Effects of the antiprogesterone RU 486 in normal women

I. Single-dose administration in the midluteal phase

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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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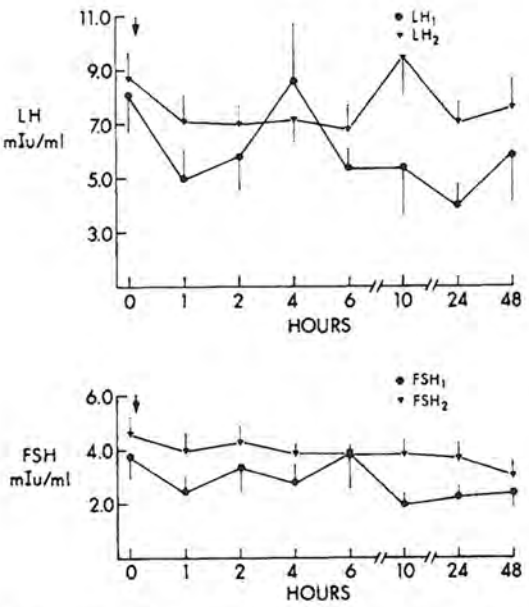

Fig. 1. LH *(upper panel)* and FSH *(lower panel)* values after a single oral dose of RU 486 ranging from 50 to 800 mg. LH_1 and FSH_1 refer to the patients with one bleeding episode after RU 486 administration and LH_2 and FSH_2 refer to patients having two bleeding episodes. No significant changes 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.

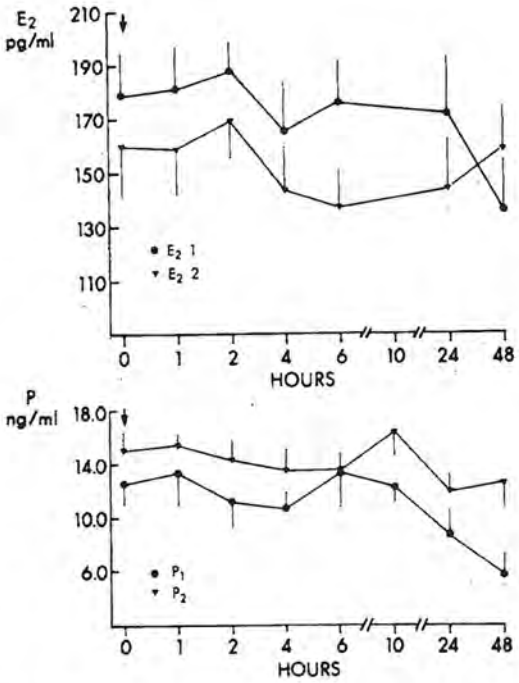

Fig. 2. *Upper panel*: Estradiol (E_2) values after a single oral dose of RU 486 ranging from 50 to 800 mg. $E_2 1$ refers to patient with one bleeding episode after RU 486 administration and $E_2 2$ refers to those with two bleeding episodes. Analysis of variance detected a significant decrease in $E_2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

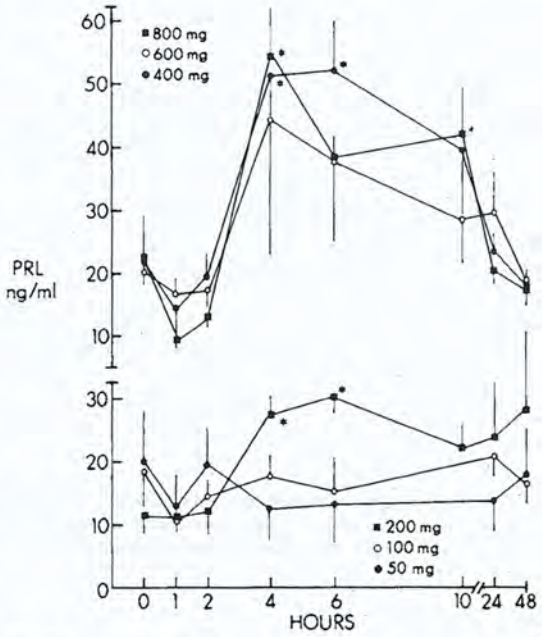

Fig. 3. Prolactin *(PRL)* levels after a single oral dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected a significant increase in *PRL* after the 50, 200, 400, and 800 mg doses. * $p < 0.05$ increase over baseline values as determined by the paired *t* test.

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, 13] 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.[6-14]

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

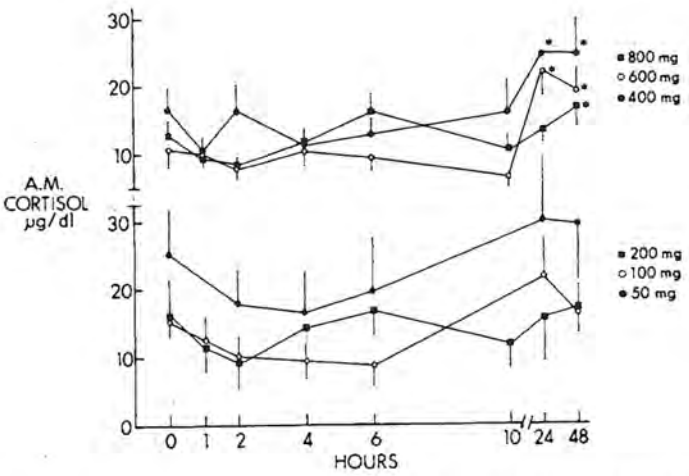

Fig. 4. Cortisol values after a single dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected a significant increase in cortisol ($p < 0.05$) after the 100, 400, 600, and 800 mg dose. * $p < 0.05$ increase over baseline values as determined by the paired t test.

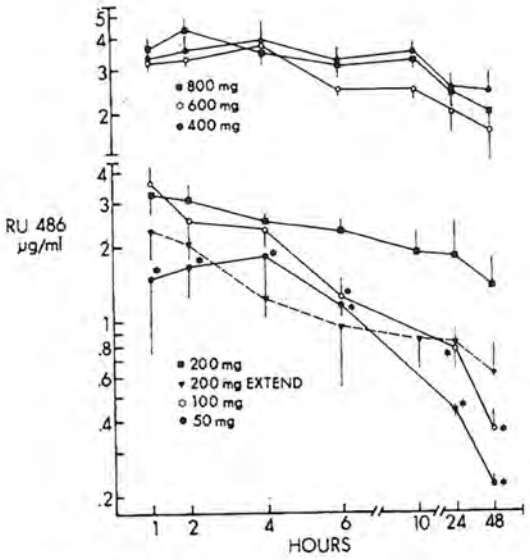

Fig. 5. RU 486 levels after a single oral dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected that all points after 50 mg dose and at 6, 24, and 48 hours after the 100 mg dose were significantly lower ($p < 0.05$) than values after the higher doses. The *200 mg extend* refers to the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

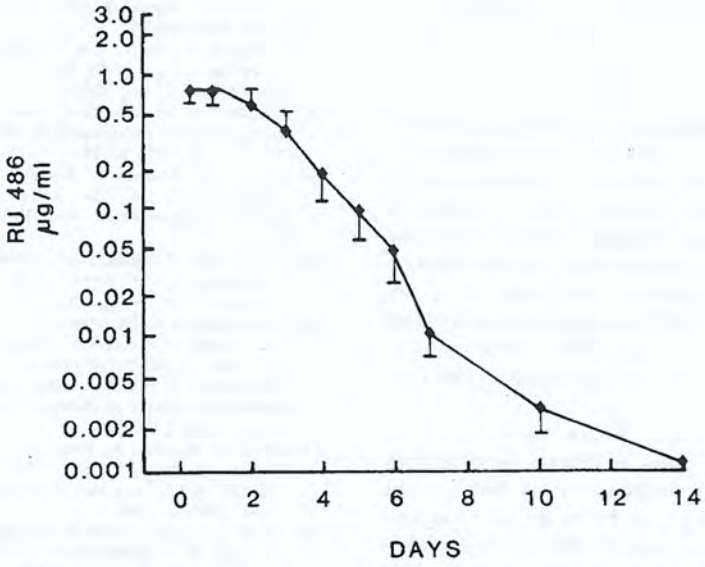

Fig. 6. RU 486 levels in the extended sampling group after a single oral dose of 200 mg RU 486. The half-life of RU 486 is 24 hours.

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-

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