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# Screening for Antiproliferative Actions of Mifepristone

## *Differential Endometrial Responses of Primates Versus Rats*

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*This laboratory has previously shown the capability of the antiprogestin, mifepristone, to noncompetitively inhibit estrogen-induced endometrial proliferation in nonhuman primates. In the following study, use of the rat uterine weight bioassay was compared against a primate (*Macaca fascicularis*) uterine bioassay to identify the noncompetitive/antiproliferative effects of mifepristone. These uterine bioassays were contrasted for reasons of identifying a comparative laboratory rodent model that could substitute for the need to use primate models in the screening of potential antiprogestins, thereby saving time, cost, and primate resources.*

*Results of the primate experiment showed that mifepristone decreased endometrial proliferation in a dose-dependent manner; importantly, this decrease occurred in the presence of sustained physiologic serum 17β-estradiol ($E_2$) levels. However, in the rat model, results showed that mifepristone altered uterine wet weight and blotted weight values only in those animals receiving pharmacological doses of $E_2$ ($p < 0.05$).*

*Based on the results summarized herein, use of this rat uterine weight bioassay as a substitute for primate models is not recommended for screening and identification of ''interesting'' antiprogestins. Apparently, the endometrial noncompetitive antiestrogenic/antiproliferative effects of mifepristone, observed repeatedly in these laboratory primates, do not operate in the rat uterine tissue.* CONTRACEPTION 1998;58:45–50 

**KEY WORDS:** RU486, antiprogestin, antiproliferative, endometrium, cynomolgus monkey, rat

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## Introduction

Mifepristone (originally produced by Roussel UCLAF, Paris, France, as RU 486) is a representative member of a new class of pharmaceutical compounds called antiprogestins. Antiprogestins have applications for use in treating clinically relevant diseases,[1–5] as well as being a contragestational agent.[6–12] Accordingly, pharmaceutical companies interested in identifying specific effects of novel antiprogestins have applied their methods of high-throughput screening (HTS), a preliminary tool for drug discovery, to the screening of antiprogestins. The benefits of using HTS include savings in time, money, and diminished ethical concerns surrounding the use of laboratory animals. However, regardless of HTS's benefits, animal models remain an essential component in the evaluation of a drug's safety and efficacy; HTS cannot characterize many factors, including: 1) bioavailability; 2) pharmacokinetics; 3) pharmacodynamics; 4) toxicity; or 5) cell, tissue, or organ specificity.[13] In addition, development of drug formulations and delivery systems often benefit from appropriate animal testing.

Previous results from this laboratory and others have suggested that mifepristone and other antiprogestins inhibit estrogen-induced endometrial growth in nonhuman primate and rabbit by a noncompetitive antiestrogenic/antiproliferative action.[14–18] As such, this laboratory has a continuing interest in examining the paradoxical, antiestrogenic effects of mifepristone. Note that mifepristone has been reported to not bind the estrogen receptor, thus resulting in the designation of noncompetitive action.[19]

Motivated by the drug discovery efficiencies cited above, experiments were designed to identify a laboratory rodent model that could be substituted for the use of primate models in the identification of new antiprogestins via drug screening trials. In this regard, a current rendition of the traditional rat uterine weight bioassays is widely accepted as one of the ''gold-standards'' for the evaluation of estrogen-in-


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duced endometrial tissue growth.[20–22] However, preliminary studies conducted in this laboratory indicated that the rat uterine weight bioassay did not respond to the noncompetitive antiestrogenic effects of mifepristone. Therefore, the goal of this study was to compare the practical use of the rat uterine weight bioassay versus an often used monkey endometrial proliferation test as a screening tool for characterizing noncompetitive antiestrogenic/antiproliferative potency of mifepristone on endometrium.

## Materials and Methods

*Reagents*

17β-Estradiol ($E_2$) and progesterone were purchased from Sigma Chemical Co. (St. Louis, MO). $E_2$ pellets were purchased from Innovative Research of America (Cat. #E121, Sarasota, FL). The mifepristone used in these experiments was purchased from the National Research Institute for Family Planning, Beijing, China. It was determined that the bioavailability and potency of the compound from this source was equivalent to genuine RU 486 supplied earlier by Roussel UCLAF.

*Primates, Rodents, and Treatments*

All Sprague-Dawley rats used in these experiments were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The animals were maintained under 14/10 hour light/dark cycle and provided water and food ad libitum. Immature female rats were ovariectomized on day 16 of age and allowed to recover for 4 days before starting hormone treatment. The animals were randomly assigned to a treatment group following ovariectomy (five per group). Hormone treatments were given for 6 days, 3 days of $E_2$ pretreatment (either $E_2$ in oil or as a pellet), followed by 3 days of $E_2$ plus mifepristone. $E_2$ was given as a daily single sc injection (0.5 μg/100 g body weight (BW), in corn oil), a dose reported to mimic the proestrus $E_2$ surge,[23] or as a single subcutaneous 0.5 mg pellet implant.[24] Mifepristone was dissolved in corn oil and administered as a daily single subcutaneous injection at the doses indicated in figures or tables. Cardiac blood samples were collected under ketamine-acepromazine induced anesthesia from a group of similarly treated rats for determination of serum $E_2$ levels.

Fifteen long-term ovariectomized cynomolgus monkeys (*Macaca fascicularis*), weighing approximately 2–4 kg, were maintained under standard conditions.[14–16] These primates were assigned to one of five treatment groups (three per group). An $E_2$-containing Silastic capsule was implanted on day 1 and left in place for 21 days by methods previously described.[15] Starting on day 1, monkeys were treated with vehicle or mifepristone for 20 days at doses indicated in the figures and tables. Femoral blood samples were collected under ketamine-induced anesthesia for determination of serum $E_2$ levels.

*Uterine Tissue Collection and Histology*

Rats were euthanized by $CO_2$ asphyxiation, the entire uterus removed, and wet and blotted weights obtained. After weighing, the uterine tissue was placed into 10% buffered formalin and processed for histological evaluation. Histology was performed on two cross sections from each rat.

Uterine biopsies were obtained from each monkey on day 21 of the experiment and processed for histological evaluation as previously described.[15] Mean endometrial thickness for each animal was obtained by averaging at least four separate determinations using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) on a Olympus BX50 microscope.

*Radioimmunoassay*

Monkey serum levels of $E_2$ were measured by radioimmunoassay (RIA) using a commercially available kit from ICN Biomedicals, Inc. (Costa Mesa, CA), and quality control standards of known potency from BioRad (Hercules, CA) as previously described.[25] All serum samples were measured in a single assay. The intra-assay coefficient of variance for the BioRad control of similar potency as the unknowns was 9%. This laboratory's historical interassay coefficient of variance for this assay is $<10\%$. The assay sensitivity for $E_2$ was 10 pg/mL.

Rat serum levels of $E_2$ were measured by Covance Laboratories Inc. (Vienna, VA). The reported intra-assay coefficient of variance and interassay coefficient of variance from Covance are 10.76% and 12%, respectively. The assay sensitivity for $E_2$ was 10 pg/mL.

*Statistical Analysis*

Number of mitotic cells, cells showing nuclear fragmentation, uterine wet weight, and uterine blotted weight, were statistically compared by analysis of variance using Instat GraphPAD Software, version 1.11a (1990). Significant differences between group means were determined by Fisher's least significant difference test, with values being considered significant at $<0.05$.

*Ethical Treatment of Animals*

All primates and rodents were housed in facilities accredited by the American Association for the Ac-

**Table 1.** Mean serum estradiol levels ± SEM (pg/mL) in ovariectomized monkeys and rats given estrogen replacement

| Treatment group | Monkey* | | Estrogen treatment | Rat† | |
|---|---|---|---|---|---|
| | Pretreatment control | Day 21 | | Pretreatment control | Day 7 |
| Vehicle | 10 ± 0 | 10 ± 0 | 0.5 μg/100 g BW | 10.7 ± 0.5 | 15 ± 3 |
| $E_2$ control | 10 ± 0 | 113 ± 6 | 0.5 mg pellet | 10.7 ± 0.5 | 2480 ± 632 |
| $E_2$ + mifepristone (0.01 mg/kg) | 20 ± 10 | 140 ± 16 | | | |
| $E_2$ + mifepristone (0.1 mg/kg) | 29 ± 19 | 214 ± 33 | | | |
| $E_2$ + mifepristone (1.0 mg/kg) | 10 ± 0 | 110 ± 33 | | | |

* n = 5 per treatment group.
† n = 3 per treatment group.

creditation of Laboratory Animal Care. These studies were approved by the Animal Care and Use Committee of Eastern Virginia Medical School and carried out according to National Institutes of Health guidelines and US Department of Agriculture regulations.

## Results

*Monkey and Rat Endocrine Analysis*

The pretreatment mean serum $E_2$ levels, as well as levels attained by day 21 in the monkey experiment, are shown in Table 1. All treatment groups receiving an $E_2$ Silastic capsule maintained serum $E_2$ levels in the physiological range. Pretreatment control and day 7 mean serum $E_2$ levels attained by the two different $E_2$ delivery methods in the rodent model are shown in Table 1. Administration of 0.5 μg $E_2$/100 g body weight (BW) maintained daily average serum $E_2$ levels in the physiologic range. In contrast, rats given the 0.5 mg $E_2$ pellet manifested pharmacologic serum levels of $E_2$.

*Antiestrogenic Effects of Mifepristone on Monkey Endometrium*

Histological results showing the effect of mifepristone on monkey endometrium are shown in Table 2. These data show that as the dose of mifepristone was increased from 0.01 mg/kg/day to 1.0 mg/kg/day, mean endometrial thickness decreased ($<0.05$), glandular morphology attained an appearance similar to that in the vehicle control animals, glandular mitotic activity decreased, and histological classification features more closely resembled that of vehicle control primates.

*Antiestrogenic Effects of Mifepristone on the 17β-Estradiol-Primed Rat Uterus*

In the first rat experiment, the antiestrogenic effects of mifepristone were assessed in animals treated with 0.5 μg $E_2$/100 g BW/day. The uteri of rats from the $E_2$ control group showed a fourfold increase in both uterine wet weight ($<0.05$; Figure 1A) and blotted weight ($<0.05$; Figure 1B) in comparison to the vehicle control group. The uterine wet and blotted weight values from all mifepristone-treated groups did not differ from their respective $E_2$ control group ($>0.05$), indicating that mifepristone did not affect the change in uterine wet weight or uterine blotted weight induced by 0.5 μg $E_2$/100 g BW.

Because a previous report suggested antiestrogenic effects of 1.5 mg mifepristone in immature rats treated with a 0.5 mg $E_2$ pellet, conflicting with these results showing that mifepristone did not induce antiestrogenic effects in rats treated with 0.5 μg

**Table 2.** Histological classification of the Cynomolgus endometrium developed under different steroid treatments

| Group (n = 3) | Thickness (mm ± SEM) | Glandular morphology | Glandular mitotic activity* | Stromal mitotic activity† | Histological classification |
|---|---|---|---|---|---|
| Vehicle | 0.92 ± 0.1‡ | Small rounded | 0/10 | 0/10 | Atrophic |
| $E_2$ control | 2.83 ± 0.3§ | Dilated tubular | 19/10 | 0.3/10 | Midproliferative |
| $E_2$ + mifepristone (0.01 mg/kg) | 2.11 ± 0.2§ | Tubular | 10/10 | 0.3/10 | Midproliferative |
| $E_2$ + mifepristone (0.1 mg/kg) | 1.64 ± 0.1‡§ | Small rounded | 3/10 | 0/10 | Weak proliferative |
| $E_2$ + mifepristone (1.0 mg/kg) | 0.67 ± 0.1‡ | Small rounded | 1.7/10 | 0/10 | Very weak proliferative |

Animals were treated with either a 3 cm $E_2$-containing Silastic implant or a vehicle-containing implant for 21 days. Daily injections of mifepristone (at doses indicated) began on day 1 and were contained for an additional 19 days. Uterine biopsies were obtained on day 21.
* Glandular mitotic activity = average number of mitotic cells/10 glands.
† Stromal mitotic activity = average number of mitotic cells/10 fields; 400×.
‡ Means with different superscripts are significantly different ($<0.05$).

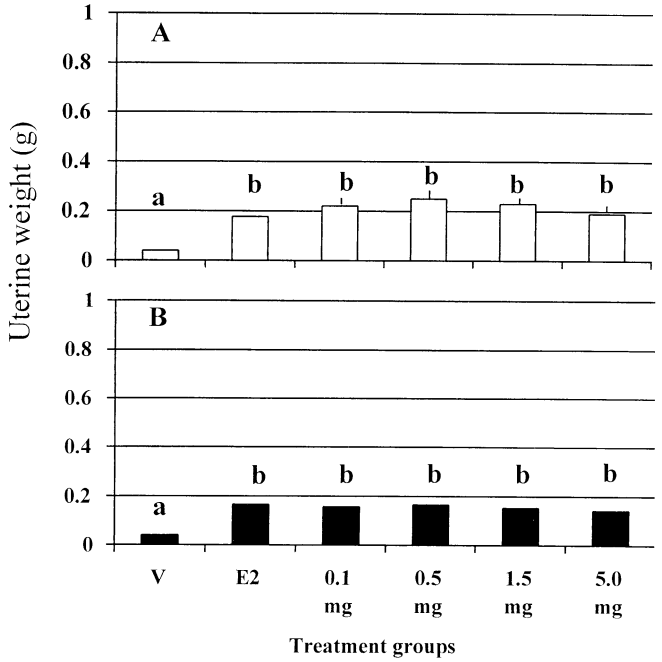


**Figure 1.** Uterine weight values attained in immature rats given 0.5 μg $E_2$/100 g BW/day and treated with mifepristone. Treatment groups are: vehicle control (V); estradiol control ($E_2$); $E_2$ + 0.1 mg mifepristone; $E_2$ + 0.5 mg mifepristone; $E_2$ + 1.5 mg mifepristone; and $E_2$ + 5.0 mg mifepristone. Each column represents the mean + SEM for five determinations. Columns with a different superscript are significantly different (<0.05). Animals treated with mifepristone did not show a decrease in uterine wet or blotted weights in comparison to their respective $E_2$ control group. Columns without bars indicate that the SEM is within the column. □, wet weight; ■, blotted weight.

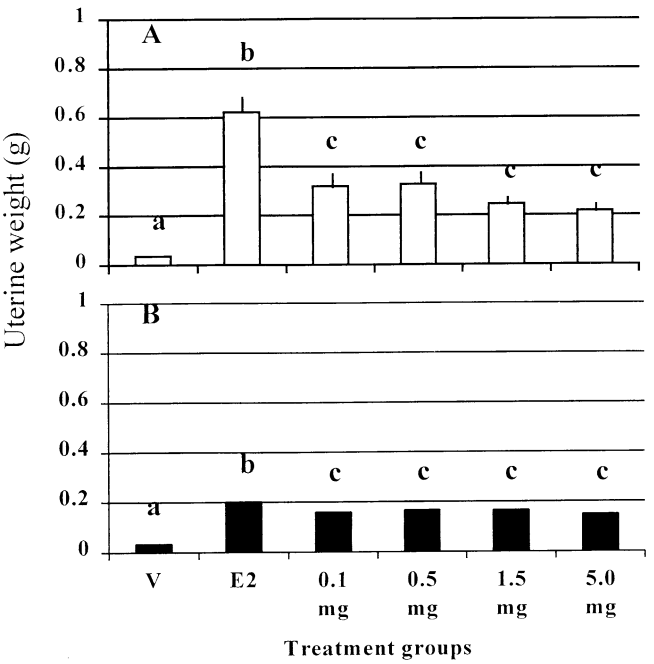


**Figure 2.** Uterine weight attained in immature rats given a 0.5 mg $E_2$ pellet and treated with mifepristone. Treatment groups are: vehicle control (V); estradiol control ($E_2$); $E_2$ + 0.1 mg mifepristone; $E_2$ + 0.5 mg mifepristone; $E_2$ + 1.5 mg mifepristone; and $E_2$ + 5.0 mg mifepristone. Each column represents the mean + SEM for five determinations. Columns with a different superscript are significantly different (<0.050. The $E_2$-treated group showed a 15-fold increase in uterine weight in comparison to the vehicle control group. This estrogen-induced weight increase was inhibited by treatment with mifepristone. Columns without bars indicate that the SEM is within the column. □, wet weight; ■, blotted weight.

$E_2$/100 g BW, a second experiment was conducted using the experimental model already published.

In the second experiment, immature rats were treated with a 0.5 mg $E_2$ pellet and mifepristone. In these rats, an ~15-fold increase in uterine wet weight and fivefold increase in uterine blotted weight were observed in the $E_2$ group in comparison to the vehicle control. In this experiment, all doses of mifepristone diminished the $E_2$-induced increase in uterine wet weight (<0.01; Figure 2A). Secondly, mifepristone, irrespective of the dose used, decreased uterine blotted weight only slightly (15% in all treatment groups; <0.05; Figure 2B). In addition to the experiments using immature rats, two similar studies were conducted using ovariectomized adult rats (~250 g). In these studies (data not shown), mifepristone did not inhibit the increase in uterine wet or blotted weights induced by either 0.5 μg $E_2$/100 g BW or 0.5 mg $E_2$ pellet (>0.05).

**Table 3.** Number of mitotic figures* (mean ± SEM) measured in ovariectomized immature rats, treated with 17β-estradiol and mifepristone

| Estradiol ($E_2$) replacement | Vehicle control | $E_2$ control | $E_2$ + 0.1 mg mifepristone | $E_2$ + 0.5 mg mifepristone | $E_2$ + 1.5 mg mifepristone | $E_2$ + 5.0 mg mifepristone |
|---|---|---|---|---|---|---|
| Immature rats + 0.5 μg $E_2$/100 g | 0.2 ± 1 | 18 ± 5 | 15 ± 4 | 14 ± 2 | 17 ± 3 | 26 ± 5 |
| Immature rats + 0.5 mg $E_2$ pellet | 0 ± 0 | 3 ± 0.5 | 4 ± 0.5 | 3 ± 0.4 | 3 ± 0.5 | 4 ± 1 |

Animals were pretreated with $E_2$ at 0.5 μg/100 g BW or 0.5 mg pellet alone for 3 days, followed by an additional 3 days of $E_2$ plus mifepristone or $E_2$ alone. Mifepristone-treated groups were not significantly different from their respective $E_2$ control group (>0.05).

* Mean number of cells/tissue cross-section/animal.

**Table 4.** Number of cells with nuclear fragmentation* (mean ± SEM) measured in ovariectomized immature rats, treated with 17β-estradiol and mifepristone

| Estradiol ($E_2$) replacement | Vehicle control | $E_2$ control | $E_2$ + 0.1 mg mifepristone | $E_2$ + 0.5 mg mifepristone | $E_2$ + 1.5 mg mifepristone | $E_2$ + 5.0 mg mifepristone |
|---|---|---|---|---|---|---|
| Immature rats + 0.5 μg $E_2$/100 g | 0 ± 0 | 10 ± 3 | 8 ± 4 | 4 ± 1 | 10 ± 3 | 11 ± 3 |
| Immature rats + 0.5 mg $E_2$ pellet | 0 ± 0 | 0.5 ± 0.4 | 0.5 ± 0.3 | 0.4 ± 0.2 | 0.5 ± 0.3 | 0 ± 0 |

Animals were pretreated with $E_2$ at 0.5 μg/100 g BW or 0.5 mg pellet alone for 3 days, followed by an additional 3 days of $E_2$ plus mifepristone or $E_2$ alone. Mifepristone-treated groups were not significantly different from their respective $E_2$ control group (>0.05).

* Mean number of cells/tissue cross-section/animal.

*Histological Analysis of Rat Uteri*

Uteri from the rat experiments were stained with H&E and histologically examined for number of mitotic figures and cells with nuclear fragmentation. A comparison of these parameters are shown in Tables 3 and 4. Within each experiment, the number of mitotic cells and cells with a fragmented nucleus in the $E_2$ control group were not significantly different from the mifepristone-treated groups (>0.05).

## Discussion

In accord with the efforts of pharmaceutical testing facilities to perform initial drug screening trials using HTS techniques for antiprogestins, experiments herein were conducted to develop a laboratory rodent model as a substitute for nonhuman primates. The immature rat uterus is frequently used by endocrine scientists as a target organ for screening of estrogenic or antiestrogenic compounds. Here this model was compared to a frequently used primate model. The findings suggest that the rat uterine weight bioassay is not an appropriate laboratory model suitable for screening antiprogestins for medical indications in which endometrial tissues grow in response to estrogen.

Results from the nonhuman primate experiment shown here confirmed the dose-response noncompetitive antiestrogenic effect of mifepristone on endometrial proliferation. These data, although not novel, further established the antiestrogenic/antiproliferative nature of mifepristone and allowed a direct comparison to the rat uterine weight model.

Contrary to these monkey data, results from the rat experiments did not illustrate the capability of mifepristone to affect estrogen-induced uterine tissue mitotic activity. In these experiments, the single dramatic effect of mifepristone observed on rat uterine tissue (ie, decreased uterine wet weight) was associated with pharmacologic serum $E_2$ levels, which raises the question of its physiologic relevance. In addition, the number of mitotic cells and cells showing nuclear fragmentation did not differ within the experiment using the 0.5 mg $E_2$ pellet. As such, the slight decrease in uterine blotted weight observed can not be attributed to mifepristone-induced decrease in mitotic activity or increased cell death.

Effects of mifepristone on estrogen-induced endometrial growth appear to be debatable. The experimental results shown here are in agreement with a previous study showing no dose-response effects of mifepristone on rat endometrial tissue.[26] Yet other authors have observed a 17% decrease in uterine wet weight of rats treated with 1.5 mg mifepristone.[23] These data were interpreted as an indication of the capability of mifepristone to inhibit $E_2$-induced increase in uterine growth; however, this assessment was made in the absence of histological evaluation.

In the presence of both physiological and pharmacological serum $E_2$ levels, failure of the rat uterine weight assay to either identify or quantify mifepristone as an antiestrogen in the control of endometrial growth illustrates the nonutility of the rat uterine weight bioassay to screen for lead antiprogestin compounds for potential noncompetitive antiestrogenic activity. From these data it cannot be generalized about other antiprogestins. In summary, the rat uterine weight bioassay, in contrast to the primate uterine assay, is not a valid in vivo model for detecting or quantifying the noncompetitive antiestrogenic/antiproliferative effects of mifepristone. Accordingly, reliance on an appropriate monkey model to demonstrate antiprogestin potency for controlling endometrial growth is recommended before undertaking clinical trials toward this therapeutic endpoint.

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