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LUTEAL PHASE CONTRACEPTION WITH MIFEPRISTONE (RU 486) IN THE RHESUS MONKEY

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Abstract: Mifepristone (RU 486), an antiprogesterone, is a promising luteal phase contraceptive agent for human use. However, at present its use is limited by the practical constraint of determining the day of ovulation for an LH+2 day administration of the drug as indicated from experimental and clinical studies. The aim of the present study was to identify the effective period of luteal phase (luteal phase window) when a single administration of mifepristone would induce antinidatory activity without disturbing menstrual cyclicity and ovulatory pattern in the rhesus monkey. RU 486 (2 mg/kg body weight in benzyl benzoate/olive oil, 1:3) was given to mated monkeys (n = 9) on cycle day 16 in the first treatment cycle (treatment group T1, n = 9), and in the following cycle on cycle day 20 (treatment group T2, n = 8). A single s.c. injection of this antiprogestin during early to midluteal phase (days 1-10 after ovulation, as determined from retrospective analysis of serum concentrations of estrogen and progesterone) provided a one hundred per cent protection against pregnancy, with no apparent side effects. There were no changes in cycle lengths ($F=3.5$; $P < 0.3$), day of ovulation ($F=1.8$; $P < 0.7$) and duration of menses ($F=3.5$; $P <0.3$) compared with the pre-treatment and post-treatment cycles. Pooled analyses of serum concentrations of estrogen and progesterone during luteal phases of T1 and T2 cycles also showed no variations with those in pre- and post-treatment cycles. Retrospective analysis revealed that administration of mifepristone during the midluteal period (days 6-10 after ovulation) induced a decrease ($P <0.02$) in mean AUC of serum progesterone concentrations along with consistent shortening ($P<0.05$) of lengths of treatment cycles as compared to pre-treatment cycles. No such changes in cycle lengths and AUCs for luteal phase progesterone were noted when mifepristone was administered on days 2-5 after ovulation. Thus, the duration of the window for early luteal phase contraception with mifepristone was found to be wider (days 2-5 after ovulation). Two monkeys became pregnant in post-treatment cycles indicating that reproductive functioning in the post-treatment cycle was not impaired. The results support the suggestion that the antinidatory principle of early luteal phase mifepristone administration offers a promising lead for the design of a novel and user-acceptable contraceptive for human use.

Key words : antiprogestin endometrial maturation early luteal phase contraception

INTRODUCTION

Normal luteal phase endometrial maturation which is essential for blastocyst implantation is mainly dependent upon progesterone. In fact, progesterone insufficiency causing inadequate endometrial secretory differentiation remains one of the well known causes of infertility (1). It is, therefore, possible that appropriate application of an antiprogestin during the post-ovulatory luteal period inhibits uterine preparation for nidation. If true, this will provide a plausible new lead in luteal phase contraception. To this end, mifepristone appears

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to be a promising contraceptive agent. Mifepristone (RU 486), a 19-nor steroid with p-dimethyl-aminophenyl group at the 11β position and 1-propyl chain at the C-17 position is an antiprogestin which binds to progesterone receptors with higher affinity than progesterone itself (2). RU 486 is indeed known to affect progesterone dependent uterine cell functions in many mammalian species including primates (2). Administration of this antiprogestin during the mid- to late-luteal phase of the cycle, however, did not appear to be very promising for several reasons : (a) The luteal phase of the treatment cycle tends to become shortened (3,4). (b) Two episodes of vaginal bleeding may be seen following mifepristone treatment. Generally, the first bleeding episode is drug induced, while the second episode is regular menstruation associated with the demise of corpus luteum (5). (c) There may be incomplete shedding of the endometrial functionalis following low dose of RU 486 administration (6). In that case, chances of blastocyst nidation remain unpredictable. (d) On the other hand, a higher dose which seemingly assures complete shedding and failure of implantation, is accompanied by a marked increase in the length of the subsequent cycle (7).

Swahn et al (8), however, demonstrated that a timed single dose, oral administration of RU 486 to healthy human volunteers on the second day after the urinary luteinizing hormone (LH) surge induced significant retardation in endometrial development without upsetting their menstrual cylicity. It appeared possible that application of mifepristone during early luteal phase can be used as a post-coital, once-a-month luteal phase contraception for human use. Subsequently, we have reported that a single subcutaneous application of mifepristone at 2 mg per kg body weight on the second day after ovulation provided effective contranidatory action without otherwise affecting menstrual cycles and steroid hormonal patterns in mated rhesus monkeys (9,10). Very recently, it has been reported that only one pregnancy occurred in a study of 21 women receiving a single treatment of 200 mg RU 486 on LH + 2 day of cycle as their only contraceptive method for 1-12 months (11).

Two issues clearly emerge from these studies. Firstly, the efficacy of mifepristone as a once-a-month contraceptive agent when given on second day after ovulation is significantly high without any untoward side effects. Secondly, it appears that the feasibility and the acceptibility of the present approach is limited at least for the Indian population, mainly because self-monitoring of ovulation using the Ovu-quick method for rapid LH test as has been done in the human study (11) is a serious practical hurdle. A more feasible method may be formulated if it can be identified that the effective length of the 'window' for luteal phase contraception with a single treatment of mifepristone is wider than is being presently viewed. The present study was therefore conducted in rhesus monkeys with an aim to assess the contraceptive efficacy of once-a-month mifepristone application (2 mg per kg body weight) given on days 16 and on day 20 in two successive, ovulatory, mated cycles. Cycle days 16 and 20 were chosen *a priori* based on the assumption that these cycle days represent the interphase of early luteal period of a 30 day menstrual cycle. Hormonal profiles, ovulatory patterns, vaginal bleeding and occurrence of implantation and pregnancy were monitored for each treatment, as well as, pre- and post-treatment cycles.

METHODS

Animal : Sexually mature, healthy and proven fertile male and female rhesus monkeys *(Macaca mulatta)* were used in this study. The details of animal housing and management have been described elsewhere (9,10,12). Briefly, monkeys were individually housed in cages having access to natural lighting and fed with standard pellet diet supplemented with fresh soaked gram, peanuts and water *ad libitum.* Menstrual cycles were monitored by daily examination of vaginal swabs, and females showing at least two consecutive ovulatory cycles of normal length (26-32 days) were used in this study. The present study was carried out in the Primate Research Facility, All India Institute of Medical Sciences.

Treatment : Nine female monkeys showing normal ovulatory cycles were cohabitated with males during cycle days 8 to 16 (cd 8-16). Success in insemination was semi-quantitatively assessed by the daily microscopic examination of vaginal smears during the mating period. A single injection of mifepristone (2 mg/kg body weight in benzyl benzoate/olive oil, 1:3) was given subcutaneously (s.c.) on cd 16 (treatment 1, T1) of the first mated treatment cycles (n = 9) of

individual females. In the second mated treatment cycles (n = 8), mifepristone was injected s.c. on cd 20 (treatment 2, T2). All monkeys were carefully evaluated for vaginal bleeding, duration of menstrual flow and resumption of cyclicity throughout the pre-treatment (PT), treatment (T1 and T2) and post-treatment (PoT) cycles. These cycles were also monitored for the serum concentrations of estradiol-17β (E) and progesterone (P) by collecting peripheral blood samples (daily during cd 8-16, and then on alternate days till menses commenced) and performing radioimmunoassays (RIAs) as described earlier (13).

Detection of ovulation and pregnancy: The procedural details for the detection of ovulation and pregnancy have been given earlier (9,10). In brief, 24 h after the peak level of serum E was taken as the day of ovulation. Pregnancy was determined on the basis of two fold criteria (9). A successful mated cycle having a serum concentration of P at 10 nmol/l or more during day 10 onwards after ovulation along with the extension of menstrual cycle was considered as a potential conception cycle. In cases, wherein cycle lengths were extended beyond 50 days, uterine palpation was done to check for the occurrence of clinical pregnancy.

Data analysis : RIA results were analysed using log-logit transformation of the data. Hormone values were log transformed for analysis and means ± 2SD were calculated. The area under the curve (AUC) for both serum levels of E and P during days 2-14 after ovulation (taking the day of ovulation as day 0) was determined and used to compare the hormone profiles. Data are shown as means ± SEM, and were statistically compared using modified t-test and Kruskal-Wallis test (14) as appropriate.

RESULTS

Table I shows the results of cycle lengths, days of ovulation and duration of menses for individual PT, T1, T2 and PoT cycles. A one hundred per cent protection against pregnancy was obtained in both treatment groups (T1 and T2) with no significant changes in cycle lengths ($F=3.5$; $P<0.3$), days of ovulation ($F=1.8$; $P<0.7$) and duration of menses ($F=3.5$; $P<0.3$) compared with those in PT and PoT cycles (Table I). Two monkeys became pregnant in mated PoT cycles indicating that reproductive functioning in menstrual cycles following the drug treatment was not impaired.

TABLE I : Effects of luteal phase mifepristone administration on cycle pattern and pregnancy outcome.

Animal number	*Cycle length PT/T1 /T2 /PoT (days)*	*Day of ovulation PT / T1/T2/PoT (cycle day)*	*Duration of menses PT/T1/T2/PoT (days)*
098	29/29/21/30	13/13/12/14	2/3/4/2
104	32/40/21/27	10/11/10/12	3/3/3/3
105	31/31/22/31	14/14/10/12	3/3/2/3
137	28/32/22/32	11/14/10/12	4/3/3/4
138	29/19/36/26	12/10/17/10	2/2/3/3
143	28/48*/22/32	12/15/11/14	4/4/3/2
146	32/51/46*/25	13/NOv/10/10	3/3/2/2
150	31/18/21/26**	13/10/13/10	4/5/5/2
152	26/23/NA/G	11/10/NA/11	4/5
Mean	29.6/32.3/26.4/28.6	12.1/12.1/11.6/11.7	3.2/3.4/3.3/2.6
sem	0.7/4.2/3.5/1.0	0.5/0.8/0.9/0.6	0.3/0.4/0.4/0.3
P	<0.3	<0.7	<0.3

PT, pre-treatment. T1, treatment 1. T2, treatment 2. PoT, post-treatment. NOv, no ovulation. *High AUC of progesterone profile during the treated luteal phase. **The monkey was mated and became pregnant in the next PoT cycle. NA, T2 was omitted. G, mated and became pregnant in the first PoT cycle. P, statistical comparison between groups using Kruskal-Wallis test.

Retrospective analyses of serum concentrations of E and P revealed that in the treatment cycles (T1 and T2) mifepristone had actually been given on different days throughout early- to mid-luteal period. 63% of T1 monkeys received RU 486 during days 2-5 after ovulation (early luteal phase), while 88% of T2 had actually received the drug during days 6-10 post-ovulation (midluteal phase). Single s.c. mifepristone treatment (n=6) during days 2-5 after ovulation induced a marginal (8.5%; P < 0.2) increase in the mean cycle length (31.8 ± 2.5 days) compared with those in corresponding PT cycles (29.2 ± 1.0 days). In this group, drug induced slight intermenstrual vaginal spotting was noted in 25% treatment cycles. However, early menses were induced in 89% monkeys when treated with single s.c. mifepristone injection during midluteal period (6-10 days after ovulation); this resulted in consistent shortening (ranging from 10-40%; P <0.05) of cycle lengths (23.6 ± 3.0 days) compared with the PT cycles (30.0 ± 0.6 days).

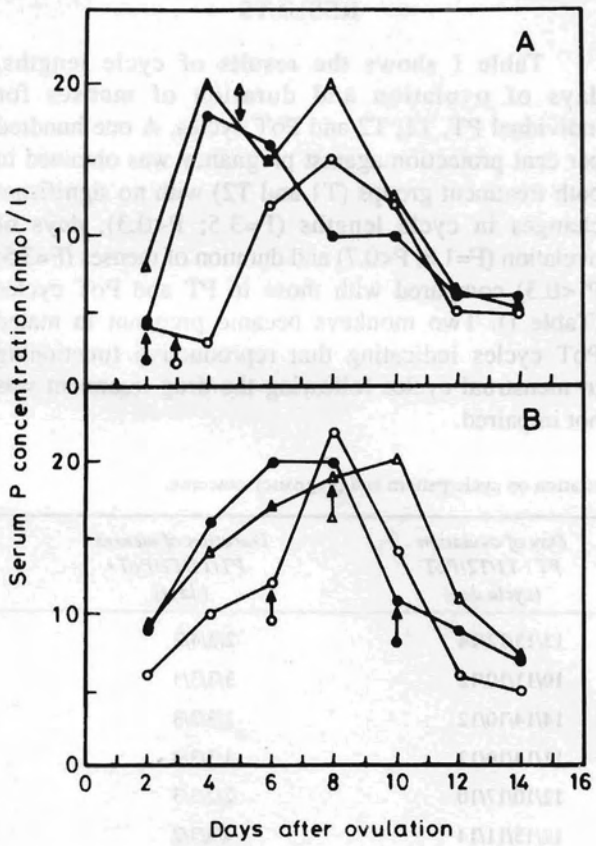

Fig. 1 : Serum concentrations of progesterone (nmol/l) during luteal phases of treatment cycles of individual mated monkeys receiving a single s.c. mifepristone (2 mg/kg body weight) injection during days 2-5 after ovulation, i.e., early luteal phase (A) and days 6-10 after ovulation ie., midluteal phase (B). Of the representative monkeys receiving the drug during early luteal phase as shown in A, #105 (-●-), #098 (-○-) and #104 (-Δ-) were treated with mifepristone respectively on day 2, day 3 and day 5 after ovulation. B shows the representative profiles on serum P concentration in #150 (-○-), #098 (-Δ-) and #105 (-●-) treated with mifepristone on day 6, day 8 and day 10, respectively. Arrow indicates the day of mifepristone injection.

Fig. 1. shows the representative profiles of serum concentrations of P in treatment cycles of monkeys receiving mifepristone on different (2-10) days after ovulation. Despite an apparent trend of transient fall followed by a small rebound rise of P concentration in peripheral circulation after mifepristone application in 47% treatment cycles, the pooled analysis of data failed to reveal any remarkable change in serum P concentrations during luteal phase of treatment cycles as compared to those in PT and PoT cycles (Fig. 2A). However, the mean AUC of serum P concentrations in luteal phase of treatment cycles receiving mifepristone during days 6-10 (Fig. 2B) was significantly less than that in PT cycles (P<0.02) and that in treatment cycles with mifepristone injection during early luteal period (P<0.05). Though no such change was noted when mifepristone was applied on days 2-5 after ovulation, there was a shift to the right, indicating a delay of 2 days in the peak rise of P concentration in the peripheral circulation during post-ovulatory period (Fig. 2B). Despite an apparent increase in serum concentration of E in 38% treatment cycles, there was no significant change in the serum concentration of this hormone in treatment cycles compared with PT and PoT cycles (data not shown).

Interestingly, the females (# 143, T1 and # 146, T2) who received RU 486 on day 1 and day 10 after ovulation showed increases (71% and 44%, respectively) in the duration of luteal phase along with higher (50-60%) AUCs for serum P compared with corresponding PT cycles. Furthermore, the concentration of serum P was increasing in T2 of # 105 till day 10, when it received the antiprogestin followed by a sharp fall in its level (Fig. 1B). However, these cycles were neither

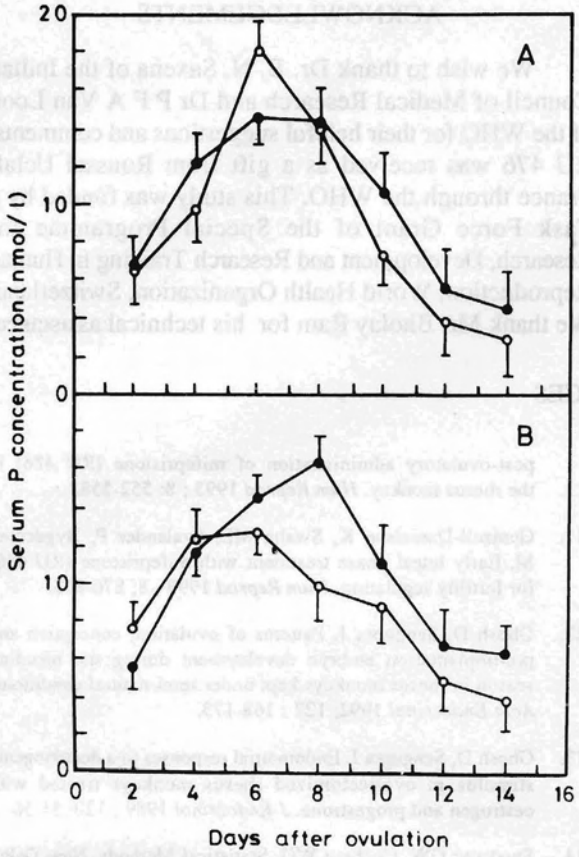

Fig. 2 : Mean (±SEM) concentration of progesterone (nmol/l) during luteal phase of (A) pre-treatment (-○-, n=9) and treatment (-●-, n=16) cycles, and (B) treatment cycles receiving single s.c. mifepristone (2 mg/kg, body weight) injections during days 2-5 after ovulation (-●-, n=6) and during days 6-10 after ovulation (-○-, n=9). Mean AUC of serum P concentrations during luteal period of treatment cycles receiving mifepristone during midluteal period (-○-) was significantly ($P < 0.05$) less than that observed in treatment cycles having mifepristone treatment in early luteal phase (-●-) as shown in B.

biochemically nor clinically pregnancy cycles, and the following ovulatory cycles were apparently normal (Table I).

DISCUSSION

The present study clearly documents that administration of mifepristone (RU 486) during early to mid luteal period is a potential method of luteal phase contraception in monkeys. A hundred per cent protection against pregnancy with luteal phase RU 486 treatment to a monkey colony having a success rate of preganancy occurrence of about 70% from natural insemination in the breeding season (12) is highly significant. Clearly, the window for luteal phase contraception with this antiprogestin is not limited to LH+2 day. It now appears that the window is indeed wider, and any day during the early luteal phase (days 2-5 after ovulation) can be used as a target with no significant changes in cycle lengths and hormone profiles. On the other hand, administration of mifepristone during the midluteal period (days 6-10 after ovulation) also provided 100% protection against pregnancy in mated cycles, the hormonal synchrony was, however, affected along with associated reduction of treatment cycle lengths.

We have earlier shown that alteration in endometrial function and hypothalamus -pituitary-ovarian axis function in the rhesus monkey showed a dose-dependent differential sensitivity to mifepristone given on second day after ovulation, and the dosage amount of RU 486 required to affect ovarian functions was higher compared with that required to influence endometrial maturation (9,10,15). It is now evident from the available literature that the effect of the antiprogestin on endometrial activity, vis-a-vis, ovarian activity is dependent upon the hormonal milieu, the duration of progesterone exposure and the state of progesterone dominance (2-5,16). Moreover, its action on both compartments is more likely during high progesterone dominance. Thus, the vulnerability of endometrial maturation and endocrine synchony being affected by mifepristone treatment is higher during the midluteal period, while it is possible to affect endometrial maturation selectively without any discernible action on the hypothalamus-pituitary and ovarian axis by applying this antiprogestin during the early luteal period of a menstrual cycle.

In conclusion, the present study shows that luteal phase contraception with mifepristone does not require a strict rigidity of its application on day 2 after ovulation. Mifepristone (RU 486) when given as a single dose during early luteal phase (days 2-5 post-ovulation) provided 100% protection against pregnancy without affecting hormonal patterns and subsequent ovulatory cycles. It is presumed that this treatment rendered

endometrial desynchronization, and thus impaired the process of implantation in the treatment cycle (8,17,18). It is also possible, as suggested by Greene et al (19) that that a lower staggered dose given during the early to mid luteal phase may even provide better results. These propositions, however, require final confirmation by open clinical trials. The antinidatory principle of early luteal phase mifepristone treatment offers a highly promising lead for further design of a novel and practical contraceptive method for human use.

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