Late follicular phase administration of mifepristone suppresses circulating leptin and FSH – mechanism(s) of action in emergency contraception?

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Abstract

Objective: Low dose mifepristone (RU486) is highly effective in emergency post-coital contraception (EC), although the mechanism(s) of action remains unclear. We studied the endocrine actions of 10 mg mifepristone administered orally as a single dose to healthy volunteers (aged 20–45 years) during the late follicular phase.

Methods: Serum levels of LH, FSH, oestradiol, progesterone, leptin, mifepristone, cortisol, and glucocorticoid bioactivity (GBA) were measured before and 1, 2, 4 and 8 h after ingestion of mifepristone on cycle day 10 or 11 (study day 1), and follow-up was continued for 10 days. Ovarian ultrasonography was performed on study days 1 and 7. Similar measurements were carried out during a control cycle.

Results: Mifepristone postponed ovulation, as evidenced by a 3.4 ± 1.1 day (means ± SD) delay (P < 0.005) in the LH surge and 3.6 ± 4.0 day prolongation of the treatment cycle (P = 0.08). During the mifepristone cycle, an LH surge was displayed by five subjects when serum mifepristone levels had declined to 9.5 ± 7.1 nmol/L. During the day of mifepristone administration, circulating GBA (P < 0.001) and leptin (P < 0.001) levels declined. On the day after mifepristone administration, mean serum FSH and leptin levels were lower than pretreatment values (3.8 ± 1.8 IU/L vs 5.2 ± 1.1 IU/L, n = 7, P < 0.05; 28.9 ± 6.7 μg/L vs 33.2 ± 9.0 μg/L, n = 7, P < 0.05 respectively), and the corresponding difference in the mean serum oestradiol concentration was borderline (452 ± 252 pmol/L vs 647 ± 406 pmol/L, n = 7, P = 0.056). In contrast to the control cycle, individual leptin levels declined during the follow-up after ingestion of mifepristone (n = 8, P < 0.01).

Conclusions: These data showed that the commonly employed dose of mifepristone for EC delays ovulation and prolongs the menstrual cycle, when given during the late follicular phase. The mechanism of action of mifepristone may include a reduction of FSH secretion via a decrease in circulating leptin.

Introduction

Emergency post-coital contraception (EC) with mifepristone is highly efficient (1–4). It was estimated that single doses of 10, 50 or 600 mg mifepristone prevented 84–86% of pregnancies in a multicentre trial (4). The action of mifepristone in EC is likely to involve several mechanisms, both endocrine and endometrial (5). Investigations evaluating the effects of late follicular phase administration of mifepristone (6–8) have revealed arrested follicular growth and a decrease in serum oestradiol concentration associated with either no change (7) or a slight decline in circulating gonadotrophin levels (6). Following preovulatory administration of 10 mg mifepristone, the luteinizing hormone (LH) surge was retarded for 2–5 days in some subjects and was not detectable in others (8). Thus, inhibition or a delay of ovulation is probably the most important single factor mediating the effect of this drug in EC (9).

The mechanism by which mifepristone perturbs ovulation remains unclear, especially as the drug is capable of inhibiting both glucocorticoid and progesterone receptors: both signalling pathways may have direct
or indirect effects on gonadotrophin secretion. For example, both progesterone and glucocorticoids are involved in the regulation of circulating leptin concentrations, which, in turn, may modify the timing of ovulation. The role of leptin appears to be age dependent, because the (pre)pubertal rise in circulating leptin concentrations in adolescent girls is associated with maturation of the hypothalamic–pituitary–ovarian axis and may act as a signal for the initiation of menstrual cycles (10, 11). After establishment of ovulatory cycles, leptin is thought to have at least a permissive role in ovulation (12). In adult women, serum leptin concentrations increase during the luteal phase of the cycle (13–15) and during the follicular phase after administration of progesterone (16). On the other hand, leptin is also regulated by glucocorticoids (17). For example, dexamethasone increases circulating leptin concentrations in both men and women, and stimulates secretion of leptin from cultured adipocytes (18, 19). Anti-glucocorticoid effects of mifepristone on adipose tissue lipoprotein lipase activity have been demonstrated previously by Ottilsson et al. (20). To the best of our knowledge, the effect of mifepristone on circulating leptin concentrations in humans and the subsequent timing of ovulation has not been investigated.

The purpose of this work was to characterize the endocrine effects of mifepristone given during the late follicular phase that potentially contribute to its action in EC. In particular, we have hypothesized that mifepristone may perturb ovulation in part by suppressing circulating leptin concentrations.

Materials and methods

Subjects

Eight healthy women between 20 and 45 years of age volunteered for the study. All had regular menstrual cycles, with cycle length varying between 25 and 32 days. None of them used hormonal contraception: four were sterilized, one used a copper-releasing intrauterine device and the remaining three used barrier methods. All were of normal weight, with body mass index varying from 19 to 26 kg/m². Prior to their participation in this study, each woman signed an informed consent document.

Protocol

Following an overnight fast, a single dose of 10 mg mifepristone (supplied by Hualian Pharmaceuticals Co. Ltd, Shanghai, China) was ingested on day 10 or 11 of the menstrual cycle. On the day of mifepristone administration (day 1), venous blood samples were collected in the morning before (0730–0930 h) and 1, 2, 4 and 8 h after ingesting the drug, daily thereafter for the next 6 days and then on day 11. The same blood sampling protocol was repeated during a control cycle without mifepristone. However, samples equivalent to those taken at 1–8 h in the mifepristone cycle were not collected on day 1 during the control cycle. The time of blood sampling varied between 0700 and 1905 h. The control cycle and the mifepristone cycle were separated by a minimum of one regular menstrual cycle. The subjects kept a diary of their menstrual cycles. The study protocol was approved by the Institutional Review Board of Helsinki University Central Hospital and the Finnish National Agency for Medicines. Pharmacokinetic data concerning 10 mg mifepristone have been published elsewhere (21).

Transvaginal ultrasonography

Transvaginal ultrasonography (US) was carried out in all participants on day 1, and again on day 7, during both the control and mifepristone-treated cycles in order to measure the diameter of the largest follicles in both ovaries. A Hitachi EUB-550 scanner (Hitachi Medical Corporation, Tokyo, Japan) with a 6.5 MHz vaginal transducer was used.

Hormone assays

Serum LH, follicle-stimulating hormone (FSH), progesterone and cortisol concentrations were measured by time-resolved fluoroimmunoassays. Commercial kits (DELFIA), manufactured by Perkin Elmer Life Sciences (Turku, Finland) were used. The detection limits reported by the manufacturer were 0.05 U/l for both LH and FSH, 0.8 nmol/l for progesterone and 15 nmol/l for cortisol. The intra-assay and interassay coefficients of variation (C.V.) were 2.0–2.4% and 3.1–4.2% for LH, 2.0–2.8% and 1.8–2.0% for FSH, 3.3–7.3% and 2.7–10.1% for progesterone and 4.5–7.0% and 3.5–5.0% for cortisol respectively. Oestradiol concentrations were determined by RIA according to the protocol from the World Health Organization (22). The quantitation limit of the RIA (55 pmol/l) is the lowest concentration that can be measured with acceptable precision (i.e. intra-assay C.V. \( \leq 20\% \)). The intra-assay C.V. varied from 4.3 to 7.4% and the inter-assay C.V. from 7.9 to 16.6%. Serum levels of mifepristone were measured by RIA preceded by Chromosorb column chromatography (Sigma, St. Louis, Missouri, USA), as described previously (23). The quantitation limit of the assay was 0.36 nmol/l. The intra-assay C.V. was 8.4% and the interassay C.V. varied from 10.3 to 13.6%. An immunoradiometric assay (IRMA) was used to measure serum leptin concentrations. The commercial IRMA kits were manufactured by Diagnostic Systems Laboratories Inc. (Webster, Texas, USA). The detection limit was 0.10 ng/ml. The intra-assay and interassay C.V. values varied from 2.6 to 4.9% and 3.7 to 6.6% respectively.

Glucocorticoid bioactivity (GBA) was measured directly from 10 μl serum samples using a recombinant
cell bioassay in which COS-1 cells are transfected with expression vectors encoding human glucocorticoid receptor and a nuclear receptor co-regulator, ARIP3, together with an appropriate reporter gene (24). In the current work, GBA values less than 15.6 nmol/l cortisol equivalents were considered undetectable.

Data analyses

Ovulation was defined to have taken place following an LH peak if an increase in progesterone of 10 nmol/l was measured and/or if signs of preceding follicular rupture were seen in US performed on study day 7. Correlations between various parameters were assessed by simple regression. Paired t-tests and ANOVA for repeated measurements followed by Scheffe’s post hoc analysis were used when appropriate. To investigate the changes in serum leptin concentrations during the follicular phase of the control and mifepristone cycles, the method of summary measures (25) was employed. In brief, linear regression lines were fitted for each individual with the day of the cycle as an independent variable and serum leptin concentration as a dependent variable. The areas under the concentration curves (AUCs) for serum oestradiol and progesterone were calculated using the trapezoidal rule (26). The AUCs and slopes of the leptin equations were subsequently compared by means of the paired t-test. A P value of ≤ 0.05 was considered significant.

Results

Effects of mifepristone on the menstrual cycle

We examined the effects of a low dose of mifepristone given during the late follicular phase of the menstrual cycle in healthy women who were followed-up during a control cycle and a mifepristone treatment cycle. We examined eight women and evidence of ovulation during the control cycle was found in seven. The

Table 1 Day of the LH peak, cycle length and AUCs for progesterone (P₄) and oestradiol (E₂) between days 1 and 7 of the control and mifepristone cycles. The data are derived from the seven subjects who ovulated during the control cycle and are expressed as means ± s.d. LH peaks during the follow-up period in the mifepristone cycle were recorded in five subjects.

<table>
<thead>
<tr>
<th></th>
<th>LH peak (cycle day)</th>
<th>Cycle length (days)</th>
<th>AUC P₄ (nmol/l per day)</th>
<th>AUC E₂ (pmol/l per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cycle</td>
<td>12.7 ± 2.4</td>
<td>26.1 ± 3.3</td>
<td>64.2 ± 55.3</td>
<td>3375 ± 862</td>
</tr>
<tr>
<td>Mifepristone cycle</td>
<td>14.8 ± 0.8†</td>
<td>29.7 ± 3.5</td>
<td>20.3 ± 15.8</td>
<td>2797 ± 1393</td>
</tr>
<tr>
<td>Difference between the cycles</td>
<td>3.4 ± 1.1†</td>
<td>3.6 ± 4.0</td>
<td>0.06</td>
<td>0.58</td>
</tr>
<tr>
<td>P value</td>
<td>0.003</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Based on the five subjects who displayed surges of LH secretion following mifepristone administration; their LH peaks occurred on cycle day 11.4 ± 1.4 during the control cycle.

Figure 1 Serum FSH, LH, oestradiol, progesterone and mifepristone levels during the late follicular phase of a control cycle (no medication; left-hand panels) and the mifepristone cycle (10 mg mifepristone on cycle day 10; right-hand panels) in a representative subject.

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subject who did not ovulate during the control cycle was removed from further analysis of the effects of mifepristone on the menstrual cycle. Data related to the timing of ovulation during the two cycles, together with the AUCs for oestradiol and progesterone between days 1 and 7 in these seven subjects are given in Table 1. The delay in the LH surge appeared to be dependent on mifepristone concentration, because LH peaks exceeding 25 IU/l became evident in five subjects only after circulating mifepristone levels had declined to 9.5±7.1 nmol/l (means±S.D.). Figure 1 shows the circulating FSH, LH, oestradiol, progesterone and mifepristone levels during the two menstrual cycles in a representative subject. Administration of 10 mg mifepristone on cycle day 10 prolonged her menstrual cycle from 25 to 28 days; her LH peak was postponed by 2 days.

Figure 2 shows circulating FSH and LH levels (means±S.D.) during the control and treatment cycles among the seven subjects who ovulated during the control cycle; in addition, serum mifepristone concentrations are shown. Serum levels of FSH were significantly different (P < 0.01, two-way ANOVA) during days 1–4 of the control and mifepristone cycles, whereas those of LH approached significance (P = 0.1, two-way ANOVA). During the mifepristone cycle, the decline in serum FSH from day 1 to day 2 was statistically significant (P < 0.05). Serum progesterone levels were significantly different (P < 0.01, two-way ANOVA), whereas those of oestradiol were not distinguishable (data not shown) between the two cycles during days 1 to 4.

Transvaginal US was performed twice during the control and mifepristone cycles. The diameter (means±S.D.) of the dominant follicle measured on study day 1 did not differ between the control and mifepristone cycles (14.5±4.4 vs 12.4±3.2 mm respectively). During the mifepristone cycle, the dominant follicle that had been visualized on day 1 persisted in three of the seven subjects, whereas ovulation had occurred prior to day 7 in the remaining four subjects. The size of the dominant follicle and the delay in the LH surge during the mifepristone cycle were not correlated.

**Effects of mifepristone on circulating GBA and leptin**

On the day of mifepristone administration, circulating GBA levels were rapidly suppressed close to, but above, the detection limit of the bioassay (Fig. 3A), whereas there was no evidence of a compensatory increase in serum cortisol occurring on the same day or the day following mifepristone administration (Fig. 3A). At approximately noon, GBA values during the mifepristone cycle and those observed without medication began to overlap, indicating that relatively short suppression of GBA is brought about by 10 mg mifepristone (Fig. 3A). This suppression of GBA (P < 0.0001, ANOVA) was accompanied by a decrease in circulating leptin concentrations (P < 0.001, ANOVA) (Fig. 3B). On the day after mifepristone administration, the mean serum leptin level was lower than the 0 h (pretreatment) value (28.9±6.7 µg/l vs 33.2±9.0 µg/l, n = 7, P < 0.05).

Individual serum mifepristone levels during the days following mifepristone ingestion are shown in Fig. 4A and the individual profiles for serum leptin during the control cycle and mifepristone cycle are shown in Fig. 4B. We next compared the changes in serum leptin concentrations during the control and mifepristone cycles by fitting linear regression lines for
each woman with leptin values from both cycles with the day of the cycle as an independent variable and serum leptin concentration as a dependent variable. There was a significant difference ($P < 0.01$) between the slopes of the regression lines between the control and mifepristone cycles (Fig. 4B). To ensure that this difference was not caused by diurnal variation in leptin, we adjusted serum leptin levels for the time of day (serum leptin concentration divided by the corresponding time of the day) and repeated the regression analyses. After this procedure, the difference between the cycles remained significant (Fig. 4B; $P < 0.05$).

**Discussion**

In the present study, we have demonstrated, as also shown previously (6–8), that preovulatory administration of mifepristone to healthy women interrupts the final stages of follicular development and postpones ovulation. Typical characteristics of mifepristone action in EC are a dose-dependent delay in the subsequent menstrual period and a risk of unintended pregnancy in cases of further unprotected intercourse following use of the drug (3, 27). The relationship between circulating mifepristone concentration and inhibition of ovulation has not been previously assessed in women. Among the present subjects, LH peaks were apparent when the mean circulating mifepristone level had declined to a mean of 9.5 nmol/l. Serum levels of mifepristone exceeded 10 nmol/l for approximately 4 days, which is well in line with the observed prolongation of the menstrual cycle following mifepristone administration. Thus, when counselling women requesting mifepristone for EC, the risk of unintended pregnancy later during the same cycle needs to be considered.

In this work, we found that administration of mifepristone was followed by a statistically significant decline in serum FSH levels, which was associated with a similar, albeit non-significant, change in serum oestradiol concentrations. A decline in circulating oestradiol levels after preovulatory administration of mifepristone associated with either a slight decline or a lack of normal FSH increase has been reported previously (6, 7). Thus, prolongation of the follicular phase and the subsequent delay in ovulation brought about by mifepristone may occur via suppression of FSH.

The precise mechanism(s) by which mifepristone affects the timing of ovulation has remained enigmatic. Mifepristone has been shown not to alter the pattern of pulsatile secretion of LH (6, 7) or pituitary responsiveness to gonadotrophin-releasing hormone (GnRH) (28). Mifepristone possesses both anti-progestogenic and anti-glucocorticoid properties, and their roles in the timing of ovulation have been investigated previously. For example, co-administration of dexamethasone and mifepristone during the late follicular phase does not abolish the mifepristone-induced inhibition of ovulation in cynomolgus monkeys (29). In healthy
Women progesterone has been shown to reverse the midcycle effects of mifepristone (30). Thus, the ability of mifepristone to perturb ovulation and FSH secretion is thought to be a result of the anti-progestogenic effect. On the other hand, we found that mifepristone significantly modified circulating levels of leptin. Accumulating evidence suggests that leptin is an important modulator of the timing of menstrual
cycles. For example, in laboratory animals, leptin is known to regulate GnRH secretion via neuropeptide Y (31, 32), and administration of exogenous leptin to fasting rodents and monkeys prevents suppression of gonadotrophin secretion (31, 32). In humans, administration of leptin to a girl with a leptin gene mutation resulted in gradual activation of gonadotrophin secretion (33), and leptin is suggested to regulate the nocturnal LH profile in the mid- to late follicular phase that precedes ovulation (34). Although adipocytes express progesterone receptors (35), progesterone does not stimulate leptin secretion of cultured adipocytes (36). On the other hand, expression and release of leptin from adipose tissue is stimulated by glucocorticoids (18, 19, 37). We found that mifepristone induced a profound suppression of circulating GBA, and have previously shown that the concentrations of mifepristone in serum and adipose tissue after a single ingested dose are similar (38). Thus, the observed suppression of leptin in the current work probably reflected an anti-glucocorticoid effect of mifepristone in fat, similar to that previously demonstrated with cultured adipocytes (39). It is of note that healthy women in the mid- to late follicular phase of the menstrual cycle display an increase or no change in leptin around noon (34), whereas we found that leptin declined during the day of mifepristone ingestion. We cannot therefore exclude the possibility that, in humans, suppression of circulating leptin concentrations following ingestion of mifepristone contributes to the subsequent and transient decrease in gonadotrophin secretion.

In conclusion, late follicular phase administration of 10 mg mifepristone delayed or inhibited ovulation in all subjects. Mifepristone may postpone ovulation via several endocrine mechanisms, suppression of circulating FSH being the most probable. The mechanisms that lead to gonadotrophin suppression following mifepristone may involve changes in circulating leptin.

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