occurs after administration of mifepristone in the luteal phase is due to a direct effect on the endometrium and occurs even if the progesterone concentration remains high, for example, when human chorionic gonadotropin (hCG) is injected to maintain the corpus luteum (Croxatto et al., 1989). The exact mechanism by which mifepris

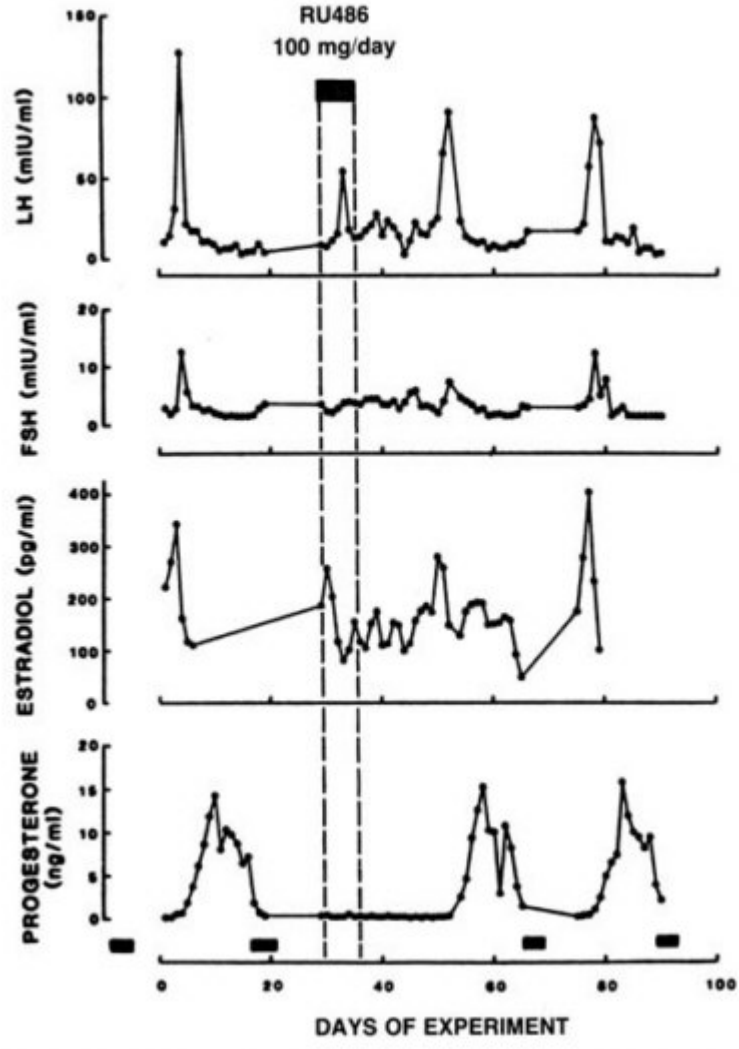

FIGURE B4.1 The effect of 100 mg/day mifepristone from days 10 to 17 of the ovarian cycle. Note that follicular development is arrested and ovulation delayed for more than 14 days. SOURCE: Shoupe et al. (1987).

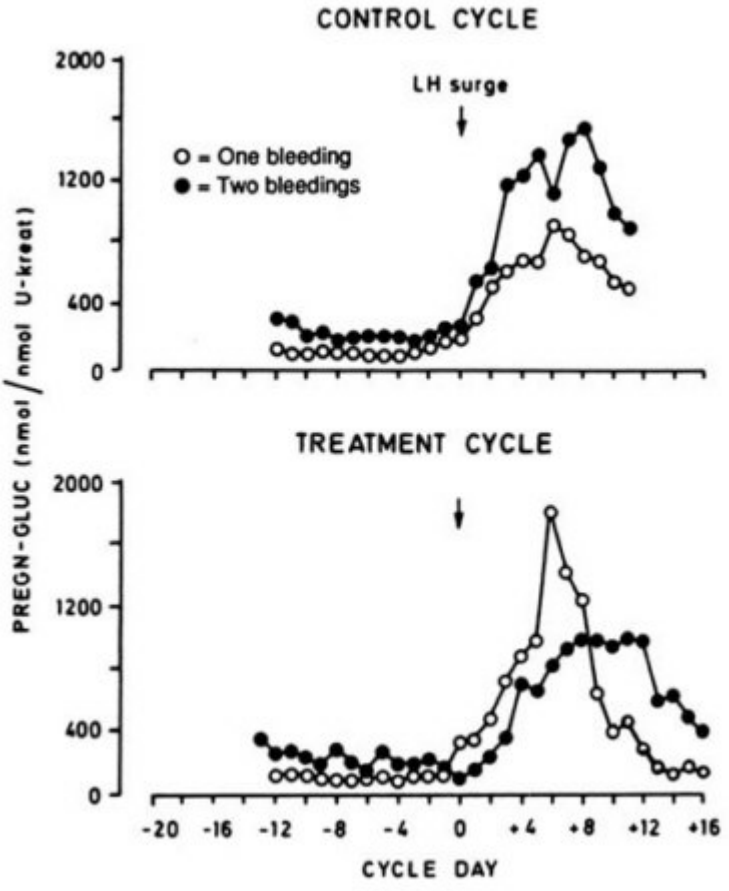

FIGURE B4.2 The effect of 50 mg/day mifepristone from days 20 to 23 of the ovarian cycle. Five women had only a single episode of bleeding, which was associated with luteal regression. In the remaining four women in whom a second episode of bleeding occurred at the time of expected menses, a normal luteal phase was observed. SOURCE: Swahn et al. (1988).

tone acts on the endometrium to induce bleeding is not entirely understood. In the early luteal phase, the development of secretory changes is retarded (Swahn et al., 1990). In the midluteal phase, changes suggestive of degeneration of the endometrium prior to menstruation occur, with shrinkage of glandular lumen, infiltration of leukocytes, and

necrosis of the blood vessels (Li et al., 1988; Swahn et al., 1988). These latter changes are of particular interest because they occur in the decidua in early pregnancy after administration of mifepristone and are associated with the reduction in prostaglandin dehydrogenase in the vessel wall (Cheng et al., 1993a). At the same time there is a striking increase in the concentration of PGE_2 as demonstrated by immunocytochemistry (Cheng et al., 1993b). Mifepristone increases the release of the prostaglandins PGF_{2a} and PGE_2 by dispersed endometrial cells cultured in vitro (Kelly et al., 1986), while the "concentration" of PGF_{2a} is increased in endometrium recovered from women who had taken mifepristone (5 mg/kg) 24 hours previously (Table B4.2). Thus antigestogens may increase the effective local concentration of prostaglandins both by provoking their release and by inhibiting their local metabolism (Kelly and Buckman, 1990).

TABLE B4.2 Concentration (pg/mg) of PGF2a and PGE2 in Endometrium Obtained from Women Who Received Mifepristone (5 mg/kg) in the Follicular (day 8–10) or Luteal (day 19–22) Phase of the Cycle, 24 Hours Prior to Sampling (N = 5–7 each group)

	Follicular Phase		Luteal Phase	
Prostaglandin	Control	Mifepristone	Control	Mifepristone
$PGF_{2\alpha}$	193 ± 37	515 ± 204	473 ± 49[a]	1458 ± 259[a]
PGE_2	310 ± 50	489 ± 80	433 ± 106	795 ± 211

[a] P 8 0.001, Mann-Whitney.

In summary, in the follicular phase of the cycle, mifepristone inhibits follicular development and ovulation through an effect on the secretion of gonadotropins but has little effect on the endometrium. Immediately after ovulation, mifepristone retards the development of a secretory endometrium by antagonizing the effect of progesterone. Late in the luteal phase, endometrial bleeding is provoked in a manner that simulates what occurs at menses after regression of the corpus luteum.

Although antigestogens do not bind to the estrogen receptor, both onapristone and mifepristone have the ability to antagonize the action of estrogen on the endometrium (van Uem et al., 1989). Endometrial atrophy occurs in castrate monkeys given mifepristone in combination with estrogen. The mechanism of this noncompetitive inhibition of estrogen is unknown but is associated with a striking up-regulation of the estrogen receptor (ER) (Neulen et al., 1990). Another antigestogen (onapristone) produces a similar noncompetitive inhibition of the effect of estrogen on the rabbit uterus (Chwalisz et al., 1991). The antagonism of estrogen is not due to binding to the ER. It is possible that the increase in ER after mifepristone is due to uncontrolled expression of ER, which

is normally inhibited by very low levels of progesterone such as are present even in the castrate animal.

POSSIBLE CONTRACEPTIVE USES OF MIFEPRISTONE

Mifepristone could be used as a contraceptive by virtue of its action on the hypothalamic-pituitary system as well as its effect on the endometrium.

Inhibition of Ovulation

In the follicular phase, when given in relatively large doses (25–600 mg) for several days, mifepristone inhibits follicular development and ovulation. In the monkey, 25 mg but not 12.5 mg once per week inhibited ovulation by preventing the development of the dominant follicle (Luukkainen et al., 1988; Danforth et al., 1989; Spitz et al., 1993). Similar experiments in women have given inconsistent results, although 25 mg/day for 14–21 days combined with norethisterone has been suggested as a possible non-estrogen-based contraceptive (Spitz et al., 1993). Good cycle control was observed, but the necessity to add a synthetic gestogen and the fact that ovulation was not always blocked are disadvantages (Kekkonen et al., 1990).

In another small study involving five women, mifepristone (50 mg/day) was given from days 9–11 of the cycle to inhibit ovulation, followed by medroxyprogesterone acetate from days 17–26 (Croxatto et al., 1991). Menstrual bleeding occurred after a second short course of mifepristone was given on days 27–29.

In these studies it was thought necessary to give a gestogen to prevent endometrial hypertrophy due to unopposed estrogen and to produce regular menstrual bleeding. However, as mentioned above, mifepristone has the paradoxical effect of antagonizing the effect of estrogen by a noncompetitive action and, hence, inducing a degree of endometrial atrophy. Two recent studies have investigated the effect of daily administration of mifepristone for 30 days in doses from 10 to 1 mg (Ledger et al., 1992; Croxatto et al., 1993). There was a dose-dependent suppression of follicular activity and ovulation. A single dose of 2 mg was the minimum dosage that suppressed ovulation consistently, whereas 1 mg/day resulted in variable effects on cycle length (Figure B4.3). In some women, ovulation apparently occurred normally; in others, there was suppression of ovulation or evidence of luteinized unruptured follicle. At doses ³2 mg, ovulation was suppressed, although follicular development, as assessed by ultrasound measurements and serum estradiol, continued. In spite of levels of estradiol that were similar to those found in the late follicular phase of the cycle, there

was no evidence of endometrial hypertrophy. The women remained amenorrheic as long as they took mifepristone. On stopping the mifepristone, an LH surge and ovulation occurred within 14 days, although the luteal phase of the following cycle was often shorter than normal, suggesting an insufficient corpus luteum.

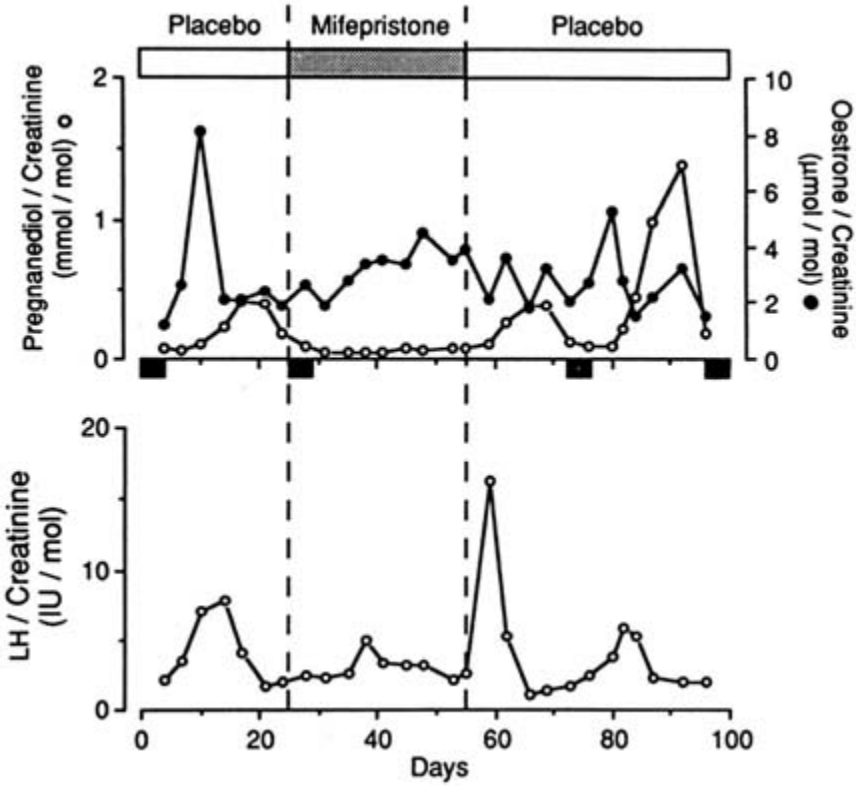

FIGURE B4.3 The effect of mifepristone (a single dose of 2 mg/day) on ovarian function. Note that during the administration of mifepristone the excretion of pregnanediol remained low, indicating suppression of ovulation. SOURCE: Ledger et al. (1992).

The mechanism by which these low doses of mifepristone prevent ovulation probably involves inhibition of the positive feedback mechanism. In the normal cycle, in the few hours prior to the onset of the LH surge, there is a rise in the concentration of progesterone due to increased secretion by the preovulatory follicle (Hoff et al., 1983). This rise probably facilitates the ability of estrogen to induce an LH surge, as indicated by the fact that it can be delayed for a few days by the administration of mifepristone in doses as low as 1 mg/day (Batista et al., 1992). That this effect is due to its antigestogen action is illustrated by the

observation that an LH surge can be induced in these women by the simultaneous injection of 5 mg progesterone. Further evidence that the antigestogen is blocking ovulation by inhibiting the LH surge is provided by the occurrence of a surge within a few days of stopping mifepristone (Ledger et al., 1992).

In summary, these studies suggest that mifepristone given in a daily dose that is 100-fold less than that used to induce abortion would be an effective contraceptive by suppressing ovulation. If further studies demonstrate no deleterious effect on the endometrium of long-term administration of mifepristone, this may provide a useful alternative to continuous gestogen (the "mini pill") without the disruptive effect on the pattern of menstruation.

Post-Coital Contraception

Because antigestogens retard the development of a secretory endometrium and induce bleeding in the luteal phase, it is likely that they would be effective at preventing or disrupting implantation of the early embryo. In two recent studies, 402 and 195 women exposed to the risk of pregnancy were given 600 mg mifepristone within 72 hours of unprotected intercourse (Glasier et al., 1992; Webb et al., 1992). No pregnancies occurred in either study, demonstrating that mifepristone is a highly effective emergency post-coital agent. Although side effects such as nausea and vomiting were much fewer than in a group of women given a large dose of the combined oral contraceptive pill ("Yuzpe" regimen), about 30 percent of the women experienced a delay of more than three days in the onset of the next menstruation (Glasier et al., 1992). This delay probably occurs in those women given mifepristone during the follicular phase, with the subsequent arrest of follicular development and ovulation. Ovulation is delayed until a new follicle is recruited. The delay in the onset of the next period is a source of concern to women who might worry that they may be pregnant. In the study by Webb et al. (1992), three women became pregnant during this time due to further acts of intercourse, indicating that it is necessary to give contraceptive advice at the time of emergency contraception.

The efficacy of mifepristone, given in the early luteal phase of the cycle, in preventing pregnancy has recently been tested in trials in Sweden (Gemzell-Daniellson et al., 1993). Twenty-one sexually active women were given 200 mg mifepristone as their sole method of contraception on the second day after the LH peak in urine. Women were studied for periods of 1–12 months. There was only one pregnancy in a total of 157 ovulating cycles. In 124 cycles, at least one act of intercourse occurred in the three days around ovulation. Menses occurred at the expected time, although in 35 percent of the cycles there

was slight bleeding for two or three days after taking the mifepristone. This preliminary study confirms that mifepristone, given at this time, is highly effective at preventing pregnancy and could be used as a once-a-month contraceptive. However, in order to avoid disruption in the cycle it must be given within two or three days of ovulation. Unless a cheap, simple, and robust method of detecting ovulation is developed, it is difficult to see how this method could be used widely.

Endometrial Contraception

An alternative approach to contraception is the administration of an antigestogen during the luteal phase in a dose sufficient to cause asynchrony of the endometrium but insufficient to disrupt ovarian function or induce menstrual bleeding. In one study in which 10 mg mifepristone was given on days 5 and 8 of the luteal phase, there was no disturbance in ovarian function (Greene et al., 1992). In seven of eight cycles there was reduced stromal edema as well as delayed development of the endometrial glands. Asynchrony of endometrial histology has been described following single doses of mifepristone during the luteal phase with an increase in the number of apoptotic bodies, suggesting programmed cell death (Li et al., 1988b). Some aspects of endometrial function were retarded (e.g., reduction of secretory material in the glands), while other aspects (e.g., reduction in stromal edema and increase in stromal mitosis) were advanced. Whether these changes, which result in desynchronization of the endometrium, are incompatible with the establishment or maintenance of pregnancy is not yet known. Large doses of mifepristone (200–600 mg) in the early luteal phase retard the development of a secretory endometrium and prevent pregnancy (see below), but have a significant failure rate when given on day 27 after implantation, has become established (van Santen and Haspels, 1987; Dubois et al., 1988; Couzinet et al., 1990). The efficacy of giving mifepristone at this stage of the cycle to prevent implantation could be tested as a late post-coital agent given more than 72 hours after coitus but earlier than the time of missed menses.

Late Post-Coital Contraception

Mifepristone induces menstrual bleeding when given in doses from 50 to 600 mg in the mid- and late luteal phases of the cycle and, hence, might be expected to prevent pregnancy by disrupting implantation. In a prospective trial, 12 women were given a single 600-mg dose of mifepristone on the day before their expected menses (day 27), and eight days later if a continuing pregnancy was diagnosed after failure of the first dose (Couzinet et al., 1990). In a total of 137 cycles of exposure

there were 22 pregnancies (16 percent); 4 (18.2 percent) were ongoing after the second dose of mifepristone. The failure rate was similar to that found in women who are given the same dose of mifepristone to induce abortion in very early pregnancy (within 10 days of a missed menstrual period) and is clearly too high to be used as a regular method of contraception (Couzinet et al., 1986). It is likely that the efficacy could be increased by giving an oral prostaglandin such as misoprostol 24–48 hours after the mifepristone, as has been used to induce abortion (Aubeney and Baulieu, 1991; Norman et al., 1991; Thong and Baird, 1992). However, there are doubts as to whether such a method would be acceptable to all women as a regular method of contraception. In a study involving more than 400 women in Edinburgh, less than 25 percent would use a method that disrupted implantation, although the vast majority were in favor of a once-a-month pill that inhibited ovulation (Rimmer et al., 1992). Presumably, the thought of willingly exposing themselves to the risk of pregnancy and then inducing a very early abortion was usually unacceptable to the majority.

Such an approach, however, could be used as a late post-coital emergency contraceptive following unplanned, unprotected intercourse. In one study, 139 women at risk of pregnancy were given 400 or 600 mg mifepristone on the day before expected menses (Dubois et al., 1988). Of the 48 who were pregnant (as detected by measurement of β-hCG in plasma) there were 9 ongoing pregnancies. The failure rate (18.8 percent) was similar to that in the studies above. The reason some women fail to abort following mifepristone is not entirely clear but may be related to incomplete shedding of the endometrium (Li et al., 1988a). However, the fact that the complete abortion rate is increased to nearly 100 percent by combining the treatment with a suitable prostaglandin suggests that inadequate uterine contractions, due possibly to lack of endogenous prostaglandins, may be responsible.

In summary, although administration of mifepristone will induce bleeding and disrupt pregnancy in the majority of women during the luteal phase, the failure rate is too high for it to be used as a regular method of contraception, although in combination with a prostaglandin it may be effective as a late post-coital pill.

FUTURE DEVELOPMENTS

The ideal contraceptive would prevent pregnancy without disrupting the ovarian or menstrual cycle. The approaches described above involve inhibition of ovulation and disruption of ovarian cyclicity or disruption of endometrial development. It has been suggested that the problems of timing the administration of mifepristone could be avoided by the daily administration of an antigestogen in a dose too

low to interfere with the hypothalamic-pituitary axis and ovarian cyclicity but sufficient to desynchronize the endometrium ("endometrial contraception"). In a recent study in which women received 1 mg mifepristone per day, endometrial development was delayed, and there were lower levels of placental protein 14 (PP14), suggesting insufficient endometrial function (Batista et al., in press). However, the follicular phase was extended and ovulation delayed. Whether doses of mifepristone 81 mg/day would induce changes in endometrium incompatible with pregnancy, while at the same time having no effect on ovarian cycles, is not yet known.

Further research is necessary in several areas before mifepristone or other antigestogens can be used as contraceptives.

Basic Research

Mode of Action

We still do not fully understand how mifepristone inhibits follicular development and ovulation. Current evidence suggests that suppression of ovarian activity is secondary to inhibition of gonadotropins at least in high doses. Why blocking the hypothalamus or pituitary receptors for progesterone should inhibit rather than stimulate LH release is not clear. In lower doses (1–5 mg), there is little if any measurable effect on gonadotropin levels; yet the rate of follicular development and ovulation is suppressed. In the luteal phase, the effects on the corpus luteum are dependent on the stage of the cycle and are inconsistent. There is really still no indication as to why luteal regression occurs only in approximately half the women who are given mifepristone in the midluteal phase.

An even more intriguing question is how mifepristone affects the endometrium. Many of the events that occur after its administration during the luteal phase simulate those that occur in response to withdrawal of progesterone at the time of luteal regression.

Mifepristone in large doses (200–600 mg) has been shown to prevent pregnancy when given in the early luteal phase of the cycle. However, the minimum dose that will prevent implantation is not known. Although abnormalities have been reported in the secretory endometrium after single doses as low as 5 mg and after daily administration of 1 mg, a dose insufficient to inhibit ovulation consistently, it is not known whether these are incompatible with the establishment of pregnancy.

Although mifepristone does not bind (at least in vitro) to the ER, studies in rabbits and monkeys have demonstrated that it will prevent the estrogen-induced hypertrophy of the endometrium. The mechanism by which this "noncompetitive" inhibition is produced is not known,

although it has been suggested that the striking dose-dependent increase in ER sets in motion an irreversible destruction of the cell.

Clinical Studies

1. When started in the follicular phase of the cycle, mifepristone inhibits ovulation and would clearly be contraceptive. However, the effects on the endometrium and on bleeding patterns of different modes of administration require investigation. The optimal amount of mifepristone given as a single weekly dose that will inhibit ovulation as is seen in the monkey has not yet been established (Spitz et al., 1993). The effect on the endometrium of chronic anovulation induced by mifepristone, given either as a single weekly dose or in smaller daily amounts, requires investigation. Is it necessary or practical to give additional progestogen to prevent endometrial hypertrophy?
2. Studies are required to establish the minimum dose of mifepristone that is effective as a post-coital agent. Used as an emergency contraceptive, it is unlikely that the dose could be reduced much lower than 50 mg because it is probably necessary to inhibit ovulation in those women who have unprotected intercourse in the first half of the cycle. However, used as a once-a-month pill in the luteal phase of the cycle, a lower dose (5–10 mg) may still be effective at preventing implantation. It will be very difficult to design clinical studies to determine the minimum changes in endometrial function that are incompatible with pregnancy. Fertility studies in primates may be necessary before it would be ethical to expose women to the risk of pregnancy.
3. The clinical efficacy of mifepristone as a late post-coital agent, in combination with an oral prostaglandin analogue such as misoprostol, requires further investigation. The acceptability of this form of "menstrual induction" as a regular form of contraception is likely to differ depending on the particular society. Moreover, although repeated administration of mifepristone on day 27 has no effect on the length of the subsequent cycle, we know little about what might happen if pregnancy had occurred (Croxatto et al., 1987). In an unpublished study sponsored by the World Health Organization (WHO), involving induction of abortion with 600 mg mifepristone and 1 mg gemeprost in 140 women within seven days of the missed menstrual period, the subsequent cycle was prolonged by a mean of seven days (Table B4.3). Moreover, there was great variability among women in the return to menstruation (median 35 days, range 2–68 days), which would make it very difficult to predict when to take the next once-a-month pill (Baird and Cameron, 1985).

In summary, although there are several studies suggesting that antigestogens have a range of antifertility properties, considerable

research is required before they could be used clinically as contraceptives. Moreover, virtually all the data in women are confined to mifepristone. The effect of other antigestogens such as onapristone, which has different pharmacological properties, requires investigation.

TABLE B4.3 Use of Mifepristone and Cervagem for Medical Abortion £35 Days

	Nonpregnant			Pregnant		
	Median	Range	(*N*)	Median	Range	(*N*)
Duration of bleeding	6	2–12	(31)	9	3–39	(140)
Time to return of meanses (days)	33	29-71	(31)	35	2-68	(135)

NOTE: 600 mg mifepristone + 1 mg cervagem; complete abortion rate: 97%.
SOURCE: WHO (1993).

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B5 USE OF ANTIPROGESTINS BEFORE 63 DAYS OF AMENORRHEA

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INTRODUCTION

Progesterone plays a central role in the establishment and maintenance of pregnancy. It is secreted by the corpus luteum during the second part of the menstrual cycle and during early pregnancy, and by the placenta after the luteal placental shift, which occurs between six and eight weeks of pregnancy. It is essential for the nidation of the conceptus and is thought to inhibit myometrial contractility, thereby ensuring that the uterus is kept in a quiescent state throughout pregnancy.

EFFECT OF MIFEPRISTONE DURING EARLY PREGNANCY

The mechanism through which mifepristone terminates early pregnancy has not been fully elucidated. Blockage of the progesterone receptor by treatment with mifepristone leads to ultrastructural changes in the endothelium of decidual capillaries, vascular damage, bleeding, decidual necrosis, detachment, and ultimately, expulsion of the products of conception (see references in Van Look and Bygdeman, 1989). It has been suggested that the first step in this series of events is an increased local concentration of prostaglandins, since mifepristone stimulates the synthesis of prostaglandin by glandular cells of the early human decidua (Smith and Kelly, 1987).

Of great importance for the clinical use of mifepristone as an abortifacient is the effect of the compound on uterine contractility. During early pregnancy the uterus is inactive, probably due to the

inhibitory effect of progesterone. Treatment with mifepristone changes the inactive uterus to an active one. The time interval between the start of mifepristone treatment and the appearance of uterine contractions is 24 to 36 hours. Simultaneous with increased contractility, the sensitivity to prostaglandin increases about five times (Swahn and Bygdeman, 1988). These data created the scientific background for combining mifepristone and a low dose of prostaglandin for termination of early pregnancy.

Mifepristone also has a ripening effect on the cervix and causes dilatation of the cervical canal (Rådestad et al., 1990; WHO, 1990). These effects do not seem to be mediated through a stimulation of endogenous prostaglandin production because they are not blocked by simultaneous treatment with prostaglandin biosynthesis inhibitors (Rådestad and Bygdeman, 1992). It is possible, however, that the antiprogestin acts by inhibiting or changing prostaglandin metabolism.

TERMINATION OF EARLY PREGNANCY

Mifepristone Alone

Clinical evidence that it was possible to interrupt early human pregnancy with mifepristone was first provided by Hermann et al. (1982) and was confirmed shortly afterwards in a dose-finding study conducted under the auspices of the World Health Organization (Kovacs et al., 1984). Although carried out in a relatively small number of women, these two studies permitted several tentative conclusions that were proven in subsequent trials. Undoubtedly, the most disconcerting findings were the relatively low efficacy, 73 percent and 61 percent, respectively, and the absence of a clear dose-response relationship. Efforts to improve the therapeutic efficacy were made by various investigators and focused on changing the daily dose and the duration of treatment (for references, see Van Look and Bygdeman, 1989). From these studies, two conclusions could be drawn. Firstly, the frequency of complete abortion decreased with advancing pregnancy, and secondly, there was no relationship between the success rate and the treatment regimen employed for women at the same stage of gestation. For pregnancies up to eight weeks the frequency of complete abortion was generally between 60 percent and 70 percent. A somewhat higher success rate could be achieved if treatment was given within the first 10 to 14 days after the missed menstrual period. For example, Couzinet et al. (1986) reported a complete abortion rate of 85 percent following treatment with up to 800 mg mifepristone for 2 to 4 days in women who were within 10 days of missed menses.

TABLE B5.1 Termination of Early Pregnancy by 600 mg Mifepristone and Vaginal Administration of 1.0 mg Gemeprost or 0.25 mg Sulprostone Intramuscularly

	French Multicenter Study (<49 days)		U.K. Multicenter Study (<63 days)
	Gemeprost (%)	Sulprostone (%)	Gemeprost (%)
Complete abortion	96.5	95.4	94.0
Incomplete abortion	2.3	2.8	5.0[a]
Ongoing pregnancy	0.4	0.6	
Hemostatic surgical procedure	0.8	0.4	1.0

[a] In the United Kingdom study, separate figures for incomplete abortion and ongoing pregnancy are not given.
SOURCE: U.K. Multicentre Trial (1990); Ulmann et al. (1992).

Mifepristone in Combination with Prostaglandin

The outcome of treatment is quite different if mifepristone is combined with prostaglandin. In the first study with the combined therapy, 25 mg mifepristone was given twice daily for three to six days. On the last day, 0.25 mg sulprostone (Schering AG, Berlin, Germany) was injected intramuscularly (Bygdeman and Swahn, 1985). The overall frequency of complete abortion was 94 percent. Success rates between 95 and 100 percent were also reported in similar studies where mifepristone was combined with vaginal administration of 0.5 to 1.0 mg gemeprost May and Baker, Dagenham, U.K., 1990 (Cameron et al., 1986; Dubois et al., 1988).

Today mifepristone is available for routine clinical use in France, Great Britain, and Sweden. The dose schedule recommended by the pharmaceutical company (Roussel-Uclaf, Paris) is a single dose of 600 mg mifepristone followed 36 to 48 hours later by 1.0 mg gemeprost. Sulprostone, which was mainly used initially, is no longer available since the intramuscular preparation has been withdrawn from the market. In Sweden and Great Britain the procedure is used through the eighth week of pregnancy (63 days of amenorrhea), whereas in France the upper limit is 49 days of amenorrhea. The clinical outcome of the treatment has been evaluated in two large multicenter clinical studies from France and Great Britain (U.K. Multicentre Trial, 1990; Ulmann, 1992). Mifepristone followed by either vaginal gemeprost or intramuscular injection of sulprostone was shown to be highly effective in terminating early pregnancy. The frequency of complete abortion was around 95 percent, and complete failures in terms of continued pregnancy occurred in about 0.5 percent of the patients (Table B5.1). The clinical events are very similar to those of a spontaneous abortion, with

bleeding and increased uterine contractility. About 50 percent of the patients have started to bleed at the time of prostaglandin treatment, and almost all within four hours thereafter. The mean duration of bleeding in the French study was eight days. In 89.7 percent of the women, bleeding lasted for 12 days or less. In 9 percent of the women the bleeding was described as severe or excessive in the 24 to 48 hours after mifepristone and 4 hours after prostaglandin therapy (U.K. Multicentre Trial, 1990). In 93 percent of the women there was little or no change in the hemoglobin value (up to 1 g/dl) before treatment and seven days after gemeprost administration, while in 1 percent of the patients there was a decrease of 2–4 g/dl. The frequency of hemostatic surgical procedures was between 0.4 and 1.0 in the two studies (Table B5.1).

Uterine pain is also common, especially during the first hours following prostaglandin treatment: 2 percent of the women reported severe pain after mifepristone administration, a frequency that increased to 21 percent two hours after gemeprost administration. The frequency had decreased to 10 percent by four hours in the British study. Clinical signs of infection were rare, or less than 1 percent. Nausea and vomiting were not uncommon during the first hours after prostaglandin therapy.

In the French study, which included more than 16,000 women, serious cardiovascular side effects were reported in four cases after sulprostone injection. They consisted of one acute myocardial infarction attributed to a coronary spasm and marked hypertension in the other three women. Since the drug has been marketed and used in more than 60,000 cases in France, two additional myocardial infarctions have occurred, one of them being fatal. The frequency of severe cardiac complications after sulprostone is thus approximately 1/20,000 cases. As a consequence, smoking more than 10 cigarettes per day, age over 35 years, and any suspicion of cardiovascular disease must be considered relative contraindications to the method. It is noteworthy that so far, no myocardial infarction has occurred when gemeprost has been used, and most likely smaller doses of prostaglandin may still be sufficient and associated with a lower frequency of this type of complication.

As indicated previously, pregnancy might continue in spite of treatment, as occurred in 0.4 to 1.5 percent of clinical trial subjects. Mifepristone is known to cross the placenta (Frydman et al., 1985), and concern has been expressed that the drug may have teratogenic potential. Since surgical uterine evacuation is recommended when the treatment fails, data on infants exposed to mifepristone in utero are rare. However, the information that does exist suggests that the antiprogestin does not cause fetal malformations (Lim et al., 1990; Pom et al., 1991).

TABLE B5.2 Clinical Management of Medical Abortion in Sweden

	First Visit	Second Visit	Third Visit	Fourth Visit
Physician				
Duration of pregnancy			Evaluation of outcome	
Information necessary			Ultrasound exam if contraceptive advice	
Sampling			Start hormonal contraception	
Nurse		Mifegyne (600 mg)	Gemeprost (1.0 mg)	
			Supervision for 4–6 hours	
			Treatment of pain	
			Rh prophylaxis	
		⊢—— 36–48 hours ——⊣		
		⊢———— 14 days ————⊣		

Practical Guidelines for Clinical Use of Mifepristone in Combination with Prostaglandin

The clinical use of the combination of mifepristone and prostaglandin for termination of pregnancy is more regulated than most other medical procedures: (1) it may be prescribed only in centers registered for pregnancy termination (in Sweden by the National Board of Health and Welfare); (2) distribution of the drug is strictly controlled; and (3) each patient must sign a consent form indicating that she is aware that the method is not risk free and does not have a 100 percent success rate. The treatment includes four visits to the clinic (Table B5.2). The patient is seen by the physician at the initial visit and two weeks after the start of treatment to evaluate the outcome of the therapy. The nurse supervises the intake of mifepristone and the four- to six-hour observation period in the outpatient ward following prostaglandin therapy.

FURTHER DEVELOPMENTS IN MEDICAL TERMINATIONOF EARLY PREGNANCY

The ideal combination of mifepristone and prostaglandin remains to be established. Studies in both pregnant and nonpregnant women have shown that the pharmacokinetics of mifepristone are nonlinear and that oral administration of the drug in single doses greater than 100 mg results in serum concentrations that differ only minimally or not at all (for references, see Van Look and Bygdeman, 1989; Puri and Van Look, 1991). Multicenter trials conducted under the auspices of the World Health Organization have shown that the same effectiveness observed with 600 mg can be achieved with either repeated small doses of mifepristone (five doses of 25 mg given at 12-hour intervals) (WHO, 1991) or a single dose of 200 mg (WHO, to be published) if combined

with 1.0 mg gemeprost. It is also likely that the dose of gemeprost may be reduced to 0.5 mg (unpublished observation by author).

Oral administration of the prostaglandin (PG) may be an advantage. The analogue 9-methylene-PGE_2 is orally active. When given in combination with mifepristone, this prostaglandin has been reported to terminate 95 percent of early pregnancies (Swahn et al., 1990). Another alternative is misoprostol (G.D. Searle Co., Chicago, Illinois). Misoprostol is licensed for sale in many countries for the treatment of gastric and duodenal ulceration. It is active by mouth, inexpensive, and stable at room temperature. If administered alone during early pregnancy, doses ranging from 200 µg to 600 µg induce significant uterine pressure. However, only 2 of 40 women given misoprostol alone aborted. When misoprostol was administered after mifepristone, there was a significant increase in both amplitude and frequency of uterine contractions, and complete abortion took place in 18 out of 21 women (Norman et al., 1991). Aubeny and Baulieu (1991) treated 100 women who were undergoing legal abortion at gestations of up to 49 days with 600 mg mifepristone followed two days later by 400 µg misoprostol by mouth. Complete abortion was found in 95 women, four women had an incomplete abortion, and in one woman the pregnancy continued despite treatment.

Recently, an extended study from France has been published (Peyron et al., 1993). It includes altogether 25 centers and 895 women with an amenorrhea of less than 50 days. The majority of women (n = 505) were treated with 600 mg mifepristone and 48 hours later, if the abortion had not occurred, 400 µg misopristol (Trial 1). In the remaining 390 women, a second dose of 200 µg misoprostol was given four hours later if the patient had not aborted. This was found necessary in approximately 25 percent of the patients (Trial II). In the first trial with the fixed dose of misoprostol, the success rate (complete abortion) was 96.9 percent. This rate is similar to that previously observed for mifepristone followed by sulprostone or gemeprost. In the second study, in which an extra dose of misoprostol was added if judged necessary, the success rate increased to 98.7 percent. In both trials the majority of women experienced uterine cramps, but only 20 percent required administration of a non-opiate analgesic. This figure is considerably lower than that reported in the United Kingdom multicenter study in 1990 in which 28 percent of women required opiate analgesic and an additional 31 percent non-narcotic analgesia following mifepristone and gemeprost. Although it is well known that the degree of pain and need for analgesic treatment vary considerably, it seems that the replacement of gemeprost by misoprostol will result in decreased pain. However, only a randomized study comparing the two treatment schedules will give definite information.

TABLE B5.3 Termination of Early Pregnancy with 600 mg Mifepristone Followed by 400 µg Misoprostol in Women with an Amenorrhea of Less Than 50 Days (N = 1286)

	Percentage of Patients
Efficacy	
Complete abortion	95.4
Incomplete abortion	2.8
Ongoing pregnancy	1.5
Hemostatic procedure	0.3
Pain	
Within four hours of misoprostol	81.5
Need for analgesia	19.6
Narcotic analgesia	0.1

SOURCE: Silvestre et al., 1993

The latest summary of the French experience of the combination of mifepristone and misoprostol for termination of early pregnancy was reported at the recent VIIIth World Congress in Human Reproduction (Silvestre et al., 1993). The report included 1,288 early pregnant women (duration of amenorrhea <50 days) who received 600 mg mifepristone followed two days later by a fixed dose of misoprostol (400 µg). The efficacy of the treatment was evaluated two weeks after the treatment by clinical examination, β-hCG and or ultrasound scan. The success rate, defined as complete expulsion of the conceptus with no need for additional surgical procedure, was 95.4 percent. Of the remaining patients, 2.8 percent experienced an incomplete abortion, in 1.5 percent the pregnancy continued, and in .3 percent a hemostatic vacuum aspiration was performed (Table B5.3) Expulsion of the conceptus occurred within four days of administration of misoprostol in 59 percent of the women and within 24 hours in 81.5 percent of the women. Almost all women experienced uterine bleeding, the mean duration being nine days, and the mean drop in hemoglobin value was .7 g/d. Blood transfusion was required in only one woman (.1 percent). All these figures are comparable to those reported for the combination of mifepristone and sulprostone or gemeprost (Ulmann et al., 1992). However, also in this study uterine pain seemed less pronounced since the frequency of analgesic treatment was only 19.6 percent and narcotic analgesic was given to only one woman.

If treatment with mifepristone in combination with gemeprost or misoprostol is compared, available data indicate that both treatments are equally effective at least up to 49 days of amenorrhea. After 49 days of amenorrhea, the efficacy of mifepristone in combination with misoprostol seems to decrease significantly (D. Baird, personal communication).

TABLE B5.4 Suggested Differences Between Mifepristone in Combination with Either Gemeprost or Misoprostol

	Mifepristone with:	
Characteristics	Gemeprost	Misoprostol
Efficacy		
<50 days	—	—
50–63 days	+?	—
Complications	—	—
Side effects, mainly uterine pain	+?	—
Cost	++	—

Complications in terms of duration of bleeding, heavy bleeding necessitating treatment, time of expulsion of the conceptus, and pelvic infections seem to be frequent, while uterine pain necessitating treatment seems lower following misoprostol in comparison to gemeprost (Table B5.4).

Once-a-month treatment with mifepristone at the time of menstruation has so far not been found effective. In case of pregnancy the failure rate is around 15 percent (for references, see Van Look and Bygdeman, 1989). To date, the possibility of adding a low dose of prostaglandin has not been evaluated. One obvious reason is that a combination of mifepristone and gemeprost, in about 15 percent of the patients, is so painful that strong analgesic treatment is needed. In a nonpregnant cycle, misoprostol in the doses used (400 µg) has very little effect on uterine contractility, and the effect of prostaglandin is not enhanced by pretreatment with mifepristone (Gemzell et al., 1990). However, if the women were treated with human chorionic gonadotropin to rescue the corpus luteum and create an endocrine situation similar to that in a pregnant cycle, mifepristone followed 48 hours later by 400 µg had a significant effect on uterine contractility (Gemzell et al., 1993). The data indicate that this combined treatment may be effective at the time of menstruation in women exposed to the risk of pregnancy. If the woman does not become pregnant, the degree of uterine stimulation is minor and should not cause any side effects. If she becomes pregnant, the degree of uterine contractility should be sufficient to expel the conceptus.

CONCLUSIONS

The need for a safe, noninvasive method of medical abortion was expressed by the World Health Organization in 1978, and the mifepristone-prostaglandin combination goes a long way towards fulfilling this

concept. Medical abortion using this combination seems to be a safe, effective, and realistic alternative to surgery. Preliminary data indicate that side effects, mainly in terms of uterine pain, may be further reduced if treatment with mifepristone is combined with a lower dose of prostaglandin. In addition, the approach may be more convenient to use if ongoing trials confirm that oral administration of misoprostol is an effective alternative to gemeprost.

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B6 USES OF ANTIPROGESTINS AFTER 63 DAYS OF AMENORRHEA

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Roussel-Uclaf, Romainville, France

RU 486 (mifepristone), the first antiprogestin available for clinical purposes, has been synthesized by Roussel-Uclaf. It possesses both antiprogesterone and antiglucocorticoid properties (Philibert et al., 1985; Ulmann et al., 1990). It is now marketed in France, the United Kingdom, and Sweden as a medical abortifacient, but has many other potential uses linked to its antiprogesterone activity. In addition, the antiglucocorticoid activity of this compound is of potential clinical interest and is currently being evaluated.

Based on the results of a large body of experience reviewed elsewhere (Bygdeman, Appendix B5), it has been recognized that RU 486 can be used as an alternative to vacuum aspiration for early pregnancy termination (up to 63 days of amenorrhea). It is given as a single oral dose of 600 mg followed 36–48 hours later by the administration of a small dose of a prostaglandin (PG) E1 analogue (gemeprost or misoprostol). In the three countries where RU 486 is currently marketed, it is approved with the following conditions:

- It must be prescribed only in centers registered for pregnancy termination.
- Its distribution must be strictly controlled.
- Each patient must sign a form indicating that she is aware that the method does not ensure a 100 percent success rate, hence, the need for a mandatory control visit 8–10 days after RU 486 intake.

Availability under these conditions allows RU 486 to be studied and approved for administration at other times during pregnancy (i.e., after 63 days of amenorrhea), which are reviewed in this paper.

RU 486 FOR SECOND-TERM PREGNANCY TERMINATION

Second-term pregnancy termination can be achieved through either surgical means (dilatation and evacuation) or medical treatment (with local or systemic prostaglandins). Prostaglandins, although efficient, induce many side effects. These side effects can sometimes be severe, hence the importance of using doses as low as possible. Previous studies with RU 486, including studies of first-term pregnancy termination, indicate that RU 486 treatment sensitizes the myometrium to the action of prostaglandins, allowing a decrease in the amount of prostaglandin necessary to induce expulsion. This observation led to several clinical trials in which the effect of RU 486 treatment prior to PG administration was evaluated as a means to allow a decrease in PG doses and to accelerate expulsion in second-term pregnancy termination. The results of the trials are summarized in Table B6.1.

These studies indicate that treatment with RU 486 prior to PGs allows a significant decrease of the PG doses and significantly shortens the induction to abortion interval. This finding is of great clinical value for the comfort of patients. In one study (Roussel-Uclaf, data on file), the duration of hospitalization was shortened by one day in the group given RU 486. In another study (Thong and Baird, 1992), which compared RU 486 and laminaria tent (Dilapan) in gemeprost-induced abortion, RU 486 resulted in a significantly shorter induction-abortion interval than Dilapan.

RU 486 FOR INTRAUTERINE FETAL DEATH (IUFD)

After a pilot study suggested that the compound alone seemed efficient to induce expulsion of the dead fetus, a placebo-controlled study was undertaken to evaluate the efficacy of RU 486, given as 600 mg for two consecutive days, in this condition. Results of this study, published elsewhere (Cabrol et al., 1990), are summarized in Table B6.2.

These results show that RU 486 alone was able to induce labor in patients with IUFD and that expulsion took place significantly earlier than in patients given a placebo.

CERVICAL RIPENING WITH RU 486 PRIOR TO SURGICAL ABORTION

Animal studies have demonstrated that RU 486 is able to mature the cervix, evidenced by an increase in cervical diameter and a decrease of cervical resistance to mechanical dilation (Stiemer and Elger, 1990; Cabrol et al., 1991). It has also been shown that RU 486 triggers the biochemical modifications that characterize the normal process of cervi

TABLE B6.1 Results of Several Controlled Studies of RU 486 Followed by a Prostaglandin for Second-Term Pregnancy Termination

		Units of PG Necessary (range)			Induction to Abortion Interval (hours, range)		
Reference	PG Used (units, route)	RU 486	(or none)	Placebo	RU 486	(or none)	Placebo
Rodger and Baird (1990)	Gemeprost (1 mg pessary every 3 hours)	3[a] (1–10)	$P < 0.01$	5[a] (2–10)	6.8 (2–67.8)	$P < 0.01$	15.8 (5.9–95.6)
R.-U.[b]	Sulprostone (0.5 mg IM every 6 hours)	2[a] (1–4)	$P < 0.01$	4[a] (2–9)	9.5 (2.2–25.8)	$P < 0.01$	22.3 (10–84)
Hill et al. (1990)	PGE_2 (1.5 mg extraamniotic once)	—		—	8.5 (5.3[c])	$P < 0.02$	18.5 (10.1)
Urquhart et al. (1989)	Prostin E_2 infusion (extraamniotic infusion)	11[d] (5–30)	$P = 0.01$	18[d] (5–45)	9.3 (4–14.3)	$P < 0.01$	12.3 (6.6–22.4)

NOTE: IM = intramuscular.
[a] Median.
[b] Roussel-Uclaf, data on file, report of study FFR/88/486/03.
[c] Standard deviation.
[d] Mean of total dose infused (mg).

TABLE B6.2 Results of Double-Blind Placebo-Controlled Study of RU 486 (600 mg/day for two consecutive days) for IUFD

	RU 486 ($N = 46$)	Placebo ($N = 46$)	P
Days of amenorrhea	199.1 (7.8[a])	197.5 (6.7[a])	NS
Duration of retention (days)	18.2 (4.1[a])	15.1 (2.5[a])	NS
Progesterone levels before treatment (ng/ml)	61.5 (12.1[a])	57.1 (7.6[a])	NS
Number (%) of women in whom labor occurred within 72 hours after first drug intake	29 (63)	8 (17)	< 0.001

NOTE: NS = not significant at .05.
[a] SEM = standard error of the mean.
SOURCE: Cabrol et al. (1990).

cal maturation (i.e., increase in water content and hyaluronic acid, and collagenase activation) (Ikuta et al., 1991). Data obtained during first- and second-term pregnancy termination, and in IUFD, have shown that RU 486 is also able to induce cervical maturation in humans. Several placebo-controlled studies have been performed to evaluate the efficacy of RU 486 in cervical ripening prior to vacuum aspiration. Their results are summarized in Table B6.3.

The table shows that except in two studies [Rådestad et al., 1988, where the compound was given at a relatively small dose, 100 mg *bid* (twice a day), with measurements 24 hours after the last dose, and study FCH/85/486/21, where RU 486 was given only 12 hours prior to the measurements], RU 486 always induced a significant increase in the cervical diameter. Information obtained from trials other than those described above includes the following:

The time lag between the last RU 486 intake and the measurement of cervical diameter is an important factor. In a Canadian study (Lefèbvre et al., 1990) where several doses of RU 486 were evaluated, the cervical modifications were always significantly more important after 48 hours than after 24 hours. A similar finding was reported by Rådestad et al. (1990).

There is a dose-response relationship between dose of RU 486 and cervical diameter 24 to 48 hours after administration. The optimal dose of RU 486 was studied in the aforementioned Canadian study, in which different groups received either a placebo or 50, 100, 200, 400, or 600 mg of RU 486, as a single administration 24 or 48 hours prior to cervical calibration. For all doses of RU 486 studied, there was a significant increase of cervical diameter, which was linearly related to dosage up to 400 mg. Interestingly, in this study the duration of the subsequent vacuum aspiration was significantly decreased in parallel with the dose

TABLE B6.3 Results of Placebo-Controlled Studies of Cervical Ripening with RU 486

Reference	RU 486 (Placebo) Dose, Time Before Measurement (number of patients per group, mean duration of amenorrhea)	Cervical Diameter (mm)		
		RU 486 (or none)	Placebo	*P*
R.-U.[a]	50 mg bid x 2 days, 48 hours (*N*= 20, 63 DA)	6.4 (1.2[b])	4.3 (1.2)	$<10^{-4}$
R.-U.[c]	600 mg once, 12 hours (*N*= 20, 70 DA)	6.4 (1.6[b])	6.3 (1.6)	NS
Rådestad et al. (1988)	100 mg bid x 1 day, 24 hours (*N*=20, 8.5 WA)	5.5 (3–11)	4.4 (3–6)	NS
R.-U.[d]	600 mg once, 48 hours (*N*=20, 76 DA)	7.5 (5–9)	6 (3–8)	<.001
R.-U.[e]	600 mg once, 30 hours (*N*=38, 68 DA)	7.0 (5–10)	6 (5–7)	<.001
Gupta and Johnson (1990)	600 mg once, 48 hours (*N*=15, 68 DA)	5 (2.0[b])	3 (1.2)	.002
Henshaw and Templeton (1991)	200 mg once, 36 hours (*N*=30, 75 DA)	6.8 (6.3–7.3[f])	5.0 (4.7–5.4)	$<10^{-5}$

NOTE: DA = days of amenorrhea; WA = weeks of amenorrhea; NS = not significant.
[a] Roussel-Uclaf, data on file: study FCH/85/486/25.
[b] Standard error of the mean.
[c] Roussel-Uclaf, data on file: study FCH/86/486/21.
[d] Roussel-Uclaf, data on file: study UK/88/486/04.
[e] Roussel-Uclaf, data on file: study UK/88/486/05.
[f] 5% confidence interval.

of RU 486 administered. For the 600-mg dose, the duration of the procedure was the shortest (8.2 minutes versus 12.3 minutes in the placebo group). A second study, undertaken by the World Health Organization, evaluated the effects of three doses (50, 100, or 200 mg) of RU 486 and a placebo on cervical diameter 12 and 24 hours after drug intake. All three doses of RU 486 significantly increased the cervical diameter, although there were no significant differences among doses (WHO, 1990). Given the short time between drug intake and cervical calibration, it is likely that the maximal effect was not observed. In this study, the ease of complementary mechanical dilation was linearly related to the dose of RU 486 (dilatation was considered easy in 10, 17, 22, and 35 percent of the patients for the placebo and in the 50-, 100-, and 200-mg groups for RU 486).

RU 486 significantly reduces the mechanical resistance of the cervix. In the study of Rådestad et al. (1988), the mean force necessary to introduce a Hegar dilator No. 9 was 21.6 N in the RU 486-treated patients versus 31.4 N in patients given the placebo ($P < .05$). Similarly, the force necessary to dilate the cervix to 8 mm was significantly reduced in women given RU 486 compared to those given placebo in several

studies (Gupta and Johnson, 1990; Henshaw and Templeton, 1991; Roussel-Uclaf-monitored studies UK/88/486/04 and UK/88/486/05).

In one study (Henshaw and Templeton, 1991), which compared RU 486 to gemeprost (1 mg pessary 3–4 hours prior to calibration), both RU 486 and gemeprost induced an identical increase in cervical diameter and a similar reduction in the resistance to mechanical dilatation. However, abdominal pain was significantly ($P < .001$) more frequent in the group treated with gemeprost (43 percent of the patients) than in the group treated with RU 486 (10 percent of the patients).

Overall, RU 486 is well tolerated. The most frequently reported side effects are abdominal pain (23.9 percent of the patients on RU 486 versus 10.9 percent on placebo), and nausea and vomiting (20.5 percent on RU 486, 13.0 percent on placebo). Approximately one-quarter of the patients in whom the drug is used for cervical ripening prior to surgical abortion experienced uterine bleeding after RU 486 intake, which was always reported as minimal or moderate but never necessitated specific treatment.

Blood loss during vacuum aspiration was usually identical in patients given RU 486 or the placebo, except in two studies (Roussel-Uclaf, data on file, study UK/88/486/04; Henshaw and Templeton, 1991) in which blood losses were significantly lower in RU 486-treated than in placebo-treated patients. In one of these studies, blood loss was 225 ml (range: 110–825 ml) in the placebo group, and 115 ml (range 60–500 ml) in the group given 600 mg of RU 486 ($P = .001$). In the second study, blood loss was 320.8 ml [95 percent confidence interval (CI): 243.2–398.5 ml] in the group given placebo, 137.3 ml (95 percent CI: 96.7–178.0 ml) in the group given 200 mg of RU 486, and 115.3 ml (95 percent CI: 93.2–143.6 ml) in the group given 1 mg gemeprost ($P < .0001$).

Abdominal pain tended to be less frequently reported postoperatively in patients given RU 486 than in those given placebo (27.1 versus 40.4 percent of the cases). Postoperative bleeding was semiquantitatively assessed in some studies and was comparable in RU 486- and placebo-treated patients. Similarly, there was no difference in the duration of postoperative bleeding.

Thus, RU 486 appears to be a safe and efficient means for cervical ripening prior to vacuum aspiration. It seems better tolerated than prostaglandins, but must be given 36–48 hours prior to the surgical procedure, instead of 3–4 hours for PGs.

RU 486 FOR LABOR INDUCTION

Studies in ewes (Burgess et al., 1992) and monkeys (Wolf et al., 1989) indicate that at term, RU 486 induces uterine contractions and enhances the myometrial sensitivity to oxytocin. Newborns from mothers treated

with RU 486 were normal and actually grew faster than newborns from untreated mothers. This can be explained by an increase in milk output in mothers treated with RU 486, which is likely to be secondary to the suppression of progesterone-induced inhibition of prolactin. In humans, it has been demonstrated that RU 486 crosses the placental barrier (Frydman et al., 1985), so it is mandatory to evaluate the consequences of cortisol blockade in the newborn.

TABLE B6.4 Induction of Labor with RU 486 (200 mg/day for two consecutive days) or a Placebo

	RU 486 (*N* = 57)	Placebo (*N* = 55)	P
N (% of women with spontaneous onset of labor)	31 (54.4)	10 (18.2)	<.001
Interval between day 1 and onset of labor (hours, SD)	51.7 (26.7)	74.5 (39.5)	<.001
Mean (SD) total dose (IU) of oxytocin[a]	2.0 (2.2)	4.7 (3.0)	<.0001
Number of cesarean sections	18	18	NS
Neonatal tolerance *N* (% of infants)			
• With Apgar score below 7			
• At 1 minute	5 (8.8)	4 (7.3)	NS
• At 5 minutes	0	0	
• With umbilical vein pH below 7.20	4 (7.0)	3 (5.4)	NS

NOTE: IU = intrauterine; NS = not significant; SD = standard deviation.
[a] For women who delivered vaginally.
SOURCE: Frydman et al. (1992).

A pilot study (Frydman et al., 1992) evaluated the efficacy and safety of RU 486 for the induction of labor in cases of postdate pregnancies or other medical indications for labor induction. Table B6.4 summarizes the results of this study.

The table confirms that RU 486 is able to induce labor. It was well tolerated by both the newborn and the mother, and in particular, the number of hypoglycemic episodes up to 48 hours after birth was identical in both groups (three and five episodes in the RU 486and the placebo-treated groups, respectively). Dose-finding studies are currently in progress to determine the minimal dose of RU 486 necessary to induce labor. Once the dose is determined, a large-scale study will be undertaken to confirm the good tolerance to the compound by newborns.

In conclusion, it appears that the myometrial consequences of progesterone blockade can be utilized for various purposes during pregnancy, and antiprogestins such as RU 486 appear as interesting therapeutic innovations in an area where available efficient drugs are either

absent or poorly tolerated. Because of the controversy surrounding abortion in several countries, it is necessary that antiprogestins are first approved as medical abortifacients before being approved for their other obstetrical indications, as was the case for RU 486 in France, the United Kingdom, and Sweden. Since these other obstetrical indications are limited to inpatient use, the approved antiprogestins can be distributed and prescribed following the same procedures as those followed for medical pregnancy termination, which limits the risk of improper use.

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B7 COMMENTS ON SESSION II

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I would like to review with you what we have heard this morning and what I think are some of the key points. I will begin with a discussion of the use of mifepristone (RU 486) for first-trimester abortion.

USE OF MIFEPRISTONE AND PROSTAGLANDIN FOR FIRST-TRIMESTER ABORTION

Need for Medical Supervision

The first scientific issue relates to the implied danger of mifepristone. A recurrent theme in review articles to date is that mifepristone for abortion requires "strict medical supervision" and that hospital facilities are required. There is, to my knowledge, no scientific foundation for either assertion. Antiabortion elements have used the call for strict medical supervision to imply that the drug is dangerous. Extensive documentation confirms that abortion with mifepristone alone or with misoprostol is very safe and poses no unique medical challenges to a gynecologist or family physician. Routine medical care is warranted. Instead of strict medical supervision, what is required is strict administrative supervision to ensure that each abortion is completed. This follow-up is a logistical, not a medical, challenge.

Second, appropriate medical backup for the small percentage of women who have an incomplete abortion with heavy bleeding is suction curettage on an outpatient basis. Operating room facilities and general anesthesia are not necessary. Because patients may develop heavy bleeding during nights or weekends, facilities for suction curettage

should be available around the clock. While hospital emergency departments are open at all hours, they are often very expensive—and very hostile—locations to perform suction curettage. Your committee might want to consider after-hour availability of suction curettage in clinics or physician's offices that might be dispensing antiprogestins.

Spontaneous abortion is a prototype for expulsion of uterine contents without medical supervision, and the morbidity and mortality are negligible. Over the past five years, I have been in charge of the emergency room in the largest maternity hospital in North America, where I have personally supervised the care of about 2,000 patients with spontaneous abortions each year. The morbidity is really negligible. So, with medical supervision in a doctor's office, the safety should be even greater.

Acceptability of Medical Versus Surgical Abortion

Given that we are going to be using prostaglandins along with RU 486, how can we make this regimen less clumsy, less cumbersome? A very interesting study from France published in the fall of last year showed that among women who had received this combination regimen of mifepristone plus sulprostone, three times as many women were unsatisfied with this regimen as with the curettage offered under either local or general anesthesia. This shows that we have a long way to go in making this more palatable for women in Europe and in the United States.

Number of Visits Required

The second issue that generated debate this morning is the practice of four visits for mifepristone plus prostaglandin abortions. The scientific basis for two of these visits is debatable. This practice evolved in countries with very different health care systems for abortion services than the United States. In France, the United Kingdom, and Sweden, abortions take place in publicly supported facilities. In contrast, most abortions in the United States take place in free-standing abortion clinics. The response of public hospitals in the United States to the *Roe v. Wade* decision in 1973 was tantamount to default. Moreover, since 1977, the federal government has been prohibited from paying for abortions.

The European model of abortion provision is unlikely to be compatible with the United States health care delivery system because of the cumbersome requirement for multiple visits. In the United States, abortion services are currently limited largely to metropolitan areas, with more than 80 percent of U.S. counties having no provider. For

example, there is only one provider in South Dakota, and more than half of all Wyoming women who have abortions cross state lines for care. Women disadvantaged by lack of transportation or difficulties related to child care costs will find it difficult to make four separate visits for a first-trimester abortion. For example, this might require over 1500 miles of round-trip travel for many women in rural America. Use of this regimen would effectively deprive these women of this option, if one assumes that they could afford such care.

Medical science supports the need for two contacts with a health care provider for mifepristone abortion. The first would be for counseling, evaluation, and initiation of treatment. A second contact is important to confirm that the abortion has been completed and, if not, to perform a curettage. A second visit could be done at the initial facility or, if the travel distances were prohibitive, at a provider closer to home. A quantitative pregnancy test or a vaginal ultrasound examination can be used for this purpose. Women with a failed attempted abortion might be referred back to the initial provider.

Medical decisions about antiprogestins should be based on scientific evidence. Importation of abortion practices from dissimilar health care systems, without firm evidence of benefit, may be counterproductive. At the present time, a single visit to an abortion provider poses a hardship for many women in the United States. If mifepristone abortion is offered only in the European model, the service will be so cumbersome and expensive that many U.S. women will not have access to it. Rather than improving access to safe, legal abortion, this model would aggravate the existing disparity in access to service for disadvantaged women. To bring antiprogestins to our country and then to place them out of reach of women would be a cruel hoax.

I believe that randomized controlled trials could be done comparing the safety and acceptability of mifepristone abortions with varying numbers of visits. This would place clinical recommendations on a more firm scientific footing.

Appropriate Dose of Mifepristone

Third, we have heard discussed the appropriate dose of antiprogestins for the various indications. A common theme is the fact that we don't really know what the optimal dose is, and that is because we still lack sophisticated pharmacokinetic studies. As we have heard from several speakers, the dose-finding studies today have tended to use arbitrary doses with round multiples, such as 100, 200, 600 mg. They featured small sample sizes and, as we have heard from Dr. Nieman (see Appendix B3), have looked at relatively few outcome measures.

We need elegant pharmacokinetic studies correlating serum levels,

areas under the curve, and outcomes. What we now appear to know is that the 600-mg dose of mifepristone that we have been using appears far in excess of what is actually required, as you heard from Dr. Bygdeman this morning (see Appendix B5).

Does the dose make a difference? The pharmacology is very, very difficult. We don't see a linear relationship between dose and serum levels. Likewise, we don't see a linear relationship between dose and response. Nonetheless, it has been shown that the regimen does make a difference, and I will share with you our experience in Los Angeles.

Our experience using 13 different regimens (nearly all with mifepristone alone) can be divided into three broad categories: a single 600-mg dose, divided doses for a week, and all other regimens. Using regression analysis, we found three factors predictive of success for abortion: (1) body mass index, (2) initial beta human chorionic gonadotropin level, and (3) the regimen. The seven-day regimen was about twice as likely to result in failure as the other regimens and about six times as likely to fail as the single 600-mg dose. Each of these differences was highly statistically significant.

Side Effects

There is no free lunch in medicine, and we pay a price for everything we do. What should be the role of the prostaglandins with RU 486? After publication of the pivotal paper of Drs. Bygdeman and Swahn in 1985, the routine use of prostaglandins with RU 486 became common. We know that most of the noxious side effects and, indeed, the serious morbidity and mortality related to this regimen, result from the prostaglandins and not the RU 486—an example of the tail wagging the dog. The same week that a woman died in France with the RU 486 and prostaglandin regimen, we had a maternal death in Los Angeles of a 37-year-old woman who received a single, vaginal E2 suppository for abortion.

Since the prostaglandins cause most of the noxious side effects, the question we must ask ourselves is whether increasing the efficacy from the 80 to 90 percent range to the 90 to 100 percent range is worth the inconvenience, expense, and morbidity involved.

SECOND-TRIMESTER ABORTIONS

An important point that we heard raised this morning is that RU 486 may be most useful for augmenting second-trimester abortions. This has not received much attention in the United States. Premedication 36 to 48 hours before beginning an induction abortion cuts the time in half, and the important advantage here is that this now makes induction abortion

potentially an outpatient procedure instead of an inpatient procedure. At the present time in the United States, dilatation and evacuation (D&E) dominates second-trimester abortions, but requires substantial skill, in contrast to induction abortions. However, induction abortions are very expensive because they require hospitalization. Now we have the option with RU 486 for outpatient second-trimester labor induction.

CONTRACEPTION

The relative value of the antiprogestins for contraception in comparison to the current low-dose oral contraceptives cannot be judged until we know about the long-term effects of, let's say, continuous administration of an antiprogestin. The health benefits of low-dose oral contraceptives are really quite compelling. So, on public health grounds, one could make a much stronger case for using contemporary oral contraceptives as opposed to antiprogestins.

POST-COITAL CONTRACEPTION

It has been shown by our colleagues in Edinburgh and Manchester that mifepristone has great promise as a post-coital contraceptive. In these two randomized controlled trials sponsored by the World Health Organization, about 600 women have received RU 486 without a single failure. Importantly, there was much less nausea and vomiting with this regimen as compared with the birth control pill regimen widely used in North America. The downside, however, was a substantial delay in onset of next menses.

CERVICAL RIPENING

We have also seen that mifepristone will soften and dilate the cervix, and its side effects are fewer than with prostaglandins. However, as you heard this morning, the need for prolonged pretreatment—36 to 48 hours—limits the usefulness of this method and makes this less convenient than osmotic dilators.

So, osmotic dilators, such as the traditional laminaria, Lamicel, which is a polyvinyl alcohol sponge impregnated with magnesium sulfate, or the newer Dilapan, which is hygroscopic plastic, will continue to dominate practice here.

Finally, I think we need to hear more about work with RU 486 analogues, other antiprogestins such as onapristone, lilopristone, and literally scores of other pristones, that await study.

SUMMARY

In summary, I would leave you with six points. First, with regard to the safety issue, I think that issue has largely been resolved. Second, I think we all concur that better dose-finding studies are needed. Third, we need a way to come up with a less cumbersome regimen for administering RU 486, particularly in conjunction with a prostaglandin analogue. Fourth, I think a great potential use for RU 486 and similar drugs is to augment second-trimester abortions. Fifth, another very exciting potential is use as a post-coital contraceptive. Sixth, and finally, we need much more work, such as we have heard this morning, with these other scores of compounds.

B8 USE OF ANTIPROGESTINS IN THE MANAGEMENT OF ENDOMETRIOSIS AND LEIOMYOMA

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ABSTRACT

Pelvic endometriosis and uterine leiomyomas (fibroids) are the two most common disorders in women during reproductive age. Infertility, pelvic pain, and uterine bleeding are major clinical manifestations. Although their pathogeneses are unclear, both conditions are ovarian steroid dependent, and tumors are endowed with receptors for estrogen (ER) and progesterone (PR). The rationale for inducing regression of these tumor growths with an antiprogestin was formulated soon after the demonstration of the ability of RU 486 to interrupt early pregnancy in women. It was followed by a series of short-term studies showing the effectiveness of RU 486 in inhibiting ovulation, inducing luteolysis, and disrupting endometrial integrity in normally ovulatory women. Concurrently, clinical trials were conducted to determine the beneficial effect and safety of long-term daily administration of RU 486 in patients with endometriosis and fibroids.

For *symptomatic endometriosis*, in a pilot study, six patients were treated with a daily dose of 100 mg of RU 486 for three months. The treatment resulted in marked improvement of pain scores without discernible change in the extent of endometrial implants. In subsequent studies with a reduced dose (50 mg/day) and extended duration (six months) of RU 486 administration, both pain and endometriosis scores improved significantly.

In patients with *leiomyomas*, administration of RU 486 at a daily dose of 50 mg for three months induced a decrease in leiomyoma volume by 22 percent at one month, 40 percent at two months, and 50 percent at three months. When the dose of RU 486 was reduced

to 25 mg/day, the decrease of leiomyoma volume was virtually identical to that observed with the 50-mg dose. Further decreasing the dose to 5 mg/day was less effective, with an overall reduction of tumor volume by 25 percent. There was a decrease of immunostaining for PR but not for ER in leiomyomas treated with RU 486.

In all studies, amenorrhea with early to midfollicular range of estradiol levels was induced, and no change in bone mineral density was noted. Ovulatory cycles resumed four to six weeks after completion of treatment. Side effects were observed, which included atypical hot flushes, mild elevation of serum transaminase, and antiglucocorticoid effects at higher doses. Thus, an antiprogesterone may provide a novel mode of long-term (years) medical management for pelvic endometriosis and uterine leiomyomas. Future studies of lower doses and correlation of individual responses with the status of steroid hormone receptors, growth factors, anatomical sites, and vascularity may be helpful in predicting maximal responses of individual patients.

INTRODUCTION

Pelvic endometriosis and uterine leiomyomas (fibroids) are the two most common disorders in women during reproductive age. Infertility, pelvic pain, and uterine bleeding are major clinical manifestations, and hysterectomies are frequently resorted to. Although their pathogeneses are unclear, both conditions are ovarian steroid dependent, and tumors are endowed with receptors for estrogen (ER) and progesterone (PR). The rationale for inducing regression of these tumor growths with an antiprogestin was formulated soon after the demonstration of the ability of RU 486 to interrupt early pregnancy in women (Baulieu, 1989). It was followed by a series of short-term studies showing the effectiveness of RU 486 in inhibiting ovulation, inducing luteolysis, and disrupting endometrial integrity in normally ovulatory women (Schaison et al., 1985; Liu et al., 1987; Garzo et al., 1988; Luukkainen et al., 1988; Roseff et al., 1990). Concurrently, clinical trials were conducted to determine the beneficial effect and safety of long-term daily administration of RU 486 in patients with endometriosis and fibroids.

ENDOMETRIOSIS

Endometriosis is a common disease, affecting as many as 1 in 15 women of reproductive age (Barbieri, 1990). The incidence is much higher among women with infertility (25 percent; Jones and Rock, 1976). In this condition, functioning endometrial gland and stroma have migrated outside the uterine cavity. These ectopic endometriotic

implants are infiltrating lesions involving reproductive organs, peritoneum, bladder, and rectosigmoid colon. On rare occasions, implants may be found outside the abdominal cavity (e.g., in the lung). The depth of infiltration into the fibromuscular tissue of the pelvis is strongly correlated with the severity of pelvic pain. Superficial implants, on the other hand, are found more frequently in patients with infertility (83 percent; Cornillie et al., 1990). Dysmenorrhea, dyspareunia, and pelvic, back, and rectal pain are common symptoms. Definitive diagnosis is based on the visualization of endometriotic implants by laparoscopy.

The histologic features and the density of immunostaining of PR and ER are heterogeneous as contrasted to the uterine endometrium (Metzger et al., 1993). Approximately half of these implants are in phase with normal endometrium, indicating nonuniformity in hormone responsiveness among implants (Lessey et al., 1989). Nonetheless, the growth of endometriosis is stimulated by the cyclic ovarian steroids estrogen (E2) and progesterone (P4), and in the absence of these steroids (e.g., after ovariectomy), endometriosis undergoes regression (Shaw, 1992).

Current *medical treatments* of endometriosis depend on suppression of ovarian function and induction of endometrial atrophy. *Danazol*, an isoxazole derivative of 17α-ethynyltestosterone, has been used for the treatment of endometriosis for the past 15 years. The drug has multiple and diverse effects on reproductive tissues. The main biological properties of Danazol are binding to androgen receptors and suppression of the hypothalamic-pituitary axis, resulting in acyclic ovarian function (Dmowski et al., 1983). Although Danazol is effective in the subjective improvement of symptoms and decreasing the growth of implants, the androgenic, anabolic, and hepatic side effects, such as hirsutism, voice deepening, weight gain, edema, headache, and increase in serum transaminases, are major drawbacks to its clinical use. The second effective treatment is the use of a gonadotropin-releasing hormone (GnRH) agonist to down-regulate the pituitary-ovarian axis, thereby inducing hypoestrogenism. The beneficial effect of a GnRH agonist on endometriosis is similar to that of Danazol, but it is devoid of androgenic and anabolic effects. However, the profound hypoestrogenic state and the associated consequence of severe hot flushes and a reduction of bone mass are major limitations for its long-term use (Matta et al., 1988a; Rummon et al., 1988). Both Danazol and a GnRH agonist are approved by the Food and Drug Administration for treatment of endometriosis of six-month duration. It is apparent that the development of novel, effective, and long-term medical treatments with little or no side effects would be a significant advance in the management of symptomatic endometriosis.

Antiprogestin in the Treatment of Endometriosis

Pilot Study

Rationale. Since ectopic endometrial tissue contains both ER and PR, and is sensitive to the hormonal agents that affect these receptors (Lessey et al., 1989), it prompted us to evaluate whether the antiprogestin RU 486 could have some beneficial effects in women with symptomatic pelvic endometriosis (Kettel et al., 1991).

Protocol. Six normal-cycling women with pelvic pain due to endometriosis participated in the pilot study. Their mean age was 29 years (range: 17–40 years). Four of them had failed other hormonal treatments, and none had taken any hormonal medication for at least two months prior to the study. All patients used barrier contraceptives throughout the study. Informed consent was obtained prior to enrollment.

RU 486 was given as two 50-mg tablets per day for three months, starting on cycle day 1. Staging of the disease was assessed at pre- and posttreatment laparoscopy by the American Fertility Society (AFS) Revised Classification (AFS, 1985). Pelvic pain was graded as minimal (+), mild (++), moderate (+++), or severe (++++).

Baseline blood samples were obtained in the early follicular phase of the pretreatment cycle and again during the last month of treatment. Ovarian function was monitored by daily determinations of estrone 3α-glucuronide (E_1G), an estrogen metabolite, and pregnanediol 3α-glucuronide (PdG), a progesterone metabolite, on the first void urine samples throughout the treatment period. Values were compared with data collected from 13 regularly cycling women during the menstrual cycle. In addition, 24-hour frequent sampling (10–20 minutes) for luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and cortisol measurements was performed in the early follicular phase of the pretreatment cycle and in the third month of treatment. Analytical methods are well established in our laboratory.

Outcome. All women became amenorrheic during treatment. Concentrations of PdG were acyclic and remained relatively constant throughout the treatment period. After an initial transient rise in E_1G levels during the first month of treatment, values comparable to the early to midfollicular phase range of normal-cycling women (Figure B8.1) were maintained. Serum estradiol (E_2) and estrone (E_1), testosterone (T), and androstenedione (A) as well as serum follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and prolactin were unchanged. LH pulse amplitude (but not frequency) was increased

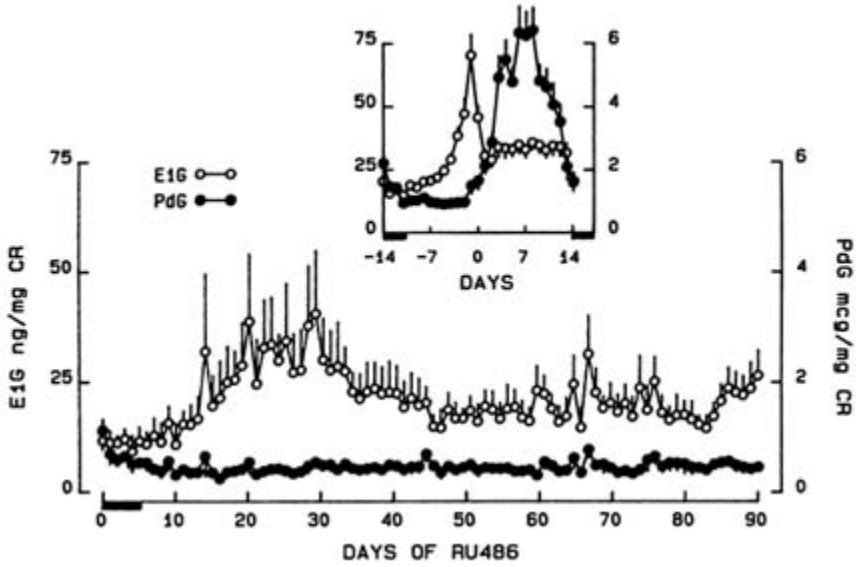

FIGURE B8.1 Mean (±SE) urinary estrone 3α-glucuronide (E_1G) and pregnanediol 3α-glucuronide (PdG) normalized to creatinine (CR) in six women during treatment with RU 486, 100 mg/day for three months. *Insert:* mean (±SE) daily urinary E_1G and PdG levels in 13 normally cycling women. Data are centered around the day after the E_1G peak (day 0).

($P < .02$), suggesting an antiprogesterone effect on the hypothalamic-pituitary. After cessation of RU 486, regular menstrual cycles ensued and the first episode of vaginal bleeding occurred within three to six weeks in all subjects.

Twenty-four-hour mean cortisol ($P < .01$) and ACTH ($P < .05$) concentrations were increased after RU 486 treatment. This rise in cortisol and ACTH was most apparent during the early morning hours (2:00 a.m. to 4:00 p.m.) and became indistinguishable during times of secretory quiescence (4:00 p.m. to 2:00 a.m.) (Figure B8.2). With cosinor analysis, the normal circadian rhythm of cortisol and ACTH secretion was maintained, with no change in acrophase (7:36 a.m. versus 7:35 a.m.) after treatment. These findings indicate that RU 486, at 100-mg daily dose, induced an antiglucocorticoid effect.

Treatment resulted in an improvement of pelvic pain in all subjects (Table B8.1). This pain relief persisted over a one- to two-year follow-up interval in three of six patients; pain recurred to a lesser extent in one subject; and two subjects successfully conceived after completing therapy. Follow-up laparoscopy was accomplished in five patients. Endometriotic implants had resolved in only one subject and persisted in all others (Table B8.1). Three subjects reported atypical flushes, and one

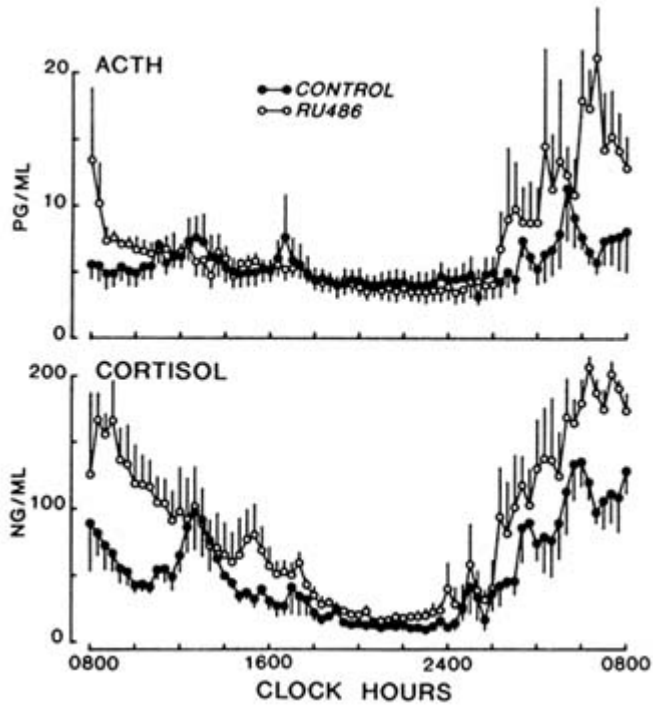

FIGURE B8.2 Mean (±SE) 24-hour secretory patterns of ACTH and cortisol before and after treatment with RU 486 at 100 mg/day for three months.

TABLE B8.1 Six Women with Symptomatic Endometriosis Before and After Treatment with RU 486 (100 mg/day for three months)

		AFS Stage[a]		Pelvic Pain	
Subject	Age (years)	Before	After	Before	After
1[b]	26	III	III	++++	+
2	17	I	I	+++	
3	34	I	I	+++	+
4[b]	40	I	I	++	
5	33	I	O	+++	++
6	24	II		+++	+

[a] The American Fertility Society revised classification of endometriosis.
[b] Conceived.

subject complained of anorexia and fatigue during the first four to eight weeks of treatment. No other significant side effects were reported. These preliminary findings offered promise for future investigations using *lower doses* and *longer-term* therapy with RU 486, with the aim of avoiding the antiglucocorticoid effect and allowing sufficient time for the

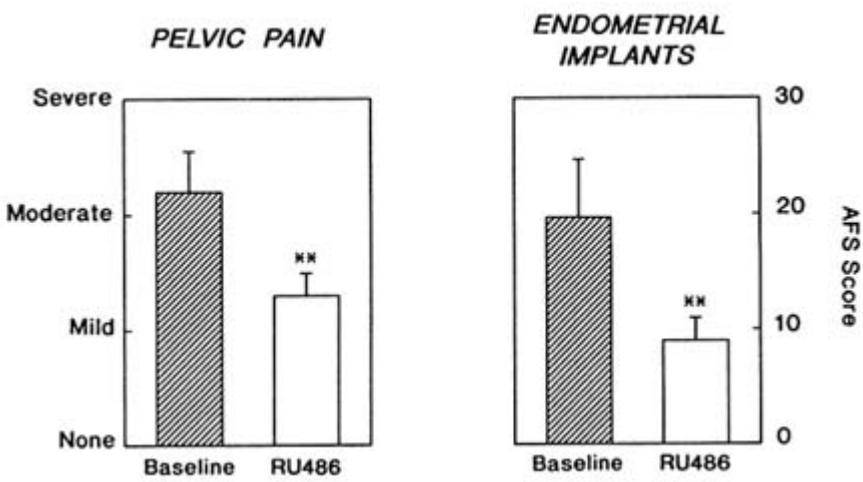

FIGURE B8.3 Clinical scores for pelvic pain and AFS scores for endometriotic implants before and after six months of RU 486 treatment at 50-mg daily dose (**$P < .05$).

resolution of endometriotic implants, which was not seen in this three-month trial.

Long-Term, Low-Dose Studies

The purpose of this study was to test the efficacy of a reduced dose of RU 486 (50 mg/day), and extended duration of therapy (six months), on symptomatic endometriosis, and to dissociate the antiglucocorticoid effect of RU 486. Nine women with symptomatic endometriosis and previous failure in medical treatment (either no improvement or dropout due to side effects) participated in this study. Daily symptom inventories and urine collections were obtained during baseline, treatment, and recovery cycles. Each subject underwent pre- and posttreatment laparoscopy, and bone mineral density (BMD) determinations. Blood samples for hormone analysis were obtained weekly for four weeks and monthly thereafter. Seven subjects underwent 24-hour frequent sampling (every 10 minutes), before (early follicular phase) and during the last month of therapy.

Outcome. All patients became amenorrheic during therapy and exhibited anovulatory urinary E_1G and PdG profiles. All subjects reported a significant decrease in pelvic pain ($P < .02$) and dysmenorrhea ($P < .03$), which lasted throughout therapy (Figure B8.3). Laparoscopic assessments of endometriotic implants by AFS scores (two observers)

showed decreases in eight of nine subjects (19.8 ± 4.9 to 9.4 ± 1.7, $P < .05$) (Figure B8.3). BMD of the lumbar spine and femur remained constant throughout therapy (Figure B8.4). Serum levels of LH increased during the first month of treatment [11.4 ± 2.6 to 25.3 ± 7.3 IU (international units) per liter, $P < .05$], with a concomitant increase in testosterone (1.07 ± 0.3 to 1.90 ± 0.4 nmol/liter, $P < .05$).

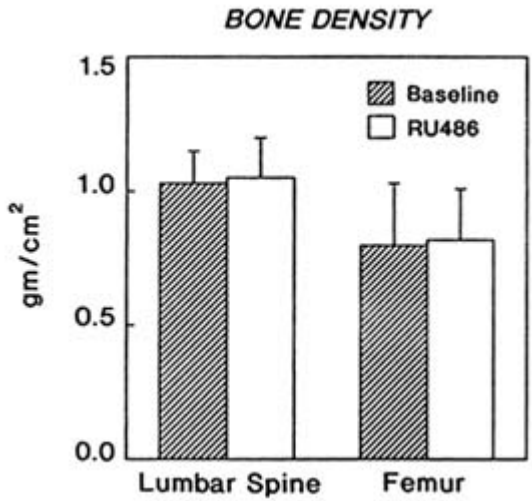

FIGURE B8.4 Bone mineral density of the lumbar spine and femur determined by dual photon x-ray absorptiometry before and after six months of RU 486 treatment at 50-mg daily dose.

Frequent sampling studies demonstrated no change in LH pulse frequency or amplitude. Twenty-four hour mean serum cortisol was unchanged (164.4 ± 13.1 versus 166.2 ± 12.3 nmol/liter) with preservation of the normal circadian rhythm (Figure B8.5). No other hormonal changes occurred.

Side Effects. Two patients developed transient, mild increases in liver transaminase, which normalized at one month. Atypical, mild hot flushes were noted in these patients. No other significant adverse effects were noted. This study demonstrates the effectiveness of long-term, low-dose RU 486 in achieving a condition of ovarian acyclicity and improving both pain and extent of disease without inducing an antiglucocorticoid effect. Thus, RU 486 may provide a safe and well-tolerated alternative for the medical management of endometriosis.

A *follow-up study*, using a 5-mg daily dose for six months, was conducted in patients with symptomatic endometriosis. In this ongoing study, four patients have completed treatment (three are ongoing). All four patients have had improvement of pelvic pain, amenorrhea, and no

side effects observed. These preliminary observations have prompted us to extend this study to a larger number of patients with the hope of developing a long-term (years) therapy using antiprogestins in symptomatic endometriosis when fertility is not an issue.

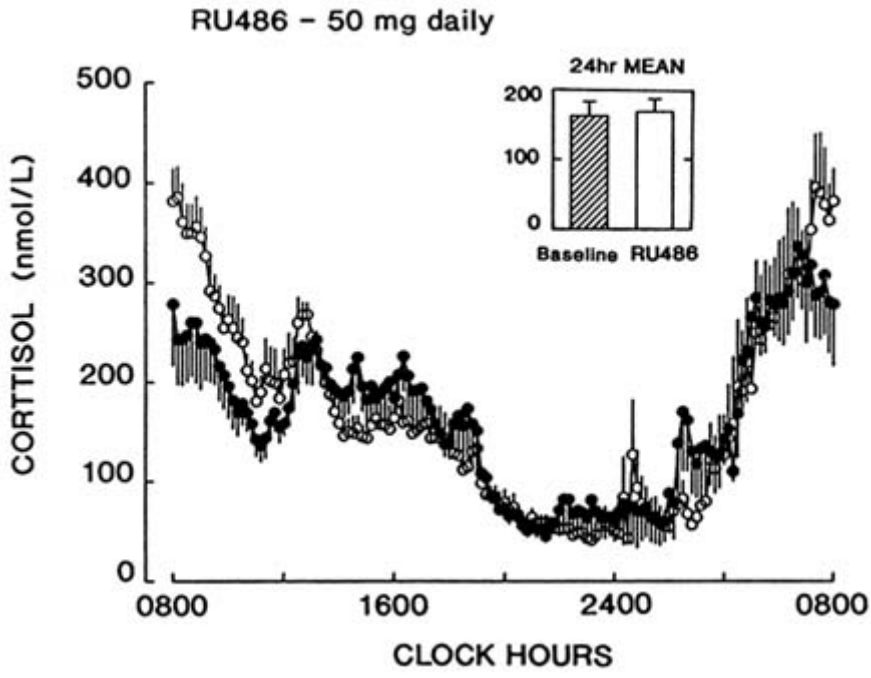

FIGURE B8.5 Twenty-four-hour serum cortisol levels measured at 20-minute intervals before (•) and after (○) six months of RU 486 treatment. *Insert*: 24-hour mean cortisol values before and at the end of RU 486 treatment.

In summary, RU 486 at a 50-mg dose, and possibly at a 5-mg dose for a period of six months has resulted in impressive therapeutic effectiveness. Side effects were limited to slight and reversible elevation of liver transaminase in about 20 percent of the patients, which is similar to the side effects of danazol. The major advantage of this treatment modality may be the potential long-term use (years) in the "moderation" of symptomatic endometriosis, thereby circumventing a hysterectomy and bilateral ovariectomy, particularly in women of late reproductive years.

LEIOMYOMA (FIBROID TUMOR)

Uterine leiomyomas are common pelvic tumors occurring in up to 20 percent of women over 30 years of age (Buttram and Reiter, 1981). Leiomyomas represent one of the most frequent indications for opera

tive procedures in women of reproductive age: 600,000 women had hysterectomies in 1991, with an annual cost exceeding $5 billion (Carlson et al., 1993). Although the mechanisms of tumorigenesis are unknown, evidence suggests that leiomyomas are ovarian steroid dependent (Buttram and Reiter, 1981; Lumsden et al., 1989; Rein et al., 1990). Receptors for both estrogen and progesterone have been identified in leiomyomas, and their content is significantly greater than those found in the myometrium (Lumsden et al., 1989; Rein et al., 1990). Pituitary-ovarian down-regulation by GnRH agonists, the only medical treatment currently available, results in 50 percent regression of leiomyomas in women of reproductive age (Friedman et al., 1987; Perl et al., 1987; West et al., 1987; Andreyko et al., 1988; Kessel et al., 1988; Lumsden et al., 1989; Schlaff et al., 1989; Rein et al., 1990; Friedman et al., 1991). The major limitation of the treatment modality is severe hypoestrogenism, with consequent severe hot flushes and bone loss. Treatment is approved by the U.S. Food and Drug Administration for a duration of six months only.

Since leiomyomas and endometriosis share ovarian steroid dependency, we reasoned that RU 486 may have an inhibitory effect on the growth of leiomyomas. That progesterone may play a role in leiomyoma growth is suggested by the finding of a higher mitotic count in leiomyomas obtained during the secretory phase than in the proliferative phase of the menstrual cycle (Kawaguchi et al., 1988). Additionally, when the GnRH agonist and a progestin are coadministered, the expected regression of leiomyoma size observed with the GnRH agonist alone is not achieved (Friedman et al., 1988). Here, we report studies showing an inhibitory effect of RU 486 on the growth of uterine leiomyomas.

Antiprogestin in the Management of Leiomyoma— Dose-Response Studies

Subjects. Thirty-four normally cycling women between the ages of 18 and 45 years with symptomatic uterine leiomyomas were recruited for this study. None of the subjects had taken any hormonal medications for at least three months prior to the study. All subjects used barrier contraception. These subjects participated in the following experiments:

RU 486, 50-mg daily dose for three months ($N = 10$)

RU 486, 25-mg daily dose for three months ($N = 17$) (7 underwent uterine blood flow studies); and

RU 486, 5-mg daily dose for three months ($N = 7$).

Clinical and Laboratory Assessments. Each subject had a pelvic sono

gram performed prior to initiation of drug therapy and monthly thereafter. This was performed either vaginally or abdominally, with each tumor measured in three dimensions. Subjects were given RU 486 beginning on days 1-3 of the menstrual cycle. Baseline and monthly blood samples for complete blood count and chemistry panel were obtained. Serum samples for hormonal evaluation were obtained before and after initiation of therapy, daily for one week, weekly for four weeks, and monthly thereafter. LH, FSH, E_2, E_1, A, T, TSH, progesterone (P), dehydroepiandrosterone (DHEA), DHEA-sulfate, cortisol, and prolactin were determined by in-house radioimmunoassays.

Bone mineral density, determined by dual photon x-ray absorptiometry of the spine and hips was assessed (50-mg dose only) before and at the end of therapy. Uterine arterial flow was analyzed by a duplex sonography combining real-time imaging and pulsed Doppler velocimetry by transvaginal scanning. Doppler waveforms were computed and expressed as vascular resistance index (RI) (De Ziegler et al., 1991).

Histology and Receptor Protein Studies. Leiomyomas and myometrial tissue were obtained at surgery from six RU 486-treated patients and six untreated patients in the follicular phase of the cycle. Tissues were fixed in neutral-buffered formalin for 24 hours and embedded in paraffin. Mounted sections (8 µm thick) were used for immunohistochemical analysis (Garcia et al., 1988; Lessey et al., 1989). Briefly, after treatment with pronase-phosphate buffered saline (PBS) solution, sections of tissue were incubated with primary antibody for 30 hours. Secondary antibody, biotinylated goat antirat immunoglobulin G, was applied for 30 minutes, followed by tertiary antibody, streptavidin-alkaline phosphatase for 30 minutes. Samples were developed in McGrady's reagent. Pronase treatment was not required with the PR primary antibody. PR antibody was used at 1:5 dilution. The ER antibody was not diluted. For control, a duplicate section of each slide was stained in a similar manner, except that the primary antibody was eliminated.

Levels of ER and PR were evaluated using an image analysis software program (Image, program provided by Wayne Rasband, National Institutes of Health). This computer program captures an image from the microscope and provides a quantitative analysis of staining intensity. An arbitrary numerical unit is assigned to each slide and its nonimmune control. For each immunostained section, the adjacent nonimmune control was subtracted and the difference reported.

50-mg Dose

Outcome. At the 50-mg dose, a decrease in uterine leiomyoma volume in response to RU 486 is demonstrated. Reductions of 22 percent

at 4 weeks, 39 percent at 8 weeks, and 49 percent at 12 weeks were observed (Figure B8.6). This effect is comparable to that achieved with the GnRH agonist after a six-month treatment (35–50 percent decrease) (Friedman et al., 1987, 1991; Perl et al., 1987; West et al., 1987; Andreyko et al., 1988; Kessel et al., 1988; Lumsden et al., 1989; Schlaff et al., 1989; Rein et al., 1990). Schlaff et al. (1989) have noted that GnRH agonist treatment is accompanied by a significant decrease of both myometrial volume and leiomyoma volume, as assessed by magnetic resonance imaging. In fact, the nonleiomyoma volume, presumably representing normal myometrium, was more responsive than leiomyomas to hypoestrogenism. In our study, response was defined as a decrease in leiomyoma volume and excludes the myometrial component. When both factors are considered, the effectiveness of RU 486 in the regression of leiomyomas may be greater.

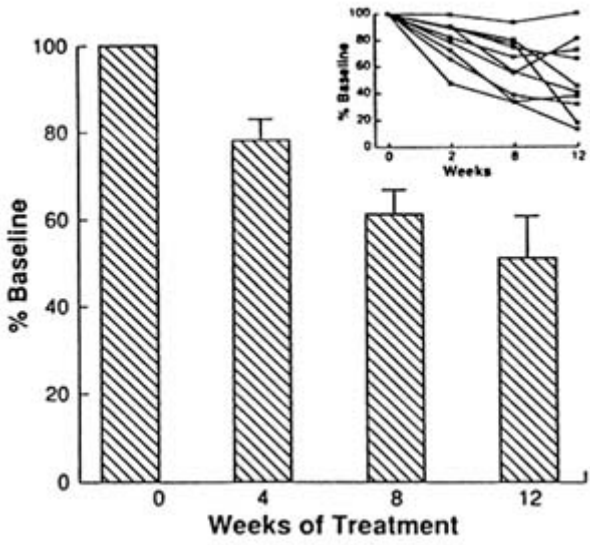

FIGURE B8.6 Percentage change in uterine leiomyoma volume in response to three months of RU 486 treatment. *Insert*: individual patient response.

Eight of ten (80 percent) patients had a significant response to RU 486 (25 percent or greater reduction in leiomyoma volume) after three months of treatment. This is comparable to a clinical response of 77 percent (25 percent or greater decrease in uterine volume) in patients treated with leuprolide (long-lasting GnRH agonist) for six months (Friedman et al., 1991). Although all but one patient displayed a decrease in leiomyoma volume, individual variations ranging from 15 percent to 90 percent in response to RU 486 were apparent (Figure B8.6

insert). Such variations in response may represent differences in vascularity, cellular composition, and biologic endowment of tumors.

With GnRH agonist treatment, 95 percent of patients in the study by Friedman et al. (1991) experienced some side effects related to the hypoestrogenism. Side effects noted with the use of RU 486 included mild, infrequent, atypical hot flashes (distinct from menopausal hot flushes) in four patients. A transient elevation in serum transaminases accompanied by joint pain was seen at the end of treatment in one patient, with rapid resolution after discontinuation of RU 486. There was no significant change in bone mineral density of the spine and hips after three months of therapy (0.8 ± 0.319 versus 0.835 ± 0.713 g/cm^2). Serum E_2 and E_1 levels remain in the early to midfollicular phase range during therapy.

A significant decrease in both leiomyoma and myometrium observed in PR staining (Figure B8.7a), with unaltered ER staining (Figure B8.7b), after prolonged exposure to RU 486 suggests that RU 486 in vivo may have a direct effect on PR number in these target tissues. The possibility that receptor occupancy by RU 486 may mask immunohistochemical localization of PR protein is unlikely but cannot be excluded (Weigel et al., 1992).

In the ovariectomized rhesus monkey model, Wolf et al. (1989) and Neulen et al. (1990) have demonstrated the ability of RU 486 to antagonize the mitogenic effects of exogenous estrogen on the endometrium. Under estradiol replacement, RU 486 treatment induced a rise in cytosolic and nuclear ER concentrations. Although the mechanisms remain unclear, these observations suggest that RU 486 may alter the functionality of the ER by acting as a noncompetitive antiestrogen (Wolf et al., 1989; Neulen et al., 1990). In our study, there was no clear effect of RU 486 on ER immunoreactivity, suggesting that if an antiestrogenic effect exists, it is likely functional in nature.

The mechanism by which continuous RU 486 acts to induce chronic anovulation is unclear. There is a transient increase in LH, A, and T levels, which may be due to the withdrawal of feedback action of progesterone on the hypothalamic-pituitary axis (see below). However, in the absence of endogenous progesterone, RU 486 may act as an agonist and thereby induce an acyclic hormonal pattern and anovulation (Dierschke et al., 1973; Liu and Yen, 1983; Gravanis et al., 1985; Collins and Hodgen, 1986; Liu et al., 1987). Alternatively, RU 486 may disrupt the hypothalamic-pituitary-ovarian axis by acting as a noncompetitive antiestrogen (Wolf et al., 1989; Neulen et al., 1990).

In summary, we have shown that an antiprogesterone that induces ovarian acyclicity also decreases leiomyoma volume. This decrease in volume is seen in the face of follicular phase levels of estrogen. Thus, an antiprogesterone may provide a novel mode of management for leiomy

omas without undesirable hypoestrogenism. This observation prompted us to conduct two subsequent studies to ascertain the minimal effective dose of RU 486.

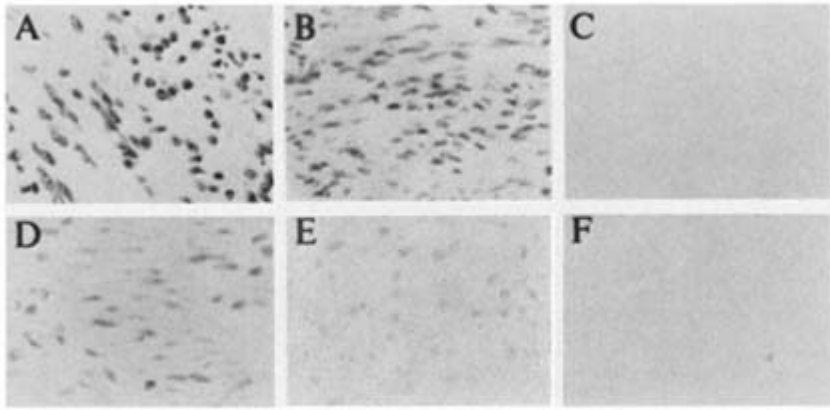

FIGURE B8.7a (A) Immunohistochemical staining of PR in leiomyomas of a control patient. (B) Immunoreactivity of PR seen in myometrium of control patient. (C) Nonimmune control of leiomyomas as in A. (D) Immunohistochemical staining of PR in leiomyomas of RU 486-treated patient. (E) Immunoreactivity of PR seen in myometrium of RU 486-treated patient. (F) Nonimmune control slide of myometrium as in D.

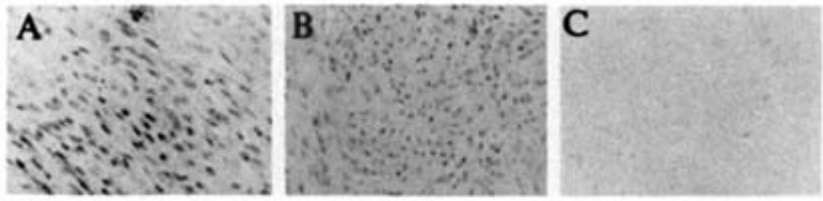

FIGURE B8.7b (A) Immunohistochemical staining of ER in leiomyomas of a control patient. (B) Immunohistochemical staining of ER in leiomyomas of RU 486-treated patient. (C) Nonimmune control slide of leiomyomas seen in A.

25- and 5-mg Doses

The effect of reduced doses of RU 486 on leiomyoma volume was evaluated in three-month treatment with a 25-mg daily dose in 17 patients, and 5 mg in 7 patients. All patients were monitored as described above, with the exception that 24-hour urinary free cortisol was used as an index of antiglucocorticoid effects instead of serum cortisol.

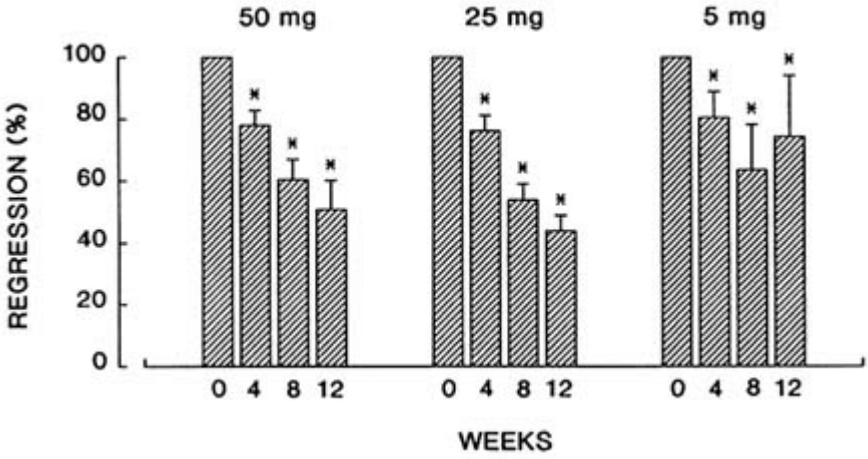

FIGURE B8.8 Comparison of percent change in uterine leiomyoma volume in response to three-month treatment of RU 486 at daily doses of 50, 25, and 5 mg.

Outcome

- *Response of Leiomyoma Volume*: The regression of the mean leiomyoma volume in patients treated with a 25-*mg daily dose* of RU 486 was 21.7 percent at one month, 56.6 percent at two months, and 68.4 percent after three months ($P < .001$). These responses were similar or greater than those observed at a 50-*mg daily dose* (Figure B8.8). At a 5-*mg daily dose,* there was a reduction of 36.5 percent after the first month, 27.2 percent after the second month, and 29.2 percent after the third month of therapy ($P < .05$) (Figure B8.8). Thus, at the lowest dose tested (5 mg), the time-dependent progressive regression of leiomyoma observed with 50-mg and 25-mg doses was no longer apparent.

  As with the 50-mg daily dose, all patients became amenorrheic during their treatment. Five patients experienced atypical hot flushes during the first month of treatment, and two patients who used the 25-mg daily dose had mild elevations of liver transaminases, which resolved within one month after they discontinued the medication.
- *Hormonal Changes*: Based on our earlier observations that RU 486 exerted multiple sites of action at the hypothalamic-pituitary-ovarian-endometrial axis (Garzo et al., 1988), we anticipated that long-term administration of RU 486 might disclose sequential antagonist and agonist effect on the reproductive axis. As with the 50-mg daily dose, levels of LH, A, and T increased during the first two weeks of treatment with the 25-mg daily dose (Figure B8.9). In contrast, a significant decline in FSH levels was evident in both 25- and 5-mg doses, which lasted for the entire course of treatment. This inhibitory effect of RU 486 on FSH

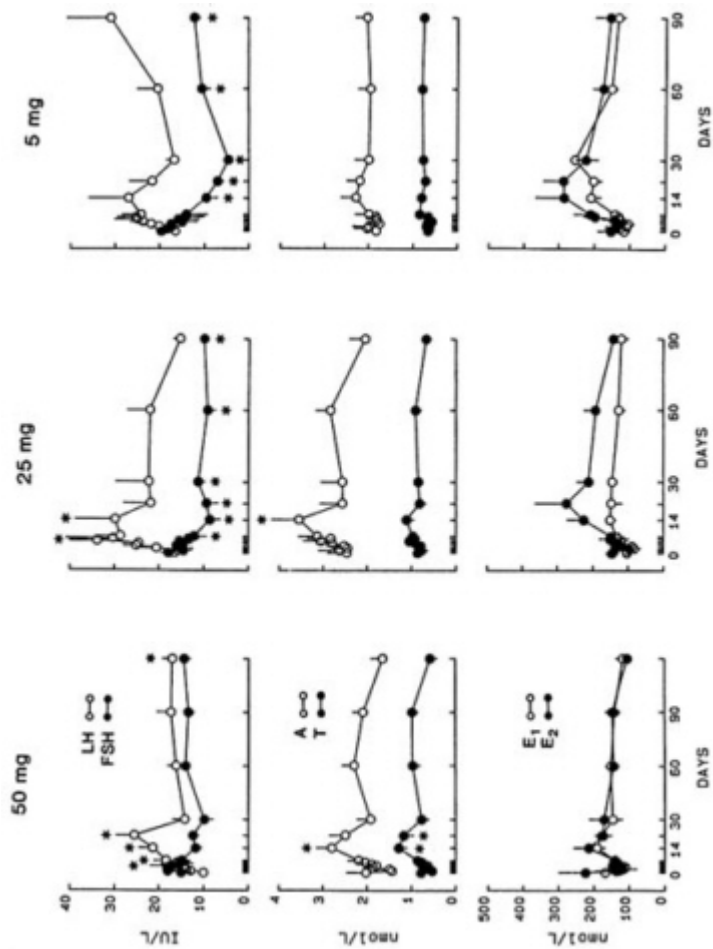

FIGURE B8.9 Gonadotropin, androgen, and estrogen levels in normally cycling patients with leiomyomas during three months of RU 486 treatment at 50-, 25-, and 5-mg daily doses [$*P < .005$ (LH), $P < .0001$ (A and T), $P < .01$ (FSH)].

levels, however, was not observed with the 50-mg dose (Figure B8.9). In the face of decreased FSH, serum E_2 and E_1 levels were maintained in the early to midfollicular phase range. These findings are entirely consistent with our previous studies, which indicated that RU 486 exerted impact at multiple sites with diverse actions (Garzo et al., 1988). Our present finding also suggests that long-term exposure to RU 486 induces a biphasic effect on different target cells in a dose-dependent manner. Further studies are essential to evaluate the specific responses of different target tissues.

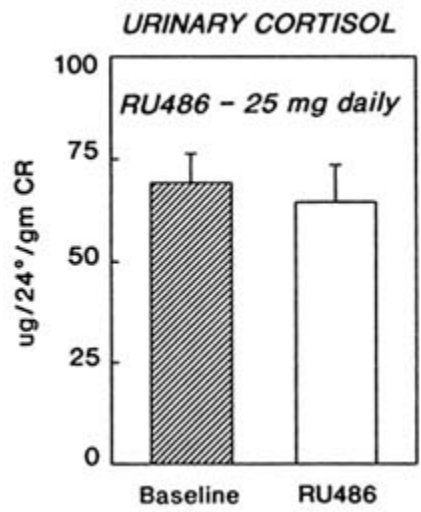

FIGURE B8.10 Twenty-four-hour urinary free cortisol levels (mean ± SE) at baseline and after three months of treatment with RU 486 at 25-mg daily dose.

At both the 25- and the 5-mg daily doses, urinary free cortisol levels were unchanged (Figure B8.10). Thus, the antiglucocorticoid action can be disassociated from the antiprogesterone action of RU 486 at these doses. A finding is critical for long-term use of RU 486 in the treatment of sex-steroid dependent tumors.

- *Uterine Blood Flow*: At the 25-mg daily dose, the change of uterine blood was evaluated. As shown in Figure B8.11, there was an increase in vascular resistance (reflecting a decrease in blood flow) recorded from the uterine arteries after three months of treatment as compared to pretreatment values recorded during the early follicular phase. This finding resembled that reported after GnRH agonist therapy for leiomyoma (Matta et al., 1988b). The mechanism(s) and significance of the increased vascular resistance in response to RU 486 are unclear at the present time.

Conclusion. RU 486 at a 25-mg dose causes a greater than 50 percent reduction, and at a 5-mg dose a small but significant decline, in myoma

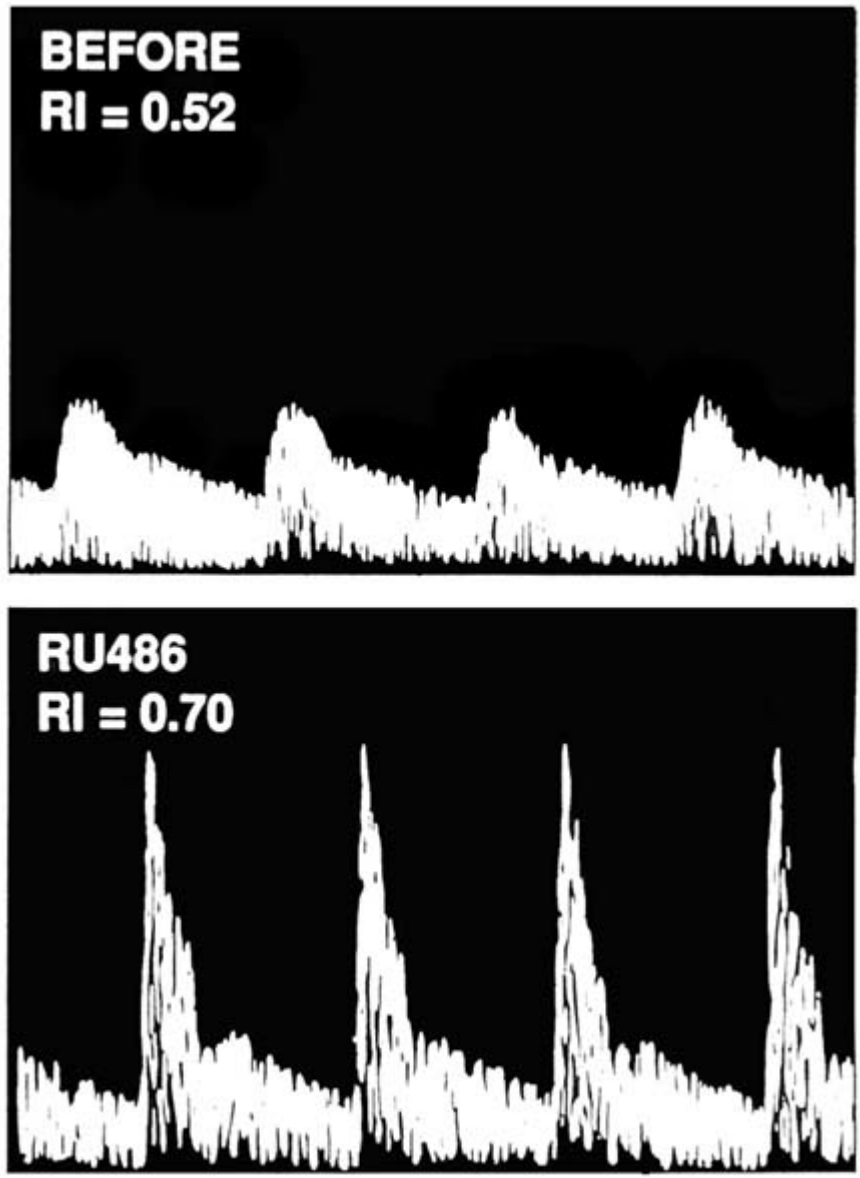

FIGURE B8.11 Doppler ultrasonography of uterine arterial flow velocity waveforms before (top) and after RU 486 treatment (bottom) in a patient with uterine leiomyoma (RI = resistance index).

volume unaccompanied by antiglucocorticoid effects. These findings provide strong support that long-term use of RU 486 may serve as an effective and safe alternative for the management of myoma and

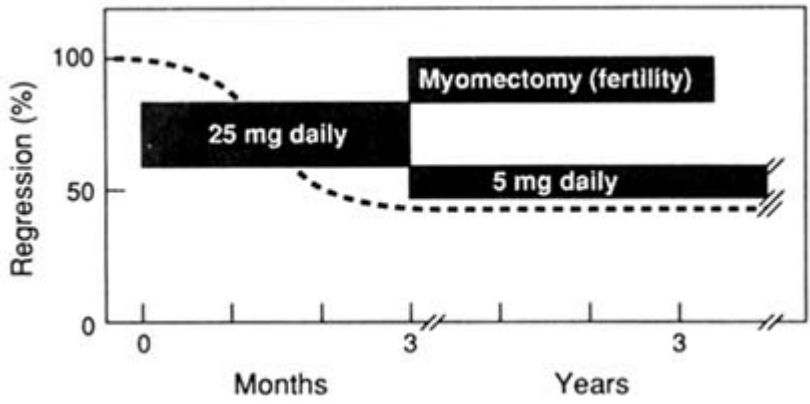

FIGURE B8.12 The therapeutic strategy of a step-down regimen for the use of antiprogestins for long-term (years) suppression in the treatment of endometriosis and leiomyoma. Myomectomy may be performed in cases of infertility associated with leiomyoma following the initial downregulation during the first three months of treatment.

endometriosis. Future experiments to test the effectiveness and safety of long-duration (years) suppression of endometriosis and myoma growth are both necessary and feasible. The therapeutic strategies for the use of antiprogestins are depicted in Figure B8.12. A step-down dose of RU 486 is proposed. At a 25-mg daily dose or lower, a 50 percent down-regulation of these lesions (and pain) is anticipated. This is followed by a reduced daily dose of 5 mg to serve in a long-term (years) maintenance of regression. If proved successful, the advantages include cost-effectiveness, circumvention of the need for a hysterectomy, and a reduction of surgical morbidity.

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B9 ANTIPROGESTINS AND THE TREATMENT OF BREAST CANCER

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INTRODUCTION

Endocrine therapy used either prophylactically or therapeutically for the treatment of locally advanced or metastatic breast cancers offers many advantages to patients whose tumors contain functional estrogen receptors (ERs) and progesterone (PR) receptors. The range of treatments defined as endocrine includes surgical ablation of endocrine glands, administration of pharmacologic doses of steroid hormones, chemical blockade of steroid hormone biosynthesis, and inhibition of endogenous steroid hormone action at the tumor with synthetic antagonists. The last of these approaches is the most widely used, making the antiestrogen tamoxifen the preferred first-line therapeutic agent for treatment of hormone-dependent metastatic breast cancer. The widespread use of tamoxifen reflects its efficacy and low toxicity, and the fact that it makes good physiological sense to block the local proliferative effects of estrogens directly at the breast. But are estrogens the only hormones with a proliferative impact on the breast and on breast cancers? This review, abstracted from one recently published (Horwitz, 1992), focuses on evidence that progesterone also has proliferative actions in the breast; on the role of synthetic progestins in breast cancer treatment; and on the preliminary data showing that progesterone antagonists may be powerful new tools for the management of metastatic breast cancer because they block the local effects of endogenous progesterone on breast cell proliferation. The reader is also referred to the excellent general review on progestin regulation of cell proliferation by Clark and Sutherland (1990).

PROGESTERONE AND THE NORMAL BREAST

Conventional wisdom holds that the mechanisms by which estradiol and progesterone regulate the proliferation and differentiation of uterine epithelial cells, apply equally to the breast. This is probably inaccurate (Anderson et al., 1987, 1989; Going et al., 1988). In the uterus, estrogens are clearly mitogenic, and the addition of progesterone to the estrogenized endometrium leads to the appearance of a secretory pattern characterized by cells engaged in protein synthesis rather than cell division (Berliner and Gerschenson, 1976). That is, in the uterus, estradiol is a proliferative hormone; progesterone is a differentiating hormone. For this reason the unopposed actions of estradiol are considered to be tumorigenic in the uterus, while the risk of endometrial hyperplasia and cancer is lowered when estrogens are combined with progestins. In fact, the combined regimen may even be protective since a decrease in endometrial cancers has been reported in women prescribed combined estrogens and progestins, compared to women receiving no treatment (Henderson et al., 1988).

However, considerable evidence has now accrued to suggest that in the epithelium of the breast, progesterone has a different influence—that, like estradiol, progesterone in the breast has a strong proliferative effect. Studies in support of this come both from experimental models and from normally cycling women. The proliferation of normal mammary epithelium in virgin mice, and the lobular-alveolar development of mammary tissues in pregnant mice, both require progesterone (Imagawa et al., 1985; Haslam, 1988). A fundamental difference in the actions of estradiol and progesterone in the breast is that the latter stimulates DNA synthesis, not only in the epithelium of the terminal bud but also in the ductal epithelium (Bresciani, 1971). The stimulating effects of progesterone on the development of mammary gland buds can be inhibited by progesterone antagonists (Michna et al., 1991).

Data from normal human mammary cells have been more difficult to obtain and are often equivocal. Compared to the increase caused by estradiol treatment (11.3-fold), progesterone treatment only marginally (2.0-fold) increases the mitotic index of normal human breast ductal epithelium maintained in intact athymic nude mice (McManus and Welsch, 1984). In fact, Mauvais-Jarvis and colleagues (Gompel et al., 1986; Mauvais-Jarvis et al., 1986) concluded, using primary cultures of epithelial cells from normal human mammary glands, that while estradiol treatment stimulates growth, progestins inhibit growth. Their data are difficult to interpret however, since the experiments using estradiol were done with cells growing in minimally supplemented medium, whereas the progestin treatment studies were done with cells in optimally supplemented medium, and any progestin growth

stimulatory effect might have been masked. In contrast to the sparse and conflicting in vitro data are studies of the mitotic rate in breast epithelial cells during the normal menstrual cycle and in women taking oral contraceptives. These data show that the highest thymidine labeling indices occur during the progestin-dominated, secretory phase of the menstrual cycle. Both the estrogen and the progestin components of oral contraceptives increase the thymidine labeling index, with progestin-only formulations exhibiting high activity (Anderson et al., 1987, 1989; Going et al., 1988). The investigators conclude that it is difficult to sustain the idea that progestins are protective in the breast (Anderson et al., 1989). It would seem that more work must be done to understand the actions of progestins in the normal breast, but that clinical decisions based on an inappropriate uterine model system are unjustified (McCarty, 1989).

PROGESTERONE AND BREAST CANCER

A discussion of the role of progestins in breast cancer must distinguish between their effects on carcinogenesis and their role in regulating proliferation of established cancers.

Progestin Agonists and Tumor Induction

Progestin agonists have been shown to be carcinogenic or to increase the incidence of spontaneous mammary tumors in dogs and mice (Frank et al., 1979; Lanari et al., 1986, 1989; Nagasawa et al., 1988; Kordon et al., 1990). In mice, results vary with the strain tested, which suggests the contribution of a genetic component; however tumorigenic effects of progestins have been observed whether or not the strain harbors the mouse mammary tumor virus (MMTV). The importance of progesterone in carcinogen-induced rat mammary cancers is documented by the early reports of Huggins et al. (Huggins and Yang, 1962; Huggins et al., 1962; Huggins, 1965), who showed that pregnancy promotes the growth of dimethylbenzanthracene (DMBA)-induced mammary tumors, and that administration of progesterone together with the carcinogen to intact rats accelerates the appearance of tumors, increases the number of tumors, and augments the growth rate of established tumors. The relationship between progestins and carcinogenesis is temporally complex. In general, progesterone administered simultaneously with or after the carcinogen enhances tumorigenesis, whereas progesterone given prior to the carcinogen inhibits tumorigenesis (Welsch, 1985). Thus, the high progesterone level associated with pregnancy can be protective if it precedes administration of the carcinogen (Russo et al., 1989). Extrapolation of these experimental models to human disease is

unclear since the only data available for the latter are epidemiologic in nature and relate hormone use, particularly oral contraceptive use, to the risk of breast cancer. The trend toward increased risk with increased duration of hormone use appears repeatedly (Meirik et al., 1986; Hulka, 1990; Schlesselman, 1990), and a possible adverse effect of progestins seems likely (Ewertz, 1988; Bergkvist et al., 1989). This is discouraging when taken together with the likelihood that in the breast, unlike the uterus, progestins enhance proliferation during the menstrual cycle.

Progestin Agonists and Growth of Established Tumors

Carcinogen-induced rat mammary tumors are a major model for in vivo studies of progestin-regulated growth (Welsch, 1985). Following ovariectomy, progesterone alone is usually unsuccessful in preventing regression of established tumors. The rapid decrease of PR levels due to estrogen withdrawal is probably a critical factor (Horwitz and McGuire, 1977). In intact animals, which more closely mimic the clinical situation, progestin agonists at moderate doses have been reported to promote tumor growth and to reverse the antitumor effects of tamoxifen (Robinson and Jordan, 1987). Thus, there exists the possibility that endogenous circulating progesterone may enhance breast cancer growth. Enigmatically, progestins at higher pharmacologic doses appear to be growth inhibitory (Danguy et al., 1980). The molecular mechanisms responsible for the opposing actions of physiologic and high-dose progestins remain unclear and are discussed later in this paper.

In vitro cell culture models designed to assess the role of progestin agonists in tumor cell proliferation have generated contradictory results. Experiments can be cited in support of any argument—that progestins stimulate (Dao et al., 1982; Hissom and Moore, 1987; Hissom et al., 1989), inhibit (Vignon et al., 1983; Chalbos and Rochefort, 1984; Horwitz and Freidenberg, 1985; Purohit et al., 1989; Poulin et al., 1990), or have no effect (Lippman et al., 1976) on growth. Explanations for the lack of a consensus are as varied as the results. Responses of cells in culture are critically dependent on the conditions in which they are grown. In a rich medium, where growth is optimized, further growth enhancement is difficult to demonstrate, while inhibitory stimuli may be exaggerated. In a deprived medium the reverse is true, although here, key co-factors may be lacking. There is no simple solution to these inherent problems. Couple this generic uncertainty with other variables including the use of different cell lines (Lippman et al., 1976; Vignon et al., 1983; Horwitz and Freidenberg, 1985), heterogeneity and genetic instability even within the same cell lines (Reddel et al., 1988; Graham et al., 1989) a burgeoning list of factors besides estradiol and progestins that directly or indirectly modulate progestin sensitivity through regulation of PR

levels (Eckert and Katzenellenbogen, 1983; Sarup et al., 1988; Katzenellenbogen, 1990; Aronica and Katzenellenbogen, 1991; Clarke et al., 1991) and the possibility that progestin-sensitive cells can generate resistant subpopulations (Graham et al., 1992) and it may be that no physiological consensus is likely to be forthcoming from using these in vitro models. These models remain invaluable, however, for the analysis of molecular mechanisms of progestin actions, as well as underscoring the complexities inherent in tumor cell biology.

Where does this leave us on the critical issue of the use of progestin agonists in breast cancer treatment? Interestingly, here there is more agreement, but the data contradict the conclusion that physiological levels of progestins are growth stimulatory. Especially at high doses, progestins appear to be antiproliferative in breast cancers. A comprehensive review of the clinical literature (Sedlacek and Horwitz, 1984; Canobbio et al., 1987; Howell et al., 1987a; Lundgren et al., 1989; Gundersen et al., 1990; Pronzato et al., 1990; Parnes et al., 1991) shows that synthetic progestins, used at pharmacologic doses for first- or second-line therapy, are as effective as tamoxifen in the treatment of advanced breast cancer. That is, in patients whose tumors are not screened for steroid receptors, approximately 30 percent have an objective, positive response. Since in addition, progestins are well tolerated and have a relatively low toxicity (Henderson et al., 1989), their use in the treatment of advanced breast cancer is experiencing a resurgence (McGuire et al., 1985, 1989). However, the mechanisms underlying the actions of intermediate and high doses of progestin agonists in breast cancer regression remain unclear when compared to their proliferative actions at physiologic doses. Although some studies suggest that PR-negative tumors respond just as well as do PR-positive tumors (implying that progesterone receptors are not involved), others suggest that methodological problems produce false PR-negative values in responders (McGuire et al., 1989) and that progesterone receptors are indeed required to obtain a response to progestins. An interesting study comparing tamoxifen therapy to therapy in which tamoxifen was alternated with medroxyprogesterone acetate (MPA) in ER-positive patients, showed a 40 percent response to tamoxifen alone versus a 62 percent response to the alternating treatment (Gundersen et al., 1990). It is postulated that when tamoxifen is cycled, its agonist properties predominate, which increases PR levels, thereby enhancing the efficacy of MPA. The same argument is made for enhancing the therapeutic efficacy of antiprogestins (see below). In general, tamoxifen inducibility of the PR is considered to be a good indicator for a positive response to hormone therapy (Howell et al., 1987a). Thus, although definitive data are still lacking, it is likely that positive responses to therapy with progestins in breast cancer are mediated by the PR in the tumors.

PROGESTERONE ANTAGONISTS AND THE TREATMENT OF BREAST CANCER

Because progestin antagonists are relatively new compounds, and because of the political controversy that surrounds them, their promise and use in the treatment of breast cancer are just beginning to be evaluated. What is the rationale for their use? What is the explanation for the paradox that both antiprogestins and high-dose progestin agonists inhibit the growth of breast cancers?

Human Breast Cancer Cell Lines

Two PR-positive human breast cancer cell lines that are phenotypically different have served as the major models for studies of growth regulation by antiprogestins. The MCF-7 cell line is classically estrogen-responsive cells; the cells are ER positive but have only low PR levels unless these are induced by estradiol (Horwitz and McGuire, 1978). The T47D cell line (Keydar et al., 1979) is more complex; it is genetically unstable (Reddel et al., 1988; Graham et al., 1989), and differs phenotypically among and within laboratories (Vignon et al., 1983; Horwitz and Freidenberg, 1985; Hissom et al., 1989), and this leads to reported differences in response to hormone treatment. One major T47D subline, clone 11, is ER positive and PR positive, and the cells respond to estradiol treatment by proliferating—a response that can be inhibited by tamoxifen (Vignon et al., 1983). Another subline, T47Dco, is estrogen resistant, and cell growth is neither accelerated by estradiol nor inhibited by tamoxifen (Horwitz et al., 1982). The response to antiprogestins is generally similar among these cell lines, but interpretation of the results differs. In general, RU 486 inhibits the growth of both T47D cells and MCF-7 cells (Bardon et al., 1985, 1987; Horwitz, 1985; Rochefort and Chalbos, 1985; Bakker et al., 1987; Gill et al., 1987; Sutherland et al., 1988). The antiproliferative effects are evident at low doses, and their magnitude correlates loosely with PR levels; T47D > estrogen-primed MCF-7 > unprimed MCF-7 (Bardon et al., 1985). That the antiproliferative effects are mediated by PR is also shown by the fact that in T47D cells, growth inhibition is confined to progestins; other steroid hormones are ineffective (Bardon et al., 1985; Sutherland et al., 1988). Moreover, the fact that RU 486 is not antiproliferative in PR-negative breast cancer cell lines also argues for a receptor-mediated mechanism of action (Bardon et al., 1985).

The antiproliferative actions of RU 486 in these models of breast cancer would support a logical treatment strategy if it were not for one disturbing fact—progestin agonists, including R 5020, also inhibit their growth. This effect of R 5020 is seen even at low doses in the T47Dco cells

(Horwitz, 1985), but requires higher doses in the clone 11 cells in which, interestingly, low doses actually protect the cells from the antiproliferative effects of RU 486 (Bardon et al., 1985). Furthermore, in the estrogen-responsive clone 11 cells, R 5020 inhibits only the estradiol-stimulated growth fraction where it is cytostatic, whereas RU 486 reduces cell numbers below the estrogen-untreated baselines, suggesting that it has a more profound cytotoxic effect (Bardon et al., 1987; Gill et al., 1987). Thus the antiproliferative mechanisms for progestin agonists such as R 5020, and antagonists such as RU 486, may be fundamentally different. R 5020 appears to have dual proliferative/ antiproliferative effects, depending on the dose that is tested, and the antiproliferative doses produce growth stasis. By contrast, RU 486 is more purely antiproliferative even at low doses, and it can produce growth regression. Indeed, ultrastructural studies show effects of RU 486 on cell and chromatin condensation and pyknosis that are consistent with the induction of cell death by apoptosis (Bardon et al., 1987). This cytotoxic effect is prevented by low doses of R 5020. To explain these results, Bardon et al. (1987) propose that there are three different mechanisms by which progestins inhibit the growth of breast tumor cells: (1) "PR-mediated cytotoxic" mechanisms that apply to antagonists such as RU 486. These are observed only in PR-positive cells by using "physiological" hormone doses, and are preventable by receptor occupancy with an agonist. This cytotoxicity is characterized by ultrastructural evidence of cell death. (2) "PR-mediated cytostatic" effects are produced by physiological doses of antagonists or agonists, and are characterized by inhibition of the growth-stimulatory actions of unrelated (nonprogestin) growth factors including estradiol. (3) "Nonspecific cytotoxic" effects are not receptor mediated and are seen with most steroid hormones at high doses. The molecular explanations that underlie these three mechanisms are unknown, but they serve as an important departure for further research. For example, the prolonged DNA occupancy time of RU 486-bound PR compared to R 5020-bound PR may account for cytotoxic versus cytostatic effects (Sheridan et al., 1988). As described above, variables like the gene or cell being tested (Tora et al., 1988), and regulation by either the A or the B receptor (Meyer et al., 1990), may dictate an agonist or antagonist response. Alternatively, it has been suggested that R 5020 is inhibitory because it is "antiestrogenic" (Vignon et al., 1983) whereas RU 486 is inhibitory through a direct antiproliferative effect involving the PR (Bardon et al., 1985). Although these explanations may begin to address the paradox that allows both R 5020 and RU 486 to be growth inhibitory in the appropriate physiological setting, it is clear that a considerable amount of research remains to be done. Preliminary analyses of cell cycle parameters show no definitive differences between agonists and antag

onists. Both appear to inhibit growth by significantly decreasing the proportion of cells in the S-phase of the cell cycle; cells accumulate in G_0/G_1 possibly due to increase in the G_1 transit time (Sutherland et al., 1988; Michna et al, 1990).

Although the majority of studies using cell culture models ascribe growth-inhibitory properties to progestins and to antiprogestins through direct effects involving PRs, contradictory results have also been reported. Given the fact that at physiological levels, progesterone is believed to be mitogenic in the normal breast (see above), it is not entirely surprising that Moore and colleagues (Hissom and Moore, 1987) consistently report proliferative effects of R 5020 at all doses in T47D cells. It is surprising, however, that RU 486 also stimulates growth in these cells (Bowden et al., 1989). The explanation for this discrepancy is unknown. However, it is now clear that T47D cells are exceptionally unstable; during prolonged time in culture, subpopulations can develop that are phenotypically different from the parental stocks, and some of these subpopulations may have responses to hormones that differ outright from the expected response. For example, some sublines or subpopulations of T47D cells respond to high doses of tamoxifen by growth stimulation (Graham et al., 1990, 1992). In the case of tamoxifen, these aberrant responses may be mediated by the presence of mutant or variant ER (Graham et al., 1990). By analogy, it is possible that the T47D cells of Moore and his colleagues have arisen from a subpopulation harboring a mutant PR. This scenario would be very interesting, but the PRs of these cells have not been analyzed in detail. Less interesting trivial explanations for discrepancies among laboratories studying growth regulation by progestins in cell culture are discussed above (see also Clark and Sutherland, 1990).

Animal Models of Mammary Cancer

The antiproliferative properties of progesterone antagonists are well documented in animal models of hormone-dependent mammary cancer. These include rats bearing DMBA-induced or nitroso-methyl urea (NMU)-induced tumors, and mice bearing the transplantable MXT tumor line. Growth of these tumors is inhibited by ovariectomy and maintained by physiological doses of estrogens (Bakker et al., 1989, 1990; Klijn et al., 1989; Michna et al., 1989a, b; Schneider et al., 1989). While treatment of rats with progestins at the time of DMBA administration accelerates tumor formation (Huggins and Yang, 1962; Huggins, 1965; Welsch, 1985), prophylactic treatment of rats with RU 486 at the time of DMBA administration delays the initial appearance of tumors from an average of 39 days to 81 days (Bakker et al., 1987). The reversal by progesterone of the inhibition of tumor induction produced by

tamoxifen can in turn be blocked by RU 486 (Robinson and Jordan, 1987). These implicate a PR-mediated mechanism. Treatment of established tumors with RU 486 for three weeks prevents their further enlargement, but tumor remission is not observed (Bakker et al., 1989). In contrast to the stasis seen with RU 486, ovariectomy leads to a decrease in tumor size (Schneider et al., 1989), which in MTX tumors is accompanied by necrosis and cytolysis of the tumor cells (Michna et al., 1989a). While ovariectomy-induced tumor regression is known to be accompanied by extensive loss of PR (Horwitz and McGuire, 1977), loss of tumor PRs was also seen with RU 486 treatment (Bakker et al., 1989). Since in the latter, the PR assay was not performed under exchange conditions or by immunologic methods, the validity of this decrease requires reexamination in light of other studies that show persistently high levels of PR in RU 486-treated human breast cancer cells (Sheridan et al., 1988). Since adrenal weights are unchanged by RU 486, participation of the antiglucocorticoid effects in the antitumor activity is considered to be unlikely (Schneider et al., 1989). This is supported by studies in human breast cancer cell lines, where the inhibitory effects of RU 486 cannot be rescued by dexamethasone (Bardon et al., 1985). Antigonadotropic effects have also been excluded as a mechanism (Schneider et al., 1989).

In established DMBA tumors, inhibition of growth by tamoxifen resembles the inhibition seen with RU 486. The two hormones have equal growth-inhibitory effects when each is used alone (Bakker et al., 1989). When the two drugs are combined, however, the inhibitory effects are additive, and tumor remission similar to that induced by ovariectomy is observed (Bakker et al., 1990). This effect of combined treatment with an antiprogestin and an antiestrogen is extremely exciting and has considerable therapeutic promise. The mechanisms underlying these effects remain unclear, but several proposals have surfaced. First, tamoxifen can have agonist actions, among which is the induction of PR (Horwitz and McGuire, 1978). A tumor with increased, or restored, PR may have greater or more sustained sensitivity to RU 486. This hypothesis could be tested by the use of an antiestrogen having no agonist activity. Second, among the physiological effects seen in RU 486-treated intact female rats are increased plasma levels of luteinizing hormone (LH), prolactin, estradiol, and progesterone, as well as the persistence of numerous and actively secretory corpora lutea associated with hypertrophic pituitaries (Bakker et al., 1989, 1990; Michna et al., 1989a; Schneider et al., 1989). It has therefore been proposed that the efficacy of simultaneous tamoxifen results from its ability to counteract the proliferative effects of the high estrogen levels.

Several newer antiprogestins, ORG 31710 and ORG 31806 (Bakker et al., 1990), and ZK 98299 and ZK 112993, have equal or greater antipro

liferative actions than RU 486 (Michna et al., 1989a; Bakker et al., 1990). In the hormone-dependent MXT-transplantable tumor model, treatment with ZK 98299 or RU 486 starting one day after transplantation led to an almost complete inhibition of tumor growth. Their effect on established tumors was equivalent to that of ovariectomy (Michna et al., 1989a; Schneider et al., 1989). In this model, the potent antiproliferative actions of the antiprogestins completely counteracted the growth-stimulatory actions of estradiol or of approximately equimolar doses of MPA, but at higher MPA doses the agonist actions of the progestin prevailed (Michna et al., 1989b). It appears that antiprogestins inhibit growth by direct antagonism of progesterone action at the tumor, probably mediated by PR. This conclusion is bolstered by the fact that the hormone-independent MXT tumor is antiprogestin resistant (Michna et al., 1989a).

In DMBA-induced tumors, ZK 98299 was more potent than an equal concentration of RU 486 (Michna et al., 1989a). It produced tumor regression analogous to that of ovariectomy, rather than the tumor stasis observed with RU 486. A similar trend was observed with NMU-induced rat mammary tumors (Michna et al., 1989a, b; Schneider et al., 1989). However, lack of comparative metabolic and pharmacokinetic data on the two antiprogestins in rats and mice makes these quantitative differences uninterpretable at present. Also of interest is the finding that strong antitumor activity was noted at 20 percent of the doses needed to obtain abortifacient actions in these rodent systems. This is important because by the use of lower doses of antiprogestins, their antiglucocorticoid effects may be mitigated. After treatment with the antiprogestins, the morphology of the hormone-dependent MXT and DMBA tumors showed signs of differentiation of the mitotically active polygonal epithelial tumor cells toward the nonproliferating glandular secretory pattern, with the formation of acini and evidence of secretory activity (Michna et al., 1989a). Based on this, it is suggested that the antiproliferative efficacy of the antiprogestins is related to their ability to induce terminal differentiation. Recall that they block tumor cells in G_0/G_1 (Sutherland et al., 1988; Michna et al., 1990). Note that an antiproliferative mechanism based on induction of terminal differentiation is fundamentally different from a mechanism involving tumor cell death. Tumor cell degradation and cytolysis were features of ovariectomy-induced regression (Michna et al., 1989a). The mechanisms underlying the antitumor effects of antiprogestational agents require further study, especially in human tissues and cells.

Human Clinical Trials

The enormous promise of progestin antagonists in treating breast cancer remains largely unexplored in clinical practice. Only two small

clinical trials using RU 486 have been reported, both from European laboratories. The first involved a series from France (Maudelonde et al., 1987) of 22 oophorectomized or postmenopausal patients in whom chemotherapy, radiotherapy, or tamoxifen and other hormonal therapy had already been used. RU 486 at 200 mg/day led to partial regression or stabilization of lesions in 12 of 22 (53 percent) women following four to six weeks of treatment. The response rate at three months had dropped to 18 percent. It is important to note that for ethical reasons, this untried therapy was used only in patients with advanced breast cancers in whom other treatment modalities had already failed. PR levels were not measured in all patients, but of the responders, 4/4 were PR positive, whereas of the nonresponders 0/4 were PR positive. In general, RU 486 was well tolerated in long-term treatment with few symptoms of adrenal dysfunction, but plasma cortisol levels were elevated. Of interest was the fact that a strong analgesic effect was observed in most of the patients with bone metastases.

The second trial, from the Netherlands (Michna et al., 1989b; Bakker et al., 1990), involved 11 postmenopausal patients with metastatic breast cancer who were treated with 200 to 400 mg of RU 486 for 3 to 34 weeks as second-line therapy after first-line treatment with tamoxifen, irrespective of the response to tamoxifen. Six of eleven patients had a short-term (three to eight months) stabilization of disease, and one had an objective response lasting five months after RU 486 treatment. Again, response was associated with the presence of PR in the tumors. In this study, which involved prolonged use of RU 486, two patients had undesirable side effects associated with the antiglucocorticoid actions of the drug. Three days of treatment with dexamethasone reversed these symptoms after RU 486 was stopped. As in the animal studies, plasma estradiol levels increased despite the fact that these women were postmenopausal, and it is suggested that the simultaneous administration of tamoxifen might be beneficial because of its ability to blockade tumor ER. Alternatively, symptoms of adrenal hypersecretion and elevated plasma estradiol levels might be reduced with concurrent aminoglute-thimide or aromatase inhibitors. By modification of the dose and time of RU 486 administration, its antiglucocorticoid effects might be further minimized (Gaillard et al., 1984), although maintenance of high sustained blood levels of the drug is likely to be important.

PROGESTIN RESISTANCE

The emergence of hormone-resistant cells eventually reduces the effectiveness of all therapies in advanced breast cancer, and progestin agonists or antagonists are unlikely to be exceptions. Until recently, this has been an unexplored field. Murphy et al. (1991) generated a subline

of T47D cells that are resistant to the growth-inhibitory effects of progestins. This was done by sequential selection in medium containing 1 μM MPA. The cells remained PR positive, but receptor levels were halved. Transforming growth factor (TGF) and epidermal growth factor (EGF) receptor mRNA levels were both increased. The investigators suggest that increased growth factor expression and action, and decreased PR levels, may be involved in the development of progestin resistance.

It is also likely that extensive heterogeneity in PR content exists within cell subpopulations of tumors that are PR positive based on analyses of solid tumors (Howell et al., 1987b) and of human breast cancer cell lines (Graham et al., 1992). Factors or treatments that lead to the selection and expansion of PR-poor or PR-negative populations would in the long run produce progestin resistance.

Recent molecular analyses of human progesterone receptors (hPRs) are beginning to address mechanisms of resistance to progesterone antagonists. These studies suggest that the term "resistance" may be inappropriate. "Resistance" implies that the tumor stops responding to the drug, and ignores it instead. This may be an oversimplification, since under appropriate conditions, progesterone antagonists can behave like agonists. Rather than ignoring the drug the cell alters its transcriptional response to the drug. How is that possible? One explanation focuses on a mutant PR. Unlike the case for other members of the steroid-receptor family, no examples of natural PR mutants have yet been reported. The explanation for this may be, that unlike mutations in androgen receptors, systemic mutations in PRs are incompatible with life. However, theoretically, acquired mutations could develop in tumors as one mechanism for the development of resistance, and a systematic search might demonstrate them. In view of this, Vegeto et al. (1992) recently showed that a synthetic hPR mutant with a 42-amino acid truncation at the C-terminus of the 933-amino acid hPR B-receptors, loses its progesterone-binding ability but retains RU 486-binding ability. This receptor mutant, when occupied by RU 486, has agonist transcriptional activity.

Additional models of resistance associated with functional reversion have emerged from our studies of progesterone antagonists as transcriptional inhibitors. These studies provide two scenarios in which antagonists can have inappropriate agonist-like effects on normal PR. We believe that the mechanisms underlying these functional switches may be analogous to mechanisms by which tumor cells become hormone resistant. The first case involves studies with the human breast cancer cell line T47D, which expresses high natural levels of PR and is stably transfected with the progestin-responsive MMTV promoter linked to the CAT reporter. In this model, PR-antagonist complexes are transcription

ally silent, and the antagonists inhibit the effects of agonists. However, if cyclic adenosine 3',5'-monophosphate (cAMP) levels are elevated, the antagonists become strong transcriptional stimulators—they behave like agonists. This functional reversal occurs only if the receptors are bound to DNA, and it does not involve hPR phosphorylation by pathways dependent on cAMP. The model we propose involves transcriptional synergism, in which a promoter that is independently regulated by cAMP-responsive factors, and by hPR, is selected for positive or negative transcription, through cooperative interactions between the two, DNA-bound factors (Sartorius et al., 1993).

The second case involves the functional difference between progesterone A- and B-receptors (Meyer et al., 1990). A-receptors occupied by progesterone antagonists are transcriptionally silent on a progesterone response element (PRE) thymidine kinase promoter-CAT reporter. By contrast, in the same cells and with the same promoter-reporter, antagonist-occupied B-receptors strongly stimulate transcription. Interestingly, this unusual property of B-receptors does not require the presence of the PRE (K.B.H., unpublished). Our working model is that transcription by antagonist-occupied B-receptors proceeds through a mechanism in which the receptors are tethered to a DNA-bound protein partner at the promoter, without being bound to DNA themselves. We have ruled out the possibility that the unknown protein is AP-1.

In sum, each of these recent experimental models suggests that "resistance" can be a condition in which tumors respond inappropriately to hormone antagonists. These studies may also explain why, in some normal target cells, antagonists have tissue-specific, agonist-like activity.

SUMMARY AND FUTURE PROSPECTS

The foregoing suggests that progesterone antagonists could have an important place in the routine management of hormone-dependent breast cancers. Our knowledge of the actions of these compounds is rudimentary, however. The following points provide an outline for future directions:

1. If endogenous progesterone has the mitogenic actions in normal breast epithelia that the current data would indicate, then it is likely that physiologic progesterone is also a mitogen in breast cancers. Blockade of endogenous progesterone with antiprogestins, especially in premenopausal women, would seem to be an important therapeutic goal. However, nothing is known about the pattern of mitosis in breast cancer cells during the normal menstrual cycle. Obtaining such data is important, and it should be possible to analyze the mitotic patterns of tumors taken from cycling patients. The problem, of course, is the difference in

mitotic indices among tumors. Ideally, each tumor should serve as its own control, with multiple samples analyzed for proliferative activity at different times of the cycle. This approach is fraught with ethical problems. However, it might be possible to obtain a fine-needle aspirate of a tumor for initial mitotic analysis, then to acutely treat the patient with RU 486 before the tumor is removed 24 hours later for reanalysis. Additionally, it is important to know whether RU 486 is cytostatic or cytolytic in human breast cancers. Electron microscopy of tumors taken from patients entered into trials may provide answers to this. However, while it is always preferable to ask biological questions using clinical tissues, much of the work on the mechanisms of the mitogenic actions of progestins and progestin antagonists will require well-controlled studies using organ-cultured human breast tumors, human breast cancer cell lines, and human tumors implanted into nude mice.

2. There are sufficient theoretical and preclinical data to justify large-scale clinical trials. Trials must include accurate measurements of ER and PR in tumors, and analysis of pre- and posttreatment levels of endogenous hormones to monitor the status of the pituitary-adrenal-ovarian axis. Progestin antagonists may be useful both for adjuvant endocrine therapy, when used either alone or in combination with tamoxifen, and for therapy of locally advanced or metastatic cancers, again when used either alone or in combination with tamoxifen. The usual issues of drug doses, metabolism, and other pharmacokinetic parameters must be addressed. Schedules in which the antiprogestins and antiestrogens are combined or alternated must be tested. Is an antiestrogen with agonist properties preferable because it induces PR, or is a pure antiestrogen preferable because it blocks the actions of elevated circulating estrogens? Is the answer different in premenopausal versus postmenopausal women?
3. The antiglucocorticoid side effects of RU 486 remain a major impediment to its long-term use. However, as the newer Schering and Organon antiprogestins show, it appears to be possible to design molecules with maximal antiprogestin activity and minimal antiglucocorticoid activity. Further, even RU 486 may be used for long-term treatment if it is combined with drugs that block adrenal steroidogenesis or prevent peripheral aromatization of adrenal steroids to estrogens. Additionally, since the antiglucocorticoid effects of RU 486 are apparently tolerable in the short term (i.e., two to three months), perhaps it is reasonable to ask if alternating RU 486 with tamoxifen maximizes its antiprogestin effects while minimizing its antiglucocorticoid ones.
4. Basic tumor biological and molecular research must continue in order for us to understand the precise molecular targets and mechanisms of antagonist action. If different antagonists target different molecular sites, which antagonist would be best for clinical use? What

are the genes in breast cells that are regulated by progestins, whose inhibition is associated with lowered cell proliferation? What is the underlying cause of the tissue-specific differences in progestin action at the breast and uterus? What is the molecular explanation for the dual agonist/antagonist effects seen on only some promoters, with only one or the other PR isoform, and in only some cells when using RU 486? Can pure progesterone antagonists devoid of antiglucocorticoid activity be synthesized? Are there mutant progesterone receptors in breast cancers?

Where do we begin? Ensuring that scientists and clinicians have access to antiprogestins, unencumbered by the Byzantine bureaucratic obstacles and the "antagonistic" political climate currently encountered in the United States, is a good place to start.

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B10 USES OF RU 486 AS AN ANTIGLUCOCORTICOID

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Physiologic exposure to glucocorticoid hormones is required for life: both deficiency and excess are lethal. It is surprising, given the importance of these steroids, that much about their action is unclear. The availability of RU 486 (mifepristone), the first potent glucocorticoid antagonist in vivo, was hailed as a potential breakthrough both to expand knowledge about glucocorticoid action and to treat glucocorticoid-dependent disease states. This paper first reviews current concepts of the physiology of glucocorticoids and conundrums arising from this knowledge; second, it summarizes studies using RU 486; and third, it suggests promising areas of research.

HOW DO GLUCOCORTICOIDS ACT?

Glucocorticoids are steroidal hormones produced and secreted into the bloodstream by the adrenal glands in response to stimulation by the pituitary hormone adrenocorticotropin (ACTH). The synthesis and secretion of ACTH are stimulated, in turn, by corticotropin-releasing hormone (CRH), which is synthesized by the hypothalamus and secreted into portal vessels so as to reach the corticotrope cells of the pituitary gland. CRH secretion is increased by a variety of stimuli, many of which can be considered biologic stressors: states of chronic and acute psychological or physical stress are presumed to increase CRH secretion (Chrousos and Gold, 1992). The system is returned to balance by the negative influence, or feedback, of high levels of glucocorticoids on both CRH and ACTH secretion. Thus, low levels of cortisol, the major glucocorticoid in people, provoke an increase in ACTH that is sustained

until cortisol levels increase; high levels of cortisol inhibit ACTH secretion.

One feature of this hypothalamic-pituitary-adrenal (HPA) axis is the presence of a diurnal rhythm, so that the ACTH nadir occurs in the early morning hours, followed by increasing levels during the night that stimulate cortisol to peak levels in the morning (around 8:00 a.m.). Both ACTH and cortisol values decline to very low levels in the evening between 10:00 p.m. and 2:00 a.m.

Glucocorticoids, like other steroid hormones, are thought to exert their biologic effects by binding to an intracellular receptor specific to each class of steroid. The steroid-receptor complex can bind specifically to sites on DNA called response elements and thereby alter rates of transcription of genes. This type of genomic response involves subsequent protein synthesis and is usually detected within hours of exposure to steroids. Importantly, the outcome may be to either increase or decrease an end point. Full expression of the expected effect is deemed a full "agonist" effect. A reduction in the observed effect after the addition of another steroid can be considered "antagonism." When given alone, the second steroid may have a suboptimal agonist effect (partial agonist) or have only antagonist effects.

A number of factors influence glucocorticoid effects on a given tissue. These include the type and amount of the glucocorticoid available and the number of intracellular glucocorticoid receptors. About 95 percent of the circulating cortisol in man is bound to corticosteroid-binding protein (CBG). Since only the free, or unbound, cortisol is available for entry into the cells, conditions that alter CBG levels may alter transiently the availability of glucocorticoids to the cells, until a new steady state is achieved by alteration in ACTH, and hence cortisol production and the free cortisol fraction. Studies in man involving agents that compete with cortisol for CBG sites, or agents that do not bind to CBG, and studies in rats, which have no corticosteroid-binding protein, must be interpreted with this in mind.

The diurnal rhythm of circulating cortisol already mentioned illustrates the concept that the plasma glucocorticoid concentration may not reflect accurately the physiologic status: circulating levels vary up to 30-fold over a day in normal individuals. For this reason, daily urine free cortisol excretion is a better index of the integrated exposure of the body to cortisol, because it derives from unbound plasma cortisol. Finally, the number of glucocorticoid receptors in a given cell population may be regulated, usually inversely, by glucocorticoid exposure, so that exposure to glucocorticoids may induce a decrease of up to 50 percent in receptor number (Hoeck et al., 1989). Also, the number of glucocorticoid receptors may vary in relation to the cell cycle in in vitro systems. Whether these observations hold in vivo is not known. For these

reasons, knowledge of the circulating level of glucocorticoids within the physiologic range does not predict well the biologic response. However, measurement of the production of cortisol, either indirectly by using urine free cortisol excretion or more directly by using radioactive or deuterium-labeled cortisol, is useful clinically to distinguish normal from excessive exposure to cortisol and to predict the presence of the signs of hypercortisolism.

The study of glucocorticoids is complicated vastly by the differences in the response of various tissues to any given concentration of steroid. In other words, a concentration or dose of steroid that elicits a maximal response in one tissue may elicit only a very small response in another tissue. Additionally, as reviewed below, although a number of measures are known to be glucocorticoid sensitive or dependent, few end points of steroid action are easy to measure in the intact animal or person, especially acutely. One exception to this is the change in plasma ACTH levels. This end point responds rapidly to changes in cortisol and is relatively easy to obtain. For these reasons ACTH is often used to assess glucocorticoid action.

The classical model of steroid hormone action described above fits many of the known effects of glucocorticoids. Other observations do not conform to this model, however. Some of these have stimulated research that has resulted in important new physiologic insight and modification of the model; other observations remain unexplained. Within the last few years the intracellular receptors for the major classes of steroid hormones have been found to be structurally related, with a high degree of homology. The site conferring specificity is the site that binds to the hormone itself (Evans, 1988). Application of techniques of molecular biology led to the discovery of two receptors that bind with high affinity to glucocorticoids, dubbed the type I and II receptors. Interestingly, the type I receptor also binds aldosterone, the principal circulating mineralocorticoid in man (Funder et al., 1988). This led to the conundrum of how aldosterone can function as the major mineralocorticoid in the presence of much higher circulating levels of cortisol. It appears that the kidney is protected from the mineralocorticoid activity of cortisol by rapid metabolism of cortisol to its 11-keto analogue, cortisone, by the enzyme 11β-hydroxysteroid dehydrogenase. Since cortisone does not bind to the type I glucocorticoid receptor, this mechanism leaves aldosterone as the specific agonist of the type I receptor in the kidney. This observation elicits questions about the regulation of this enzyme in the kidney and at other sites of glucocorticoid action. There is some indication that the enzyme activity is increased by exposure to glucocorticoids, perhaps even within the physiologic range, but dose-response relationships have not been studied extensively (Hammami and Siiteri, 1991). Similarly, the intracellular

metabolism of cortisol to other compounds with glucocorticoid activity has not been studied extensively and may contribute to glucocorticoid activity. One such possibility is the conversion of cortisol to 6β-OH-cortisol in the liver and kidney. Although at normal circulating concentrations, only a small portion of cortisol is metabolized in this way, in states of glucocorticoid excess, both urine and plasma levels of 6β-OH-cortisol increase (Clore, 1992).

One controversial area of glucocorticoid physiology is the possibility that glucocorticoids act through nonclassical receptor mechanisms, either through intracellular receptors that are not type I or II, or through binding to membrane "receptors." The evidence for this activity derives from three sources: First, in certain animal models, glucocorticoids and progestins induce changes within minutes (Hua and Chen, 1989; Orchinik et al., 1991). The time course of response, and the inability to block the response with agents that inhibit protein synthesis, suggest strongly that this is a nongenomic response (i.e., not mediated through interaction with DNA). Such plasma membrane-mediated activity has been observed with thyroid hormone (Lawrence et al., 1989) but is not considered a classic effector mechanism of steroid hormones. Second, binding studies of labeled steroids with subcellular fractions have shown specific high-affinity binding to mitochondrial and synaptosomal (cell membrane) fractions (with K_d in the nanomolar range for corticosterone). The signature of binding to a variety of steroids, the pattern of the binding curves, and the ability to isolate membranes and not cytosol, suggest that these are not type I or type II receptors. Third, some effects of glucocorticoids, such as restoration of adrenal phenylethanolamine N-methyltransferase activity after hypophysectomy (Margolis et al., 1966), require glucocorticoid doses that are far in excess of those needed to occupy all classical receptors, thus suggesting that the response is not mediated via this mechanism. Research into these currently heretical areas of steroid action promises to increase our understanding of steroid physiology.

Finally, the concept of homeostasis should be considered. When intact, laboratory animals and people tend to maintain themselves in a normal physiologic state, with outcome parameters that fluctuate within a defined "normal" range. This concept is implicit in the consideration of health and disease, since disease is often defined as the extent of variation from the physiologic norm. However, when exploring mechanisms of action or when focusing on a specific end point, experiments often disrupt physiologic mechanisms designed to maintain homeostasis. Thus, pieces of the organism, such as cells or parts of cells, are isolated, or experiments are conducted over very short periods of time so that the compensatory mechanisms are not yet operative. It is especially important to remember the principle of homeostasis when

predicting a potential clinical use of a compound based on data from in vitro or subhuman models.

Thus, in evaluating glucocorticoid action, the following principles should be kept in mind:

1. Binding of a hormone to a receptor does not predict whether the biologic response will be agonist, antagonist, or mixed. These outcomes must be determined experimentally and may vary between tissues and species.
2. Likewise, dose-response relationships do not generalize from one tissue, outcome measure, or species, to another.
3. The bioavailability, pharmacokinetics, and metabolism of the agent must be determined for each species and physiologic condition (such as gender).
4. The entire animal/person must be studied. Other known actions and unexpected effects should be sought. Initial short-term studies must be extended to evaluate chronic effects that are not initially apparent.

PHYSIOLOGIC ROLE OF GLUCOCORTICOIDS

What are the physiologic roles of glucocorticoids in people and how could RU 486 be used as an antiglucocorticoid? The importance of glucocorticoids has been inferred largely from states of deficiency and excess. Adrenal insufficiency and Cushing's syndrome represent experiments of nature that illustrate the effects of too little and too much glucocorticoid. Although concomitant mineralocorticoid excess and deficiency may cloud consideration of pure glucocorticoid effects, the leitmotif of glucocorticoid action derived from observation of these diseases reveals the involvement of nearly all tissues and many physiologic processes. Important among these are effects on fuel disposition and economy, structural catabolism (especially of bone, collagen, and muscle), and the immune system and inflammation. Effects on fuel metabolism seen in sub- and supraphysiologic exposure to glucocorticoids range from hypo- to hyperglycemia (loss of cortisol counter-regulation to insulin resistance), and lipolysis to lipogenesis with muscle catabolism. Excessive glucocorticoids reduce inflammation at the expense of increased susceptibility to infection. Structural integrity of bone, muscle, and skin is reduced by glucocorticoid excess, but does not appear to be increased in adrenal insufficiency.

Activation of the hypothalamic-pituitary axis includes increased levels of CRH and pro-opiomelanocorticotropin (POMC) products as well as glucocorticoids and other adrenal products. CRH probably has important actions on the sympathetic nervous system and may participate in apparent "glucocorticoid" modulation of reproductive, immu

nologic, and metabolic function (Chrousos and Gold, 1992). The HPA axis may be activated by stressful conditions with specific behavioral phenotypes (anxiety, major affective disorders); conversely, there is evidence that administration of components of the axis, such as CRH, glucocorticoids, and POMC products, modulates behavior.

RU 486 AS AN ANTIGLUCOCORTICOID

RU 486 represents the first clinically active glucocorticoid antagonist, and has been used to probe glucocorticoid action and to treat conditions known or suspected to be glucocorticoid sensitive or dependent. The remainder of this paper reviews these studies, and concludes with speculations about other potential uses and further studies to be performed.

The antiglucocorticoid activity of RU 486 was first demonstrated in women undergoing induced abortion (Herrmann et al., 1982) and in normal men in acute dose-response studies using ACTH/lipotropin (LPH) and cortisol levels as end points (Bertagna et al., 1984; Gaillard et al., 1984). The rationale of this experimental design is that interruption of glucocorticoid negative feedback should increase CRH, ACTH, and eventually cortisol secretion to overcome RU 486 inhibition. A consistent dose-response relationship emerged: RU 486, at daily doses of 3–6 mg/kg given for one to four days, caused a dose-dependent increase in pituitary or cortisol end points. The effect at 3 mg/kg was transient and was not observed at lower doses. Interestingly, regardless of the time of administration of RU 486 (morning versus evening), the hormonal effect was observed only in morning values, so that the diurnal rhythm was maintained and amplified. The ability of dexamethasone, 1 mg at midnight, to suppress morning cortisol values was completely antagonized by RU 486, 6 mg/kg. Although the apparent "resistance" of the HPA axis to RU 486 effects during the evening hours (8:00 p.m.–2:00 a.m.) remains unexplained, the persistence of a demonstrable effect 24 hours after morning administration of RU 486 may be explained in part by its long plasma half-life of around 20 hours.

Patients with Cushing's syndrome, studied in a similar way by using ACTH and cortisol as response measures, had different responses depending on the etiology of Cushing's syndrome. Patients with Cushing's disease, in whom an ACTH-producing pituitary tumor retains many of the normal physiologic regulatory mechanisms, responded to RU 486 with increased cortisol levels. By contrast, patients with other causes of Cushing's syndrome (in whom hypercortisolism presumably suppressed activity of normal ACTH-producing pituitary corticotropes) showed no response to RU 486 (Bertagna et al., 1986).

In principle, administration of a glucocorticoid antagonist would

represent an ideal treatment of Cushing's syndrome. The short-term studies discussed above demonstrated that the compensatory homeostatic mechanisms of ACTH-producing tumors would probably preclude effective therapy, because the tumor would likely continue to increase ACTH production. In patients with long-standing hypercortisolism not caused by Cushing's disease, however, RU 486-induced antagonism of peripheral glucocorticoid effects presumably would not be overcome. This concept was tested in a few patients with ectopic ACTH secretion and proved correct. At daily doses of 5–22 mg/kg, RU 486 reversed psychosis, hypokalemia, hypertension, weight gain, inhibition of luteinizing hormone, follicle-stimulating hormone, testosterone, thyroid-binding globulin (TBG), corticosteroid-binding protein (CBG), and T4, and restored euglycemia. In most patients there was little effect on cortisol or ACTH levels, although cortisol, but not ACTH levels, fell in an occasional patient, suggesting a potential effect on steroidogenesis (Nieman et al., 1985; Chrousos et al., 1989). The compound worked best in patients with sustained, invariant hypercortisolism. We have given RU 486 to seven such patients for six weeks to a year, with excellent results, either as preoperative preparation or while seeking to localize and remove an ACTH-secreting tumor. Three patients had no adverse side effects. Although no hepatic, hematologic, renal, or dermatologic toxicity was observed, three patients had nausea, one with prostration reminiscent of adrenal insufficiency. Two of three men developed gynecomastia (presumably because of antiandrogenic properties), and one had an unmasking of autoimmune thyroid disease (not uncommonly seen after successful reversal of Cushing's syndrome). RU 486 was discontinued in one patient with variable cortisol levels, for whom it was not possible to find an optimal daily dose. The agent was discontinued within three weeks, and dexamethasone was instituted, in three others in whom hypotension and clinical deterioration suggested either sepsis or RU 486-induced adrenal insufficiency. Of these, one developed *Pneumocystis carinii* pneumonia. Thus, while RU 486 can be an effective agent for the treatment of hypercortisolism, it is difficult to monitor therapy and adjust dose because of the temporal pattern of response, and the risk of adrenal crisis alternating with undertreatment is great in patients with variable hormonogenesis.

RU 486 has potential activity as a tumoricidal agent in tumors with glucocorticoid or progesterone receptors, and has been tested in breast cancer, leiomyomas (reviewed elsewhere in this report) and meningiomas. The effects of RU 486, 200 mg/day, given for up to a year, have been reported in 38 patients with meningioma (Lamberts et al., 1991; 1992; Grunberg, 1993). Regression of tumor size was documented by objective measures in about 30 percent of patients; in the remainder, responses were evenly divided between tumor stabilization and growth.

Pain decreased and subjective well-being improved in a majority. Signs of adrenal insufficiency developed within three weeks of initiation of therapy in about half of the patients in one study, prompting concomitant prednisone therapy (Lamberts et al., 1992). Activation of the adrenal axis was apparent, with increased urine cortisol values, and gradual increase in adrenal androgens, leading to follicular phase estrogen values, presumably via peripheral conversion. Although all women were amenorrheic during the study, one developed bleeding shortly after discontinuation of RU 486 and had endometrial hyperplasia. In the other study, 11 of 14 patients complained of mild to moderate fatigue; 5 had hot flushes (including 3 of 6 men); 3 of 6 men developed gynecomastia; 2 of 6 postmenopausal women had transient alopecia; and both premenopausal women were amenorrheic. Shortcomings of these studies include the failure to characterize tumor receptors and inclusion of a clinically diverse patient population, so that outcome could not be clearly correlated with clinical features. However, the observation that tumor size remained stable or decreased in two-thirds of individuals with previous progression indicates that RU 486 may be a promising treatment of their disease.

RU 486 has also been tested in a rat model of fibrosarcoma, where it retarded tumor growth even in intact, nonadrenalectomized animals (Laue et al., 1988b), and in human glioma cells in vitro, where it inhibited dexamethasone-induced stimulation of growth (Langeveld et al., 1992). Notably, the glucocorticoid effects correlated with the level of expression of the glucocorticoid receptor, suggesting that gliomas with high expression of glucocorticoid receptors might respond to RU 486 treatment in vivo. This concept has not been tested, however.

Other potential clinical uses for RU 486 have been suggested by disease states or by use of the agent as a probe of glucocorticoid function. Glucocorticoid-induced animal or tissue culture models of hypertension, wound healing, cataracts, inflammation, and arthritis have suggested a potential role for RU 486 in these states. Whether these results will pertain in people is largely unexplored. Apart from local/topical administration, systemic administration is likely to result in a compensatory increase in cortisol that may blunt the desired effect of RU 486.

Dexamethasone induces hypertension in rats that is effectively prevented or reduced by the addition of RU 486 but not mineralocorticoid antagonists (Kalimi, 1989). Hypertension in Cushing's syndrome can be reversed by RU 486 treatment also (Nieman et al., 1985). Taken together, these observations suggest that glucocorticoid hypertension is mediated through the type II glucocorticoid receptor. It is possible, however, that in states of marked cortisol excess, the ability of 11β-hydroxysteroid dehydrogenase to metabolize cortisol is decreased, so that some cortisol

accesses the type I receptor. A conflicting observation was made by Watlington and colleagues, who showed that cortisol-induced sodium retention and hypertension could not be reversed by RU 486 (type II receptor antagonist) or the potent type I receptor antagonists spironolactone or 9α-fluorohydrocortisone (Clore et al., 1992). This group has proposed that other intracellular metabolites of cortisol, such as 6β-cortisol, acting through another receptor, may have sodium-retaining and hypertensive effects. This concept was derived from studies using A6 cells derived from kidney of *Xenopus laevis*. 6β-OH-Corticosterone, the major metabolite of corticosterone in these cells, binds to a third type of receptor (dubbed the type IV receptor) with half-maximal occupancy at an extracellular corticosterone concentration one order of magnitude higher than the physiologic level (3 × 10-7 versus 10-8 M). Occupancy of type IV receptors is postulated to be important in the presence of high circulating glucocorticoid levels or if activity of the 6β-OH enzyme is altered. Although data regarding the presence of a type IV receptor in man are not available, other evidence for this hypothesis is the increased urinary 6β-OH-cortisol excretion in some hypertensive patients, including those with Cushing's syndrome, toxemia of pregnancy, and hypothyroidism. The concept of local renal alteration in cortisol metabolism as a cause of some forms of hypertension is an intriguing hypothesis deserving further investigation. The activity of renal 6β-hydroxylation appears to be increased by glucocorticoids, but its regulation is not well understood. In particular, the effect of RU 486 on the fractional excretion of 6β-OH-cortisol is not known.

Acute injection of RU 486 in rats increases oxygen consumption and brown adipose tissue activity, effects mediated by sympathetic activation and increased CRH levels. In lean animals, RU 486 inhibited weight gain without altering food intake (Hardwick et al., 1989). A similar preliminary study evaluating fuel metabolism after acute administration of RU 486 to men showed no effect on metabolic rate, however (Garrel, 1993), and chronic administration of RU 486, 200 mg/day, to patients with meningioma, did not alter weight (Grunberg et al., 1993). Thus, any potential lipolytic or anorexic effect of RU 486 in people remains to be elucidated.

The effects of RU 486 on inflammation and the immune system have been studied largely in animal or in vitro models involving dexamethasone-induced alterations (Emilie et al., 1984; Laue et al., 1988a; Van Voorhis et al., 1989). In these systems, RU 486 antagonizes dexamethasone effects. It is important, however, to study the effects of RU 486 in individuals not receiving exogenous glucocorticoids. One such study showed no effect of chronic (14-day) administration of RU 486, 10 mg/kg per day, on the total and differential white cell counts, or of T, B, and NK cell phenotypes in normal men. The cytotoxic and proliferative

responses of lymphocytes, the erythrocyte sedimentation rate, C reactive protein concentration and quantitative immunoglobulin G levels were unaltered (Laue et al., 1990). A similar lack of effect on blood leukocyte counts was shown in another acute study (RU 486, 400 mg; Bertagna et al., 1988). It is likely that the compensatory increase in cortisol after RU 486 administration at these doses may cancel out or mask any effects of RU 486 in an intact person. Bimodal effects on inflammation have been noted in some rat models, in which an inflammation-promoting action was abolished at higher doses. This might be explained by activation of the HPA axis. These findings suggest that studies directed to elucidating RU 486 effects on the immune system should include doses of the agent that do not increase ACTH and cortisol levels. It may be of interest also to examine effects on the immune system that might be discerned in the evening after a morning dose of RU 486, while bearing in mind the finding that cortisol levels increase after RU 486 only at certain times of day. This feature may allow enhancement of the immune system at times when cortisol levels remain "normal." Such a possibility deserves examination.

Topical glucocorticoid eyedrops cause increased intraocular pressure in the majority of patients treated for open-angle glaucoma. In the rabbit model, topical administration of RU 486 reduced intraocular pressure (Phillips et al., 1989). These observations may provide a foundation for similar work in humans, if a satisfactory vehicle can be found for the non-water-soluble RU 486. The concept is especially appealing because topical administration may limit systemic effects.

Although RU 486 may be a good probe of the physiology of the affective disorders, few data are available in this regard. The agent has been given to patients with major depression and produced mild activation of the HPA axis reminiscent of the effects seen in normal individuals (Kling et al., 1989; Krishnan et al., 1992).

Few adverse effects have been noted in studies of RU 486 using single doses or doses of up to 10 mg/kg daily for as long as seven days. RU 486, at daily doses of 200 mg, given for more than seven days has been associated with fatigue, anorexia, and nausea, in decreasing frequency. It is important to note, however, that these effects have not been uniformly reported. Although they are consistent with relative adrenal insufficiency and improve with the administration of dexamethasone or other glucocorticoids, it is not clear that they represent adrenal insufficiency. Here, too, the paucity of glucocorticoid-sensitive end points makes discrimination of this diagnosis difficult. Additionally, there are insufficient data to evaluate these events in terms of RU 486 levels, other medications, or other predisposing factors. RU 486 induced a maculopapular erythematous cutaneous eruption in 8 of 11 normal men receiving the agent at a dose of 10 mg/kg for 9 to 14 days and in 5 of 28

patients receiving treatment for meningioma, 200 mg/day (Laue et al., 1990; Grunberg et al., 1993). The cause of this spontaneously resolving rash is unknown. At higher doses, up to 22 mg/kg per day given for months to patients with Cushing's syndrome, no exanthema was seen. Nausea was common in these patients. Four of eleven patients begun on RU 486 had a deterioration in clinical condition that prompted discontinuation of the agent. Again, the diagnosis of adrenal crisis was difficult to ascertain in this setting. The antiandrogenic properties of RU 486 probably underlie reports of gynecomastia and impotence in men receiving chronic therapy with RU 486: gynecomastia occurred in two of three men with Cushing's syndrome and in four of nine men with meningioma, whereas impotence was reported at a slightly lower rate (one of three and three of nine men respectively, in the two studies).

RESEARCH DIRECTIONS FOR ANTIGLUCOCORTICOIDS

The "state of the science" regarding RU 486 invokes many questions germane to glucocorticoid action, and provokes others relating to its potential therapeutic efficacy, as follows:

1. RU 486 may be useful in better understanding renal physiology and in the treatment of some forms of hypertension. Questions in this area follow. What is the role of renal 6β-hydroxylation in the development of hypertension and the maintenance of electrolyte balance? Does RU 486 undergo 6β-hydroxylation in the kidney? Does RU 486 alter 11β-hydroxysteroid dehydrogenase activity? Can RU 486 affect absolute or diurnal rhythm in blood pressure in normal individuals or in patients with essential hypertension? What is the role of 6β-OH-cortisol in hypertensive states? Although RU 486 did not reverse hypertension caused by exogenous cortisol, could it possibly modulate 6β-hydroxylation in a subset of patients with increased activity?
2. Can RU 486 alter glucocorticoid-receptor number? (For example, if up-regulation occurs, could tumors be sensitized to glucocorticoids by previous RU 486 administration?)
3. Can RU 486 modulate immune function or inflammation when given systemically to people? Specific indicators of immune function need to be developed that can be examined over a broad dose range, including doses below the threshold of activation of the HPA axis. The responses to a variety of schedules of administration, including alternate day, once weekly, and night versus morning, should be examined for time-of-day and time-course characteristics.
4. What about local forms of administration—topical or intracavitary—for eye disease, wound healing, keloid reduction, joint abnormalities?

5. How do high-dose (i.e., supraphysiologic) steroids work to reduce edema/inflammation? If this is not mediated through classical receptors, maybe RU 486 would not induce the same effect, and RU 486 could be given with high-dose glucocorticoids to prevent iatrogenic Cushing's syndrome while allowing the desired effect.
6. What are the characteristics of the nonclassical steroid binding sites, membrane, mitochondrial, and cytoplasmic? What is their physiologic significance? Does RU 486 interact with these sites and if not, can it be used in conjunction with steroids to reduce biologic glucocorticoid mediated by classical mechanisms?
7. What about the use of RU 486 as an antiandrogen—especially topically—in the prevention or treatment of acne, for example, that would exploit both antiglucocorticoid and antiandrogen properties? What about its use to prevent balding?
8. Would RU 486 be an effective treatment of human glioma tumors in vivo?
9. Can better glucocorticoid-sensitive end points be developed so as to more efficiently and specifically evaluate the effects of both glucocorticoid agonists and antagonists?
10. RU 486 deserves further study as a modulator of fuel economy. The well-known importance of cortisol to lipid metabolism and as a counterregulatory hormone for glucose homeostasis suggests that investigation of dose-response and regimen relationships with RU 486 may be fruitful.

In conclusion, RU 486 is a promising compound deserving of further investigation as a physiologic probe and treatment for glucocorticoid-dependent states.

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B11 PRIMATE MODELS FOR THE STUDY OF ANTIPROGESTINS IN REPRODUCTIVE MEDICINE

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PART I: ACTIVITY EXPRESSIONS OF ANTIPROGESTINS

Progesterone Antagonists

The new class of hormonal compounds called antiprogestins are so named because, whether they are steroids or nonsteroids, they bind to the progesterone receptor, thereby competitively inhibiting the binding of progesterone itself (Figure B11.1). In turn, such progesterone antagonists deny the metabolic actions of progesterone within tissues having progesterone receptors. In the virtual absence of progesterone or its potent analogues, the antiprogestins themselves are very weak agonists,

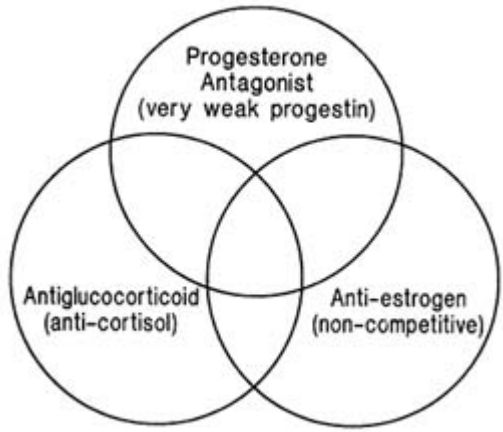

FIGURE B11.1 Competitive displacement of progesterone due to higher affinity of the antagonist and its long metabolic half-life in circulation.

expressing progestin activity (Figure B11.2), albeit less than 1/1,000 that of *levo*-norgestrel, a synthetic progestin (Gravanis et al., 1985; Koering et al., 1986; Wolf et al., 1989a) (Figure B11.3).

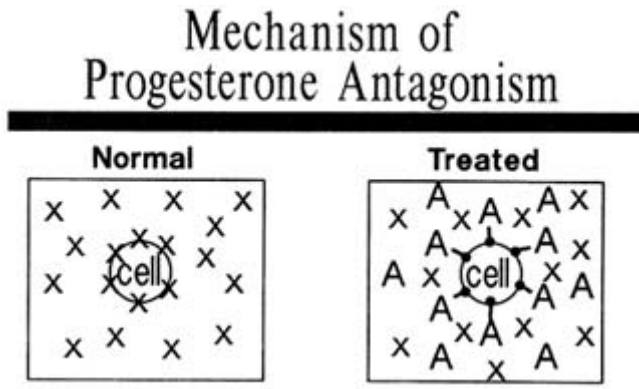

FIGURE B11.2 Multiple actions of antiprogestins.

Antiglucocorticoid Activity

In addition, certain antiprogestins are known to exert antiglucocorticoid activity upon the adrenocorticotropic hormone (ACTH)/adrenal cortex axis, therein elevating ACTH and cortisol secretion. The degree of this effect is both dose and compound specific, as well as being more pronounced during nighttime hours, when there are inherent diurnal rises in circulating ACTH and cortisol (Healy et al., 1983; Kettel et al., 1991; Chwalisz et al., 1992).

Noncompetitive Antiestrogenic (Antiproliferative) Activity

In the context of estrogen-induced mitogenesis, as it occurs in proliferative endometrium, antiprogestins can also be antiestrogens (Figure B11.4). However, since such antiproliferative actions do not arise through competitive binding of antiprogestins to the estrogen receptor (apparently postreceptor binding mechanisms intervene), the inhibition of tissue growth is called a noncompetitive antiestrogenic activity. This is very different from that achieved by tamoxifen or clomiphene (van Uem et al., 1989; Wolf et al., 1989b; Chwalisz et al., 1991). Moreover, this antiproliferative action of antiprogestins is not a manifestation of a progestin-like agonist activity. As revealed by the concentration of estrogen receptors in endometrial tissue, antiprogestins elevate the estrogen-receptor concentration by about sixfold (Neulen et al., 1990), yet paradoxically mitogenesis due to estrogen-induced growth is strik

ingly curtailed (Figure B11.5). In contrast, progestins are known to inhibit endometrial proliferation in association with a marked suppression of estrogen-receptor levels.

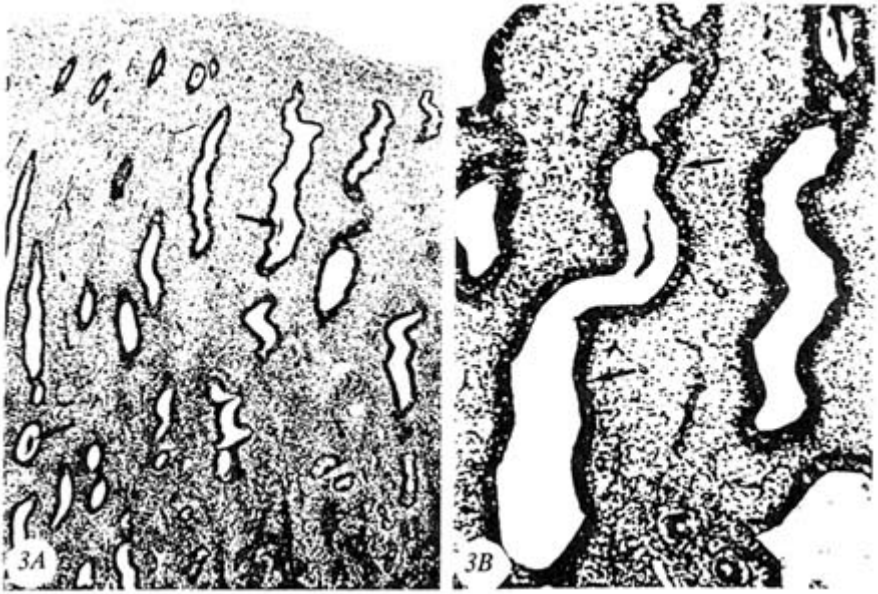

FIGURE B11.3 (A) Section of endometrium and myometrium (M) from an E_2treated castrate monkey that was given mifepristone for three days. Most of the glands have an irregular-shaped wall and a wider lumen. Secretory product (s) is present in some glands (arrows) (magnification, × 45). (B) Greater magnification of the same endometrium. The glands are dilated, and epithelium has mainly subnuclear vacuoles (arrows). Areas between the stromal elements are filled with collagen (magnification, × 130).

Sources of Antiprogestins

It is important to appreciate the limited research and clinical experience to date using antiprogestins. For example, although more than 400 chemical structures of antiprogestins have been devised, and many patented as unique chemical entities, the biological data base from which the above remarks derive largely reflects research on only two antiprogestins. Of the almost 1000 scientific manuscripts and abstracts worldwide, perhaps as much as two-thirds of these reports are on the Roussel-Uclaf (Paris) compound mifepristone (RU 486), with the bulk of the remainder from studies of the Shering AG (Berlin) compound onapristone (ZK 299).

There are substantial additional data on other antiprogestins. Both Organon AKZO (Oss, Netherlands) and Research Triangle Institute

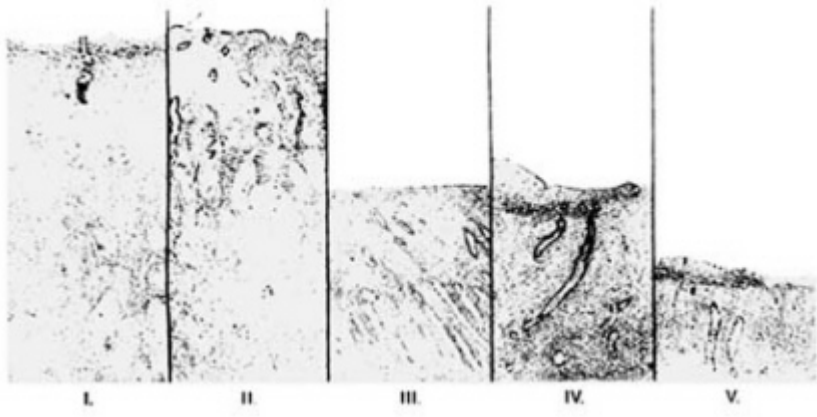

FIGURE B11.4 Sections of the endometrium resting on its myometrium from the five groups (magnification, × 40). After E_2 treatment, the endometrium is thick, the stroma dense, and the glands tubular (panel I). With progesterone stimulation, glands became tortuous and the stroma edematous (panel II). Association of P with mifepristone resulted in a midproliferative endometrium (panel III), whereas mifepristone induced a dosedependent inhibition of glandular development and endometrium growth (panels IV and V).

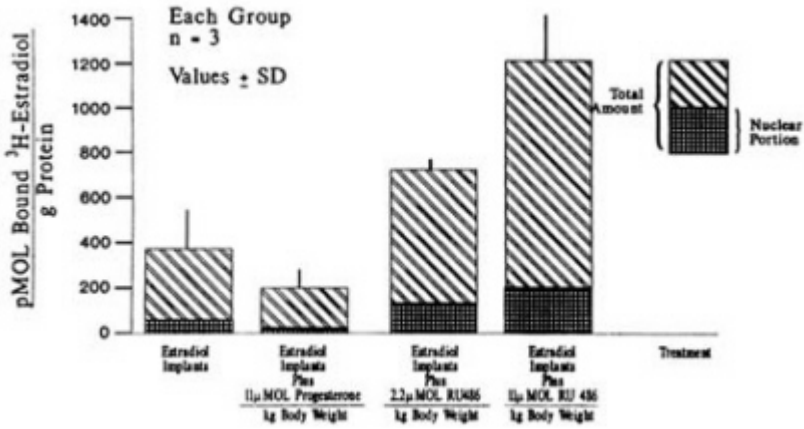

FIGURE B11.5 Endometrial estradiol-receptor concentrations measured after different treatment regimens (estradiol implants versus estradiol implants plus 11 μM progesterone, or estradiol implants plus 2.2 μM or 11 μM mifepristone).

(North Carolina) have made genuine contributions to antiprogestin research and reported preliminarily on their findings (Hodgen et al., 1993). However, a larger body of more recent data on additional antiprogestins is cloaked by proprietary interests. Hopefully, these

findings will be reported publicly soon. A Chinese version of mifepristone is also being produced for abortifacient use in that country.

PART II: NONCOMPETITIVE ANTIESTROGENIC ACTIVITY OF PROGESTERONE ANTAGONISTS

That some progesterone antagonists express other biological activities, besides being antiprogestins, was revealed by the antiglucocorticoid functions of mifepristone (Healy et al., 1983, 1985). More recently, we reported that mifepristone has a noncompetitive antiestrogenic activity that blocks estrogen-induced endometrial proliferation in primates (van Uem et al., 1989). This action of the antiprogestin was later found to be dose dependent in the presence of physiologic estradiol (Wolf et al., 1989b). Paradoxically, we found that mifepristone elevates the concentration of estrogen receptors in monkey endometrium, yet the mitogenic (proliferative) impact of estrogen on endometrial growth was negated (Neulen et al., 1990).

These observations are consistent with other monkey and human data that may substantiate this antiproliferative activity of mifepristone on primate endometrium (Kettel et al., 1991; Murphy et al., 1991; Batista et al., 1992). Apparently, other progesterone antagonists may also possess this property. For example, Chwalisz and coworkers recently reported that onapristone curtails endometrial growth (Chwalisz et al., 1991). Based on this report, we wonder how general this activity may be among a wider spectrum of antiprogestin compounds.

Below, some basic biological studies are summarized that suggest potential therapeutic uses of the antiproliferative activity of antiprogestins on uterine tissues.

Initial Evidence of Noncompetitive Antiestrogenic Activity of Mifepristone

In previous studies, mifepristone administration arrested spontaneous folliculogenesis (van Uem et al., 1989). To investigate the central versus peripheral effects of mifepristone on the ovarian/menstrual cycle, including endometrial proliferation, mifepristone was administered daily [10 mg/kg per day, intramuscular (IM)] from menstrual cycle day 3 or 7 to day 25 in six normal adult cynomolgus monkeys receiving human menopausal gonadotropin (hMG) treatment [37.5 IU (international units) per day] from days 3 to 8. Mifepristone administration with hMG/human chorionic gonadotropin (hCG) therapy did not inhibit ovarian response, as evidenced by steroidogenesis and ovulation. Nine of 23 oocytes retrieved by lavage or follicular aspiration at laparotomy after ovulation induction were morphologically classified as mature

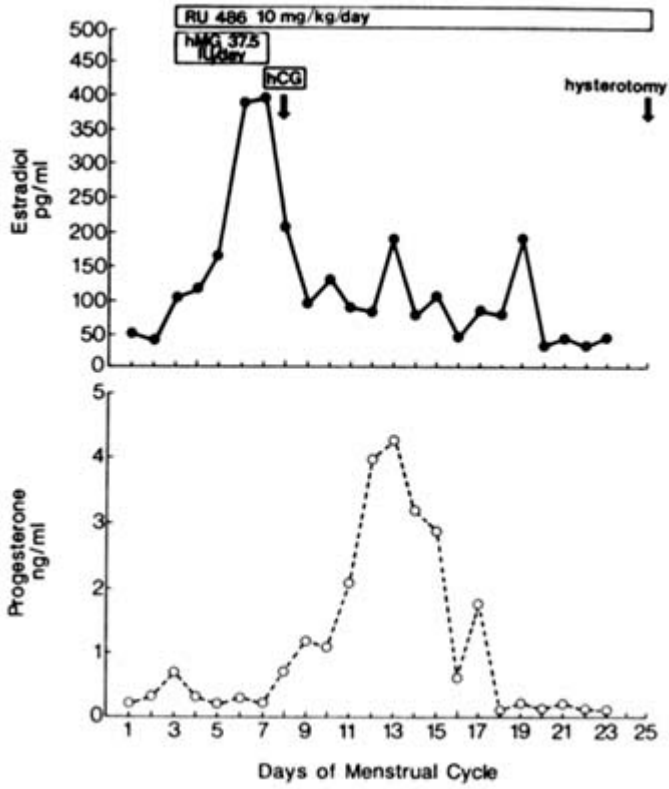

FIGURE B11.6 Representative ovarian response during the coadministration of mifepristone and hMG/human chorionic gonadotropin in a monkey. Similar hormonal patterns for estradiol and progesterone in serum were observed in all other five mifepristone-treated monkeys, as well as in both control females.

preovulatory status. Whereas endometrial biopsies performed on cycle day 25 in control monkeys revealed an in-phase mature secretory endometrium, histologic sections from mifepristone plus hMG/hCG-treated females uniformly demonstrated atrophic to weakly proliferative endometrium on cycle day 25. This was despite serum estradiol levels of > 300 pg/ml during hMG/hCG treatment (Figure B11.6). Three months after the initial 25-day study, endometrial biopsies revealed persistent atrophic endometrium even though repeated ovulation induction with hMG/hCG therapy resulted in elevated serum estrogen concentrations. The findings were observed whether mifepristone treatment began on cycle day 3 or 7. The intermenstrual interval was significantly lengthened by mifepristone treatments (28.5 ± 2.0 days for controls versus

131.3 ± 11.5 days for mifepristone treatment; $P < .01$).

In summary, mifepristone consistently blocked ovulation unless hMG/hCG was provided. It elicited a persistent retardation of early proliferative endometrium when administered daily beginning in early or midfollicular phase. The normal mitogenic effects of elevated ovarian estrogen secretion on endometrial tissue were quelled, which uniformly resulted in amenorrhea (Figure B11.3). The long-lasting action of mifepristone in these studies—causing ovulation inhibition and atrophic endometrium—may be due to the depot effect of IM injection. In addition, mifepristone did not prevent ovarian steroidogenesis, ovulation, or oocyte maturation when an ovulation induction regimen of hMG/hCG was given. These findings show that mifepristone alone prevents ovulation by diminishing pituitary gonadotropin secretion, rather than by direct effects on ovarian folliculogenesis. It induces amenorrhea by inhibiting estrogen-induced endometrial proliferation.

Dose-Dependent Blockade of the Proliferative Action of Estradiol Endometrium by Mifepristone

The noncompetitive antiestrogenic effects of mifepristone were examined using estradiol (E_2)-treated ovariectomized monkeys given mifepristone, progesterone (P), or both. The E_2-induced luteinizing hormone surge of control animals was abrogated by P, mifepristone, or both. Secretory transformation by P was inhibited by mifepristone coadministration. Mifepristone alone at a dose of 1 mg/kg induced endometrial secretory transformation, but higher doses (5 mg/kg) inhibited proliferation and secretory activity (Table B11.1). Thus, in the presence of P, mifepristone is antagonistic, but in the absence of P, it exhibits endometrial progestational effects at low doses and an antiproliferative (antiestrogenic) effect at higher doses (Figure B11.4). These data are encouraging and suggest that mifepristone should continue to be evaluated as a potential contraceptive agent acting at the pituitary or endometrial level or both.

Mifepristone-Induced Elevations of Estrogen Receptor in Primate Endometrium

We have conducted a study to investigate the effect of the antiprogestin mifepristone on estradiol-receptor concentrations in the endometrium of monkeys given physiologic estrogen replacement therapy. Estradiol-17β (E_2) silastic implants were inserted infrascapularly into 12 long-term ovariectomized cynomolgus monkeys (*Macaca fascicularis*), resulting in an average peripheral serum level of approximately 100

TABLE B11.1 Effect of RU 486 and Progesterone on Ovariectomized Monkeys

Treatment	Thickness (mm)	Gland Morphology	Epithelium	Mitotic Activity	Secretion	Stroma	Histological Phase
Control	1.5 ± .5	Tubular	Pseudo St	+++	0	Compact	Late proliferative
Progesterone (1 mg/kg/day)	1.8 ± .1	Tortuous	Mono St	+	++	Oedema	Early secretory
RU 486 (5 mg/kg/day)	0.8 ± .1	Tubular	Pseudo St	0	0	Compact	Early proliferative
RU 486-P_4 (5 mg/kg/day)	1.0 ± .1	Tubular	Pseudo St	+	0	Dense	Mid-proliferative
RU 486 (1 mg/kg/day)	1.4 ± .1	Tubular	Mono St	+	+	Dense	Interval edometrium

NOTE: St = stratification.

pg/ml E_2. On day 6 of E_2 treatment, four treatment groups were initiated:

Group I: E_2 implants only;

Group II: E_2 implants plus 11 µmol progesterone/kg body weight in sesame oil, IM, on days 6, 7, and 8;

Group III: E_2 implants plus 2.2 µmol mifepristone/kg in sesame oil, IM, on days 6, 7, and 8;

Group IV: E_2 implants plus 11 µmol mifepristone/kg, IM, on days 6, 7, and 8.

On treatment day 9, endometrial biopsies were removed by hysterotomies. Cytosolic and nuclear E_2-receptor contents of tissues were estimated by the charcoal method (Figure B11.5). In group I, the tissue contained 376 ± 123 pmol bound $[^3H]E_2$ per gram of protein; the nuclear portion of binding was about 16 percent. In group II, the tissue contained 216 ± 64 pmol bound $[^3H]E_2$ per gram of protein; the nuclear binding portion was only 8 percent. In group III, tissue contained 654 ± 47 pmol bound $[^3H]E_2$ per gram of protein; the nuclear binding portion was about 22 percent. In group IV, the tissue contained 1198 ± 172 pmol bound $[^3H]E_2$ per gram of protein; the nuclear binding portion was about 17 percent. Scatchard plot analysis indicated that the $K_{d\ app}$ of the estrogen receptor (1.04×10^{-9} M) was not altered by mifepristone. This study demonstrates that after physiologic E_2-replacement, antiprogestin treatment will cause E_2 receptor concentrations to rise dramatically; this effect was dose dependent.

Whether this noncompetitive antiestrogenic (antiproliferative) property of certain antiprogestins extends to breast cancers that are estrogen or estrogen-progestin dependent is not known. In addition, the effects of mifepristone, onapristone, and other progesterone antagonists on estrogen-dependent physiological functions, such as bone density and lipid-related cardiovascular health, remain to be evaluated, especially in the context of long-term regimens.

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B12 ANTIPROGESTOGENS: PERSPECTIVES FROM A GLOBAL RESEARCH PROGRAM

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Special Programme of Research, Development and Research Training in Human Reproduction World Health Organization, Geneva

ABSTRACT

Because of their unique ability to block the action of progesterone at the cellular level through binding to the progesterone receptor, antiprogestogens are proving to be one of the most significant developments in endocrinology in recent years. In this paper we assess the current status—and make some suggestions on future directions—in the area of antiprogestogen research from the perspective of an international, public sector research program that supports basic, animal, and clinical studies with these compounds in some 16 countries worldwide. Five aspects in particular are reviewed, namely: the nature and number of currently available antiprogestational compounds, the (in) adequacy of the animal models used, the mechanisms of action, the possible clinical uses, and the prospects of antiprogestogens. The focus of our discussion is on the potential applications in fertility regulation since this is the area in which most of the research to date has been conducted. The results of this research leave little doubt that it is no longer a matter of "the antiprogesterones are coming" but that the "antiprogestogens have come to stay." The time has come for scientists, policymakers, and the general public to acknowledge this fact.

INTRODUCTION

Studies by, among others, Fraenkel (1903), Loeb (1907), and Bouin and Ancel (1909a,b) at the beginning of this century strongly suggested that the corpus luteum was an endocrine organ and intimately involved

in the establishment and maintenance of pregnancy. Convincing proof of this concept was furnished in 1929 when Corner and Allen demonstrated that injections of corpus luteum extracts into rabbits, ovariectomized shortly after mating, resulted in the development of a progestational condition similar to that observed in early pregnancy. Attempts to isolate the active principle from these extracts were successful five years later, in 1934, when four laboratories announced independently the isolation of the pure corpus luteum hormone, progesterone (Allen and Wintersteiner, 1934; Butenandt and Westphal, 1934; Hartmann and Wettstein, 1934; Slotta et al., 1934).

Like other steroid hormones, progesterone exerts its effects through interaction with intracellular receptors that, after binding the hormone, undergo a "transformation" or "activation" process. Although the molecular details of this transformation have not been established unequivocally, the process apparently imparts to the transformed receptors the ability to interact with nuclear components with high affinity. Modification of the expression of the hormonally regulated genes is thought to be the result of this interaction between activated receptors and specific nuclear acceptor sites (for review see, for example, Savouret et al., 1990).

Progesterone receptors are found primarily, albeit not exclusively, in the organs of the female reproductive tract and in the pituitary and hypothalamus, in keeping with the hormone's central role in female reproductive physiology. Other normal tissues in which receptors have been demonstrated include, inter alia, the cerebral cortex, thymus, and muscle cells of uterine arteries (Savouret et al., 1990), and more recently, endothelial cells of decidual blood vessels (Wang et al., 1992). Progesterone receptors have also been detected in some benign (e.g., meningioma) and malignant tumors (e.g., breast, kidney, prostate, and endometrial cancer), which is why progesterone antagonists have been proposed for the treatment of some of these conditions.

Recognition of the indispensability of progesterone for pregnancy establishment and maintenance in most, if not all, mammalian species including the human, led to the search for compounds with antiprogestational activity (i.e., substances that would prevent the biological effects of progesterone on its target organs). Given the multifaceted role of progesterone in the reproductive process, such antiprogestational compounds could be expected to have a number of potential applications in human fertility regulation, including termination of pregnancy, induction of missed menses, post-coital contraception, and possibly also as once-a-month drugs and ovulation inhibitors.

The Special Programme of Research, Development and Research Training in Human Reproduction (the Special Programme) was established in 1972 by the World Health Organization to coordinate, promote,

conduct, and evaluate international research in human reproduction. Recognizing the potential of safe and effective antiprogestational drugs in fertility regulation, the Special Programme started to provide support to this area of research very soon after its inception and has continued to do so in a growing manner to the present day. Work supported in the 1970s included some of the studies by Baulieu and his colleagues on the cyclical changes of estrogen and progesterone receptors in the human endometrium (Bayard et al., 1978) and the demonstration that the C-11 position of progesterone was critical in the hormone's interaction with its receptor (Holmes et al., 1981). As described below, most of the antiprogestogens reproted to date have a bulky substituent in this C-11 position of the steroid molecule.

Research on antiprogestogens, in particular mifepristone, was started by the Special Programme in 1983, shortly after results from the first clinical trial with the compound had been published (Herrmann et al., 1982). The outcome of the Special Programme's first study—a dose-finding study in which mifepristone was given alone for termination of early pregnancy (Kovacs et al., 1984)—was somewhat unexpected. The compound's efficacy in inducing complete abortion was lower than anticipated, a finding later confirmed by numerous other investigators. Subsequent work, supported by the Special Programme, led to the discovery that mifepristone increases the sensitivity of the uterus to prostaglandins and that sequential treatment with the antiprogestogen and a low dose of a prostaglandin analogue such as sulprostone could terminate early pregnancy in about 95 percent of women (Bygdeman and Swahn, 1985). Since then, the research of the Special Programme has been focused on examining various combination regimens of mifepristone and different prostaglandin analogues, and on determining the lowest effective doses. Concomitantly, studies have been initiated on other possible uses of mifepristone in fertility regulation, including ripening of the cervix, induction of missed menses, prevention of implantation, and post-coital contraception. In addition, support is being given to research aimed at finding new, and possibly more potent and "pure," antiprogestogens.

The purpose of this paper is to assess the current status—and make some suggestions on future directions—in the area of antiprogestogen research from the perspective of an international, public sector research program that supports such work in about 16 countries worldwide (Figure B12.1), which together conducted a total of 32 research projects during 1992 involving mifepristone and other antiprogestogens (Figure B12.2). Five aspects in particular are reviewed, namely: the nature and number of currently available compounds, the (in)adequacy of animal models used, the mechanisms of action, the possible clinical uses, and the prospects of antiprogestogens. Because of the mandate of the Special

FIGURE B12.1 Countries in which nonclinical (lighter stippling) and clinical research on antiprogestogens is being supported by the Special Programme of Research, Development and Research Training in Human Reproduction at the World Health Organization.

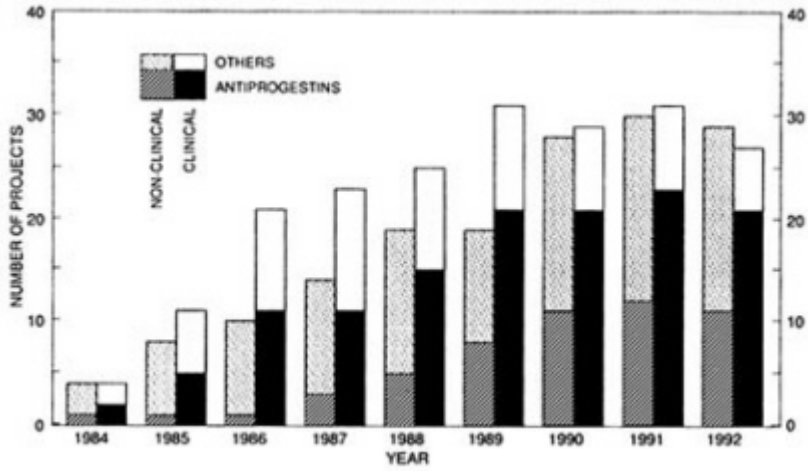

FIGURE B12.2 Number of ongoing, nonclinical, and clinical research projects on antiprogestogens and other postovulatory methods of fertility regulation supported during the period 1984–1992 by the Special Programme of Research, Development and Research Training in Human Reproduction at the World Health Organization.

Programme, which is focused on human reproduction research, and also because most of the antiprogestogen research conducted to date has been in this area, our comments relate predominantly to the possible applications of these compounds in fertility regulation. Potential uses outside this field are addressed in other papers of this report. Greater detail about antiprogestogens in general and about the Special Programme's research with these compounds in particular can be found in our earlier reviews (Van Look and Bygdeman, 1989; Puri and Van Look, 1991; Van Look and von Hertzen, 1992a,b, 1993).

COMPOUNDS

Compounds with antiprogesterone activity can be grouped into two main categories: (1) steroid enzyme inhibitors that prevent the biosynthesis of progesterone at the level of the steroid-producing cells, and (2) progesterone-receptor blockers (designated as "antiprogestogens" in this paper) that interfere with the binding of progesterone to its cellular receptor in target tissues.

Progesterone Synthesis Inhibitors

Progesterone biosynthesis in steroid-producing tissues proceeds by reactions involving removal of a C_6 fragment from cholesterol to form

pregnenolone, which in turn is converted into progesterone by the Δ^5-3β-hydroxysteroid dehydrogenase enzyme complex (3β-HSD) (Dorfman, 1973). Several synthetic progestogens have been shown to be capable of inhibiting ovarian 3β-HSD activity (Shinada et al., 1978), but clinical exploitation of this "antiprogestational" effect has been prevented by the intrinsic progestational activity of these compounds. Of more clinical interest is the series of steroidal 3β-HSD inhibitors that includes azastene, trilostane, and epostane. Cyanoketone also belongs to this series, but this compound is unsuitable for fertility regulation because it causes irreversible inhibition of 3β-HSD (Goldman, 1967).

Although azastene, trilostane, and epostane are competitive inhibitors of 3β-HSD, the compounds differ with regard to their relative potency in vivo as inhibitors of adrenal or ovarian/placental steroidogenesis. Azastene and epostane appear to be preferential inhibitors of ovarian and placental steroidogenesis rather than of adrenal hormone production, at least in primates (Schane et al., 1978; Creange et al., 1981). Epostane is the more potent of these two compounds; hence virtually all of the studies in primates, including the human, have been done with this inhibitor. A detailed review of these studies has been published (Van Look and Bygdeman, 1989).

As one might expect, the clinical effects of epostane administration are not unlike those resulting from treatment with a progesterone-receptor blocker such as mifepristone. Repeated dosing during the midluteal phase of the cycle causes premature menstruation in the majority of subjects, whereas daily treatment from the beginning of the cycle has been shown to modulate or inhibit follicular development and ovulation, depending on the dose used (Rannevik et al., 1988).

When given to pregnant women over a period of several days, the drug induces uterine contractions and increased myometrial reactivity to prostaglandins. In the two largest efficacy trials conducted (Birgerson et al., 1987; Crooij et al., 1988), a seven-day course with epostane (four times 200 mg/day) resulted in 89 (84 percent) complete abortions among 106 women with amenorrhea of less than 56 days. The limited experience that is available suggests that, as with mifepristone, the complete abortion rate can be increased significantly by complementing the epostane treatment with a prostaglandin analogue in a sequential regimen (Webster et al., 1985). Like mifepristone, administration of epostane during the second trimester of pregnancy augments uterine sensitivity to exogenous prostaglandins and significantly shortens the time needed to induce abortion with extra-amniotically administered prostaglandin E_2 (Selinger et al., 1987).

Epostane and related molecules were synthesized by the pharmaceutical company Sterling-Winthrop. When this firm was taken over by the Eastman-Kodak company, epostane was no longer made available to

clinical researchers, and further development of the compound was discontinued. Although epostane appears to be a preferential inhibitor of the ovarian and placental 3β-HSD enzyme, this compound—along with others like it—is probably less suitable for chronic administration than the more specific progesterone-receptor blockers because of possible inhibitory effects on adrenal corticosteroid synthesis. For occasional short-term use, however, such as in the termination of early pregnancy, a potent 3β-HSD enzyme inhibitor could be a potential alternative to an antiprogestogen. Also, combining a 3β-HSD inhibitor with a progesterone-receptor blocker may result in a powerful synergistic effect, as has been demonstrated in the pregnant guinea pig (Elger et al., 1988).

In view of the foregoing it would seem worthwhile to pursue the development of epostane or related compounds as a possible substitute for—or complement to—progesterone-receptor blockers. An active search for 3β-HSD inhibitors that are more potent than epostane or azastene is currently in progress in the People's Republic of China.

Progesterone-Receptor Blockers

As described by Ulmann et al. (1990), progesterone-receptor blockers were discovered by chance rather than by design. Following the invention of a method of synthesizing 11β-substituted steroids (Bélanger et al., 1981; Teutsch et al., 1988) and the observation that some of these 11β-substituted compounds behaved as glucocorticoid antagonists, a formal research project for the development of glucocorticoid antagonists was launched at Roussel-Uclaf toward the end of 1979. Mifepristone (RU 38486, later shortened to RU 486) was produced a few months later, in April 1980 and, when tested for in vitro binding to the five classes of steroid receptors, was found to possess high affinity not only for the glucocorticoid receptor but also for the progesterone receptor, and low affinity for the androgen receptor (Philibert, 1984). The ability of antiprogestogens to interact with both the progesterone and the glucocorticoid receptors is probably related to the more than 50 percent homology in amino acid composition that exists between these two receptors in their C-terminal end (i.e., the region thought to be involved in steroid binding) (Misrahi et al., 1987). Subsequent tests in various animal models soon revealed that mifepristone possessed both antiglucocorticoid and antiprogestational activities. Thus, the scientists at Roussel-Uclaf had inadvertently managed to produce the progesterone antagonist long awaited by investigators and clinicians interested in birth control.

Following the initial discovery, several hundred compounds with antiprogesterone activity were synthesized, not only at Roussel-Uclaf (Teutsch, 1984, 1985), but also by other pharmaceutical companies

including Schering AG (Wiechert and Neef, 1987) and Organon (Kloosterboer et al., 1988) and by private institutions (Cook et al., 1990). Other companies such as Upjohn, ICI, and Jenapharm also have been granted patents describing antiprogestational drugs but, as far as we are aware, have not published any biological data on these compounds in the scientific literature. Most of the molecules shown to possess antiprogestational activity have a bulky substituent at C-11, usually a dimethylaminophenyl group as found in mifepristone, lilopristone (ZK 98 734), and onapristone (ZK 98 299) (Figure B12.3). In some of the Organon compounds (e.g., ORG 31167 and ORG 31343) the dimethylaminophenyl group is situated at C-18 (Kloosterboer et al., 1988).

From the published literature it appears that only a few of the many compounds synthesized to date have been evaluated to any significant extent in biological screening models and, to our knowledge, only three of them have been given to humans, namely, mifepristone, lilopristone (which has been used in a dose-finding study for termination of early pregnancy; Swahn et al., 1993), and onapristone (which is about to enter Phase II studies). The available preclinical data suggest that there can be marked differences between antiprogestogens in their antiprogestational and antiglucocorticoid potencies in vitro and in vivo, even for compounds that are very comparable in chemical structure. For example, lilopristone, which differs from mifepristone only in the structure of the 17α side chain (Figure B12.3), is reported as having a much reduced antiglucocorticoid activity (Table B12.1). The two Organon compounds referred to above also appear to possess reduced antiglucocorticoid activity (Kloosterboer et al., 1988). Since no "pure" antiprogestogens have been described to date, it is unclear whether the ancillary antiglucocorticoid activity present in currently available antiprogestogens has a modulatory influence—either positive or negative—on the antiprogestational potency. In the case of lilopristone, the more favorable antiprogestational/antiglucocorticoid potency ratio found in animal studies did not make the compound more potent in the termination of early pregnancy in the human compared to mifepristone (Swahn et al., 1993).

The results obtained to date with mifepristone leave little doubt that antiprogestogens will make a significant impact on fertility regulation in obstetrics and gynecology and, possibly also, in other branches of medicine. Data from animal work also suggest that antiprogestogens may differ among themselves in terms of the relative effects they have on different target tissues, but this requires confirmation in the human. Current research on antiprogestogens suffers from the fact that all the compounds that have been studied to any significant extent possess antiglucocorticoid activity, and there is an urgent need therefore to produce molecules in which these two activities—the antiprogestational and the antiglucocorticoid—have been separated. The potential advan

tages of having pure, and preferably also more potent, antiglucocorticoids and antiprogestogens are manifold.

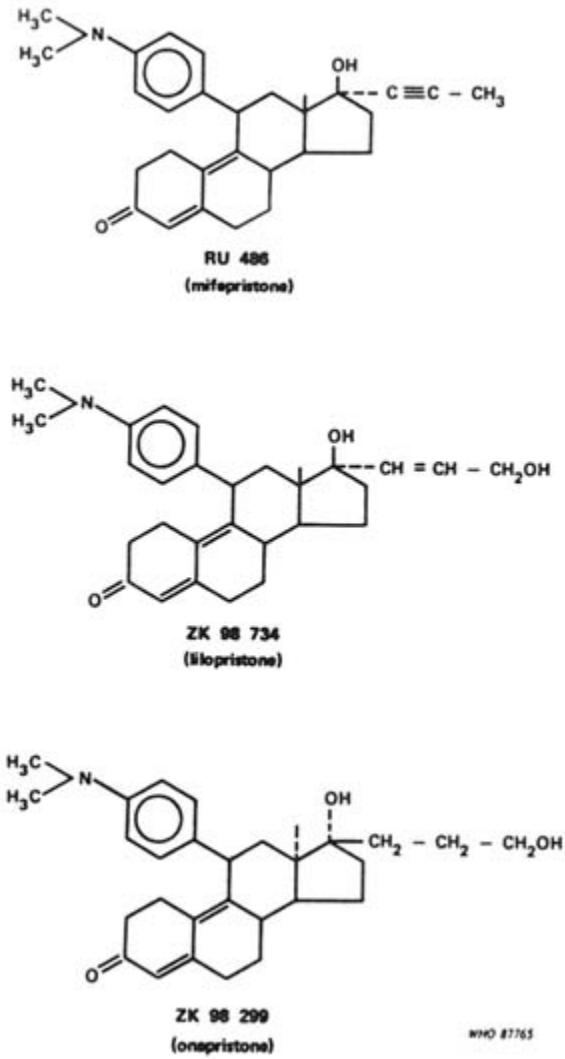

FIGURE B12.3 Chemical structures of the three best-known antiprogestogens.

Availability of selective glucocorticoid- and progesterone-receptor blockers will provide basic researchers with powerful tools to study the effects of selective receptor blockage at the cellular level and thus provide further insights into the mechanism of action of these compounds. From a clinical point of view, pure antiprogestogens that are

devoid of antiglucocorticoid activity are almost a sine qua non if they are going to be used for long periods of time, such as in the treatment of breast cancer or endometriosis, or as a daily contraceptive pill. On the other hand, pure antiglucocorticoids need to be developed that have no effect on the menstrual cycle and early pregnancy, and hence could also be used in countries where a compound with dual antiglucocorticoid and antiprogestational activities may not be made available becuase of, for example, restrictive abortion legislation. Attempts are under way to produce more selective molecules by using information from structure-activity relationships of existing compounds (Philibert et al., 1989) and from computer modeling studies of the stereochemical complementarity of steroid hormones and cavities between base pairs in DNA (Hendry and Mahesh, 1992).

TABLE B12.1 Antiprogestational and Antiglucocorticoid Activities of RU 486, ZK 98 734, and ZK 98 299

Parameter	RU 486	ZK 98 734	ZK 98 299
Relative binding affinity (R 5020 = 100%) for progesterone-receptors using rabbit uterine cytosol (%)	68	72	19
Antiglucocorticoid activity (%)[a]	100	4	5
Abortifacient activity			
Rats (days 5–7 post coitum)			
3 mg/day	4/4[b]	4/4	4/4
1 mg/day	2/4	4/4	4/4
0.3 mg/day	0/4	1/4	0/4
Guinea pigs (days 43-44 post coitum)			
30 mg/day	4/9	6/6	7/9
10 mg/day	3/9	5/7	5/9
1 mg/day	1/7	5/8	0/9

[a] Based upon reversal of dexamethasone-induced tyrosine aminotransferase activity in cultured rat hepatoma cells.

[b] The abortion figures indicate the number of animals aborting versus the number of animals treated. Compounds were administered by the subcutaneous route in an oil base once daily on days 5–7 post coitum in rats and on days 43– 44 in guinea pigs.

SOURCE: Based on Puri and Van Look (1991).

ANIMAL MODELS

Identification of potential antiprogestogens generally involves, in the first instance, determination of the in vitro binding affinities of the newly synthesized compounds for the progesterone receptor and receptors of other steroid hormones. Compounds showing high binding affinity for the progesterone receptor are then assessed in appropriate animal models to determine if they are agonists or antagonists. The models used for this purpose and for evaluating the full hormonal and

antihormonal profile of new compounds have been described on several occasions (see, for example, Philibert et al., 1985) and are not discussed here. In general, the animal models used have been fairly reliable in predicting the effects of mifepristone in the human in spite of some major differences between humans and even their closest relatives, the nonhuman primates, in important areas such as the pharmacokinetics of the compound and the type of placentation.

Receptor Binding

Antiprogestogens have been demonstrated to bind to progesterone-receptor preparations from a variety of species including rat, rabbit, calf, marmoset, bonnet monkey, and human (for review see Van Look and Bygdeman, 1989). In the chicken, where progesterone has a different role than in mammals, mifepristone does not bind to the oviductal progesterone receptor (Groyer et al., 1985) but to an immunologically distinct macromolecule, possibly ovalbumin, that does not bind progesterone (Moudgil et al., 1986; Eliezer et al., 1987). However, the chicken glucocorticoid receptor binds mifepristone very well (Groyer et al., 1985). Absence of competition by mifepristone for binding of progesterone to its uterine cytosol receptor has also been reported for the hamster (Okulicz, 1987) and the tammar wallaby (Fletcher and Blandon, quoted in Baulieu, 1989).

In the case of the chicken, Benhamou et al. (1992) have shown that the absence of mifepristone binding is due to the presence of cysteine at position 575 in the hormone binding domain of the progesterone receptor. Substitution of this cysteine by glycine—but not by methionine or leucine—generated a mutated receptor that bound the antiprogestogen. In fact, all receptors that bind mifepristone, including those for glucocorticoids and androgens, have a glycine residue at the corresponding position. The progesterone receptor in the hamster, like that in the chicken, has a cysteine. Substitution of the corresponding glycine residue at position 722 by cysteine in the human progesterone receptor abrogated the binding of mifepristone but not that of a progesterone agonist, whereas the same substitution in the glucocorticoid receptor resulted in a loss of binding of both dexamethasone and mifepristone. Interestingly, the studies of Benhamou and colleagues also showed that antagonism is not per se an intrinsic property of an antihormone, because one human progesterone-receptor antagonist acted as an agonist when tested with a mutated receptor.

About 1 percent of women given mifepristone alone for induction of abortion do not respond to the antagonistic action of the antiprogestogen (Baulieu, 1989); vaginal bleeding is not induced in these subjects and the pregnancies continue, seemingly unaffected. Since these

women can be assumed to have normal responsiveness to progesterone as evidenced by their ability to conceive, it would be of interest to examine whether in these cases a mutation at position 722 of the progesterone receptor might be responsible for the lack of responsiveness to mifepristone. Further research in this area may also contribute to the designing of more potent and more selective compounds—antagonists as well as agonists—through a better understanding of the factors that determine the nature of the response when a ligand interacts with a receptor.

Plasma Protein Binding

One important aspect that needs to be kept in mind when evaluating animal data is the fact that, in contrast to the human, no animal species appears to have a plasma protein with binding affinity for antiprogestogens. In the human, on the other hand, mifepristone is transported in the circulation bound to orosomucoid, an α_1-acid glycoprotein that acts as a high-affinity, limited-capacity binding protein (Philibert et al., 1986). Orosomucoid becomes saturated at mifepristone concentrations exceeding 2.5 μM (Heikinheimo et al., 1987); drug in excess of this concentration is probably bound with low affinity to albumin, and hence is available for metabolism and extravasation into tissues (Lähteenmäki et al., 1987). The presence of a plasma carrier protein in the human is the likely explanation for the divergent pharmacokinetic behavior of mifepristone between the human and other mammalian species (Deraedt et al., 1985). For example, plasma clearance of the compound in man (0.02 liter/hour per kilogram of body weight) is much lower than in rats and monkeys (3.0 and 1.5 liter/hour per kilogram of body weight, respectively). Administration of orosomucoid to rats reduces the clearance rate (Tremblay et al., 1989).

Lilopristone also exhibits binding to a serum protein that is not displaced by cortisol, dihydrotestosterone, or progesterone, but only by high concentrations of lilopristone or mifepristone (unpublished data of Heubner and Pollow, quoted by Henderson, 1987), suggesting that this antiprogestogen also is bound to orosomucoid. In contrast, onapristone does not appear to bind to this plasma protein (K. Chwalisz, personal communication, November 4, 1992) and, consequently, has a much shorter plasma elimination half-life (2–4 hours) compared to mifepristone (20–24 hours). It is unclear whether the absence of binding of onapristone to orosomucoid is due to this compound's configurational inversion at positions C-13 and C-17 relative to natural steroids (Figure B12.3).

The availability of compounds that do not bind to a plasma carrier protein and hence have a relatively short half-life could represent an

advantage in some situations; for example, in the use of an antiprogestogen as a late luteal, once-a-month contraceptive where a "spillover" effect into the next cycle is to be avoided. To develop such compounds, further research into those molecular characteristics of antiprogestogens that determine their binding to carrier protein(s) may prove useful.

Pregnant Guinea Pig Model

One animal model that has been employed extensively, particularly by Elger and his colleagues at Schering AG, for the study of the abortifacient potency and mechanism of action of antiprogestogens is the guinea pig in advanced stage of pregnancy (i.e., around day 43–44 post coitum) (Table B12.1). The reasons for selecting this species and stage of pregnancy as a model of pregnancy and uterine motor function in the human have been described by Elger et al. (1987). Studies in this model and similar research in the human (Bygdeman and Swahn, 1985) led to the development of the sequential treatment regimen of mifepristone followed by prostaglandin, now used clinically in France, Great Britain, and Sweden as a nonsurgical method for early pregnancy termination (for review see, for example, Van Look and von Hertzen, 1992a). Subsequently, the same group of workers also reported a marked synergism between antiprogestogens and epostane, and between antiprogestogens and tamoxifen in inducing abortion in the pregnant guinea pig model (Elger et al., 1988). From these observations they postulated that the "estrogen background" may exert an inhibitory influence on the onset of uterine contractions in antiprogestogen-treated animals.

In order to examine if a similar effect exists in the human, a study was undertaken with the support of the Special Programme in five hospitals in Beijing, China [Wu, Clinical study of mifepristone in combination with tamoxifen and 15-methyl-prostaglandin F_{2a} methyl ester in the termination of early pregnancy (provisional title), in preparation]. A total of 990 women with amenorrhea of up to 49 days were given a single dose of 200 mg of mifepristone followed, 72 hours later, by a vaginal suppository of 1 mg of dl-15-methylprostaglandin $F_{2\alpha}$ methyl ester. In addition, half the women received tamoxifen (20 mg twice a day for two days starting at the time of mifepristone intake), and the other half received placebo tablets. The results of this trial did not confirm the synergistic effect of tamoxifen observed in the guinea pig. In fact, the complete abortion rate in the tamoxifen group (91.5 percent) tended to be lower than in the placebo controls (93.4 percent), and the interval between prostaglandin administration and expulsion of the amniotic sac was significantly ($P < .02$) longer in women given the antiestrogen. These results point to the need for further evaluation of the guinea pig

model, particularly for studying synergism and antagonism for different compounds given in combination with antiprogestogens. It would be of interest, therefore, to examine in the human the efficacy of other combination regimens with synergistic activity in the guinea pig, particularly the combination of mifepristone with a 3β-HSD enzyme blocker.

MECHANISMS OF ACTION

The manner in which antiprogestogens exert their pharmacological actions has not been fully elucidated. To date most of the research has focused on the effects on the pregnant uterus, and relatively little work has been done to explain actions such as, for example, the noncompetitive antiestrogenism or the inhibition of pituitary gonadotropin secretion. Work supported by the Special Programme has been concentrated primarily on the changes induced by antiprogestogens in three areas, namely, prostaglandin metabolism, myometrial gap junctions, and progesterone- and estrogen-receptor concentrations in decidua and trophoblast (for review see Van Look and von Hertzen, 1993).

Prostaglandin Metabolism

Initial studies established that mifepristone administration resulted in an increase in the sensitivity of the uterus to exogenous prostaglandins followed by the onset of spontaneous uterine contractility, which reached a maximum level about 36–48 hours after the start of therapy (Bygdeman and Swahn, 1985; Swahn and Bygdeman, 1988). Subsequent work using the pregnant guinea pig as the animal model showed that the antiprogestogen-induced increase in myometrial sensitivity to exogenous prostaglandins appears to be due to a reduction in the catabolism rather than to a stimulation of the synthesis of prostaglandins (Brooks et al., 1990; Kelly and Bukman, 1990). Very recent experiments have demonstrated that the same applies to the human (Cheng et al., 1993). In particular, it was shown that the concentration of prostaglandin dehydrogenase, the enzyme that catalyses the first step of prostaglandin metabolism, was significantly reduced in decidual tissue obtained during surgical abortion in women treated 12, 24, or 36 hours earlier with a single dose of 200 mg of mifepristone. The activity of the enzyme was also lower in the chorionic villi of mifepristone-treated women than in controls, but this reduction was not significant. Immunohistochemistry, using a monoclonal antibody specific for prostaglandin dehydrogenase, confirmed the results of the functional assays of enzyme activity. In addition, the investigators were able to confirm immunohistochemically that the reduction in prostaglandin dehydrogenase activity was accom

panied by an increase in the concentration of prostaglandin E, particularly in perivascular cells, and a decrease in the concentration of prostaglandin E metabolites in these cells.

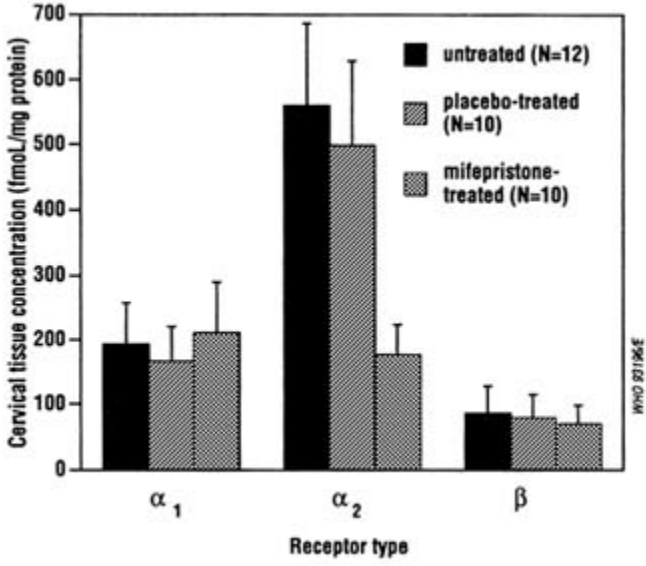

FIGURE B12.4 Effect of mifepristone on adrenergic receptors in cervical tissue specimens of early pregnant women.
SOURCE: Based on Kovacs and Falkay (1993).

The exact significance of the elevated prostaglandin concentration in perivascular cells after mifepristone treatment is not clear, but the importance of this reaction may lie in the regulatory role of these cells in controlling ingress of leukocytes. To examine this possibility, studies are ongoing to quantify the effect of mifepristone treatment on the number of cells in the decidua that express the leukocyte common antigen, CD 45. It is of interest to note in this context that a marked leukocyte infiltration has been reported in an electron microscopy study of guinea pig cervical tissue after administration of onapristone (Hegele-Hartung et al., 1989).

The results described above on the effects of antiprogestogens on prostaglandin metabolism suggest that compounds capable of inhibiting prostaglandin catabolism such as inhibitors of the dehydrogenase enzyme could have abortifacient activity similar to antiprogestogens. Further research is required to examine this possibility. Additional research is also needed on the role played by adrenergic receptors in mediating the actions of antiprogestogens on the uterus. In both the rabbit (Falkay, 1990) and the human (Figure B12.4; Kovacs and Falkay, 1993), mifepristone treatment is associated with a marked reduction of

the α_2-adrenoreceptor concentration in cervical tissue, but the functional significance of this change remains to be determined.

Myometrial Gap Junctions

There is now overwhelming evidence that gap junctions between myometrial cells play a key role in enabling the myometrium to behave as a functional syncytium by providing metabolic and electrical coupling between the individual cells (for review see, for example, Garfield et al., 1988). It seemed of interest, therefore, to evaluate the effect of antiprogestogens on the presence, distribution, and functional characteristics of gap junctions. To this end, immunocytochemical and immunoblot studies were carried out using a specific antibody to the gap junction protein, connexin 43. In addition, the degree of cell-to-cell coupling between the myometrial cells was assessed by measuring electrical input resistance with intracellular glass microelectrodes and by monitoring the spread of an intracellularly injected dye to neighboring cells.

The results of these studies, carried out by Garfield and his group in myometrial tissues of pregnant guinea pigs, rats, pigs, and humans (see Van Look and von Hertzen, 1993, for references) confirmed that gap junctions (connexin 43 content) in the myometrium and intercellular coupling of myometrial cells increase significantly in preterm labor induced by antiprogestogen treatment. Thus, compounds that affect the formation and/or function of gap junctions may prove useful to replace or complement antiprogestogens for induction of abortion and labor, and research aimed at finding such agents may prove rewarding.

Estrogen and Progesterone Receptors

The Special Programme has supported research on the distribution and characteristics of estrogen and progesterone receptors in decidua and trophoblast following mifepristone treatment in early pregnancy. These studies have shown that progesterone-receptor levels in cytosol of mifepristone-exposed decidua were less than half those found in placebo-treated controls; nuclear binding sites for progesterone, on the other hand, did not change (Shi et al., 1992). Estrogen-receptor levels in both nucleus and cytosol of decidual samples taken 12 hours after a single dose of 200 mg of mifepristone were about twice the concentration found in placebo-treated controls. Other workers (Zaytseva et al., 1993) have also reported increases in estrogen-receptor concentrations at 36 hours after three doses of 25 mg of mifepristone given at 12-hour intervals and after a single dose of 600 mg. In addition, Shi et al. (1992) found a marked increase in the dissociation constants of the progester

one binding sites in both cytosol and nucleus following mifepristone treatment, an unexpected finding that is being studied further.

Immunohistochemical staining of mifepristone-exposed decidua revealed that antiprogestogen treatment resulted in increased staining for both progesterone and estrogen receptors in specimens presumed to represent decidua parietalis because of the absence of invading cytotrophoblast cells. The increased staining was most noticeable in the decidual vessels and stromal cells, and weak to absent in glandular epithelial cells. In specimens with invading cytotrophoblast cells (decidua capsularis), immunostaining of progesterone and estrogen-receptors was weaker than in decidua parietalis and not influenced by mifepristone treatment (Shi et al., 1993a). The finding of a seemingly lower progesterone-receptor content in decidua capsularis may explain the observation (Wu et al., 1990) that prolactin production and morphological decidualization in this part of the decidua are not affected by mifepristone treatment, in contrast to the decidua parietalis. Steroid binding assays on villous cytosol failed to demonstrate the presence of a specific progesterone-binding component, and immunostaining for progesterone receptor was weak in both villous and extravillous trophoblasts (Shi et al., 1993b).

The above results indicate that in addition to its ability to compete with progesterone for the cellular hormone receptor, mifepristone may influence, either directly or indirectly, the concentration and affinity of progesterone binding sites in the decidua. These effects are most marked in the decidua parietalis, which suggests that this tissue, and particularly its blood vessels, may be the primary target site of antiprogestogens.

Increases in estrogen-receptor concentration following mifepristone treatment have also been observed in the decidua and myometrium during antiprogestogen-induced premature delivery in rhesus monkeys (Haluska et al., 1990) and in estrogen-treated, ovariectomized rhesus monkeys (Wolf et al., 1989; Neulen et al., 1990). In this latter model, mifepristone has been demonstrated to antagonize the mitogenic effects of estrogen on the endometrium, which has led to the concept that mifepristone possesses a functional, noncompetitive antiestrogenic effect. A similar antiestrogenic action has been observed in the case of onapristone (K. Chwalisz, personal communication, November 4, 1992). It is at present entirely unclear through which mechanism antiprogestogens exert this antiestrogenic action. Equally unclear is the role, if any, that this functional antiestrogenism may play in the effects of antiprogestogens on endometriosis (Kettel et al., 1991), uterine leiomyomas (Murphy et al., 1993), and possibly, breast cancer (Romieu et al., 1987). In fact, there is no convincing evidence as yet that antiprogestogens possess antiestrogenic activity in the human, although the obser

vation that mifepristone (50 mg daily for three months) administered in an attempt to induce regression of uterine leiomyomas was associated with an initial rise in the plasma level of luteinizing hormone and clinical menopausal symptoms (hot flashes) in the face of follicular phase levels of estrogen, supports a possible functional antiestrogenic effect in the human too.

TABLE B12.2 Suggested Indications of Areas in Which Antiprogestogens May Be of Clinical Value

1.	*Antiprogestational Effect*	
	Gynecology	
	•	Cervical ripening (e.g., prior to curettage or vacuum aspiration)
	•	Endometriosis (?)
	•	Uterine leiomyomas (?)
	•	Breast cancer (?)
	•	Endometrial cancer (?)
	•	Ectopic pregnancy[a]
	•	Premenstrual tension[a]
	Obstetrics	
	•	Therapeutic pregnancy termination in second or third trimester (therapeutic abortion, intrauterine fetal death, compromised pregnancies)
	•	Cervical ripening/induction of labor at term
	Fertility Regulation	
	Other	
	•	Meningioma (?)
2.	*Antiglucocorticoid Effect*	
	•	Hypercortisolism (selected cases)
	•	Depression (selected forms) (?)
	•	Ocular hypertension (?)
	•	Wound healing (?)

[a] Currently available data suggest little or no use of antiprogestogens in this condition.

Further research is also needed to unravel the mechanism by which continuous mifepristone administration acts to induce chronic anovulation. As shown by the recent work of Ledger et al. (1992) and of Croxatto et al. (1993), daily mifepristone doses of 10, 5, and 2 mg, but not of 1 mg, are capable of suppressing the final stages of follicular maturation and ovulation, suggesting the possible use of antiprogestogens in oral contraception.

POSSIBLE USES

Since its discovery in early 1980, mifepristone has been suggested as of potential use in a wide range of conditions but, as shown in Table B12.2, the clinical evaluation of the compound's therapeutic potential in many of these indications has been either slow or nonexistent for a variety of reasons and, consequently, many question marks remain.

As described in other papers in this volume and in earlier reviews

(e.g., Puri and Van Look, 1991), the gynecological and obstetrical applications in which antiprogestogens have been shown of value include ripening of the pregnant cervix prior to pregnancy termination, sensitization of the uterus to prostaglandins in second-trimester abortion, and induction of labor in cases of intrauterine fetal death and in compromised or normal pregnancies. Available data suggest that antiprogestogens have no place in the conservative treatment of ectopic pregnancy (see Puri and Van Look, 1991, for references) or in the treatment of premenstrual tension (Schmidt et al., 1991).

Potential uses of antiprogestogens in fertility regulation (Figure B12.5) are multiple and have been detailed in other papers in this report and earlier reviews (e.g., Van Look and von Hertzen, 1992a). To date, most of the research has focused on the use of mifepristone in combination with a prostaglandin analogue (represented by the hatched vertical bars in Figure B12.5) for pregnancy termination. Such a sequential mifepristone-prostaglandin regimen has been shown effective also for menses induction (see Baird, Appendix B4) and can be expected to be an efficacious once-a-month contraceptive. Mifepristone alone, without adjuvant prostaglandin, has shown promising results as an anti-implantation agent and in emergency contraception (see Baird, Appendix B4). Other potential uses include once-a-week contraception, ovulation inhibition (in a sequential regimen with a progestogen), and as a daily "mini-pill."

Confirmed and suggested applications of the antiglucocorticoid property of currently studied antiprogestogens include the palliative treatment of hypercortisolism due to Cushing's syndrome, certain forms of depression and of glaucoma, and in wound healing. However, as discussed earlier, it would be preferable if compounds were developed that were pure glucocorticoid antagonists devoid of antiprogestational activity.

The potential therapeutic range of antiprogestogens is wide, but as indicated above, much research still needs to be undertaken before the true value of these compounds can be determined.

PROSPECTS

Because of their unique ability to block the action of progesterone at the cellular level through binding to the progesterone receptor, antiprogestogens are proving to be one of the most significant developments in endocrinology in recent years. To date, clinical research has been dominated very much by studies on the termination of pregnancy, the indication for which the drug has been registered in four countries so far (France, China, Great Britain, and Sweden). However, as evidenced by the papers in this report and our brief review, antiprogesto

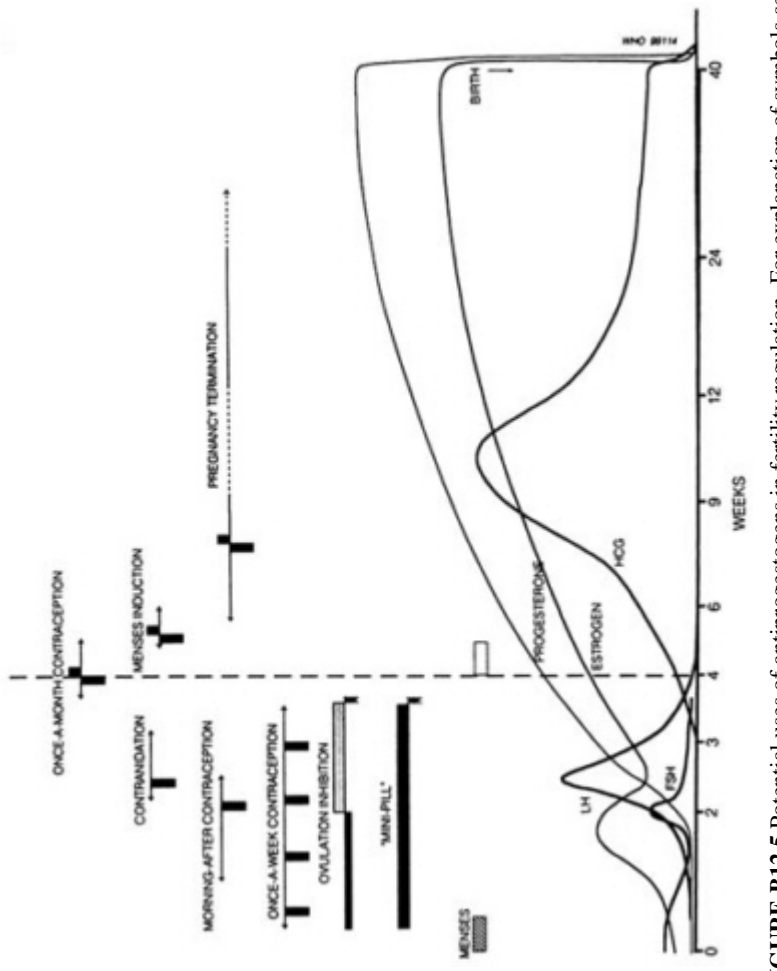

FIGURE B12.5 Potential uses of antiprogestogens in fertility regulation. For explanation of symbols see text.

gens are not merely "abortion pills"; they have a variety of other potential applications in several fields, including fertility regulation. Indeed, the ability to prevent pregnancy may well turn out to be a much more significant characteristic of these compounds than their abortifacient activity. Moreover, because of their antiprogestational properties and their apparent functional antiestrogenism, antiprogestogens are likely to have several therapeutic uses in the fields of obstetrics and gynecology and of reproductive health care.

In 1985, Healy and Fraser warned the medical community through the editorial pages of the *British Medical Journal* that "the antiprogesterones are coming." Since then, these compounds have been the subject of intense basic and clinical research, and the results obtained have left little doubt that "antiprogestogens have come to stay" (Van Look and von Hertzen, 1992a). The time has come for scientists, policymakers, and the general public to acknowledge this fact.

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