The effectiveness of a sequential regimen consisting of mifepristone, 10 mg/day for 15 days, followed by nomegestrol acetate (NOMA), 5 mg/day for the next 13 days, for inhibiting ovulation and maintaining regular bleeding cycles was assessed in 10 surgically sterilized volunteers who were followed for one pretreatment and three treated cycles. Hormonal determinations in blood and urine, ovarian ultrasonography, bleeding records in all cycles and an endometrial biopsy taken on day 22–25 of the third treatment cycle were used to monitor the effects of treatment. During treatment, 24 monophasic (no sustained progesterone rise above 12 nmol/l) and six biphasic cycles were recorded. Nine follicular ruptures were detected echographically in these 30 treated cycles, five of which occurred in monophasic cycles. All follicular ruptures occurred on days 1–7 of NOMA treatment. Echographic and endocrine features of ovulatory cycles were both present in only four treated cycles (13.3%). Development of a secretory endometrium was achieved in all cases, but it was always irregular. Regular withdrawal bleeding occurred in all subjects and no adverse reactions were recorded. The ovarian and endometrial effects of this regimen justify testing its contraceptive effectiveness in phase 2 clinical trials.

Key words: contraception/mifepristone/nomegestrol acetate/ovulation inhibition/women

Introduction

An antprogesterin–progestin sequential regimen has been proposed as an oral contraceptive method devoid of exogenous oestrogen and able to inhibit ovulation while maintaining regular bleeding cycles. In this regimen the antiprogestin is given for 15 days and the progestin for the next 13 days. This treatment cycle is repeated continuously without pill-free intervals.

The concept of this method arose from the demonstration that mifepristone, given during the follicular phase, arrests further follicular development and postpones ovulation (Liu et al., 1987; Luukkanen et al., 1988; Ledger et al., 1992; Croxatto et al., 1993, 1995). Thus, under mifepristone administration, ovulation is prevented but sufficient endogenous oestradiol is produced to stimulate endometrial growth. The progestin given following the antiprogestin course should have three effects: (i) prevent ovulation by a negative feedback on gonadotrophin secretion; (ii) antagonize temporarily the endogenous oestrogens; and (iii) transform the oestrogen-primed endometrium into a gestational endometrium, with consequent bleeding upon withdrawal of the progestin. The reinstatement of the antiprogestin, on the day after the last progestin pill is taken, should reinforce the effect of the progestin withdrawal upon the endometrium, ensuring the onset of bleeding, at the same time that it would prevent follicular escape.

Previous phase 1 studies, using mifepristone, 5 mg per day, as the antiprogestin, and norethisterone (Kekkonen et al., 1990, 1993) or medroxyprogesterone acetate (Kekkonen et al., 1993, 1995; Croxatto et al., 1996) as the progestin, have shown that this regimen inhibits ovulation but not at sufficiently high rates to achieve an acceptable contraceptive efficacy if it were used by unprotected women. A luteinizing hormone (LH) rise, follicular rupture or the rise of progesterone occurred in some treatment cycles, always during the administration of the progestin. Contrary to expectation, in some instances, the progestin appears to trigger a pituitary gonadotrophin surge when the effect of the antiprogestin wanes (Kekkonen et al., 1993, 1995; Croxatto et al., 1996). This surge fails to produce a full ovulatory response in some cases, presumably in those in whom the follicle has not reached maturity.

Alterations in the bleeding pattern are associated with several highly effective contraceptive methods and they reduce their acceptability. The bleeding pattern observed with this regimen has proved to be quite regular in all cases and this has encouraged attempts to improve the rate of ovulation suppression.

In the present study, we investigated the efficacy of mifepristone, 10 mg per day for 15 days, to ensure arrest of follicular growth, followed by the progestin nomegestrol acetate (NOMA) for 13 days. At the dose of 5 mg per day, NOMA has been reported to have potent gonadotrophin-suppressing activity and to inhibit ovulation (Bazin et al., 1987; Couzin et al., 1996). Because of the carry-over effect of this regimen into the next cycle, this phase 1 study encompassed three successive treatment cycles.

© European Society of Human Reproduction and Embryology
Materials and methods

A total of 10 healthy sterilized women, regularly cycling, mean age 36 years (range 30–40) and mean weight 59 kg (range 45.5–67.0) volunteered for the study. Subjects were admitted after giving informed consent. The study was approved by the Ethics Committee of ICMER and by the Eastern Virginia Medical School Institutional Review Board.

This was an open, non-randomized, phase 1 clinical study, in which each volunteer was her own control. Each subject was studied for one baseline cycle, three consecutive 28-day periods of treatment and one post-treatment cycle. During each period of treatment, each volunteer received mifepristone (RU486; Roussel-Uclaf, Romainville, France), 10 mg/day, on days 1 to 15 of the cycle and nomegestrol acetate, NOMA (Lutennyl; Laboratorios Silesia S.A., Santiago, Chile) 5 mg/day, on days 16 to 28 of the cycle. The entire sequence was reinitiated on the day following the last NOMA pill, regardless of the occurrence of menses.

Each subject recorded pill ingestion time, bleeding, spotting, any symptoms, concurrent illness and other drug intake. Haematology and serum chemistry (serum glutamic oxalacetic and pyruvic transaminase, lactate dehydrogenase, alkaline phosphatase, bilirubin, total protein, cholesterol, uric acid, urea nitrogen, glucose, inorganic phosphate and calcium) were assessed at admission, at the end of treatment and after the post-treatment cycle. Blood samples were taken twice a week throughout the study from each volunteer to determine oestradiol and progesterone concentrations. A first morning urine sample was collected daily for measuring LH concentration. Ovarian and uterine echography, using an Aloka SS D 620 ultrasound system, with a 5-MHz vaginal probe, were performed twice a week throughout the study, to assess follicular growth and endometrial thickness. An endometrial biopsy was taken in the third cycle of treatment, on day 7 to 10 of NOMA intake, to assess endometrial histology according to the criteria of Noyes et al (1950) or Maqueo (1980).

Hormones in plasma and LH in urine were measured according to the procedures and with the reagents supplied by the World Health Organization. The lower limits of sensitivity for oestradiol, progesterone and LH assays were 100 pmol/l, 1.9 nmol/l and 1.6 IU/l respectively. The ranges of the intra-assay coefficients of variation of low, medium and high pool, for oestradiol, progesterone and LH respectively. Creatinine concentration assay coefficients of variation were 12–21%, 12–15% and 8–16% for the low, medium and high pool, for oestradiol, progesterone and LH respectively. The ranges of the intra-assay coefficients of variation of the low, medium and high pool, for oestradiol, progesterone and LH were 6–7%, 5–10% and 4–6% respectively. The ranges of the inter-assay coefficient of variation of the low, medium and high pool, for oestradiol, progesterone and LH were 100 pmol/l, 1.9 nmol/l and 1 IU/l respectively. The ranges of the inter-assay coefficient of variation of the low, medium and high pool, for oestradiol, progesterone and LH were 6–7%, 5–10% and 4–6% respectively. The ranges of the inter-assay coefficient of variation of the low, medium and high pool, for oestradiol, progesterone and LH were 6–7%, 5–10% and 4–6% respectively.

Data analysis

The following working definitions were used in the analysis of the data:

Length of the cycle and of the phases: cycle length was calculated counting from the first day of menses until the day preceding the next menstrual-like bleeding, both inclusive. The day of maximum LH rise in urine, followed by at least a doubling of progesterone concentrations, or the first day in which the follicular echo-image disappeared, was designated day 1 of the luteal phase.

Follicular rupture: abrupt disappearance or a reduction in size of at least 50% of the echo-image.

Ovulation: follicular rupture followed by plasma progesterone concentration over 12 nmol/l, in at least two samples taken during the luteal phase.

Table I. Effect of mifepristone–nomegestrol acetate sequential regimen on ovarian function

<table>
<thead>
<tr>
<th>Cycle</th>
<th>n</th>
<th>Highest oestradiol concentration (pmol/l) (mean ± SE)</th>
<th>Highest progesterone concentration (nmol/l) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulatory*</td>
<td>10</td>
<td>711 ± 74</td>
<td>41.3 ± 3.9</td>
</tr>
<tr>
<td>Treatment</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biphasic</td>
<td>6</td>
<td>23.6 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Ovulatory</td>
<td>4</td>
<td>561 ± 70a</td>
<td>22.0 ± 4.9</td>
</tr>
<tr>
<td>LUF**</td>
<td>2</td>
<td>401 ± 141</td>
<td>26.8 ± 1.1</td>
</tr>
<tr>
<td>Monophasic</td>
<td>24</td>
<td>360 ± 31b</td>
<td></td>
</tr>
<tr>
<td>Folllicular rupture</td>
<td>5</td>
<td>416 ± 74c</td>
<td>15.3 ± 2.9***</td>
</tr>
<tr>
<td>No follicular rupture</td>
<td>19</td>
<td>357 ± 34b</td>
<td>5.8 ± 1.1</td>
</tr>
</tbody>
</table>

* All biphasic.

**Luteinized unruptured follicle.

***In each of these five cycles, progesterone level was above 12 nmol/l only in a single sample.

†Not significantly different from baseline cycle.

Significantly different from ovulatory treated cycle. Analysis of variance (ANOVA), P < 0.025.

‡Not significantly different from ovulatory treated cycle.

Significantly different from baseline cycle; ANOVA, P = 0.0072.

Results

Ovarian function

All baseline cycles were ovulatory (Table I, Figures 1 and 2). The highest plasma oestradiol concentrations were in the range 399–1171 pmol/l (mean ± SE: 691 ± 250 pmol/l), the luteal phase was 13.2 days on average (range: 10–15), and the highest progesterone concentrations were in the range 32–75 nmol/l (41.3 ± 3.9). The largest follicular diameters recorded in basal cycles prior to follicular rupture were in the range 15–21 mm (18.4 ± 0.7).

Throughout treatment, nine follicular ruptures were detected in five of the 10 participants, twice in each of four women and once in another. Three of these five women presented ovulatory cycles, one ovulated twice and two ovulated once each (Figure 1). The other five of these nine follicular ruptures were not followed by a luteal phase (see below) and were detected in three women.

The frequency of ovulatory cycles during treatment was greatly reduced (Figure 2). Follicular rupture was inhibited in 21 of the 30 cycles (70%). However, five of the nine follicular ruptures did not meet the endocrine criteria for ovulation, therefore 26 of the 30 cycles (86.6%) were considered anovulatory. Only six cycles were biphasic and four of these were

H.B. Croxatto et al.
Effect of mifepristone–nomegestrol acetate sequential regimen on urinary luteinizing hormone (LH) concentrations and follicular rupture. Shaded and open lanes represent the periods of treatment with mifepristone, 10 mg/day for 15 days, and nomegestrol acetate, 5 mg/day for 13 days, respectively. The solid line shows LH concentrations, and arrows indicate the time when abrupt disappearance or a reduction in size of at least 50% of the echo-image of the leading follicle was observed. OV = follicular rupture followed by sustained rise in progesterone concentrations; FR = follicular rupture not followed by sustained rise in progesterone concentrations.

Ovarian function during mifepristone–nomegestrol acetate sequential regimen, as in Figure 1. Each bar represents the number of subjects with biphasic (shaded bars) and monophasic cycles (open bars) during one baseline, the three treatment and one post-treatment cycles. Closed symbols represent the number of ovulatory cycles observed at each period.

Contraceptive potential of RU486–NOMA sequential regimen

Figure 2. Ovarian function during mifepristone–nomegestrol acetate sequential regimen, as in Figure 1. Each bar represents the number of subjects with biphasic (shaded bars) and monophasic cycles (open bars) during one baseline, the three treatment and one post-treatment cycles. Closed symbols represent the number of ovulatory cycles observed at each period.

Figure 3. Highest plasma oestradiol and progesterone concentrations during mifepristone–nomegestrol acetate sequential regimen, as in Figure 1. Open symbols correspond to highest concentrations detected in ovulatory treated cycles.

The largest follicular diameters observed on days 13–15 of mifepristone were in the range 10–20 mm and they reached 15–25 mm prior to follicular rupture. Maximal follicular diameters, on days 13–15 of mifepristone, in cycles in which no follicular rupture was detected were in the range 4–17 mm, excluding the enlarged follicle, with a median of 8.6 mm. Three enlarged follicles, with maximal diameters of 26, 28, and 29 mm were observed during treatment; all of them grew over 25 mm after the onset of NOMA intake and disappeared spontaneously at the time of the next menses.

A rise in LH concentrations was detected in urine, in 23 cycles during treatment. The magnitude and/or the sharpness of the surge was lower than in the baseline, in most cases. Follicular rupture occurred on the same day of the highest value only in three cycles, and two of them were considered ovulatory according to previously described criteria. In the other six cycles, the LH rise preceded follicular rupture by several days. In contrast, a clear LH peak was observed in two monophasic cycles (Figure 1). Creatinine concentrations in urine during treatment were not different from those in the baseline cycles.

Progesterone concentrations in biphasic cycles (n = 6) were significantly decreased (P = 0.0072) in comparison with those observed in the control cycles (Table I). The highest progesterone concentrations (Figure 3) observed in each of the four ovulatory cycles (22.0 ± 4.9 nmol/l, range: 12.7–34.8) were also significantly below (P = 0.04) the range observed during their corresponding control cycles (37.4 ± 1.5 nmol/l, range: 35.3–41.8).
The highest oestradiol concentrations (Figure 3) in ovulatory treated cycles (561 ± 70 pmol/l, range: 383–723) were not different from those in their respective baseline cycles (685 ± 6124 pmol/l, range: 440–831) but were significantly higher ($P < 0.025$) than in monophasic cycles (369 ± 31 pmol/l, range: 177–781). Highest oestradiol concentrations below 440 pmol/l were recorded in three women in the three treated cycles, in two women in two cycles and in another two women in a single cycle. In one cycle, oestradiol concentrations increased at the end of the NOMA period, remaining high during the 15 days of mifepristone intake (700–800 pmol/l), decreasing within the first 3 days of the next NOMA period. These oestradiol concentrations accompanied the growth of one enlarged follicle that attained a maximal diameter of 29 mm.

Cycle length and bleeding pattern
The first treated cycle (30–33 days in range), but not the second one (26–29 days), was longer than the baseline (24–30 days) due to a prolonged follicular phase ($P = 0.0001$). The third treated cycle (27–34 days) and its luteal phase were also longer than the baseline, probably due to the lack of an ensuing antiprogestin treatment period ($P = 0.0001$). The post-treatment cycle (16–30 days), in most cases, was shorter than the baseline ($P = 0.0237$).

The bleeding pattern during treatment was regular (Figure 4). Bleeding started 4, 4 and 6.5 days (median) after the first, second and third progestin treatment period respectively. As shown in Figure 4, the duration of bleeding episodes during treatment cycles (mean ± SD: 4.8 ± 1.6 days, range: 2–9) was not different from that of the baseline (5.3 ± 1.9 days, range: 2–9). Total absence of breakthrough bleeding or spotting was observed.

Endometrial morphology
The maximal endometrial thicknesses attained during the treatment cycles were lower than those observed during the baseline cycles, independent of the monophasic or biphasic profiles (Table II). Endometrial biopsies taken on days 7–10 of the third progestin treatment period showed disturbed development in all cases. All samples presented secretory signs but with a heterogeneous development of the glands, coexisting straight glands with stratified epithelium and coiled glands lined by high or low cylindrical epithelial cells, with secretion in vacuoles of basal and/or apical localization, and/or in the lumen. This glandular development was accompanied by a dense stroma, with infrequent oedematous areas in most cases, in which no vascular development or signs of focal predecidual reaction were observed.

Recovery cycles
All cycles were biphasic and associated with echographic features of the ovulatory cycle. These cycles were shorter than the baseline due in some cases to a shortening of the follicular phase and in others of the luteal phase. The maximal endometrial thicknesses in these cycles, although higher than in treatment cycles, were still below the growth attained in baseline cycles.

Side-effects
No untoward reactions or side-effects were recorded, and laboratory tests were within the normal range at the end of treatment.

Discussion
The sequential treatment tested affected ovarian function and endometrial development in all women in all cycles tested, albeit to a different degree, which ranged from total suppression of both follicular rupture and luteinization, or either one, to partial suppression of luteal-phase progesterone concentrations.
Some monophasic cycles were also associated with lower oestradiol peak concentrations. Except for the occurrence of follicular rupture in monophasic cycles, these polymorphic changes in ovarian function are to be expected from previously described effects of the antiprogestin on the pituitary–gonadal axis (van Uem et al., 1989; Croxatto et al., 1995). Follicular growth was partially inhibited in the majority of cycles during mifepristone treatment, and ovulation was less likely to occur in cycles exhibiting a stronger inhibition. The mechanism of this antifolliculotrophic effect of mifepristone remains to be disclosed, although the results of two recent studies, in which doses of 10 mg/day (Kazem et al., 1996) or 50 mg/day (Messinis et al., 1997) were used, suggest it is more likely a direct effect on the ovary rather than a disturbance of gonadotrophin secretion.

The absence of a clear pre-ovulatory LH peak in urine suggests either that oestradiol concentrations did not reach the critical level to exert a positive feedback or that a central effect of treatment interfered with the positive feedback of oestradiol on LH secretion (Baird et al., 1995) The design of the study does not enable us to conclude whether this effect is due to antiprogestin, progestin or their interaction. Whatever the case, this effect persisted in the post-treatment cycle, since an LH surge, similar to that seen during pretreatment, was observed in only one case.

Maximal endometrial thickness was decreased during treatment. Even in the four ovulatory cycles with oestradiol peaks as high as in basal cycles, the thickness values found were below the range of basal cycles. It is most likely that this reflects the antiproliferative effect of mifepristone (van Uem et al., 1989; Cameron et al., 1996; Neulen et al., 1996). On the other hand, 10 of the 24 monophasic cycles had maximal endometrial thickness values below 10 mm, whereas all six biphasic cycles had 10 mm or more. This difference did not reach statistical significance, probably due to the low number of biphasic cycles. Nevertheless, it suggests that the lower oestradiol concentrations encountered in monophasic cycles also contributed to a decreased endometrial proliferation.

The morphology of the endometrium, exposed to the exogenous progestin for 6 to 9 days, showed discrepant development among neighbouring glands as well as between the glands and stroma. The heterogeneous development of the glands was accompanied by scarce stromal development, in which vascular development and focal predecidual reaction failed to develop. This irregular development of the endometrium differs from that described for combined and sequential contraceptive pills (Dallenbach-Helweg, 1980; Maqueo, 1980). The changes in the stroma reflect a low to mild oestrogenic stimulus, followed by a sluggish response to the progestin. Glandular development was neither hyperplastic nor atrophic, as seen with the sequential and high-dose combination pills. The irregular development of glands suggests an uneven effect of the antiprogestin and/or the progestin on their target cells. Nevertheless, the most advanced development of glands was always far ahead of the stroma and behind that required for the synchrony with the embryo.

The occurrence of withdrawal bleeding, 2 to 5 days after reinitiating the antiprogestin pill intake, was highly predictable. The lengths of bleeding episodes were not different from those recorded before treatment. Breakthrough bleeding, intermenstrual spotting or amenorrhoea were not seen.

An acceptable contraceptive efficacy can be expected from the observed effects of this regimen on ovarian function and endometrial development. From the data, 13% of ovulatory cycles are to be expected with this regimen. Since follicular rupture occurs 7 to 13 days before restarting the antiprogestin intake, and implantation occurs 7 to 9 days after follicular rupture, if implantation should take place, it would be as early as 4–6 days before the next antiprogestin intake and as late as 1–3 days during progestin intake. In addition, endometrial stroma is delayed and the development of glands is highly irregular, therefore, it is unlikely that the endometrium would be receptive. Cervical mucus, which was not evaluated in this study, may be also affected by this treatment. Since follicular rupture takes place during the period of treatment with the progestin, it is likely that, in most instances, cervical mucus will be hostile to spermatozoa before and at the time of ovulation (Chretien et al., 1991).

No serious adverse side-effects have been reported by women using the antiprogestin–progestin sequential regimen during this or previous studies (Kekkonen et al., 1990, 1993, 1995; Croxatto et al., 1996). Peak oestrogen concentrations in monophasic cycles were lower than in baseline cycles, however, enough oestradiol was secreted to stimulate endometrial growth, to prevent breakthrough bleeding and to render the endometrium responsive to the progestin and to its withdrawal. Ovarian cysts, reported for the continuous 10 mg mifepristone regimen (Croxatto et al., 1993), were not seen with this regimen, and the few enlarged follicles observed disappeared at the time of next menses.

Based upon the results of this study and previous clinical experience with these steroids, the present mifepristone–nomegestrol acetate sequential regimen is expected to offer high contraceptive efficacy, excellent bleeding control and to be safe and relatively free of side-effects. It affords a definite advantage for women who cannot use the combined pill and for those who have low tolerance to bleeding disturbances.

Acknowledgements

We thank Roussel-Uclaf and Laboratoires Theramex, for supplying mifepristone and nomegestrol acetate respectively, and the World Health Organization Matched Reagents Programme for the immunoassay reagents. We also wish to thank Mrs. A.Brandeis, Mrs. G.Bravo, Mrs. C.Lados and E.Nuñez for their technical assistance. Support for this study (CSA-94–159) was provided by the Contraceptive Research and Development Program, Eastern Virginia Medical School, under a Cooperative Agreement with the United States Agency for International Development (USAID). The views expressed by the authors do not necessarily reflect the views of USAID or CONRAD.

References

H.B. Croxatto et al.


Received on January 27, 1998; accepted on September 9, 1998