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Studies in reproductive endocrinology

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values ($r=0.42$, $p<0.01$). Nevertheless, the latter parameter did not add to the prediction of the outcome.

HISTOLOGICAL STUDIES

Pathological aspects of the products of conception were examined in 61 cases. A total of 38 represented spontaneous elimination and 23 were obtained by vacuum extraction. Three subjects eliminated an empty sac and in four the sac showed no fragments of embryo. These seven cases probably represent instances of blighted ova. The remaining patients eliminated fragments of necrotic decidua compacta, gestational sac and embryo.

The histological findings were different whether decidual material was obtained spontaneously or after vacuum aspiration (Fig. 3a & b). In both cases, the decidua compacta showed necrosis and hemorrhage in some areas while the decidua spongiosa and the trophoblastic villi were intact and showed only minimal fibrotic areas. In the RU486 responders, the decidua compacta showed much larger areas of necrosis and diffuse hemorrhagic lakes. Nevertheless, there were small zones where the decidua compacta was intact. In contrast, in the non-responders, both necrotic and hemorrhagic areas were minimal. In contradistinction to the findings observed in normal spontaneous abortion (12), only partial necrosis of the decidua compacta was evident in both responders and non-responders.

FOLLOW-UP

Of the 124 women who received the mailed questionnaire, 25 did not reply. Of the 99 women who replied, 83 had chosen hormonal contraception and had regular bleeding patterns. In the 16 remaining cases, 3 were pregnant again (one requested a further course of RU486), 4 experienced irregular bleedings since the abortion and 3 had one episode of abnormal bleeding. One of the latter subjects, considered initially as a success, had a curettage performed 2 months later because of abnormal bleeding. This showed residual decidual material.

All the responders were very satisfied with the method. Their only criticism was the fact that the process took too long. The non-responders were understandably very disappointed, especially in view of all the publicity received by the drug in the media.

DISCUSSION

Mifepristone or RU486, the antiprogesterone compound, was able to induce abortion in 50 to 86% of early pregnancies, when given within 7 weeks of amenorrhea from the last menstrual period. Although the number of subjects studied is small, the four therapeutic regimens gave different results, 60 and 50% in the two first groups, 86 and 80% in Groups III and IV, respectively. The difference was significant at $p<0.01$. If considered in terms of



FIGURE 3a

Decidual histology in a patient who failed to abort with RU486. The sample was obtained by vacuum aspiration on the 8th week of amenorrhea. The decidua spongiosa appears intact with normal tortuous glands filled with secretion. There are no hemorrhagic areas in this layer and the structure appears intact. (x 100)



FIGURE 3b

Decidual histology in a patient who responded to RU486. The products of abortion were eliminated four days after commencement of therapy, at 46 days of amenorrhea. The decidua compacta contains numerous areas of necrosis (n) and hemorrhagic lakes (h). (x 10)

the total dose administered to the patient, a dose-dependent effect was not evident. Nevertheless, the lowest dose regimen of 50 mg per day for 7 days gave the poorest results. It has also recently been shown that doses as high as 800 mg gave a comparable 85% success rate (5). No dose-dependent effect was found by the authors, whether they used 400, 600 or 800 mg regimens (5).

From previously published studies (3-5,13), it appeared clearly that the earlier the therapy after a missed period, the higher is the rate of effectiveness. We did not find the same results. However, the results of all types of therapy beyond the 7th week of amenorrhea, are very poor (33%) (14).

In many previous studies, if evidence of complete abortion is not present, surgical intervention was undertaken at an early stage. We adopted a more conservative approach and performed serial βHCG levels and ultrasonographies. If a partial abortion was obvious with evident decrease in HCG levels, the completion was obtained within one or two weeks. However, it must be emphasized that this conservative attitude can only be undertaken under careful medical supervision. Indeed, the only patient who required blood transfusion did not return to the clinic between Days 14 and 21. She experienced heavy bleeding during this week. Also patients are unhappy with a long wait. From a practical approach, it could be stated that if HCG levels are above 500 mUI/ml by Day 14, a vacuum aspiration must be undertaken.

When the total group of patients was considered, the stepwise discriminant analysis indicated that the βHCG value and the ovular sac diameter on Day 1 were the best indicators and could predict the final outcome of therapy with 86.4% accuracy. In fact, in all groups, 40 of the 46 patients with an ovular sac of less than 10mm in diameter aborted. The predictive value of the conceptus size was not evident in another study (15). It must be stressed, however, that the conceptus size should be evaluated in mm of the largest diameter and not in weeks of pregnancy, as is usually the case.

Although the number of days of amenorrhea and the E2 levels on Day 1 were significantly different between responders and non-responders, these parameters, as well as P and cortisol, were not useful for predicting the outcome.

The age of pregnancy evaluated in days of amenorrhea was also not a good predictor of the outcome. The exact time of ovulation and implantation in natural conception cycles vary greatly (16). This finding which is observed in women with previous regular cycles stresses the difficulties of generalizing the method to women with previous irregular cycles. Therefore, the evaluation of pregnancy duration according to days of amenorrhea is not accurate. As already mentioned, the conceptus size assessed by the sac diameter measurement by ultrasonography reflects more closely the degree of pregnancy development. Thus, it is not surprising that the sac diameter appeared more accurate than days of amenorrhea for

predicting the outcome in the discriminant analysis. However, a significant correlation was found between these two parameters on Day 1. RU486 measurements were only useful to confirm the actual ingestion of the drug by the patients and were also a poor predictor of the outcome.

Could we find an explanation for the failures?

The mechanism of action of RU486 in termination of pregnancy appears to be mainly a local effect on the endometrium. The competitive inhibition of progesterone at the receptor site (6) leads to sloughing of the endometrium and hemorrhage (17). This endometrial effect has also been observed in HCG-induced pseudo-pregnancy. Increasing doses of HCG cannot overcome the endometrial effect of RU486 and the bleeding response always occurs (18). The antiprogesterone also induces softening of the uterine cervix (4,5), stimulates endometrial prostaglandin secretion which induces myometrial contractions (19).

In the present study the histological changes were confined to the decidua compacta whilst the decidua spongiosa remained unaffected. These findings should be contrasted to those observed in spontaneous abortion where complete necrosis of compacta and spongiosa is usually evident (12).

In the failures, the necrosis of the compacta was far less extensive than in the successes. The reason for the localized necrosis of the compacta layer could be related to its great vascular surface with high drug concentrations in some areas surrounding the vessels. Thus, variations in the distribution of RU486 in the different zones of the endometrium could explain some of the failures.

Since the decidua spongiosa is unaltered by RU486, these cells continue to nourish the blastocyst despite necrosis of the decidua compacta. The absence of necrosis of the spongiosa layer may account for the failure of the drug to uniformly induce abortion. Therefore, the abortifacient properties of RU486 may be optimal if the drug is administered prior to the trophoblast spreading into the spongiosa, i.e. before Day 29 (20). Further studies are needed to confirm this hypothesis. One failure was a very early pregnancy with an initial level of ßHCG of 65mIU/ml and a conceptus size of 12mm. Presumably implantation must have been deep within the decidual endometrium. In this case, high plasma levels of RU486 attest to the fact that RU486 was taken.

The fact that the incidence of abortion was only 60% in Group I, with high RU486 doses, and that a failure occurred in Group III on a lower dose regimen in a very early pregnancy, suggests that an ideal ratio of antiprogesterone to progesterone activity might be important to ensure therapeutic effectiveness of the drug. In this regard it should be noted that RU486 demonstrated progesterone agonistic activities in anovulatory women or in menopausal-estrogen primed women (21), both situations

characterized by progesterone deficiency. Thus, in common with other antihormones, RU486 exerts progestational agonistic effects in the absence of progesterone (21,22). Therefore, high doses of RU486 might exert a partial agonistic effect in the presence of low levels of P. Nonetheless, we found no correlation between plasma levels of RU486 and P.

It is unlikely that failures could be related to inter-individual variations in absorption of the drug, since plasma levels were not different between responders and non-responders. However, there could have been differences in the metabolism of RU486 in the two groups. After oral absorption, RU486 is metabolized to mono- and di-demethylated as well as hydroxylated compounds. These have lower relative binding affinities to the progesterone receptor (23). Forty-eight hours after ingestion of a single dose, these metabolites reach higher plasma concentrations than the parent compound (23). A lower level of active compounds could have been responsible for the failures. RU486 metabolites were not measured in our study.

Side effects of RU 486 have been recorded in this study, as well as in previous series (3-5,13). The most common side effects were nausea and fatigue, which are also observed in the early stage of a normal pregnancy. Abdominal cramps were observed in 20.9% of the women and reflect the abortive process. When compared to prostaglandin-induced abortion, this side effect is far less frequent with RU486 (24). The most severe problem observed in this and in other studies was the severe bleeding which occurred in 15.3% of the women. The analysis of these cases did not show any difference in age of pregnancy, initial cortisol or other hormonal values.

Cortisol levels rose with the four therapeutic regimens, although the increase was higher in Group I. Indeed, in the latter group, the rise exceeded the normal morning cortisol range. A dose-dependent rise in cortisol has been documented in human studies and is presumably related to the antiglucocorticoid effect of the drug at the receptor level (7). As a consequence of the receptor blockage, ACTH and cortisol production increase (7). However, this increase in cortisol was of short duration and the levels returned to basal values with termination of treatment. Despite the rise in cortisol, there was no clinical or biochemical evidence of hypocortisolism. The decrease in blood pressure observed in 4 cases was apparently related to heavy bleeding and not due to glucocorticoid deficiency. Moreover, 10 days' administration of RU486 to dogs, showed that adrenal function is retained, despite the rise in cortisol (25).

Notwithstanding the ethical problems related to abortion, it is acknowledged that a medical method of pregnancy termination which is successful in 80% to 90% of subjects is of great interest in view of the 40 to 50 million abortions reported yearly worldwide (26). Nevertheless, regarding the prolonged bleeding observed in some women and the risk of failure despite the onset of bleeding,

the use of this antiprogesterone compound should be presently restricted to centers where close medical supervision and adequate medical health services are available.

Recent studies indicate that addition of a small dose of prostaglandins to a single dose of RU486 greatly increases the efficacy and abortion occurs in about 95% of users (27). This combined therapy is therefore recommended in further studies.

However, our findings suggest that ultrasonography can be used as a screening test and detect the cases where the sac diameter is under 10 mm. In those cases, the doses of RU486 given in Groups III and IV gave a 98% success rate and therefore no prostaglandin addition should be required in those instances. On the other hand, when the sac diameter is equal to or over 10 mm, the addition of prostaglandin might be required.

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Effects of the antiprogesterone RU 486 in normal women

II. Administration in the late follicular phase

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RU 486, a synthetic steroid with antiprogesterone receptor activity, was used to investigate the importance of progesterone on gonadotropin secretory dynamics in the midcycle of the normal menstrual cycle. Six normally cycling women were followed for three consecutive cycles. During each cycle, blood samples were obtained beginning on day 10 and continued until menses. After a control cycle, 100 mg RU 486 was given orally between days 10 and 17. The patients were followed for a posttreatment cycle with no medication. When RU 486 was given before the midcycle, the luteinizing hormone surge was delayed by 15.0 ± 2.1 days after ingestion of the last pill, resulting in cycles of 40.6 ± 2.6 compared with 28.0 ± 2.3 days ($p < 0.01$). During RU 486 administration and at the time a normal luteinizing hormone surge was anticipated, an attenuated luteinizing hormone/follicle-stimulating hormone surge was noted that was not followed by a rise in progesterone. After the attenuated surge a normal luteinizing hormone/follicle-stimulating hormone level occurred, with a normal rise in progesterone. Estradiol levels during RU 486 administration decreased during treatment, indicating a possible direct action of RU 486 on the ovary. (AM J OBSTET GYNECOL 1987;157:1421-6.)

Key words: RU 486, menstruation, gonadotropin

The precise mechanisms that underlie the processes of ovulation are not known, but two factors play important roles. The rapid rise of estrogen through a positive feedback action on the hypothalamic-pituitary unit triggers the midcycle surge. Also, the small increase in preovulatory progesterone levels[1] appears to amplify the pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) at midcycle.[2-4] RU 486, a synthetic steroid with antiprogesterone receptor activity,[5] was used to assess the importance of progesterone on midcycle secretory dynamics.

Material and methods

Six women ages 21 to 34 years with cycle length 28 ± 2 days were followed for 3 consecutive cycles. During each cycle, blood was drawn starting on day 10 and continued daily until the next menses. Bleeding records were kept during the study period. After the pretreatment cycle, 50 mg of RU 486 was administered twice a day (50 mg tablets supplied by Roussel-Uclaf, Romainville, France) from day 10 through 17. The selection of this dose was based on a dose-response study.[6] During the posttreatment cycle the patient received no medication, but blood sampling continued as in the two previous cycles.

Blood samples were allowed to clot and sera were separated and stored at −20° C until assayed for LH, FSH, estradiol (E_2), and progesterone by previously reported radioimmunoassay.[7-9] Computer assistance by ClinFo program and BMDP Statistical Software[10] was used for statistical analysis with area under the curve, the Kruskal-Wallis nonparametric test, the paired t test, and one- and two-way analysis of variance when appropriate. The area under the curve was determined by the trapezoidal rule and included values on days −2 through +2 after the LH surge for determination of LH and FSH, days −2 through 0 for E_2, and days 0 through +14 for progesterone.

Results

Administration of RU 486 during the late follicular phase delayed development of a normal LH surge an average of 15.0 ± 2.1 days from the time of the last tablet (range 10 to 21 days). This resulted in a follicular phase of 28.0 ± 2.3 days, which was significantly longer than the follicular phase in the control cycle of 14.6 ± 1.3 days ($p < 0.005$). During the treatment

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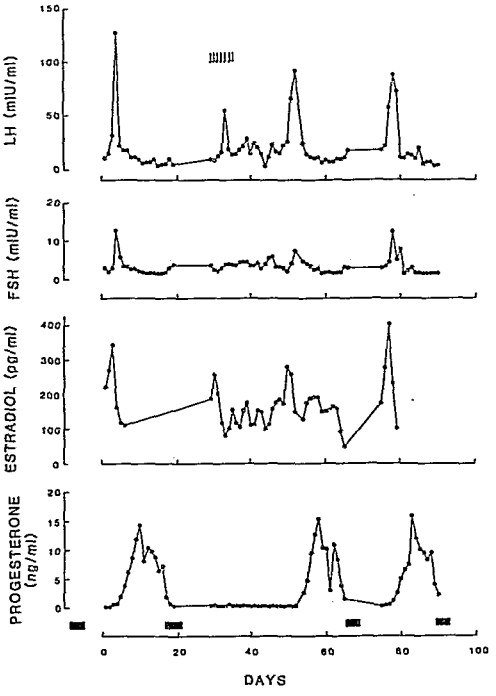


Fig. 1. LH, FSH, and progesterone values over the 3-month study period in a typical patient. *Arrows* indicate treatment days and *solid bars* indicate bleeding days. An attenuated surge occurs during the time of RU 486 administration and is followed by a normal LH surge.

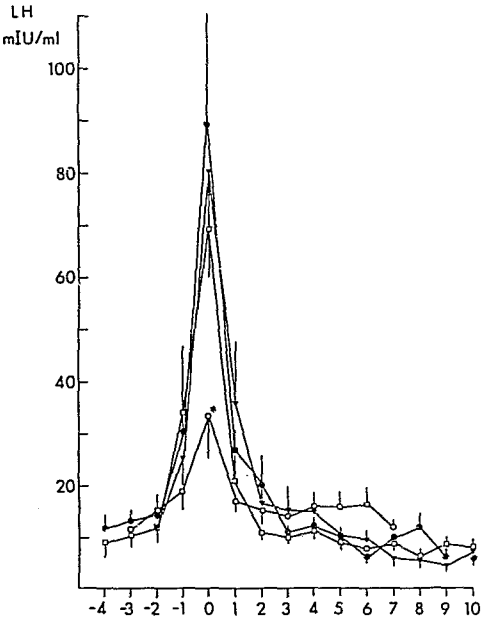


Fig. 2. LH values normalized to the day of the LH surge during the pretreatment month (▲), the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). The values of the attenuated surge are significantly lower on day 0 than before treatment ($p < 0.05$).

month, the interval between the normal LH surge and the occurrence of menses was 13.8 ± 1.2 days and not different from the pretreatment luteal phase length. The total treatment cycle length after RU 486 administration averaged 40.6 ± 2.6 days, compared with 28.0 ± 2.3 days during the pretreatment cycle ($p < 0.01$; Table I).

The bleeding pattern and serum LH, FSH, progesterone, and E_2 values of a representative subject are shown in Fig. 1. In the pretreatment cycle shortly after blood sampling began, the normal LH surge was followed by a rise in serum progesterone levels compatible with a normal luteal phase pattern. The length of this subject's pretreatment cycle was 29 days. During the following cycle RU 486 was administered during days 10 through 17. On day 14 into the treatment cycle, and during the time an LH surge was expected, an attenuated LH surge was noted that was smaller than the normal LH surge of the pretreatment cycle and was not followed by any rise in progesterone levels.

Nineteen days after the attenuated surge, a normal LH surge was noted that was followed by a rise in serum progesterone compatible with a normal luteal phase. There was no vaginal bleeding between the time of the attenuated surge and the treatment normal surge. The next bleeding episode occurred after the fall in serum progesterone, and this resulted in a total cycle length for the treatment cycle of 49 days. During the posttreatment cycle, a normal LH surge occurred on day 13 and the total cycle length was 26 days. All six patients demonstrated similar patterns.

Fig. 2 shows the mean (±SEM) LH values for all six patients normalized to the day of the LH surge (day 0). The LH surge of the pretreatment cycle, the treatment normal surge, and the surge during the posttreatment cycle are similar in magnitude. However, the attenuated surge occurring during RU 486 administration was significantly less. Kruskal-Wallis one-way analysis of variance detected LH values on day 0 to be significantly lower than control values ($p < 0.05$). These data were also confirmed by calculation of areas under the LH curve (Fig. 3).

Similar data for FSH are shown in Figs. 3 and 4. Values of FSH on days 0 and +1 were significantly smaller ($p < 0.001$) during the attenuated surge com-

pared with pretreatment values as determined by one- and two-way analyses of variance. Calculation of the areas under the FSH curves also confirmed these findings (Fig. 3).

E_2 values normalized to the day of the LH surge are shown in Fig. 5. When taken together, values on days -1 and 0 were significantly less ($p < 0.005$) than pretreatment values. Areas under the curve for E_2 during the attenuated surge were significantly less than the pretreatment, normal treatment, and posttreatment surge values.

Fig. 6 shows the progesterone values normalized to the day of the LH surge. Progesterone values preceding the attenuated surge were significantly lower ($p < 0.01$) on days -1 and 0 compared with pretreatment and posttreatment values when analyzed by analysis of variance. Progesterone levels from days 1 through 8 after the attenuated LH surge were also significantly lower ($p < 0.01$) than pretreatment values. However, once a normal LH surge occurred after RU 486, it was followed by a normal rise in progesterone during the next 14 da. The total area under the progesterone curve after the treatment normal surge was not different from the values after the pretreatment or posttreatment surges, whereas the area under the curve after the attenuated surge was significantly less ($p < 0.01$).

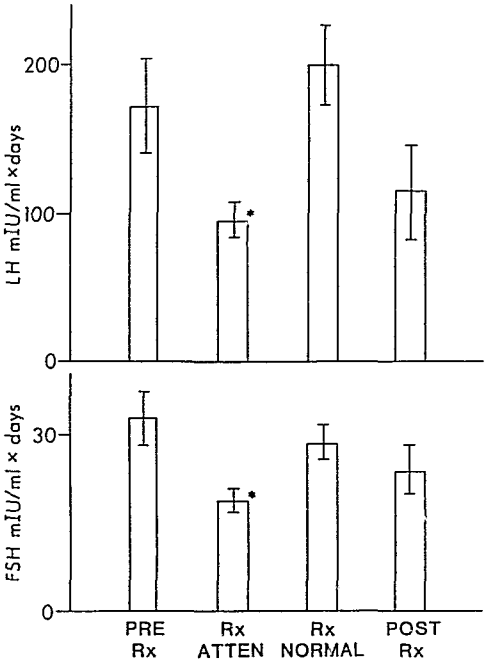


Fig. 3. Areas under the LH and FSH curve in the pretreatment month *(PRE-Rx)*, during the attenuated LH surge *(Rx ATTEN)*, in the normal LH surge occurring during the RU 486 treatment cycle *(Rx NORMAL)*, and in the posttreatment month *(POST Rx)*. $*p < 0.05$.

Comment

Administration of RU 486 during days 10 through 17 of a normal menstrual cycle prevented the development of a normal LH/FSH surge and delayed formation of a new surge by an average of 15 days. This confirms the ability of RU 486 to effectively block the midcycle gonadotropin surge, delay follicular development, and prolong menstrual cycle length.[11-14] This effect is most probably related to its antiprogesterone activity and not its antiglucocorticoid activity, as this effect is not altered by concomitant administration of dexamethasone in primates.[13] Our data support the possibility that progesterone antagonism affects the hypothalamic-pituitary axis by inhibiting the normal positive effect of progesterone on gonadotropin secretory dynamics at midcycle. RU 486 has been reported to have a mild progestational effect, and similar to the suppressive effect of progestins in oral contraceptives, the effect of RU 486 on midcycle gonadotropin may be due to progesterone agonism.[13] However, the dominant effect of RU 486 is that of an antiprogesterone, and it is more likely that suppression of the LH surge is due to progesterone antagonism.

In addition, there was a significant reduction in E_2 and progesterone levels before the day of the attenuated LH/FSH surge. These data suggest that RU 486 may have a direct inhibitory effect on ovarian steroidogenesis. In vitro studies support a direct action of RU 486 on ovarian E_2 and progesterone production.[15, 16] However, it may also be the case that lower FSH levels inadequately stimulate ovarian E_2 production. A decline in FSH levels after RU 486 was noted in this study and in other studies.[6]

The 15-day delay from the time of the last tablet to the onset of a normal LH surge suggests that recruitment of another dominant follicle is required to initiate ovulation after what appears to be ablation of the dominant follicle by RU 486 administration. This is supported by recent ultrasonographic studies of follicular development after RU 486.[14] However, it is plausible that the dominant follicle may not be ablated as such and may merely be arrested temporarily and undergo spontaneous recovery after RU 486 administration ceases. Further ultrasonographic studies may better characterize follicular development.

Successful blockage of the LH/FSH surge has been reported in humans[11] and in monkeys[12, 13] when treated in the late follicular phase. These data confirm the abil-

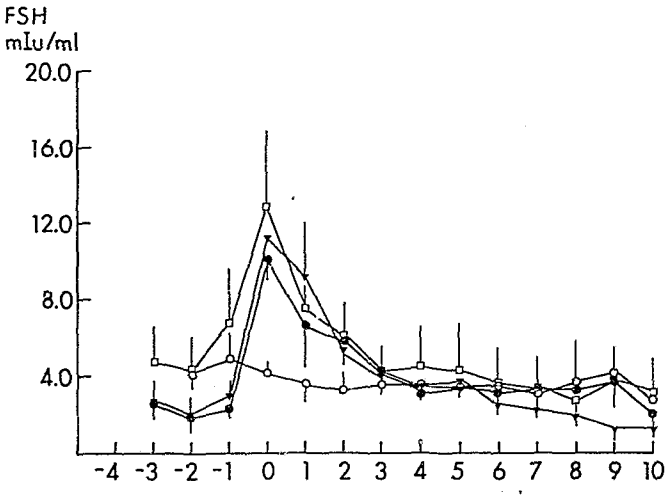


Fig. 4. FSH values normalized to the day of the LH surge during the pretreatment month (▼) the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). FSH values on days 0 and +1 were significantly less ($p < 0.001$) during RU 486 treatment than before treatment.

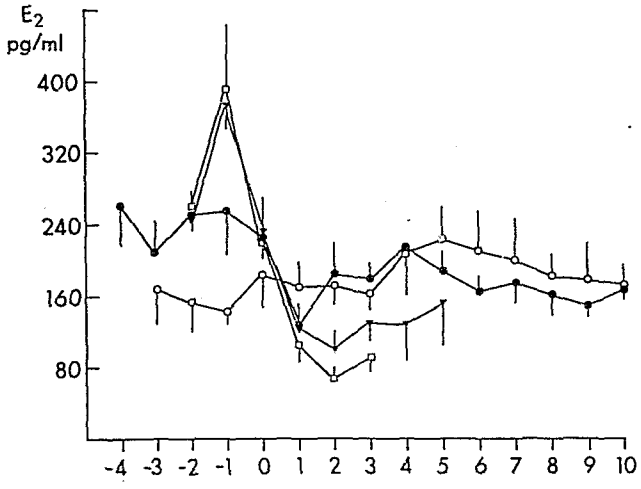


Fig. 5. E_2 values normalized to the day of the LH surge during the pretreatment month (▼), the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). E_2 values on days −2, −1, and 0 were significantly less ($p < 0.005$) during the attenuated response than at any other time.

ity of RU 486 to suppress the midcycle LH/FSH surge and support its development as a contraceptive agent. However, whether the mechanism for this action of RU 486 is primarily due to its suppressive effect on the ovary[11, 15, 16] or to progesterone antagonism at the hypothalamic-pituitary axis will require further study.

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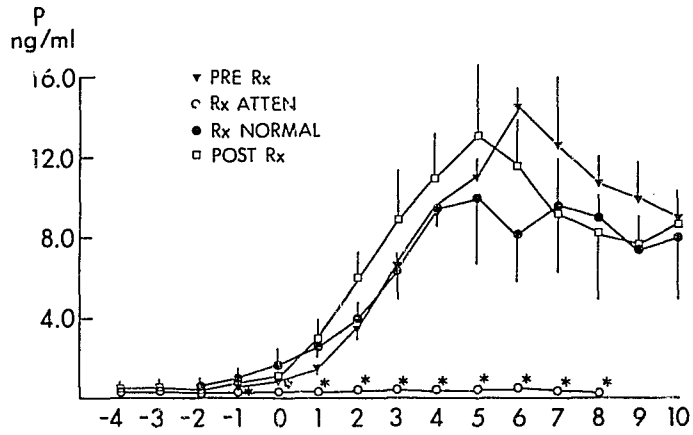


Fig. 6. Progesterone (*P*) values normalized to the day of the LH surge during the pretreatment month (▼), the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). Progesterone levels from days −1 and 0 during the attenuated response were significantly lower ($p < 0.01$) compared with pretreatment and posttreatment values. Progesterone levels from days 1 through 8 after the attenuated LH surge were also significantly lower ($p < 0.01$) than pretreatment values.

Table I. Effect of RU 486 on follicular and luteal phases of the normal menstrual cycle

	Month 1	*Month 2* (treatment month)*	*Month 3*
Total length of cycle	28.8 ± 0.7	40.6 ± 2.6†	25.2 ± 0.9
Follicular phase	14.6 ± 1.3	28.0 ± 2.3‡	13.5 ± 1.9
Luteal phase	15.2 ± 0.8	13.8 ± 1.2	13.5 ± 4.0

Data are $\bar{x}$ ± SEM.
*Discounting the attenuated LH surge.
†$p < 0.01$.
‡$p < 0.005$.

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Response to intermittent RU486 in women*†‡

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Objective: To determine the effects of intermittent administration of the antiprogestin RU486 on ovarian function.

Design: Three different regimens of RU486 were tested.

Participants: Nine healthy regularly menstruating volunteers protected by an intrauterine device or surgical sterilization.

Interventions: Two groups of three women each received 10 mg or 50 mg RU486 at weekly intervals for 5 weeks. Another three women received 50 mg RU486 for 3 consecutive days at 10-day intervals for 80 days.

Main Outcome Measures: Serum E_2, P, and RU486 levels. Ovarian ultrasound (US) and serum LH and FSH in select subjects.

Results: The predominant effect was partial inhibition of E_2 secretion and suppressed P levels. During a total aggregate of 16 treatment months, there were seven episodes of elevated P levels; however, US did not always indicate the occurrence of normal ovulation.

Conclusion: Intermittent RU486 administration can interfere with normal follicular development and function, but its clinical application may require a more effective dose and/or timing of administration. Fertil Steril 1993;59:971–5

Key Words: RU486, mifepristone, intermittent administration, follicular development, E_2 and P responses, ultrasonography

Administration of the antiprogestin RU486 to women in the midfollicular or late follicular phase of the menstrual cycle delays the LH surge and postpones ovulation. Estradiol levels fail to increase, and follicular development is slowed down or arrested. Collapse of the dominant follicle has also been observed. After cessation of RU486, there is resumption of follicular growth or new follicular recruitment (1–4). The prolongation of the follicular phase varies from 10 to 14 days. It has been shown that neither the precise amount of RU486 (administered in doses ranging from 25 to 100 mg) nor the time period of its administration during the midfollicular or late follicular phase appears to be critical for the delay in LH surge to be manifest. The time interval from ingestion of the last pill to the LH surge remains remarkably constant (4).

In view of its ability to arrest follicular development and delay the LH surge, attempts have been made to develop RU486 as a contraceptive agent.

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To be successful, such a regimen must be administered either continuously or at regular intervals to prevent the emergence of a dominant follicle.

An intermittent RU486 regimen has been used to suppress ovulation in monkeys, and a clear dose-response effect emerged. Administration once per week of 25 mg by the oral route blocked the expected midcycle LH and FSH surge, and P levels remained undetectable. Progesterone inhibition, however, was not evident when half the dose of RU486 was used (5). We have evaluated a similar protocol in normal women. Unlike the report in monkeys, however, we observed a wider range of responses in normal women.

MATERIALS AND METHODS

Subjects

A total of nine normal weight Chilean women whose ages ranged from 26 to 36 years were studied.

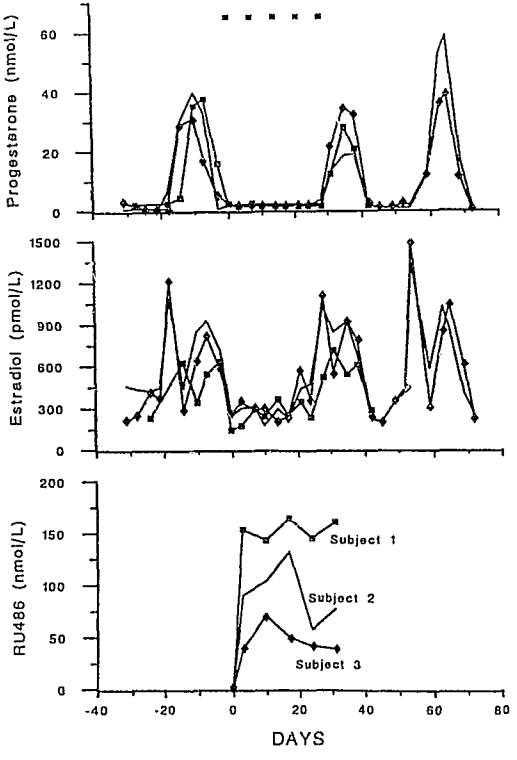


Figure 1 Response to once weekly administration or RU486 (10 mg) in subjects 1, 2, and 3. Serum levels of P, E_2, and RU486 are shown in the upper, middle, and lower panels, respectively.

They all had regular menstrual cycles of 26 to 36 days and were using intrauterine devices for contraception or had been sterilized. The study was approved by the Investigation Review Board of both the Population Council and the Chilean Institute of Reproductive Medicine, and all subjects gave signed informed consent. The subjects were reimbursed for the cost of transportation but received no other financial remuneration. The subjects were divided into three groups. Groups 1 and 2 received 10 mg or 50 mg RU486, respectively, at weekly intervals for 5 weeks. Each tablet was taken under supervision at the clinic. Group 3 subjects were given 50 mg/d RU486 for 3 consecutive days every 10 days. This regimen was repeated on eight occasions. Tablets were given to the subject to take at home. In all subjects, treatment was initiated on days 1 to 3 of the menstrual cycle. In subjects from groups 1 and 2, blood samples were taken immediately before RU486 administration and then after 3 days. Blood samples were also taken two times per week for 1 month before and after the treatment period. In group 3 subjects, blood samples were taken every 2 to 3 days during treatment and thereafter at variable intervals for 40 to 50 days. On those days when RU486 was administered, blood samples were taken before tablet ingestion. In subjects from group 3, follicular diameter was measured by ultrasound on each occasion when a blood sample was withdrawn, and an endometrial biopsy was taken on the day after ingestion of the last tablet.

Methods

Luteinizing hormone, FSH, E_2, and P determinations were performed using reagents and methods supplied by the World Health Organization (6). The intra-assay coefficients of variation (CVs) for high values ranged from 6% to 8% and the interassay CVs from 12% to 18%. Circulating levels of RU486 were determined by RIA preceded by column chromatography as previously described (7). Follicular growth was monitored by pelvic ultrasonography using an ADR 4000 SC Sector Model Ultrasound System with a 3.0-MHZ endfire abdominal transducer (Advanced Technology Laboratories, Inc., Bellevue, WA). Only measurements of the leading follicle were considered relevant and are reported.

RESULTS

None of the subjects had any untoward effects consequent to RU486 administration. Compared with the pretreatment and post-treatment control

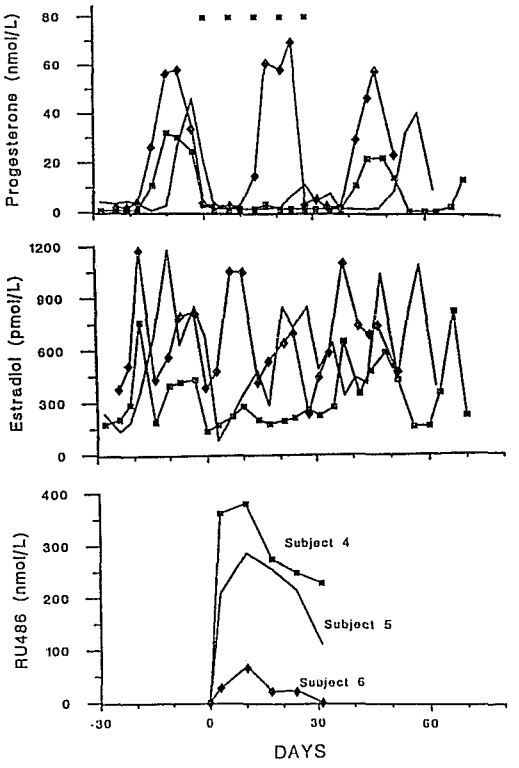


Figure 2 Response to once weekly administration of RU486 (50 mg) in subjects 4, 5, and 6. Serum levels of P, E_2, and RU486 are shown in the upper, middle, and lower panels, respectively.

cycles, E_2 levels were decreased in group 1 subjects during RU486 administration. Progesterone levels also failed to rise (Fig. 1). However, 72 hours after the last dose of RU486, at a time when circulating RU486 levels in the three subjects ranged from 37.3 nmol/L to 161.7 nmol/L (16.0 ng/mL to 69.4 ng/mL), P levels had increased and rose progressively to reach values compatible with ovulation and corpus luteum (CL) function. This was preceded by increased E_2 levels on the day of the last dose of RU486 administration in all three subjects. Had the protocol design been extended for another week to encompass an additional dose of RU486, P elevation would have been apparent before the last administration of RU486. These results indicate that luteal transformation of the dominant follicle could not be postponed longer than 29 days by this RU486 regimen.

In subject 4 from group 2, E_2 levels were decreased. Progesterone levels were suppressed during treatment and only increased 14 days after the last dose of RU486 administration (Fig. 2). Subject 5 had a minimal and short-lived increase in P levels at the very end of treatment, indicative of defective luteinization. In subject 6, however, E_2 levels were not suppressed and normal luteal phase P levels, suggestive of ovulation and CL function, were observed during the period of RU486 administration (Fig. 2). Maximum circulating RU486 levels measured in subjects 4, 5, and 6 were 379.8 nmol/L, 284.3 nmol/L, and 68.9 nmol/L, respectively.

In subjects 7 and 8 in group 3, E_2 oscillated within midfollicular or late follicular phase levels, and P was suppressed throughout treatment (Fig. 3A and B). In the same period, the growth of the dominant follicle was arrested for approximately 2 weeks after reaching a diameter of 10 to 15 mm in subject 7 (Fig. 3A) or 20 to 25 mm in subject 8 (Fig. 3B). After collapse of the dominant follicle, a new follicle emerged and followed the same pattern in the opposite ovary either immediately or after a quiescent period. Luteinizing hormone surges occurred 14 and 16 days after the last dose of RU486 and this was followed by a rise of P. Subject 9 (Fig. 3C) presented an LH surge around day 30 of treatment, which was preceded by a rise in E_2 and was followed by an elevation of P levels. Ultrasound showed increasing diameter of the follicle up to 40 mm with an abrupt decrease to 25 mm on the day preceding the LH surge without disappearance of the echonegative image throughout the luteal phase. This subject had another increase in P up to 20 nmol/L (6.29 ng/mL) immediately after the final administration of RU486. This episode was preceded by the growth of a follicle up to 38 mm without a concomitant increase in E_2 levels. No distinct LH surge was detected before the P elevation and disappearance of the echographic image of the follicle. In these three subjects circulating RU486 levels measured 24 hours after pill intake ranged from 725 to 6,300 nmol/L (311 to 2,704 ng/mL).

The endometrial biopsy taken on day 73 of treatment revealed an inactive endometrium in subjects 7 and 8 with very few and straight glands, low epithelial cells, and edematous undifferentiated stroma. In subject 9, P levels were just beginning to increase on the day the biopsy was taken. Glands were tortuous with a wide lumen, the epithelium was pseudostratified with patches of ciliated and secretory cells, and the stroma presented irregular and incomplete differentiation.

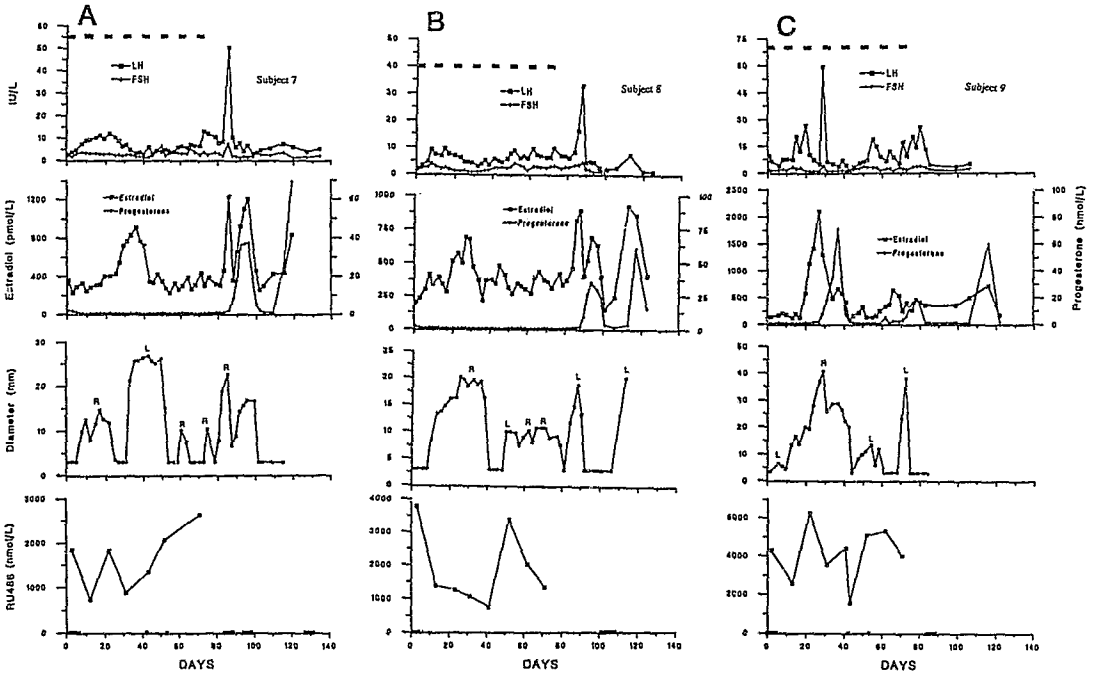


Figure 3 Response to RU486 (50 mg/d for 3 days) administered every 10 days in subject 7 (A), subject 8 (B), and subject 9 (C). Serum LH and FSH, E_2 and P, follicular diameter, and serum RU486 levels are shown, respectively, from top to bottom. L and R indicate dominant follicular development in left and right ovary, respectively. Black bars on the x axis indicate episodes of menstrual bleeding.

DISCUSSION

In this study, variable responses to RU486 were observed. These ranged from suppression of luteal phase P levels to endocrine profiles characteristic of ovulatory menstrual cycles, but which were not uniformly associated with ultrasonographic images of normal follicular development and ovulation. These results should be contrasted with those obtained in the monkey in which consistent inhibition of ovulation occurred and a dose-response effect was clearly manifest (5). Weekly administration of 10 mg RU486 to normal women appears to be at the threshold of being effective for suppression of follicular development. Indeed, follicular development was delayed, but luteinization was evident despite the last dose of RU486 administration. Treatment with 50 mg RU486 once per week suppressed follicular development only in the two subjects with the highest circulating RU486 levels. These results suggest that suppression of follicular development might be dependent on the dose and circulating levels of RU486.

It is possible that variations in RU486 bioavailability could account for the variable degree of ovarian suppression. The protocol was not designed for detailed pharmacokinetic analysis, and it is not possible to draw conclusions on any relationship between RU486 levels and biological responses with the 3-day regimen. However, with the highest dose schedule, there was still an endocrine profile compatible with CL function in subject 9 who showed the highest circulating RU486 levels.

It has been previously demonstrated that 50 mg RU486 for 3 consecutive days administered in the mid or late follicular phase did indeed postpone ovulation (4). Moreover, Batista et al. (8) have shown that even as low a dose of RU486 as 1 mg administered daily for 5 days in the preovulatory phase after ultrasonic evidence of development of the dominant follicle was able to successfully delay and abolish the LH surge and ovulation. Hence it is unlikely that the primary reason for the failure of RU486 to inhibit gonadotropic and ovarian function consistently is due to insufficient dosage. In the

study of Batista et al. (8), when the treatment was extended to 15 days, RU486 could not uniformly suppress follicular development and an LH/FSH surge occurred in four of the six women. Therefore the RU486 dose of 1 mg/d seems to be a threshold dose for effectively suppressing ovulation. Serum RU486 concentrations were not determined in this study (8).

A further reason for the failure to obtain consistent inhibition of ovarian function could be related to differences in drug metabolism in the women. This has been observed in studies on the antiglucocorticoid activity of RU486 in dogs (9) and could also apply to our subjects because there were marked differences in RU486 levels among the women in each group. The levels of RU486 observed in subjects from groups 1 and 2 were, nevertheless, in accordance with the half disappearance time of RU486 of 20 to 25 hours (10). Finally, mutation in the P receptor could also account for the variable response (11).

Despite the increases in P, it is not possible to conclude that ovulation occurred during treatment. The rise could have been a consequence of luteinization of an unruptured follicle. In the one subject from group 3 who demonstrated P elevation during treatment, the ultrasonography was not typical of ovulation.

During RU486 administration, E_2 levels were in the early follicular phase range in subjects from groups 1 and 2. However, in group 3 subjects, E_2 levels were higher and equivalent to those observed in the midfollicular or late follicular phase. This may lead to manifestations of unopposed estrogen action; however, this assumption was not substantiated by endometrial biopsies taken at the end of the treatment period.

The present results indicate that intermittent RU486 administration is unable to produce a consistent inhibition of ovarian function. However, because of the long half-life of RU486, it is able to temporarily suspend progression of the follicular phase. It may also cause follicular dysfunction, which according to echographic and endocrine data manifests either as cystic growth, failure to rupture, or premature or insufficient luteinization. The changes in ovarian function with intermittent administration of RU486 are reminiscent of those seen with progestin-only minipill contraceptive regimens. The contraceptive efficacy of progestin-only contraceptives relies on the thickening of cervical mucus and possible endometrial asynchrony when they fail to inhibit ovulation. Whether intermittent RU486 administration aiming to suppress ovarian function will also have potential as a contraceptive by additional backup mechanisms remains to be proven.

Acknowledgment. The RU486 used in this study was kindly provided by Roussel UCLAF, Paris, France.

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VARIABLE EFFECTS OF RU 486 ON ENDOMETRIAL MAINTENANCE IN THE LUTEAL PHASE EXTENDED BY EXOGENOUS hCG*

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SUMMARY

This study was designed to assess the features and conditions for endometrial bleeding induction with the synthetic antiprogestin and antiglucocorticoid RU 486 during hCG-induced prolongation of the luteal phase. Eighteen healthy, surgically sterilized women and another five women with an intrauterine contraceptive device (IUD) participated. All subjects received hCG which was injected daily in increasing doses (500 to 15 000 IU) from day 9 to day 15 of the luteal phase. Ten subjects received hCG alone, and groups of three to 16 subjects received hCG combined with RU 486 (25, 50, 100, 200 or 400 mg/day). RU 486 administration was commenced on day 12 following the LH surge and given either for 1, 4 or 7 consecutive days. In certain cycles, tamoxifen (20 mg/day) was given for 4 consecutive days with hCG, or with hCG and RU 486. All treatment cycles were separated by one or two resting cycles. Frequent blood samples were taken to monitor the endocrine response.

Treatment with hCG alone or with the various combinations of RU 486 produced similar serum levels of oestradiol and progesterone which were equivalent to those observed during early pregnancy. With hCG alone, the onset of bleeding was on day 21–24 after the LH surge, coinciding with the drop in oestradiol and progesterone. With RU 486 doses of 50 mg/day or more, an early bleeding episode almost invariably occurred on day 14–17 after the LH surge in the presence of high circulating steroid levels. In contrast, 25 mg/day RU 486 for 4 days failed to induce this early onset of bleeding in three out of six cases. Tamoxifen did not cause early bleeding and failed to potentiate the effect of RU 486. In 43% of the cycles treated with RU 486, a second bleeding episode

* These results were presented in part in abstract form at the 7th International Congress of Endocrinology, 1984 and at the 67th Annual Meeting of the Endocrine Society, 1985.

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occurred on days 22–24 after the LH surge coincident with the drop of steroids. This late bleeding was less frequent (17%) in the subgroup receiving a total dose of 700 or 800 mg RU 486. In those cycles that exhibited a second bleeding episode coincident with the fall in progesterone, endometrial biopsies, taken shortly after the initial early bleeding had stopped, showed a mixed proliferative and secretory endometrium, suggestive of incomplete shedding. In contrast, only proliferative endometrium, compatible with complete shedding, was evident in cycles characterized by the absence of a second bleeding episode. It is concluded that during maintenance of corpus luteum function with exogenous hCG, RU 486 can induce endometrial bleeding despite high circulating progesterone and oestradiol levels. With the lower doses of RU 486 this bleeding is often accompanied by incomplete sloughing of the endometrium leading to a second bleeding episode at the time of corpus luteum demise.

In the human non-conceptional cycle, there is a progressive decrease in circulating progesterone levels towards the end of the luteal phase which leads to endometrial shedding. If implantation occurs, however, human chorionic gonadotrophin (hCG) from the developing trophoblast stimulates progesterone secretion from the corpus luteum, thus preventing the occurrence of menstruation during early pregnancy. The administration of hCG to normal women during the luteal phase can simulate the events of early pregnancy (Kaiser & Geiger, 1971; Mishell *et al.*, 1974) and provides an acceptable model to assess drugs that may interfere with corpus luteum response to hCG or endometrial response to progesterone.

RU 486 [17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)-oestra-4, 9-dien-3-one], a synthetic 19-norsteroid derivative, has been shown to have antiprogestational and antiglucocorticoid actions in both man and experimental animals (Philibert, 1984; Herrmann *et al.*, 1982; Gaillard *et al.*, 1984). We reasoned that women with hCG-induced prolongation of the luteal phase would be ideal for studying the action of RU 486 and other antiprogestins *in vivo*. Utilizing this model we have reported that exogenous hCG in combination with RU 486 extends the life-span of the functional corpus luteum but fails to prevent endometrial bleeding (Croxatto *et al.*, 1985). This was subsequently confirmed by other investigators (Yen *et al.*, 1987; Garzo *et al.*, 1988). With the lower doses of RU 486 two bleeding episodes were observed, one during treatment and the second within 10 days of the end of treatment. This report covers a wider range of RU 486 doses, its combination with the antioestrogen tamoxifen, and the results of endometrial biopsies performed in an attempt to understand the nature of the two bleeding episodes.

MATERIALS AND METHODS

Study design

This study was performed on 23 regularly cycling women aged 24–40 years, five of whom were using an IUD. The remaining 18 had previously undergone surgical sterilization. The nature and aims of the protocol was explained to all the subjects who then gave their consent. Each woman underwent three to five treatment cycles with one or more resting cycles after each treatment cycle. Treatment consisted of hCG alone or hCG combined with RU 486, tamoxifen, or both these latter two drugs. In all treatment cycles, the day of

Table 1. Occurrence of early and or late bleeding episodes following treatment with RU486

Treatment	Treated	Number of cycles: With early bleeding only	With early and late bleeding	With late bleeding only
hCG only	10	0	0	10
hCG + RU 486 25 mg/d × 4	6	2	1	3
50 mg/d × 4	6	2	4	0
100 mg/d × 4	16	5	11	0
100 mg/d × 7*	6	5	0	1
200 mg/d × 4*	12	10	1	1
100 mg/d × 1*	3	1	1	1
200 mg/d × 1	3	0	3	0
400 mg/d × 1	6	1	5	0
hCG + RU 486 25 mg/d × 4 + tamoxifen 20 mg/d × 4	6	1	12	3
100 mg/d × 4 + tamoxifen 20 mg/d × 4	4	3	1	0
hCG + tamoxifen 20 mg/d × 4	6	0	0	6

In all cycles hCG was administered each morning from day 9 to 16 after the LH surge using progressively increasing daily doses of 500, 1000, 1500, 3000, 6000, 10 000 and 15 000 IU. Treatment with RU 486 started in the morning of day 12 after the LH surge. Tamoxifen was given in a dose of 10 mg every 12 h for 4 consecutive days commencing in the morning of day 12 after the LH surge.

* Endometrial biopsies were performed in six subjects receiving RU 486 100 mg/d × 7, six subjects receiving RU 486 100 mg/d × 4, and in one subject receiving RU 486 100 mg/d × 1.

the LH surge was determined by measuring LH daily in early morning urine samples using Higonavis (Mochida Pharmaceutical Co., Tokyo, Japan). This day was designated as day 0. In all treatment cycles, daily hCG administration (APL Ayerst Laboratories, Inc. or Pregnyl, Organon Inc.) was commenced on day 9 following the LH surge. Progressively increasing doses (500, 1000, 1500, 3000, 6000, 10 000 and 15 000 IU) were injected i.m. each morning from days 9 to 15. RU 486 administration commenced on day 12 after the LH surge at doses of 25, 50, 100, 200 or 400 mg/day. RU 486 was given for 1, 4 or 7 consecutive days. When given for 1 day RU 486 was administered as a single dose and when given for several days, half the RU 486 dose was given at the time of the hCG injection and the remainder in the evening. In certain treatment cycles, tamoxifen (20 mg/day) was given for 4 successive days commencing on day 12 after the LH surge. Tamoxifen was given alone or with RU 486 (25 or 100 mg daily for 4 days). The various treatment regimens together with the number of treated cycles are shown in Table 1.

During all treatment cycles, careful records were maintained of all episodes of vaginal bleeding. In those cycles in which hormonal parameters were determined, blood samples were taken each morning before drug administration from day 9 to day 17 and on days 19, 21, 23, 25 and 27 following the LH surge for the measurement of LH-hCG, oestradiol, and progesterone. Blood samples were taken from 10 subjects receiving hCG alone and from six subjects in each group receiving hCG combined with RU 486 doses of 50, 100 and 200 mg/day for 4 days. Hormonal determinations were not performed in the remaining treatment cycles. In 13 women who were not subjected to blood sampling, an endometrial

biopsy was performed 72 h following the cessation of the initial bleeding episode to determine the completeness of endometrial shedding induced by RU 486 (Table 1). None of these latter 13 women had an IUD.

Methods

All hormones were measured in plasma, utilizing the reagents and procedures supplied by the World Health Organization Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology (Hall, 1978). hCG levels were determined using a cross-reacting LH RIA.

The results of hormone assays were analysed using the BMDP statistical software (Dixon *et al.*, 1983). A one-way analysis of variance (BMDP7D) was used to determine mean statistical differences between the various groups. When appropriate, a two-way analysis of variance for repeated measures (BMDP2V) was employed to determine significant differences over time and between hCG administration and the various doses of RU 486. When insufficient numbers were available the Chi-squared analysis was used.

RESULTS

Hormonal

LH-hCGs

During hCG treatment there was a progressive increase in immunoreactive hCG levels (as measured in the LH assay). This rise was not different in any of the treatment groups. The peak was evident on the day following cessation of hCG administration. Thereafter levels decreased progressively with a half disappearance time of approximately 48 h. By day 23 following the LH surge, mean values were within the range of basal LH seen in the normal luteal phase.

Oestradiol 17β

On day 9 following the LH surge, mean ± SD oestradiol levels were 536 ± 349, 664 ± 352, 536 ± 257, and 499 ± 169 pmol/l when hCG was given alone or with RU 486 in doses of 50, 100 and 200 mg respectively. Oestradiol rose progressively during hCG administration in all treatment cycles, attaining the highest levels with the last dose or 1 or 2 days later. The highest peak levels attained were 1215 ± 499, 1380 ± 499, 1054 ± 437 and 1189 ± 341 pmol/l, respectively (Fig. 1a). These values are higher than those noted in the normal menstrual cycle and are comparable to those seen during early pregnancy. Following cessation of hCG therapy, oestradiol levels decreased and by day 21 following the LH surge values were 602 ± 441, 389 ± 250, 242 ± 106 and 220 ± 139 pmol/l, respectively. There were no differences in oestradiol responses with any of the treatment regimens.

Progesterone

On day 9 following the LH surge, the mean ± SD progesterone levels were 38·2 ± 11·1, 37·5 ± 12·1, 50·2 ± 19·4 and 49·6 ± 28·0 nmol/l when hCG was given alone or with RU 486 in doses of 50, 100 and 200 mg/day respectively. A progressive increase in progesterone levels occurred during hCG administration in the control and RU 486 treatment cycles;

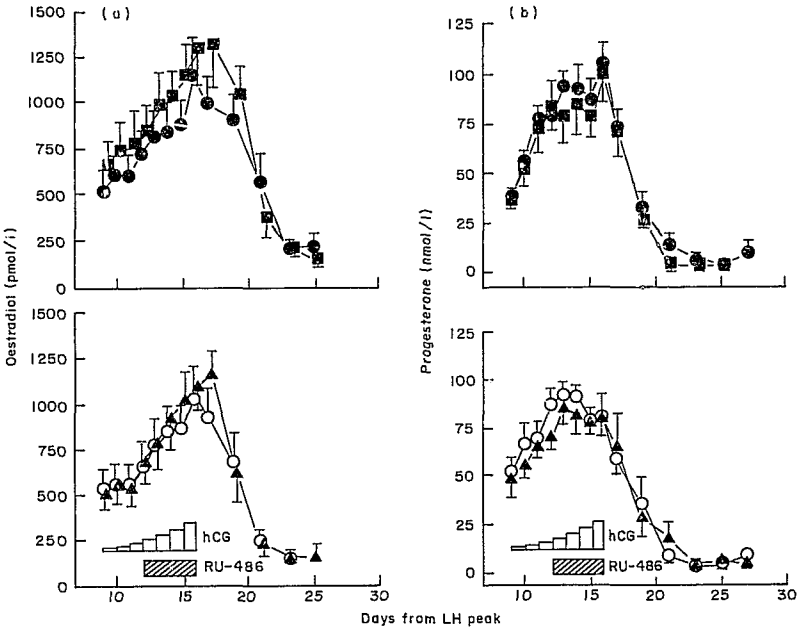


Fig. 1. a, Serum oestradiol and b, progesterone responses to (upper panel) ●, hCG alone and ■, hCG combined with 50 mg RU 486 per day × 4 days and (lower panel) to hCG and RU 486 doses of ○, 100 mg and ▲, 200 mg/day × 4 days.

peak levels attained were 107·5±31·2, 93·5±36·6, 100·5±13·0 and 94·4±24·5 nmol/l, respectively (Fig. 1b). Following cessation of therapy, progesterone levels gradually decreased and by day 23 following the LH surge mean levels were below 2 ng/ml in all groups. Statistical analysis showed that there were no differences in progesterone levels in the different treatment groups except for day 14 following the LH surge. On this day, mean progesterone values were lower in the cycles treated with RU 486 than in those in which hCG was administered alone ($P < 0{\cdot}05$ with the 200 mg dose, $P < 0{\cdot}01$ with the 50 mg and 100 mg doses).

Bleeding patterns

In 29 of 68 cycles treated with RU 486, two menstrual bleedings differing in timing after the LH surge were recognized. One occurred around day 14 and the second around day 23. They have been designated early and late bleeding, respectively. The time of onset of each bleeding relative to the LH peak and their associated oestradiol and p ogesterone levels are listed in Table 2 for those regimens in which hormonal determinations were available. In all of these cases the onset of early bleeding coincided with high oestrogen

Table 2. Timing of early and late bleeding episodes and their associated steroid levels

Treatment		*n*	Days from LH peak	E_2 (pmol/l)	P (nmol/l)
Onset of early bleeding					
hCG only		0	—	—	—
hCG+RU 486	50 mg	6	16·0±0·4	1189±411	73·8±10·8
	100 mg	6	15·0±0·0	866±121	76·6±7·3
	200 mg	6	14·6±0·3	980±173	81·4±10·5
Onset of late bleeding					
hCG only		10	22·4±0·3	210±37	6·7±1·3
hCG+RU 486	50 mg	4	24·0±0·6	190±54	3·2±0·6
	100 mg	5	24·8±0·8	255±92	3·2±0·6
	200 mg	0	—	—	—

Values shown as mean±SD.

RU 486 was given daily for 4 days in the doses indicated.

n, Number of subjects demonstrating early and/or late bleeding episodes.

A total of 10 subjects received hCG alone and six subjects in each group received hCG with RU 486 doses of 50, 100 and 200 mg/day.

E_2, Oestradiol.

P, Progesterone.

and progesterone levels in plasma while late bleeding coincided with the low levels typical of the end of the luteal phase.

When hCG was given alone, bleeding occurred only on days 21–24 following the LH surge at a time when steroid levels were low. The proportion of treated cycles that presented early and/or late bleeding is shown in Table 1. With the 25 mg RU 486 dose without tamoxifen, three of the six subjects had late bleeding only and the others had early bleeding only or both episodes. The same total dose, i.e. 100 mg, given as a single dose, failed to elicit a response in one-third of the cycles. With 200 or 400 mg RU 486 total dose given as a single dose or distributed over 4 days there were no failures to respond but a high proportion of cycles (23 of 31) had two bleeding episodes. A further increase of the total dose of RU 486 to 700 mg (100×7 days) or 800 mg (200×4 days) increased the proportion of cycles with early bleeding only (15 out of 18). These various responses were clearly dose related as seen in individual analyses of single subjects treated with different regimens in successive cycles. Using the Chi-squared analysis, it was found that the proportion of cycles responding with early bleeding only was significantly higher ($P<0·001$) in the groups treated with total doses of 700–800 mg (15 out of 18) than in the groups treated with total doses of 200–400 mg (eight out of 31). Tamoxifen at the dose tested failed to induce early bleeding by itself or to potentiate either dose of RU 486.

Endometrial histology

An endometrial biopsy was performed 24–72 h after cessation of the early bleeding in 13 hCG-RU 486 treatment cycles (Table 1). Only proliferative endometrium was evident in

five out of six cycles treated with 200 mg RU 486 for 4 days, and in one cycle treated with 100 mg for 7 days. On the other hand, with the RU 486 dose of 100 mg for 4 days, the histology showed a mixed proliferative and secretory pattern in five out of six cycles. All seven biopsies showing proliferative endometrium belonged only to subjects who did not have a late bleeding episode and five out of six biopsies showing mixed proliferative and secretory endometrium were taken from subjects who did have a late bleeding episode.

DISCUSSION

These results confirm that increasing doses of hCG administered during the normal luteal phase induce a state of pseudopregnancy prolonging the functional lifespan of the corpus luteum (Kaiser & Geiger, 1971). This is associated with a progressive rise in oestradiol and progesterone to levels higher than those seen in the normal luteal phase and are compatible with those observed during early pregnancy (Mishell *et al.*, 1974). Following cessation of hCG treatment, steroid levels decreased and bleeding occurred 21 to 24 days after the midcycle LH surge when these values had decreased substantially. This indicates that the luteal phase had been prolonged by 8 or more days.

When RU 486 in doses exceeding 25 mg/day was administered with hCG, menstrual bleeding was evident soon after RU 486 administration. At the time of this menstrual bleeding, mean oestradiol and progesterone levels were at their peak and comparable to those of early pregnancy. The RU 486 dose of 25 mg/day was at the threshold since it inconsistently produced this early bleeding. The addition of the antioestrogen, tamoxifen, did not potentiate the bleeding-inducing effect of this threshold dose of RU 486.

With the decline in steroid levels following termination of hCG administration, a further bleeding occurred in the majority of subjects who received RU 486 doses of 200 and 400 mg; however, with the 700 or 800 mg total dose a late bleeding was recorded in less than 20% of the cycles. In an attempt to explain these bleeding patterns, an endometrial biopsy was performed 24 to 72 h after cessation of the initial bleeding in 13 hCG-RU 486 treated cycles. The absence of a late bleeding was in all cases associated with the presence of proliferative endometrium indicating that complete endometrium shedding had occurred. This explains the absence of withdrawal bleeding. On the other hand, when a late bleeding episode did occur, it was associated with a mixed proliferative and secretory pattern shortly after the end of the initial bleeding episode. This suggests that incomplete shedding had occurred and accounts for the further bleeding coincident with the fall in progesterone levels. This interpretation however does not fit with the uniformity of endometrial sloughing found in cynomolgus monkeys treated with RU 486 (5 mg/kg/day i.m.) in the mid luteal phase (Chillick *et al.*, 1986). The bleeding pattern induced by the lowest doses of RU 486 in the present study was unchanged by the addition of the antioestrogen, tamoxifen, and was altered only by increasing the total dose of RU 486 administered.

Circulating oestradiol levels were not changed by RU 486 treatment. Moreover, progesterone levels were only transiently decreased by RU 486 for 1 day. The precise significance of this finding is questionable and the results suggest that when given in association with hCG, RU 486 does not have a major effect on the secretion or metabolism of oestradiol and progesterone. The implication is that bleeding and shedding of the endometrium resulted from the direct action of RU 486 at the level of this tissue. Animal studies have shown that RU 486 acts directly on the endometrium to inhibit

progesterone action on this tissue (Chang *et al.*, 1985; Chen *et al.*, 1984; Rauch *et al.*, 1985). Clearly, this study does not exclude the possibility that RU 486 may also have a direct effect at or above the level of the pituitary (Shoupe *et al.*, 1986; Gravanis *et al.*, 1985; Schaison *et al.*, 1985), but since exogenous hCG was administered, any potential pituitary role is of little relevance in this model.

In conclusion these results indicate that RU 486 can induce endometrial bleeding and shedding despite the administration of exogenous hCG in doses sufficient to maintain high circulating oestradiol and progesterone levels and corpus luteum function. The ED50 dose of RU 486 appears to be 25 mg/day and doses exceeding this produced endometrial bleeding, always associated with high steroid levels. In many of the cycles treated with hCG and RU 486 further bleeding occurred in association with the fall in steroid levels. The endometrial histology shortly after the cessation of the initial bleeding indicates that this is due to incomplete endometrial shedding. The occurrence of this phenomenon appears related to the dose and may disappear on increasing the total dose RU 486.

ACKNOWLEDGEMENTS

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THE PROGESTERONE ANTAGONIST RU 486

A Potential New Contraceptive Agent

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Abstract Since progesterone supports endometrial nidation of the fertilized ovum, a progesterone antagonist would theoretically block this process and thus have contraceptive potential. We have explored the ability of RU 486, a newly developed competitive progesterone antagonist, to function as a contraceptive agent.

A single oral dose of 10 mg per kilogram of body weight given in the midluteal phase consistently induced menses within 72 hours in women with normal cycles and no risk of pregnancy. Bleeding was not prevented by administration of human chorionic gonadotropin in the midluteal phase. This suggested that giving a single dose of RU 486 late in the menstrual cycle might be an effective contraceptive strategy.

This concept was tested in monkeys. When given to rhesus females on day 25 of the cycle, a single intramuscular dose of RU 486 (5 mg per kilogram) prevented pregnancy. The vehicle-treated control animals had a 28 percent pregnancy rate ($P<0.05$ by chi-square analysis). No side effects were noted in women or monkeys.

These data suggest that a progesterone antagonist such as RU 486 has the potential to be an effective, safe, and convenient contraceptive agent. Further work will be necessary to assess the safety of long-term monthly administration and to define the optimal dose and time of administration in women. (N Engl J Med 1987; 316:187-91.)

A SAFE, convenient, and effective method of fertility control has been sought for centuries. No currently available form of birth control, however, is ideal. Oral steroidal contraceptives must be taken daily for maximal effectiveness, and unacceptable side effects increase both with age and with tobacco use.[1,2] Intrauterine devices are associated with pelvic inflammatory disease and subsequent decreased fertility.[3,4] Barrier methods (condom and diaphragm), although largely free of untoward side effects, have high failure rates related mainly to poor compliance.[5]

Since progesterone is necessary for nidation of the fertilized ovum, a progesterone antagonist, in theory, could interrupt the hormonal support of the endometrium and function as a contraceptive agent. RU 486, or 17 β-hydroxy-11 β (4-dimethylaminophenyl)-17 α (1-propynyl)estra-4,9-dien-3-one, a 19-norsteroid, binds strongly to the progesterone receptor and antagonizes progesterone action in vivo in rat and rabbit bioassays[6] and in nonhuman primates.[7] The activity of the compound as an abortifacient has been studied in rhesus monkeys[8] and women,[9,10] but its potential application as a contraceptive agent in women remains unexplored.

The present studies were designed to evaluate the contraceptive potential of RU 486. The ability of a single midluteal-phase dose to induce menses in women was established. Human chorionic gonadotropin (hCG) was also given concurrently to test whether RU 486 could induce menses in the presence of the enhanced corpus luteal function characteristic of early pregnancy. Finally, the antifertility properties of the drug were tested directly in female rhesus monkeys.

From the Developmental Endocrinology Branch and the Pregnancy Research Branch, National Institute of Child Health and Human Development, Bethesda; the Veterinary Resources Branch, Division of Research Services, National Institutes of Health, Bethesda; the Center for Biomedical Research, Population Council, New York; and Laboratoire Hormones, Université de Paris, Paris, France. Address reprint requests to Dr. Nieman at Bldg. 10, Rm. 10N262, National Institutes of Health, Bethesda, MD 20892.

METHODS

Subjects and Approval

Eighteen women (mean age, 31 years; range, 22 to 41) were studied. All had regular menses (mean interval, 29 days; range, 26 to 31) and were within 15 percent of ideal body weight. None were at risk of pregnancy, and none were taking hormonal contraceptives or other drugs.

Approval for the studies in women was obtained from the U.S. Bureau of Drugs and Biologics and the National Institute of Child Health and Human Development (NICHHD) Clinical Research Subpanel of the National Institutes of Health. Each woman gave informed consent. Approval for the studies in animals was obtained from the NICHHD Animal Research Committee.

Preparation of RU 486

RU 486 was provided by Roussel-UCLAF, Paris, France. Scored tablets containing 50 mg of micronized powder were administered to women. Pure crystalline powder was prepared for injection into animals as outlined below.

Hormone Assays

Plasma concentrations of progesterone[11] and luteinizing hormone[12] were determined by radioimmunoassay. The intraassay and interassay coefficients of variation were 7.6 and 10.3 percent for progesterone and 2.3 and 7.6 percent for luteinizing hormone.

Effect of hCG Administration on RU 486-Induced Vaginal Bleeding

The ability of RU 486 to induce menses despite persistent progesterone support of the endometrium was examined by administering hCG concurrently with RU 486. Eighteen women were studied during two consecutive menstrual cycles. During the first cycle, blood was drawn for measurement of progesterone on days 3 and 6 and then daily from day 9 until seven days after the anticipated onset of the second cycle. Serum luteinizing hormone was measured daily beginning on day 9 until the surge in luteinizing hormone was detected. That day was designated luteal-phase day 0. Subjects were then randomly assigned to one of three treatment groups, each having six women. Treatment was begun on luteal-phase day 6, six days after the surge in luteinizing hormone; hCG (2000 IU) or 0.9

percent sodium chloride (1 ml) was given intramuscularly on luteal-phase days 6, 7, and 8. RU 486 (10 mg per kilogram of body weight) or placebo was given orally on luteal-phase day 7. Group 1 received RU 486 and hCG. Group 2 received RU 486 and saline injections, and Group 3 received both placebo tablets and saline injections. During the next cycle, blood samples were obtained for measurement of progesterone on days 3, 6, 9 through 15, 18, 21, 25, and 28. Each woman recorded the date of onset, the duration, and the quality of any vaginal bleeding. Measurements of serum creatinine, calcium, potassium, sodium, chloride, bicarbonate, and glucose, liver-function tests, and complete blood counts were performed before and within 48 hours after administration of RU 486.

Contraceptive Efficacy of RU 486 in Female Rhesus Monkeys

In studies of the contraceptive efficacy of RU 486 in rhesus monkeys, 40 fertile female animals were exposed to light–dark cycles of 12 hours and were housed in single cages with free access to monkey chow and water. Vaginal bleeding was monitored by visual inspection of the genitalia or by swabbing the vagina. Each female was caged with a male of established fertility on days 11, 12, and 13 of her reproductive cycle, the days corresponding to the usual time of ovulation in this colony. Fecund cycles were arbitrarily defined as cycles of 25 to 33 days in which mating occurred. The presence or absence of a pregnancy was established by abdominal palpation 15 and 30 days after mating.

Monkeys were randomly assigned to treatment or control groups. On day 25 of each cycle, the treatment group received a 1-ml intramuscular injection of RU 486 (5 mg per kilogram) in a solution of 70 percent ethanol and 30 percent water. The control group received 1 ml of vehicle.

Statistical Analysis

Results are presented as means ±SE or ±SD, as indicated. Comparisons between groups were done by chi-square test or by the Student t-test. The Student t-test for paired variables or analysis of variance was used to compare results within groups.

Results

RU 486-Induced Vaginal Bleeding

Vaginal bleeding began within 72 hours of administration of RU 486 in all women (Fig. 1 and Table 1). Bleeding was not prevented by administration of hCG. The menses lasted for an average of four days (range, two to five). It was characterized as being similar to a normally timed menstrual period by the wom-

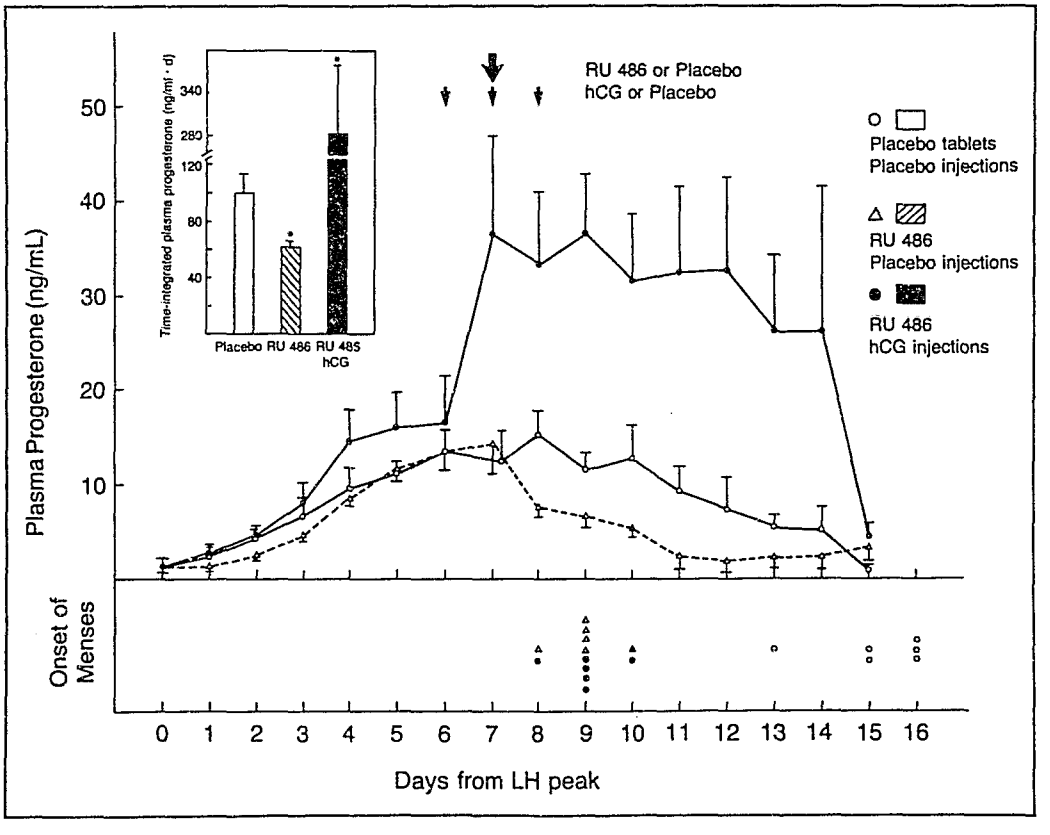


Figure 1. Effects of Placebo, RU 486, and RU 486 plus hCG on Mean (±SEM) Plasma Progesterone Levels, Time-Integrated Plasma Progesterone Levels (Luteal-Phase Days 6 to 15), and the Onset of Menses.

The arrows indicate days of drug or placebo administration. The asterisks in the inset indicate a significant increase or decrease ($P<0.05$) as compared with the placebo group.

en who received RU 486 alone and by three of the six women who received concomitant hCG. The other three women who received hCG and RU 486 characterized the bleeding as light or initially heavy. In women who received placebo pills and injections, menses occurred at the expected time, on luteal-phase day 15 (range, 13 to 16).

The effect of RU 486 on the next menstrual cycle was also examined. In the group taking RU 486 alone, the next menses occurred after a normal interval of 30±2 days (mean ±SD) in three women and after a shortened interval of 13±3.5 days in the other three. The episode of bleeding after the short interval tended to be lighter than usual. In the group treated with hCG and RU 486, vaginal bleeding occurred after a "normal" interval (on day 25 and day 34) in two women and after a short interval (8 to 10 days) in four. In both groups, the length of the cycle after the short-interval bleeding episode was 27 to 30 days. (One woman did not have a subsequent menses because of pregnancy.)

Effect of RU 486 and hCG on Corpus Luteal Function

Serum progesterone levels on luteal-phase day 6 were similar in all groups and reflected normal corpus luteal function (Fig. 1 and Table 1). Women who received RU 486 alone (on luteal-phase day 7) had a significant decrease in serum progesterone by luteal-phase day 10 ($P<0.05$). Women who received hCG plus RU 486 had significantly increased plasma progesterone concentrations by luteal-phase day 10 ($P<0.05$).

Calculation of the integrated plasma progesterone levels from luteal-phase day 6 (the first day of treatment) to day 15 (the end of the luteal phase in all control subjects) yielded similar results. The group given both RU 486 and hCG had a significant increase in time-integrated post-treatment progesterone secretion as compared with the placebo group (280±49 vs. 101±15 ng per milliliter [mean ±SE]; $P<0.01$). The group given RU 486 had decreased plasma progesterone concentrations as compared with the placebo group (65±5 vs. 101±15 ng per milliliter [mean ±SE]; $P<0.05$). (To convert progesterone values to nanomoles per liter, multiply by 3.180.)

The nature of the subsequent menstrual cycle correlated with the response of plasma progesterone levels to RU 486. The three women with a normal subsequent menstrual interval after RU 486 alone had markedly suppressed progesterone levels (<2.5 ng per milliliter) within four days of the onset of the RU 486-induced menses. In the women with intermenstrual bleeding, plasma progesterone levels were not suppressed to the same degree until after the ninth day following the post-RU 486 bleeding episode. This fall in plasma progesterone occurred within two days of the intermenstrual episode of bleeding, 17 to 22 days after the surge in luteinizing hormone.

Results of serum chemistry studies and complete blood counts were unchanged and no side effects were observed during the course of the study. Progesterone levels followed a normal ovulatory pattern in the cycle after an intermenstrual episode of bleeding.

Contraceptive Efficacy of RU 486 in Female Rhesus Monkeys

Treatment with RU 486 alone allowed pregnancy to occur in 9 of 32 fecund cycles. RU 486 treatment prevented regnancy in 17 fecund cycles ($P<0.05$ by chi-square analysis).

Post-treatment cycles were significantly longer in the RU 486-treated group than in the controls (61±33 vs. 40±18 days [mean ±SD]; $P<0.01$).

Discussion

A single oral dose of RU 486 (10 mg per kilogram) given in the midluteal phase consistently caused men-

Table 1. Clinical Data on the Three Groups of Women.

Patient No.	Treatment*	Plasma Progesterone†	Onset of Post-Treatment Menses	Duration of Post-Treatment Menses	Interval to Ensuing Menses
		ng/ml	*luteal-phase day*	*days*	
Group 1					
1	RU 486 and hCG	11.2	9	3	10
2	RU 486 and hCG	9.4	8	3	25
3	RU 486 and hCG	17.6	9	3	10
4	RU 486 and hCG	12.8	9	3	8
5	RU 486 and hCG	33.6	10	4	34
6	RU 486 and hCG	23.4	9	2	9
		18±9.2‡	9±0.6‡	3±0.6‡	16±10.9‡
Group 2					
7	RU 486 and saline	9.6	9	3	30
8	RU 486 and saline	14.9	9	4	17
9	RU 486 and saline	8.0	8	5	11
10	RU 486 and saline	12.4	9	4	11
11	RU 486 and saline	21.2	10	3	32
12	RU 486 and saline	14.3	9	5	28
		13.4±4.7‡	9±0.6‡	4±0.9‡	21.5±9.6‡
Group 3					
13	Placebo and saline	20.8	16	4	30
14	Placebo and saline	15.9	16	3	34
15	Placebo and saline	7.7	15	4	27
16	Placebo and saline	18.5	13	5	28
17	Placebo and saline	12.7	16	4	27
18	Placebo and saline	5.4	15	3	27
		13.5±6.1‡	15.2±1.2‡	3.8±0.8‡	28.8±2.8‡

*The subjects received hCG (2000 IU intramuscularly) or normal saline on luteal-phase days 6, 7, and 8 and received RU 486 (10 mg per kilogram) or placebo tablets on luteal-phase day 7.

†A single blood sample was drawn before injection on luteal-phase day 6. To convert progesterone levels to nanomoles per liter, multiply by 3.180.

‡Mean ±SD.

strual bleeding in women. This effect occurred despite concomitant administration of hCG and despite elevated progesterone levels, suggesting that continued support of the corpus luteum (and hence the endometrium) by hCG cannot overcome the antiprogestin effects of RU 486 on the endometrium. The absence of pregnancies in monkeys given a single dose of RU 486 in the late luteal phase supports this hypothesis and suggests that RU 486 is a potentially effective method of fertility control. If complete luteolysis is induced, the timing of the subsequent menstrual bleeding is dependent on the RU 486-induced menses. Since bleeding follows RU 486 administration by about 72 hours, regular cycles could theoretically be maintained if the drug were given three days before the desired day of menses.

The luteolytic properties of RU 486 are dose-dependent. Complete luteal regression has been observed in 20 percent of women given approximately 0.5 mg per kilogram per day for four days in the midluteal phase.[13] The proportion increased to 60 percent at a dose of approximately 2.0 mg per kilogram. The remaining subjects had no evidence of luteolysis. Larger single midluteal-phase doses (10 to 25 mg per kilogram) uniformly induced luteolysis but of varying degrees.[14] Luteolysis occurred in all six women in the current study, but it was complete in only three. Further investigation will be necessary to determine whether complete luteolysis can be consistently induced by altering the dose or time of administration of RU 486.

The way in which RU 486 impairs corpus luteal function is unclear. The ability of exogenous hCG to block RU 486-induced luteolysis suggests that this phenomenon occurs through inhibition of gonadotropin secretion. Progesterone can decrease the frequency of luteinizing hormone pulses,[15] and careful studies performed during the luteal phase suggest that progesterone reduces secretion of luteinizing hormone in the normal cycle.[16,17] Schaison and coworkers have demonstrated a decrease in the amplitude of the luteinizing hormone pulse in women given RU 486 during the luteal phase.[13] These findings suggest that RU 486 may have a progesterone-agonist effect on the hypothalamic–pituitary unit. Impeded agonist properties of RU 486 have been described in other systems.[18,19] The design of our study, however, does not allow us to exclude the possibility that RU 486 has direct effects on ovarian function.

If RU 486 is to be useful as a contraceptive agent taken once a month, the duration of the subsequent menstrual cycle must not be radically altered. Prolongation of the cycle following a late-luteal-phase dose of RU 486 in rhesus monkeys was observed in our study and another.[20] This phenomenon appears to be dose-dependent and reflects an increase in the length of the follicular phase.[20] Since the plasma half-life of RU 486 is long[21] and a high parenteral dose was given to monkeys, the drug may still have been present in the follicular phase and may have delayed oocyte maturation. Women receiving a lower oral dose of the drug in the midluteal phase, on the other hand, generally have subsequent cycles of normal length.[14] Normal subsequent cycle lengths in women seem to depend on complete luteolysis — a phenomenon that should be considered in attempting to define the optimal dose regimen.

The level of serum progesterone at the time of RU 486 administration is an important variable. The drug does not cause withdrawal bleeding in anovulatory women,[13,14] probably because of the absence of a progesterone effect on the endometrium. Since menses are not induced by RU 486 in the absence of luteal-phase levels of progesterone, verification of luteal-phase status before the administration of the drug would be ideal. There is currently no simple way to do this.

No untoward side effects were observed in the women in this study, and no toxicity was noted in any of the monkeys. This is consistent with the safety of single-dose or short-term administration previously reported by others.[9,10,13,22,23] In addition, no adverse effects were noted in one subject during nine weeks of treatment with RU 486 at daily doses of up to 20 mg per kilogram.[24]

These preliminary studies suggest that RU 486 holds promise as a safe and effective form of fertility control that can be administered once a month. This approach should be free of many of the shortcomings and untoward effects associated with currently available contraceptives. Further work will be necessary to assess the safety of long-term monthly administration of RU 486 and to define the optimal dose and time of administration in women.

We are indebted to Ms. Penny Colbert, Ms. Mary Hall, and Ms. Allyson Williams for their assistance in preparing the manuscript, to Roussel-UCLAF for providing RU 486, to both the inpatient and the outpatient nursing staff for carrying out the studies in women, and to Mr. Garland Brown and Mr. George Coleman for invaluable help in conducting the studies in monkeys.

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MEDICAL INTELLIGENCE



DIAGNOSIS OF CHLOROQUINE CARDIOMYOPATHY BY ENDOMYOCARDIAL BIOPSY

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CHLOROQUINE and hydroxychloroquine are antimalarial agents that are often used in the treatment of collagen vascular diseases. However, both drugs can cause a toxic skeletal-muscle myopathy, the histology of which has been carefully described.[1-9]

Chloroquine toxicity results in cardiomyopathy in animals, but documentation of cardiac involvement in humans has been limited to evidence of conduction abnormalities in one patient, congestive heart failure in another patient, and the finding of vacuolar degeneration of cardiac muscle during two autopsies.[1,4-8] This report describes the light and electron microscopical findings in chloroquine and hydroxychloroquine myocardial toxicity in two patients, as revealed by endomyocardial biopsy. The histologic features included numerous large secondary lysosomes, myeloid bodies, and curvilinear bodies — findings that are virtually identical to those in skeletal-muscle myopathy due to chloroquine and hydroxychloroquine and that are considered to be diagnostic of chloroquine cardiomyopathy.[1-9]

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CASE REPORTS

Patient 1

A 58-year-old white woman had been given a diagnosis of discoid lupus erythematosus 30 years earlier. She had received 200 mg of hydroxychloroquine a day for 10 years and 500 mg of chloroquine a day for 6 years. She presented with symptoms of progressive proximal-extremity weakness and of congestive heart failure with progressive lower-extremity edema and ascites that was refractory to diuretic therapy. Physical and laboratory examinations revealed evidence of complete heart block and of a restrictive cardiomyopathy. Right and left heart catheterization demonstrated a diastolic equilibration of right and left heart pressures, a dip-and-plateau pressure tracing from the right ventricle, a left ventricular ejection fraction of 50 percent, unremarkable coronary arteries, and a cardiac–thoracic ratio at the upper limit of normal. Biopsy evaluations of the vastus medialis muscle, the sural nerve, and the endomyocardium were performed. Subsequently, the chloroquine was discontinued, and the symptoms improved. Follow-up endomyocardial biopsies were performed six weeks and seven months after the discontinuation of chloroquine.

Patient 2

A 59-year-old white man with systemic lupus erythematosus had been treated with 400 mg of hydroxychloroquine a day for two years. He presented with signs and symptoms of congestive heart failure, including progressive dyspnea on exertion, orthopnea, and dependent edema. An endomyocardial biopsy was performed. The hydroxychloroquine was then discontinued, and the symptoms improved. Follow-up biopsies were not performed.

METHODS

Endomyocardial biopsy specimens were obtained from the right ventricle in both cases. The especially large biopsy specimen from Patient 1 was divided into three portions. One portion was snap-frozen. Frozen sections were stained with hematoxylin and eosin and were reacted with the pan-T-cell marker T11, the pan-B-cell marker Leu-14, and the macrophage marker Leu-M3 by means of an indirect immunoperoxidase technique.[10] A second portion of the specimen was fixed in Hollande's solution and embedded in paraffin. Ribbons of sections were stained with hematoxylin and eosin, Movat's pentachrome, Masson's trichrome, and sulfonated Alcian blue. The third portion of the biopsy specimen was fixed in buffered glutaraldehyde, postfixed in buffered osmium tetroxide, and embed-

Effects of the antiprogesterone RU 486 in normal women

I. Single-dose administration in the midluteal phase

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Los Angeles, California, New York, New York, Uppsala, Sweden, and Helsinki, Finland

The response to a single oral dose of the antiprogesterone RU 486 was studied in the midluteal phase in 26 normal women. Each subject received a dose between 50 and 800 mg RU 486 on days 6 to 8 after the luteinizing hormone surge and blood samples were taken over the following 48 hours. Another group of five patients received a single oral dose of 200 mg RU 486 and blood sampling was extended for 14 days. Menses were induced in all women but one within 3 days after RU 486 administration. Two distinct patient populations emerged. In nine of the subjects, there was a single bleeding episode and the treatment cycle was significantly shorter ($p < 0.05$) than the following cycle. In 16 of these 25 patients a second bleeding episode occurred 19.0 ± 0.8 days after the luteinizing hormone surge. The total treatment cycle was significantly prolonged ($p < 0.05$) when compared with the following cycle. In the group with a single bleeding episode, there was a significant decline in follicle-stimulating hormone, estradiol, and progesterone over the 48-hour sampling period, but there was no change in these values in the group with two bleeding episodes. These two groups could not be separated on the basis of RU 486 dose or serum levels. After the four higher doses, there was a dose-dependent rise in serum prolactin. There were no alterations in mean cortisol values with the three lower doses, but there was a significant increase at 24 and 48 hours after the higher doses. Serum levels of RU 486 were maximal between 1 and 4 hours and the half-life of serum RU 486 was determined to be 24 hours. (Am J Obstet Gynecol 1987;157:1415-20.)

Key words: RU 486, contraception, gonadotropins, steroids

The synthesis and development of RU 486, a 19-norprogestin derivative, is considered a major breakthrough in steroid endocrinology[1] and has opened a new era in fertility control. Beyond its potential use in contraceptive technology, the antiprogestational[1] and antiglucocorticoid[2-4] activities of RU 486 serve as a unique tool for investigation of hormone action.

In early reports, RU 486 has been noted to interrupt the luteal phase in women[4-6] and in monkeys[6] and to terminate early pregnancy[7,8] RU 486 has also been shown to have an affinity for the glucocorticoid receptor that is about four times higher than that of dexamethasone and has been shown to have antiglucocorticoid activity. After a single oral dose of RU 486, there is a transient increase in adrenocorticotropic hormone.[2,3]

The aim of this study was to investigate the effect of a single oral dose of RU 486 on bleeding, gonadotropin, and steroid patterns given to healthy women in the midluteal phase. In addition, the pharmacodynamics of this compound were studied with a recently developed radioimmunoassay.

Material and methods

Thirty-one healthy women, whose ages ranged from 22 to 35 years, were selected for participation in this study. They had regular menstrual cycles (28 ± 3 days) and were within 15% of their ideal body weight. They either had been surgically sterilized or had used a barrier method for contraception. The volunteers had not taken any steroid medication within the last 6 months.

On the tenth day of the menstrual cycle, each patient began to undergo daily blood sampling for determination of the day of the luteinizing hormone (LH) surge. Six to eight days after the LH surge, the volunteers were asked to report at 7:30 AM, after an overnight fast, and to refrain from smoking after midnight for administration of medication.

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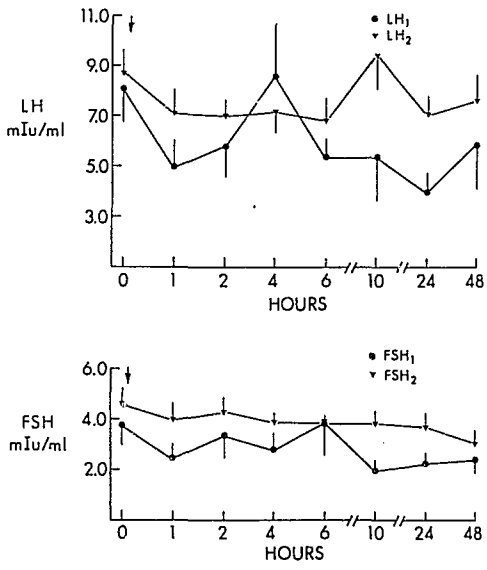


Fig. 1. LH *(upper panel)* and FSH *(lower panel)* values after a single oral dose of RU 486 ranging from 50 to 800 mg. LH_1 and FSH_1 refer to the patients with one bleeding episode after RU 486 administration and LH_2 and FSH_2 refer to patients having two bleeding episodes. No significant changes in LH were noted in either group. Analysis of variance detected a significant decrease in FSH_1 ($p < 0.001$) over the 48-hour sampling period.

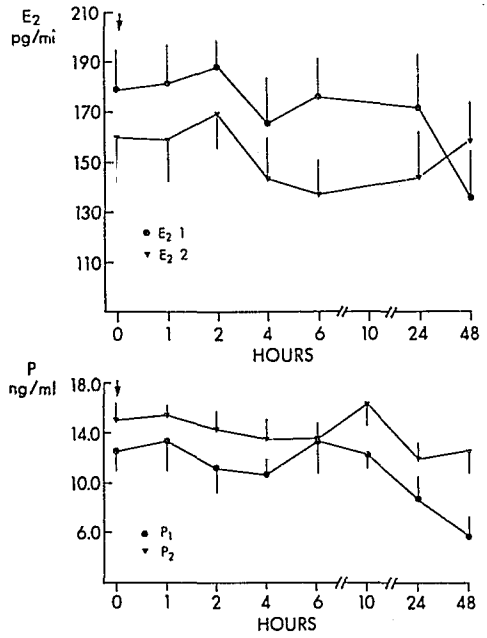


Fig. 2. *Upper panel*: Estradiol *(E_2)* values after a single oral dose of RU 486 ranging from 50 to 800 mg. $E_2 1$ refers to patients with one bleeding episode after RU 486 administration and $E_2 2$ refers to those with two bleeding episodes. Analysis of variance detected a significant decrease in $E_2 1$ ($p < 0.05$) over the 48-hour sampling period. *Lower panel*: Progesterone *(P)* values after a single oral dose of RU 486 ranging from 50 to 800 mg. P_1 refers to patients with one bleeding episode after RU 486 administration and P_2 refers to those with two bleeding episodes. Analysis of variance detected a significant decrease in P_1 ($p < 0.005$) over the 48-hour sampling period.

Subjects received either 50, 100, 200, 400, 600, or 800 mg of RU 486 (50 mg tablets supplied by Roussel-Uclaf, Romainville, France) at 8:00 AM. All groups comprised four subjects except for the two highest-dose schedules where five subjects were studied in each group. No smoking or food was permitted for 4 hours after ingestion of the medication and vital signs were recorded every 30 minutes for 6 hours. Blood samples were obtained through an indwelling catheter at −½, 0 +½, 1, 2, 4, 6, 10, 24, and 48 hours. A further group of five subjects received 200 mg as a single oral dose and blood sampling was continued daily for 1 week and then was performed at 10 and 14 days (extended sampling group).

These blood samples were assayed for prolactin, estradiol, progesterone, cortisol, LH, follicle-stimulating hormone (FSH), and RU 486 by previously described radioimmunoassay methods.[9-12] Normal serum AM cortisol values are 10 to 30 μg/dl. Two different radioimmunoassays were used to measure RU 486. The first was the method of Salmon and Mouren.[11] The second utilizes chromosorb column chromatography.[12] This latter, more specific assay was used only to measure RU 486 levels in the samples from the extended sampling group. The antiserum against RU 486 used in both assays was donated by Roussel-Uclaf. In addition, SMA-18 and complete blood count were performed at 0, 4, 10, 24, and 48 hours.

Results were analyzed by analysis of variance with the use of BMDP Statistical Software.[13] One- and two-way analysis of variance and Student's *t* test were used to determine statistical differences between groups.

Results

Clinical features. Menses commenced in all patients but one within 1 to 3 days after RU 486 administration (Table I). In nine of these 25 subjects (two who received 50 mg, two who received 100 mg, one who received 200 mg, one who received 400 mg, and three in the 800 mg group), this represented the only bleeding episode. The mean length of the treatment cycle in these nine subjects was significantly less ($p < 0.05$) than that of the following cycle (Table I).

In the remaining 16 subjects, a second bleeding episode was reported. In these subjects the initial bleeding episode occurred at a similar time after the LH surge as in the previous group, but the duration of bleeding was significantly shorter ($p < 0.05$). The full duration of the treatment cycle in those subjects who had two bleeding episodes (with the onset of the second bleeding episode used as a reference) was 32.9 ± 1.1 days. This was longer ($p < 0.05$) than the following cycle (Table I).

In none of these subjects were any adverse effects encountered. Furthermore, electrolytes, hepatic function, and blood count did not change in any subject. In one subject who received the 600 mg dose, bleeding did not occur until 56 days after treatment.

Hormonal parameters. The hormonal profiles of four patients (two from each group) were excluded from analysis because of incomplete data. In those 14 subjects who had two bleeding episodes, there was no change in serum LH, FSH, estradiol, or progesterone over the 48-hour sampling period. However, in those seven subjects who had a single bleeding episode, analysis of variance showed a significant decline in estradiol ($p < 0.05$), FSH ($p < 0.001$), and progesterone ($p < 0.005$) (Figs. 1 and 2).

There were no significant changes in LH, FSH, estradiol, or progesterone when hormonal results were expressed according to the dosage schedule of RU 486 and independent of the bleeding pattern. Analysis of variance showed that there was a significant increase in prolactin ($p < 0.05$) with the 50, 200, 400, and 800 mg dosages. When compared with the pretreatment levels, the peak values were greatest between 4 and 6 hours ($p < 0.05$). Values returned to normal by 24 hours (Fig. 3).

Analysis of variance showed a significant increase ($p < 0.05$) in mean cortisol values after the 100, 400, 600, and 800 mg doses. When compared with baseline values, the cortisol level rose significantly ($p < 0.05$) at 24 hours after the 600 and 800 mg doses and at 48 hours with all three higher doses (Fig. 4).

RU 486 levels. When measured directly after diethylether extraction, the serum levels of RU 486 reached a maximum between 1 and 4 hours after ingestion of all doses (Fig. 5). All RU 486 levels were significantly lower with the 50 mg dose than with the five higher-dose regimens ($p < 0.05$). The RU 486 levels at 6, 24, and 48 hours were also significantly lower after the 100 mg dose than with the four higher doses ($p < 0.05$). RU 486 levels were the same with doses of 200 to 800 mg. With doses of ≥200 mg, mean serum RU 486 levels fell minimally, and even after 48 hours values were still above 1.4 μg/ml. On the other hand, after the 50 and 100 mg doses, RU 486 values did decrease and were under 0.4 μg/ml at 48 hours. There were no differences in the RU 486 levels between the groups with one or two bleeding episodes.

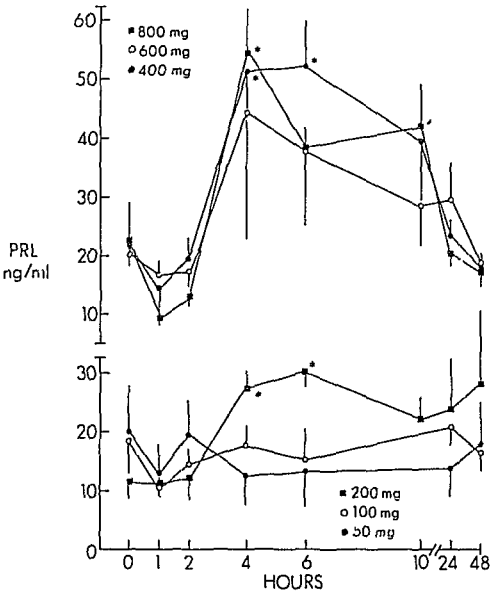


*Fig. 3. Prolactin (PRL) levels after a single oral dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected a significant increase in PRL after the 50, 200, 400, and 800 mg doses. * $p < 0.05$ increase over baseline values as determined by the paired t test.*

The serum levels of RU 486 in the extended sampling group are shown in Figs. 5 to 6. Chromosorb column chromatography was used in these samples and the values were lower than those with corresponding 200 mg dosage schedule. Nevertheless, after 4 hours the disappearance curves with both assays were parallel. Presumably, the values after chromatography represent true RU 486 concentrations. The half-disappearance time was 24 hours (Fig. 6).

Comment

Administration of RU 486 as a single oral dose from 50 to 800 mg in the early luteal phase was well tolerated. The induction of menses within 3 days is in accordance with results of previous studies.[5,6,15] This vaginal bleeding, which occurred despite high levels of estradiol and progesterone, implies that RU 486 acts directly on the endometrium to produce shedding regardless of any effect it may have on hormonal levels. A similar bleeding pattern has also been documented in nonpregnant women during concomitant treatment with RU 486 and human chorionic gonadotropin in the luteal phase.[9-11]

A total of 33% of our subjects displayed only a single

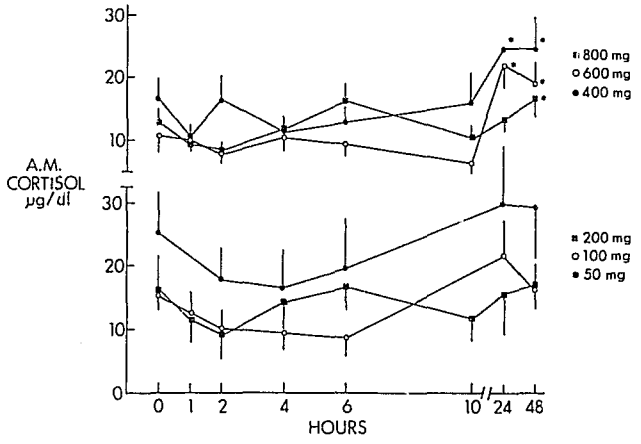


Fig. 4. Cortisol values after a single dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected a significant increase in cortisol ($p < 0.05$) after the 100, 400, 600, and 800 mg dose. * $p < 0.05$ increase over baseline values as determined by the paired t test.

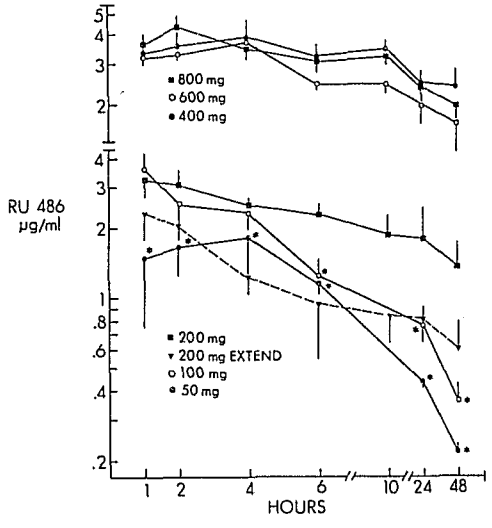


Fig. 5. RU 486 levels after a single oral dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected that all points after 50 mg dose and at 6, 24, and 48 hours after the 100 mg dose were significantly lower ($p < 0.05$) than values after the higher doses. The *200 mg extend* refers to the extended sampling group that is also depicted in Fig. 6.

bleeding episode, whereas in the remaining subjects a second bleeding episode occurred. This phenomenon appeared to be independent of the dose or serum concentration of RU 486. The hormonal profile in these two groups was distinctly different. In those subjects with two bleeding episodes there were no changes in LH, estradiol, or progesterone levels. In addition, if the commencement of the second bleeding episode is regarded as the termination of the cycle, the luteal phase appeared to be prolonged by RU 486. In contrast, in those subjects who had a single bleeding episode there was a progressive significant decline in serum FSH, estradiol, and progesterone levels over 48 hours of sampling time with a concomitant shortening of the luteal phase and cycle length. This suggests that under certain circumstances RU 486 can be luteolytic. Similar observations have been made by other workers.[6, 15] Although it is possible that the decline in steroid levels may represent the fall seen in the latter part of the normal menstrual cycle, this is unlikely since no corresponding fall occurred in the group with two bleeding episodes.

The decrease in the serum progesterone level could be related to a direct action of RU 486 on ovarian steroidogenesis.[17] However, it could also represent an effect on the hypothalamic-pituitary axis. We did observe a decrease in FSH in one group of patients but were unable to detect a decrease in LH secretion. Recently, with more frequent sampling we also observed a reduction in amplitude and frequency of LH pulses during RU 486 administration in the luteal phase.[18] Other workers have also described a reduction in LH pulse amplitude consequent to RU 486.[15]

Nieman et al.[6] observed menstrual bleeding in normal women within 72 hours of a single oral dose of RU 486 (10 mg/kg body weight) given in the midluteal phase. In the patients treated with RU 486 on luteal day 7, they report a decrease in the serum progesterone level by luteal phase day 10 and the onset of vaginal

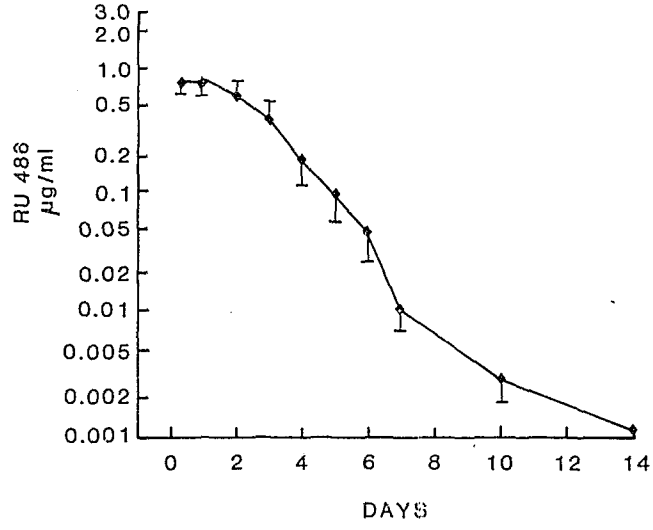


Fig. 6. RU 486 levels in the extended sampling group after a single oral dose of 200 mg RU 486. The half-life of RU 486 is 24 hours.

Table I. Effects of RU 486 administration

Bleeding episodes	*N*	*Onset (days after LH surge)*	*Days of bleeding*	*Treatment cycle length (days)*	*Posttreatment cycle length (days)*
1	9	9.8 ± 0.6	3.4 ± 0.3	25.2 ± 1.4*	34.0 ± 2.1
2	16	9.6 ± 0.5	6.0 ± 0.8†	32.9 ± 1.1	26.6 ± 1.7
		19.0 ± 0.8	4.2 ± 0.3		

Values are mean ± SD.
*$p < 0.05$ when compared with posttreatment cycle length.
†$p < 0.05$ when compared with group with one bleeding episode.

bleeding within 72 hours of treatment in all patients. They also described two patient groups as regards to the onset of menses, which may correlate with our two patient groups.

Circulating RU 486 levels measured by immunoassay showed that this synthetic steroid was absorbed rapidly. Doses of 50 and 100 mg gave lower blood levels than higher doses. However, no clear dose response was evident since 200 to 800 mg RU 486 gave similar blood levels. The immunoassay used to determine these circulating levels is nonspecific and measures metabolites, including the monodemethylated and didemethylated compounds as well as the alcoholic derivative.[11,12] These metabolites seem to have a smaller biologic activity.[19] For this reason we developed a more specific radioimmunoassay using preliminary chromosorb chromatography. With this modification, values were lower but gave a parallel disappearance curve. Values of RU 486 measured by immunoassay after chromosorb chromatography give values similar to those determined by high-pressure liquid chromatography.[12] The half-life of RU 486 was 24 hours. The long half-disappearance of RU 486 suggests that there is binding to plasma proteins. Recent studies have shown that RU 486 binds to an α_1-globulin.[19]

The late rise in cortisol, noted at 24 and 48 hours after ingestion of the 400, 600, and 800 mg doses, suggests the presence of a mild degree of antiglucocorticoid activity with these doses. Nevertheless, mean values remained within the normal range. The late increase seen after 24 and 48 hours, without any change during the initial 10 daytime hours after ingestion of the drug, would be consistent with the work of Gaillard et al.,[3] who demonstrated that the antiglucocorticoid activity is seen only during the early morning hours when adrenocorticotropic hormone secretion is high.

Rakoff and Yen[16] reported a transient increase in prolactin in estrogen-primed women receiving in-

tramuscular progesterone after oophorectomy. This would imply a positive effect of progesterone on prolactin secretion, which is in contrast to the transient increase in prolactin we noted after treatment with an antiprogesterone. Healy et al.[20] described a decrease in prolactin after treatment with RU 486 in monkeys (10 mg/kg).[20] However, these were castrated monkeys who had steroid replacement via estradiol and progesterone capsules, which produce basal hyperprolactinemia. Within 1 hour after parenteral RU 486 administration, these elevated prolactin levels were decreased. Thus the effect of RU 486 on prolactin secretion varies and may depend on the mode of its administration, the dose, the species, and the prevailing steroid concentrations.

The reason why two subgroups emerged in this study is not evident. They could be differentiated by both bleeding patterns and hormonal levels. Both groups received RU 486 on days 6 to 8 after the LH surge and there were no differences in RU 486 serum levels between the two groups. Thus these two patterns of response could not be differentiated on the basis of time of treatment, dosage of RU 486, or circulating RU 486 levels.

RU 486 has been proposed as a once-a-month pill.[6] Our data would support the value of RU 486 for this use. However, the emergence of two patient groups suggests that further characterization of these distinctive patterns is necessary.

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Late Luteal Phase Administration of RU486 for Three Successive Cycles Does Not Disrupt Bleeding Patterns or Ovulation*

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ABSTRACT. RU486, a 19-nor steroid, binds with high affinity to the receptors for progesterone and glucocorticoids, blocking the actions of these hormones on their target tissues. We conducted studies to determine whether RU486 administered at the end of the luteal phase would disturb the menstrual rhythm, ovulation, or hormonal parameters in the treatment and posttreatment cycles. The first study was done in six surgically sterilized women during two consecutive cycles. RU486 [17β-hydroxy-11β-(4-dimethylaminophenyl)17α-(1-propynyl)estra-4,9-dien-3-one; 100 mg/day] was given for 4 consecutive days, commencing on days 23–27 of the first cycle. Menstrual bleeding occurred by the second day of RU486 administration in all women and was indistinguishable from their usual bleeding pattern. The onset of this bleeding was advanced by RU486 administration, since it entailed shortening of the luteal phase with prolongation of the following follicular phase. Serum LH, FSH, estradiol, and progesterone levels were normal in five of the six women in both the treatment and posttreatment cycles.

The second study was conducted in 10 women who were not exposed to the risk of pregnancy. RU486 (100 mg/day) was given for 4 consecutive days, commencing 4 days before their expected menses for 3 successive cycles, preceded and followed by 2 placebo-treated cycles. Bleeding patterns were indistinguishable during the RU486 and placebo cycles. Late luteal phase administration of RU486 consistently produced menstrual bleeding within 1–3 days of drug administration. Daily early morning urinary LH excretion in 6 women and estrone glucuronide and pregnanediol glucuronide excretion in 5 women during both placebo and RU486 cycles were consistent with luteinization, suggesting ovulation and appropriate corpus luteum function.

We conclude that RU486 has no major effect on menstrual cycle events if given at the time of the natural progesterone withdrawal that occurs before menses in nonpregnant women. (*J Clin Endocrinol Metab* **65:** 1272, 1987)

RU486 [17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)estra-4,9-dien-3-one), a 19-nor steroid synthesized by Roussel-UCLAF in Paris, is a progesterone receptor antagonist in both human and animals (1, 2). RU486 administered to subhuman primates or to women at the time when progestational endometrium is present can induce endometrial bleeding, with partial or complete shedding of this tissue (3–10). The fact that this occurs despite high serum progesterone and estradiol levels supports the concept that this effect of RU486 is due to a direct action on the endometrium.

Administration of RU486 at the time of spontaneous progesterone withdrawal is unlikely to affect the menstrual cycle if the only action of the drug is to deprive target tissues of the action of progesterone. Thus, administration of RU486 at or close to the time of expected menses in women should ensure their occurrence with no other consequence to menstrual cycle events. This study was designed to test this proposition. This issue became more relevant since one report indicated that vaginal administration of RU486 during the luteal phase to unmated monkeys increased the interval from menses to ovulation to an average of 32 days (11). An additional report indicated that a single im injection of RU486 in the luteal phase increased the subsequent mean intermenstrual interval in monkeys up to 82 days through prolongation of the follicular phase (5). In this study we administered RU486 in the late luteal phase in up to three successive cycles to evaluate its effects on the hormonal profile and bleeding patterr of those and subsequent menstrual cycles.

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Materials and Methods

Study design

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Administration of RU486 at the time of spontaneous progesterone withdrawal is unlikely to affect the menstrual cycle if the only action of the drug is to deprive target tissues of the action of progesterone. Thus, administration of RU486 at or close to the time of expected menses in women should ensure their occurrence with no other consequence to menstrual cycle events. This study was designed to test this proposition. This issue became more relevant since one report indicated that vaginal administration of RU486 during the luteal phase to unmated monkeys increased the interval from menses to ovulation to an average of 32 days (11). An additional report indicated that a single im injection of RU486 in the luteal phase increased the subsequent mean intermenstrual interval in monkeys up to 82 days through prolongation of the follicular phase (5). In this study we administered RU486 in the late luteal phase in up to three successive cycles to evaluate its effects on the hormonal profile and bleeding pattern of those and subsequent menstrual cycles.

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Materials and Methods

Study design

Two studies were performed on 16 regularly cycling women. Investigational Review Board approval for both studies and

informed consent from each participant were obtained, and the women were compensated for participating in the study.

Six women, aged 32–37 yr, who had previously undergone surgical sterilization, participated in study 1. This study comprised a treatment and a posttreatment cycle. Each woman received RU486 commencing on days 23–27 of the treatment cycle in a dose of 50 mg twice daily for 4 days, between 0900–1000 and 2100–2200 h. Blood samples were collected on Monday, Wednesday, and Friday each week in both the treatment and immediate posttreatment cycles for the assay of LH, FSH, estradiol, and progesterone.

Ten women, aged 18–28 yr, who were not sexually active participated in study 2. They were studied during seven successive cycles. Placebo (in the form of a starch pill formulation) was administered in the initial two cycles and RU486 in the following three cycles. This was then followed by two further placebo-treated cycles. The women were not told when placebo or drug was administered. Placebo or RU486 in a dose of 50 mg was given near the end of the luteal phase every 12 h for 4 days. The first tablet was given 4 days before the expected day of menses. Daily early morning urine samples were collected throughout the seven cycles for the assay of LH, estrone-3α-glucuronide and pregnanediol-3α-glucuronide. Careful records were made of all menstrual bleeding episodes.

Assays

Hormones in serum and LH in urine were measured in duplicate, using reagents and procedures supplied by the WHO Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology (12). All serum samples from an individual woman were analyzed in the same RIA. The lower limits of sensitivity for the LH and FSH assays were 1.5 and 0.8 IU/L, respectively, and those for the estradiol and progesterone assays were 7 pmol/L and 0.5 nmol/L, respectively. The intraassay coefficients of variation for high values in the LH, FSH, estradiol, and progesterone assays ranged from 6–8%, while the interassay coefficients of variation ranged from 11–12%. Estrone-3α-glucuronide and pregnanediol-3α-glucuronide were determined in urine by previously described methods (13–16). The lower limits of sensitivity for the urinary LH, estrone-3α glucuronide, and pregnanediol-3α-glucuronide assays were 1.6 IU/L, 8.4 pmol/L, and 0.3 nmol/L, respectively. The intra- and interassay coefficients of variation in these three assays ranged from 6–12%. The early morning urine result correlates with the complete 24-h urine collection and can be used to monitor ovarian function (14). The rise in pregnanediol glucuronide is a reliable index of ovulation and corpus luteum function (15). Urinary LH was measured in six women, and urinary pregnanediol and estrone glucuronide in five. Because of the large number of samples and in view of the consistency of the results when these assays had been completed, the measurements were stopped at this point.

The results were analyzed using BMDP statistical software (17). Both Student's t test and one-way analysis of variance were used to compare the lengths of the cycle or its phases. Peak levels of each hormone were compared across the placebo and treated cycles as well as across subjects using two-way analysis of variance. $P < 0.05$ was considered statistically significant.

Results

Study 1

Bleeding commenced within 30–48 h after ingestion of the first dose of RU486. The mean (±SD) duration of the RU486 cycle was 26 ± 1.4 days, significantly less than the lengths of the prior and succeeding cycles, which were 29.7 ± 2.3 and 30.7 ± 1.9 days in duration, respectively ($P < 0.05$). The length of the follicular phase in the RU486 cycle was 14.2 ± 1.7 days compared to 17.4 ± 1.7 days in the succeeding cycle ($P < 0.01$). Corresponding luteal phase durations were 11.8 ± 1.3 and 13.2 ± 0.8 days, respectively ($P < 0.05$). The timing of RU486 administration was such that it anticipated the onset of bleeding, thus producing an apparent shortening of the luteal phase with prolongation of the succeeding follicular phase. The duration of bleeding was not different in the preceding, RU486, or succeeding cycles.

In five of the six women serum progesterone rose in the posttreatment cycle to levels indicating normal corpus luteum function (Figs. 1 and 2A). Serum LH, FSH, estradiol, and progesterone values in the sixth woman are shown in Fig. 2B. In this woman progesterone failed to increase in the posttreatment cycle in spite of a LH peak indicating that ovulation had occurred. However, before RU486 administration in this woman there appeared to be corpus luteum insufficiency, since her serum progesterone level only rose to 6 ng/mL (19.1 nmol/L). Her LH, FSH, and estradiol patterns, however, were normal in both cycles.

Study 2

Urinary LH excretion as well as estrone-3α-glucuronide and pregnanediol-3α-glucuronide excretion are shown in Figs. 3–5. In all cycles there were normal LH surges (Fig. 3), and there were no statistically significant differences in the heights of the LH peaks or intervals between successive LH surges in the RU486- or placebo-treated cycles. In the majority of the cycles, a rise in estrone glucuronide was evident in the follicular phase, and peak excretion occurred just before or coincident with the LH surge. Thereafter, estrone glucuronide levels decreased and rose again in the luteal phase, subsequently declining before the onset of bleeding (Fig. 4). The luteal phase rise in pregnanediol glucuronide in all cycles is consistent with luteinization, suggesting ovulation and appropriate corpus luteum function (Fig. 5). There was no statistically significant difference in peak estrone or pregnanediol glucuronide excretion between RU486- and placebo-treated cycles.

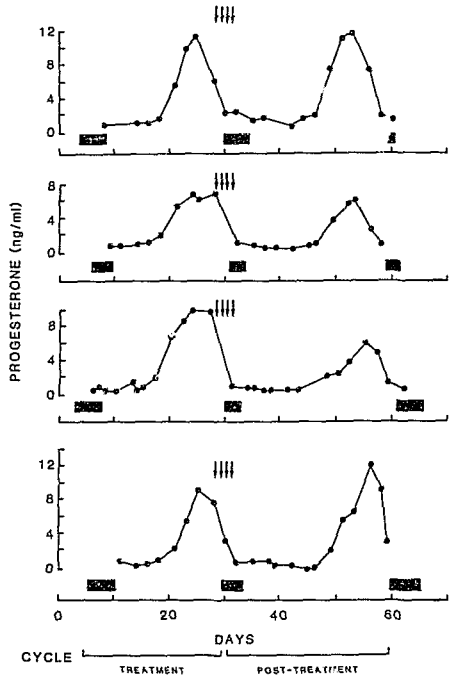


FIG. 1. Serum progesterone levels in four women who received RU486 commencing on days 23–27 of the first cycle. In this and subsequent figures, the duration of bl eding is indicated by the *black rectangles*, and RU486 administration is indicated by *arrows*. The rise of progesterone in the succeeding cycles is compatible with ovulation and corpus luteum function. To convert the serum progesterone values to SI units, multiply by 3.180 = nmol/L.

In 28 of 30 cycles, bleeding began within 1–3 days after commencement of RU486 administration and in 24 of these 28 cycles it began on the third day of RU486 administration. Bleeding started 1–10 days after initiation of placebo, with no particular day exhibiting a higher frequency. There was a small but statistically significant shortening ($P < 0.05$) of the RU486 treatment cycles compared to the lengths of the first, third, and fourth placebo cycles (Table 1). Duration of menstrual bleeding was similar in the placebo and treatment cycles.

The menstrual rhythm was disturbed in only one woman. Analysis of the hormonal profile in this women (Fig. 6) indicated that RU486 had been administered very early in the luteal phase in the second and third cycles, soon after the LH surges and at a time of maximum elevation of urinary estrone glucuronide excretion when pregnanediol glucuronide excretion was high or rising. There was an initial episode of bleeding on the second day of RU486 administration in the second treatment cycle and a subsequent episode when urinary steroids decreased. In the third treatment cycle only a single

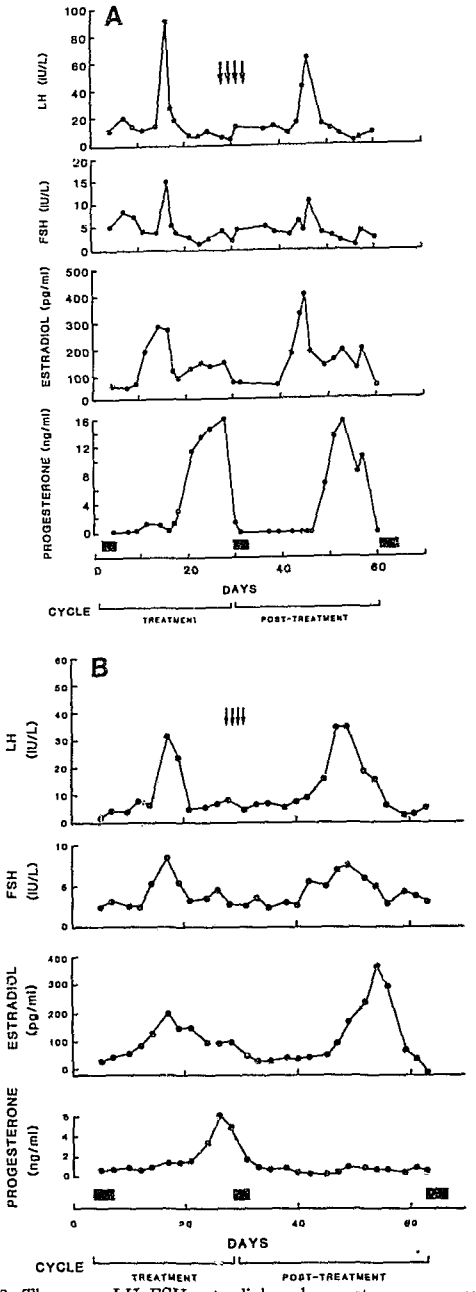


FIG. 2. The serum LH, FSH, estradiol, and progesterone responses in the RU486 and succeeding cycles in subject 5 (A) and subject 6 (B). The hormonal profiles are normal in both cycles in subject 5. In subject 6 there was an impaired progesterone response in the RU486 cycle and no progesterone rise in the succeeding cycle. To convert to SI units = estradiol pg/mL × 3.671 = pmol/L; progesterone ng/mL × 3.180 = nmol/L.

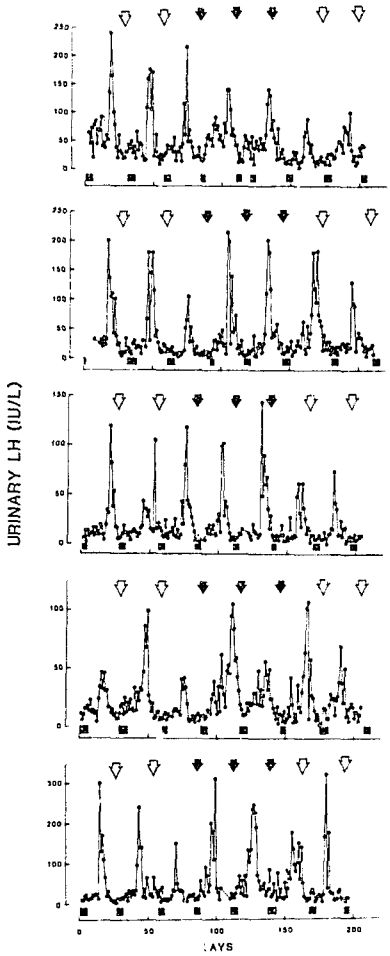


FIG. 3. Daily morning urinary LH levels in the initial five women during two placebo, three RU486, and a further two placebo cycles. In all instances normal LH surges are present. There are no consistent differences in the magnitude of increase and intervals between successive LH surges in either the placebo or RU486 cycles. The *open arrows* indicate the time of placebo administration and the *closed arrows* RU486 administration in this figure and Fig. 4 and 5.

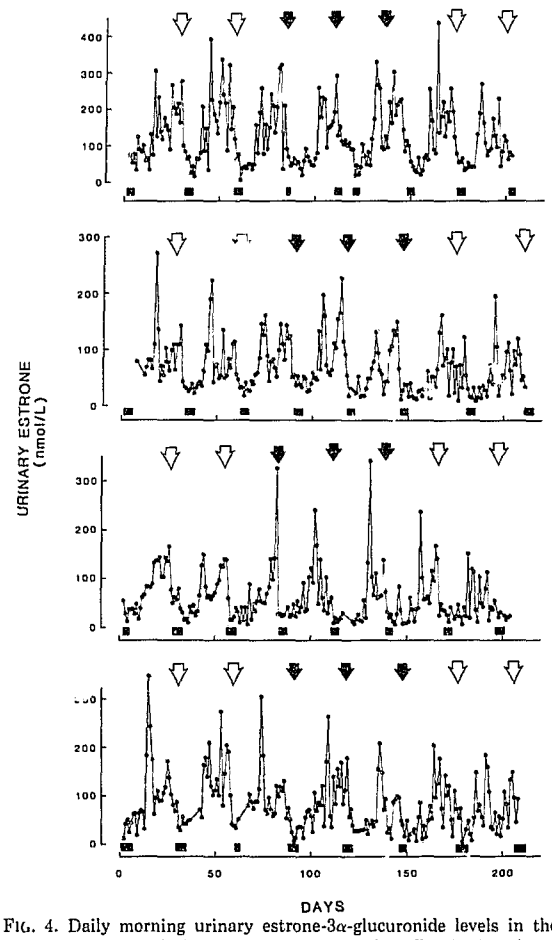


FIG. 4. Daily morning urinary estrone-3α-glucuronide levels in the initial four women during seven successive cycles. The rise in urinary estrone glucuronide in all cycles is indicative of follicular development.

delayed episode of bleeding occurred 13 days after commencement of RU486. This was the only woman in whom an altered bleeding pattern was evident. In another woman spontaneous bleeding began a few days early and before the commencement of RU486 administration. For this reason, RU486 was not given in that particular cycle. In both studies RU486 was well tolerated, and there were no adverse effects.

Discussion

We found that when RU486 was given 4 days before the expected menses it consistently produced onset of bleeding within 1–2 days. Based on previous observations on the induction of endometrial bleeding with RU486 in women with hCG-extended luteal phases (4), it was estimated that if RU486 was started 4 days before the expected menses, effective progesterone receptor blockade would develop in association with the fall in circulating progesterone. Bleeding started earlier than predicted, implying that progesterone receptor blockade did, in fact, occur. This early onset of bleeding consequent to RU486 was reflected in a shortening of the treatment luteal phase and corresponding prolongation of the post-treatment follicular phase. In the placebo-treated cycles there was a wide variation between the expected and actual dates of onset of menses. In contrast, RU486 administration, as used in this study, regularized the onset of bleeding and the interbleeding intervals. This

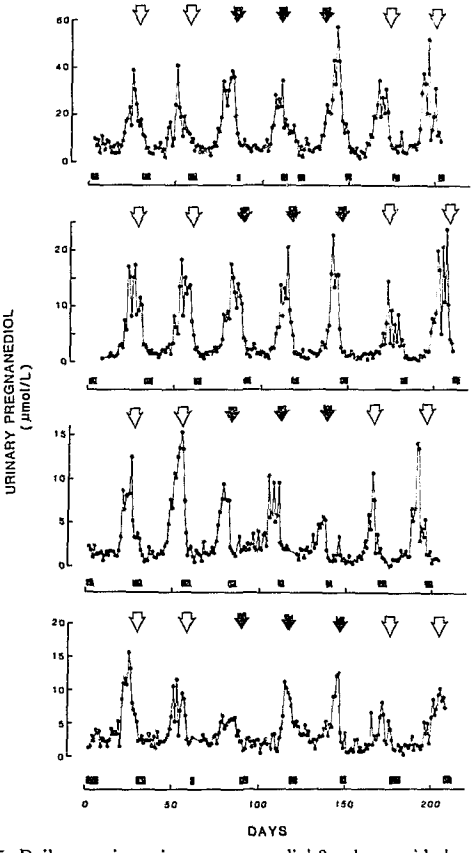


FIG. 5. Daily morning urinary pregnanediol-3α-glucuronide levels in the same four women as those in Fig. 4. The elevation of urinary pregnanediol glucuronide is compatible with ovulation and corpus luteum function.

TABLE 1. Duration of successive cycles in women receiving placebo or RU486 in the late luteal phase

Cycle	Treatment[a]	No. of cycles	Duration (days)[b]
First	Placebo	10	31.3 ± 1.1
Second	Placebo	10	28.9 ± 0.6
Third	RU486	10	27.9 ± 0.6
Fourth	RU486	10	27.7 ± 0.4
Fifth	RU486	9[c]	27.7 ± 0.4
Sixth	Placebo	10	31.1 ± 0.8
Seventh	Placebo	10	29.7 ± 0.9

[a] Placebo or RU486 (50 mg) was given twice daily for 4 days, commencing 4 days before the expected day of menses.

[b] Values shown are the mean ± SEM. The mean duration of the first, sixth, and seventh placebo-treated cycles was significantly longer than that of RU486-treated cycles ($P < 0.05$).

[c] One cycle excluded, since RU486 was taken too early in the luteal phase and two bleeding episodes occurred.

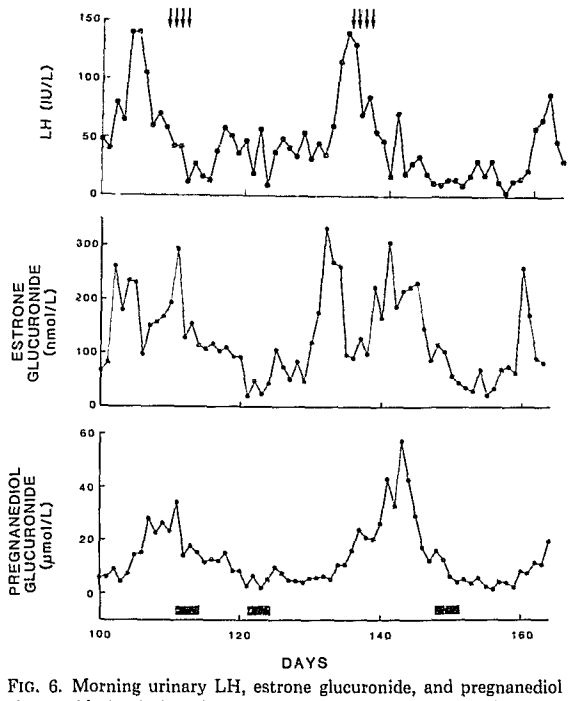


FIG. 6. Morning urinary LH, estrone glucuronide, and pregnanediol glucuronide levels in subject 1 during the second and third RU486 cycles. In both cycles, RU486 was administered soon after the LH surge at a time when urinary estrone glucuronide and preganediol values were high. This resulted in one episode of bleeding within 48 h after RU486 administration in the second cycle and a further episode occurring in association with the fall in urinary steroid levels. In the third RU486 cycle only a single episode of bleeding occurred 13 days after RU486 administration.

effect was highly predictable and has the potential of regulating cycle duration.

The rise in serum progesterone and in urinary pregnanediol glucuronide excretion is consistent with luteinization, suggesting ovulation and appropriate corpus luteum function. In a parallel study using the same dose of RU486, also administered over 4 days in the late luteal phase in a single cycle, we found normal follicular development in the posttreatment cycle by ultrasound (R. Sitruk-Ware, unpublished data). Thus, RU486 administered at the end of the luteal phase does not disturb the menstrual cycle rhythm and is associated with normal luteal function.

The prolongation of the follicular phase subsequent to RU486-induced bleeding in conjunction with LH peak intervals of normal duration is of interest. It implies dissociation between bleeding and initiation of a new cycle. In other words, the biological clock that times the occurrence of the next LH peak is not necessarily synchronized with the end of progesterone action. The re-

ported effect of RU486 on LH pulse frequency and amplitude conforms with the idea that it also blocks centrally located progesterone receptors (7, 18, 19). One obvious difference between the natural end of the normal luteal phase and the hypothetical decrease in progesterone binding to its receptor when RU486 is given, as in this study, is concerned with the participation of estrogens in the two situations. While RU486 administration selectively affects the response to progesterone, spontaneous luteolysis affects the actions of both hormones on target tissues. Thus, the fall in progesterone is sufficient to start endometrial bleeding, whereas the onset of a new cycle requires a decrease in both estrogen and progesterone.

In only one woman did disruption of the normal menstrual rhythm occur. Analysis of her hormonal profile indicated that RU486 had been given early in the luteal phase when urinary LH, estrone glucuronide, and pregnanediol glucuronide excretion were high. We previously found that the midluteal phase administration of RU486 in a single dose from 50–800 mg also produces menstrual bleeding within 1–3 days despite high progesterone levels; further bleeding frequently coincides with the fall in progesterone (3). Similar events occur when exogenous hCG is given in the luteal phase to simulate early pregnancy. RU486 has the ability to induce endometrial bleeding despite the high circulating estradiol and progesterone levels consequent to hCG treatment. A further bleed occurs in association with the decline in steroids (4).

Thus, maintenance of a normal cycle rhythm critically depends on the precise time of RU486 administration. Midfollicular phase RU486 administration prolongs the follicular phase and delays the LH surge, ovulation, and bleeding (19, 20). In the rhesus monkey a single injection of RU486 in the luteal phase also delays subsequent follicular maturation and prolongs the following follicular phase (5). However, in that study the drug was dissolved in alcohol, which significantly prolonged its half-disappearance time (21). Administration of RU486 at the time of the natural progesterone withdrawal that occurs before menses in nonpregnant women does not disrupt the menstrual rhythm or the hormonal profile of that and succeeding cycles. These results are consistent with the view that the effects of RU486 on menstrual cycle events may be attributed to its antiprogestin action.

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CONCLUDING REMARKS

Many of the predictions made in this thesis have been confirmed. It has now been established beyond question that isolated gonadotropin deficiency is indeed a heterogeneous disease due to deficiency of GnRH (1)[***] . The administration of pulsatile native GnRH has the ability to restore fertility in males and to induce ovulation in females with this syndrome (1). In addition, the mutated gene responsible for the X chromosome-linked form of Kallmann's syndrome, one of the commonest causes of isolated gonadotropin deficiency, has been cloned and sequenced. It is localized to the terminal part of the short arm of the X - chromosome in the Xp22.3 region (2).

The clinical use of potent GnRH agonists is now well established and used in situations where medical hypogonadism is required (3). In addition to acute intermittent porphyria, such situations include endometriosis, uterine fibroids, prostate and breast cancer and certain forms of precocious puberty. The major drawback, is that the hypogonadism consequent to long-term agonist administration, may produce accelerated bone loss due to the estrogen deficiency. Some form of add back therapy should be considered when long-term GnRH administration is used in situations such as endometriosis, uterine fibroids and even in intermittent porphyria.

With regard to the antiprogestin mifepristone, it has been shown that when the administration of mifepristone is followed within 36 - 48 hours by a prostaglandin such as misoprostol, the rate for successful termination of pregnancy reaches 95% in women with amenorrhea of less than 49 days. This combination is now marketed in several countries including France, Sweden, Britain and China (publications 16 and 17). The FDA have given their approval for its use in the U.S. and this will presumably occur during 1997. Mifepristone, in combination with a prostaglandin,

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[***] The number in brackets refers to the list of references cited at the end of this section.

has been used with great effect in subjects with menses delay to prevent implantation and pregnancy (4).

It should be emphasized, however, that mifepristone is more than just an agent to promote medical abortion and has widespread potential application both as an antiprogestin and antiglucocorticoid. Mifepristone acts as an effective "morning after pill" when used within 72 hours of unprotected intercourse. Although it is also possible that antiprogestins may be used as monthly menses regulators, to date this has not been satisfactorily accomplished (reviewed in publications 16 and 17).

Is there any possibility that mifepristone could still be used as a contraceptive? We have shown in monkeys that luteal phase administration of mifepristone has contraceptive potential (publication 26). However in that study high doses of mifepristone were used. Mifepristone delays endometrial development so that the fertilized ovum fails to implant. The endometrium is considerably more sensitive to mifepristone than the pituitary or ovary (reviewed in publication 17). In view of this, antiprogestins could be given in low doses which would prevent endometrial maturation. Evidence from studies in both animals and women indicate that this may be achieved (reviewed in publication 17). Thus antiprogestins may be developed as contraceptive agents which would act by delaying endometrial development and preventing implantation without altering hormone events or bleeding patterns of the cycle. This would be of great benefit to women who are reluctant for medical or personal reasons to use classical oral contraceptives.

Mifepristone could potentially be used as an antiglucocorticoid. It has a place in the treatment of Cushing's syndrome due to adrenal carcinoma and ectopic-ACTH secretion. In addition to its use in tumors containing steroid receptors, it may play a role in the prevention of viral

diseases in humans including AIDS and possibly in the treatment of burns, depression, some forms of hypertension and in patients with arthritis. Administered locally, it may lower intraocular pressure and thus have a role in the treatment of glaucoma. Many of these potential indications were reviewed in publications 16 and 17. Few of these were contemplated when the chemical structure of mifepristone was published in 1981.

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APPENDIX A:

DECLARATION IN TERMS OF RULE G28

1. ***Candidate's contributions to the publications.***

   A detailed analysis of my contribution to each of the submitted publications is provided in Appendix B.

2. ***Submission of publications for another degree.***

   None of these publications were included as part of another degree.

3. ***All the work was carried out while I was employed full-time at:***

   a) Department of Endocrinology & Metabolism, Hadassah University Hospital, Jerusalem, Israel (publications 1, 2, 7 - 9).

   b) Department of Endocrinology & Metabolism, Shaare Zedek Medical Center, Jerusalem, Israel (publications 3 - 6,10 - 13).

   c) Center for Biomedical Research, Population Council, New York, U.S (publications 14 - 30).

APPENDIX B

CANDIDATE'S CONTRIBUTION TO SUBMITTED MANUSCRIPTS

1. GROUP 1 (publications 1, 2 , 7 - 9).

These studies were all conducted at Hadassah University Hospital in Jerusalem when I was a fellow in the Department of Endocrinology under Dr. David Rabinowitz. I evaluated the patients, performed the majority of the studies, participated in the radioimmunoassays and wrote up publications 1, 7 and 8. In publications 2 and 9, I wrote the initial draft together with Dr. Julian Bell, who was also a fellow under Dr. David Rabinowitz.

2. GROUP 2 (publications 3 - 6, 10 - 13).

These studies were conducted when I directed the Department of Endocrinology and Metabolism at Shaare Zedek Medical Center in Jerusalem. In all these studies, I initiated the research, designed and planned the protocols and executed the research programs. Dr. Zylber-Haran headed the laboratory and Mr. Trestian was the senior technician. They conducted all the hormonal assays under my supervision. Dr. LeRoith, who at the time was an Endocrinologist at the Central Hospital of the Negev in Beersheva, performed all the clinical studies in publication 11 and some from publication 13. The subjects studied in publication 11 were referred by Dr. Potashnik. All blood samples collected in Beersheva were sent to Jerusalem and were analyzed in my laboratory. The determination of sex hormone binding globulin in publication 11 was performed by Dr. Dunn at the National Institutes of Health in Bethesda, MD. The studies on sleep in publication 12 were conducted in the sleep laboratory of Dr. Lavie from the Technion in Haifa, Israel.

The following physicians, Drs. Hirsch, Laufer, Palti, Polishuk, Rosen and Schenker, all referred patients to my clinical research center for study. During the course of these

studies some physicians in training in Internal Medicine, Obstetrics and Gynecology, and Surgery spent an elective period of 6 months in my Department. These included Drs. Cohen, Calderon and Halperin. Dr. Almaliach, a final year medical student from the Hebrew University did an elective period in my department. All these individuals helped with the clinical management and evaluation of the patients and performed some of the tests. I wrote up publications 3 - 6, 10, 12 and 13. Publication 11 was written up jointly with Dr. LeRoith.

3. GROUP 3 (publications 14 - 30).

Publications resulting from these studies were conducted while I was a Senior Scientist at the Center for Biomedical Research, at The Population Council in New York. The Population Council has no facility to conduct clinical studies. Consequently all studies are done in collaboration with other clinical centers. I selected the clinicians and the sites where the above mentioned studies could be performed. I had a key role in initiating and designing all the protocols. Consequently my contribution to all these clinical studies was critical and major. I coordinated the projects and monitored their ongoing evaluation by visiting these various clinic sites at very frequent intervals.

The clinical studies with the GnRH agonist were performed at the Clinical Research Center, Rockefeller University Hospital by Dr. Anderson and I assisted him in the execution of these studies.

The studies on antiprogestins were performed at the following centers:

- Santiago, Chile by Dr. Croxatto and Ms Salvatierra.
- University of Southern California School of Medicine, Los Angeles, CA by Drs. Grunberg, Mishell, Shoupe and Madkour.

- Hopital Necker, Paris by Dr. Sitruk-Ware.

- National Institutes of Health, Bethesda, MD by Drs. Nieman and Loriaux.

Measurement of mifepristone and its metabolites was carried out in Helsinki, Finland by Dr. Heikinheimo.

I played an important part in the analysis and interpretation of the results of all these collaborative studies. My precise participation in the publication of these manuscripts can be divided up as follows:

a) I wrote the manuscripts for publications 16 - 19, 26 and 30 which were then reviewed by the co-authors.

b) The following manuscripts were written up jointly by myself and the senior investigator as follows:

- Publications 14 and 15 with Dr. Anderson.

- Publication 22 with Dr. Grunberg.

- Publications 23, 25 and 29 with Dr. Shoupe.

- Publication 24 with Dr. Sitruk-Ware

- Publication 27 with Dr. Croxatto.

c) The initial drafts of the remaining three manuscripts were written by Dr. Nieman (publications 21 and 28) and Dr. Heikinheimo (publication 20). I reviewed, criticized and amended the manuscripts.

Other co-authors listed in publications 1 - 30 played only a very minor role in the execution of the studies, analysis of the results or drafting of the manuscripts.

Letters from co-authors testifying to my contribution to the studies are enclosed in in a letter submitted to the Dean of the faculty of Medicine.

Author Spitz I M
Name of thesis Studies In Reproductive End Ocrinology Spitz I M

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