The effects of 1-month administration of asoprisnil (J867), a selective progesterone receptor modulator, in healthy premenopausal women

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BACKGROUND: Asoprisnil (J867) is a novel selective progesterone receptor modulator (SPRM) that exhibits partial agonist and antagonist activities and tissue selective effects. This double-blind, dose-escalation study was conducted to evaluate the effects of asoprisnil in 60 regularly cycling premenopausal women. METHODS: Asoprisnil or placebo was administered orally for 28 days starting at the beginning of the menstrual cycle in doses of 5 mg once daily (QD), 5 mg twice daily (BID), 10 mg QD, 25 mg QD, 25 mg BID and 50 mg BID. Within each dose group, two women were randomized to placebo and eight to asoprisnil. Progesterone concentrations indicative of luteinization were defined as at least one progesterone measurement during the luteal phase exceeding 3.5 ng/ml. RESULTS: Asoprisnil consistently prolonged the menstrual cycle at doses $10 mg QD. However, the effects on luteal phase progesterone indicative of luteinization were inconsistent and lacked dose dependency. Asoprisnil suppressed periovulatory estradiol but not below follicular phase levels. No significant changes were observed in cortisol and prolactin. Asoprisnil was well tolerated. CONCLUSIONS: Asoprisnil reversibly suppressed menstruation at doses $10 mg QD irrespective of the effect on luteal phase progesterone concentrations indicative of luteinization. It induces amenorrhea primarily by targeting the endometrium in the absence of estrogen deprivation.

Key words: asoprisnil/amenorrhea/endometrium/menstrual cycle prolongation/selective progesterone receptor modulator

Introduction

Asoprisnil (J867), its active metabolite J912, and a structurally related compound asoprisnil ecomate (J956) are novel selective progesterone receptor modulators (SPRMs) that exhibit partial agonist and antagonist activities in animals and humans (Chwalisz et al., 2000; Elger et al., 2000; DeManno et al., 2003). Asoprisnil (benzaldehyde, 4-[(11b,17b)-17-methoxy-17-(methoxymethyl)-3-oxoestra-4,9-dien-11-yl]-1-oxime) is the first SPRM to reach an advanced stage of clinical development for the treatment of uterine leiomyomata (Chwalisz et al., 2003, Chwalisz et al., 2004). Both asoprisnil and J912 show progesterone receptor (PR)-specific and tissue-selective effects in in vitro and animal models. Receptor binding studies demonstrate high binding affinity of asoprisnil for the PR, reduced binding affinities for glucocorticoid and androgen receptors, and no binding affinity for the estrogen receptor (Elger et al., 2000; DeManno et al., 2003). These data are consistent with transactivation assays in various cell lines as well as in studies in animals (DeManno et al., 2003). Asoprisnil exhibits only marginal antiglucocorticoid activity in transactivation assays (DeManno et al., 2003). Under in vivo conditions in rats and monkeys, the antiglucocorticoid activity of asoprisnil was less, compared with mifepristone (DeManno et al., 2003). In male rats, asoprisnil showed mixed androgenic and antiandrogenic properties (DeManno et al., 2003).

PR-mediated responses were studied in different animal models, including rabbits, guinea pigs and non-human primates (Chwalisz et al., 2000; Elger et al., 2000; DeManno et al., 2003). In the rabbit endometrium, both asoprisnil and J912 exhibited partial (mixed) agonist and antagonistic effects, depending on the absence or presence of progesterone (Elger et al., 2000; DeManno et al., 2003). Partial PR agonist/antagonist effects were also observed in cycling and ovariectomized guinea pigs (DeManno et al., 2003). Unlike classical progesterone antagonists (PAs), mifepristone and onapristone, asoprisnil showed only marginal labour-inducing activity during mid-pregnancy and was completely ineffective in inducing preterm parturition in the guinea pig (Elger et al., 2000; DeManno et al., 2003). This is most likely due...
to the presence of its intrinsic progesterone agonistic activity. In non-human primates, asoprisnil at high doses completely eliminated menstrual cyclicity and induced endometrial atrophy in the presence of follicular phase estradiol (E2) concentrations (DeManno et al., 2003). These studies also demonstrated tissue-selective effects of asoprisnil, with the endometrium as the preferred target.

This double-blind, dose-escalation study evaluated the effects of asoprisnil on menstrual and ovarian cyclicity, and safety parameters in normal premenopausal women during 1-month oral administration.

Materials and methods

Women studied

Healthy, cycling volunteers between the ages of 18 and 45 years having weight within 20% of normal range were recruited for the study. Inclusion criteria included women with a history of at least three regular menstrual cycles of 25 to 35 days prior to study enrollment; good general health on the basis of medical history and physical examination; no oral contraceptives within 2 months of study commencement; normal ECG, biochemical and hematological profile; negative pregnancy test; and negative screen tests for recreational drugs, hepatitis and HIV. In addition, all women had a pelvic examination, transvaginal ultrasound and Papanicolaou (Pap) smear. Subjects with undiagnosed abnormal uterine bleeding, uterine fibroids, endometrial polyps, or an ovarian cyst greater than 3 cm at screening were excluded, as were those with FSH concentrations above the normal premenopausal range.

All enrolled women had to agree to use double barrier contraception (condom, sponge, or diaphragm, with spermicidal foam or jelly) throughout the study and until the onset of the first post-treatment menstrual period unless surgically sterilized. The study was conducted at two independent Phase I clinical research study sites. The study was performed according to the ethical principles of the Declaration of Helsinki (1989 revision). The Institutional Review Board (IRB) at each of these sites approved the protocol, and each woman signed the IRB-approved written informed consent prior to the screening evaluation. The study was conducted between January 1999 and March 2000.

Study design

This was a Phase I, randomized, double-blind, placebo-controlled, dose-escalation study of asoprisnil administered for 28 days. Asoprisnil or placebo capsules were administered orally starting during the first 4 days of the menstrual cycle. The study included six dose groups: 5 mg once daily (QD), 5 mg twice daily (BID), 10 mg QD, 25 mg QD, 25 mg BID and 50 mg BID. Each dose group consisted of 10 women, eight of whom received asoprisnil whereas the remaining two received placebo. The effect of asoprisnil on cycle length was the primary pharmacodynamic outcome of this study. Various endocrine parameters, endometrial biopsy results, and endometrial thickness were considered as secondary pharmacodynamic outcomes.

Asoprisnil capsules were supplied in doses of 5 mg and 25 mg. The placebo capsules were indistinguishable from those of asoprisnil. Upon successful enrollment, women within each dose group were sequentially assigned subject numbers that encoded the random assignment of the woman, via a randomization schedule, to one of the treatments (i.e. asoprisnil or placebo). Study drug was supplied to the site packaged in sealed kits that contained the appropriate number of bottles of asoprisnil or placebo.

Doses were taken in the morning after fasting; the second dose of the BID doses was taken in the evening ~12 h after the first dose. The women were confined to the testing facility for two intervals. The first confinement began on Day −1. The initial dose was administered on the following day (Day 1); and the women were discharged on the morning of the next day (Day 2). The women returned to the testing facility on Day 5 and then every three days for study drug dispensing, clinical evaluation, safety assessment and blood sampling. The second confinement began on Day 27 and continued until discharge on Day 30. With all dose schedules, the last dose was taken on the morning of Day 28.

A baseline transvaginal ultrasound was performed during the pretreatment cycle on Day 21, repeated during the treatment period on the same menstrual cycle day, and once again during the post-treatment period on Day 37 if menses had not occurred. The occurrence of ovarian cysts >3 cm was assessed at each transvaginal ultrasound examination. An endometrial biopsy was performed using a Pipelle (R) catheter on the same day of the treatment cycle as the ultrasound. Initially, it was proposed to interpret the endometrial biopsy on the basis of the Noyes criteria (Noyes, et al., 1950). However, because of mixed progesterone agonist/antagonist effects of asoprisnil on the endometrium, leading to asynchronous differentiation of endometrial epithelium and stroma, the biopsy results could not be interpreted on this basis. Instead, the biopsies were reanalyzed according to a new classification system developed by TAP Pharmaceutical Products Inc., Lake Forest, IL, Diagnostic Cytology Laboratories, Indianapolis, IN, and an expert panel of gynecological pathologists. This new classification, which was based on Blausstein’s Pathology of the Female Genital Tract (Kurman and Mazur, 1994; Kurman and Norris, 1994), included two new categories describing specific effects of asoprisnil on the endometrium. The first category, ‘non-physiologic secretory effects’, is characterized by weak secretory effects on endometrial glands without any mitotic figures and variable effects on endometrial stroma ranging from stromal compaction to focal predecidual changes. The second category, ‘secretory pattern, mixed type’, differs from the first category by the presence of isolated mitotic figures in endometrial glands.

Menstrual diaries

Each woman completed a daily diary throughout the study in which she recorded any uterine bleeding, the time of study drug administration, and any adverse events. The staff reviewed this diary at each clinic visit.

Hormonal analyses

LH, E2, estrone (E1) and progesterone were measured on Days −1, 1, 5, 14, 17, 23 and 28; FSH, prolactin, free testosterone (FT), and sex hormone-binding globulin (SHBG) were measured on Days −1, 14 and 28. Blood collections for cortisol (C) and dehydroepiandrosterone sulphate (DHEA-S) determination were taken at 7 am on Days 1, 5, 11, 17, 23, 28 and 29; cortisol was also measured at 8 pm on Days 1, 28 and 29. All hormonal assay analyses were conducted at Esoterix Inc. (former Endocrine Sciences, Calabasas Hills, CA), who developed all hormonal assays used in this study.

FSH, LH and prolactin were determined by an immunochromimetric assay developed by Esoterix Inc. This method utilizes paired monoclonal antibodies to provide highly sensitive and specific measurements. SHBG was assayed by a highly sensitive immunoradiometric assay. The intra-assay and inter-assay precision of protein hormones and SHBG assays ranged from 4 to 8% [coefficient of variation, (%CV)], respectively.
E2 and E1 were measured by radioimmunassay (RIA) after extraction and LH20 column chromatography. Samples were extracted with hexane:ethyl acetate (80:20). The recovery of each sample, which was monitored by adding [3H]E2 before extraction, was ~70%. Progesterone was measured by RIA after extraction with hexane:ethyl acetate (80:20) with a recovery >90%. C was measured directly in serum by RIA. A non-crossreactive steroid was used to displace C from cortisol binding globulin. Samples were diluted before assay to a concentration of C suitable for accurate measurement. DHEA-S was measured as DHEA by RIA after enzymolysis of the DHEA sulfate without extractions and chromatography. Total serum testosterone is measured by RIA after extraction with hexane:ethyl acetate (80:20) and column chromatography using AI203 micro-columns. Equilibrium dialysis was used to determine FT. Intra-assay variation of steroid hormone assays ranged from 2–6% and the inter-assay variation ranged from 2–17% depending on the hormone concentration. There was no crossreactivity with asoprisnil and its metabolites in the E2, E1, C, DHEA and progesterone RIAs. However, there may have been cross-reactivity in the FT assay with asoprisnil or one of its metabolites as observed in subsequent studies. The assays used to measure total and free testosterone are currently being validated versus a more specific method (high-performance liquid chromatography, gas chromatography/mass spectrometry).

**Safety parameters**

Liver function was determined by total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, lactate dehydrogenase and alkaline phosphatase. Blood urea nitrogen and creatinine were evaluated to assess renal function. In addition, all women had complete blood counts, serum biochemical determinations including albumin, total protein, glucose, chloride, potassium, sodium, thyroid-stimulating hormone (TSH) and total thyroxin (total T4), as well as urinalysis. These tests were performed between 8 and 10 am during the screening visit, on Days 1, 5, 11, 17, 23 and 27 of the treatment period and on Day 30 during the post-treatment visit. Pregnancy testing was performed during screening and the visits on Day –1, 11, 20, 27 and 30. Clinical and safety assessments including monitoring for adverse events and use of concurrent medications, as well as vital signs and electrocardiogram (ECG), were frequently performed.

**Data analysis and statistical methods**

For all analyses, the results from the placebo subjects of the six dosing regimens were pooled, giving a total of 12 women who received placebo.

The effect of asoprisnil on the menstrual cycle was measured by comparing the cycle lengths for the different dosing regimens. Cycle length was defined as the number of days from the beginning of the woman’s baseline menses during which dosing started to the first day of her next menses (during or after the 28 days of treatment). Mean cycle length was calculated for each dosing regimen and comparison was made using a one-way analysis of variance (ANOVA) with dosing regimen as the factor. For this and all following ANOVAs, pairwise comparisons to placebo, a comparison of 10 mg QD to 5 mg BID, and a test for linear effect of total daily dose were also made within the framework of the ANOVA model. All P-values <0.05 were regarded as significant unless otherwise specified.

 Serum luteal phase progesterone concentrations during the treatment cycle were used to determine the presence of corpus luteum formation. The cycle was arbitrarily defined as indicative of luteinization if at least one progesterone measurement exceeded 3.5 ng/ml during treatment. These results were cross-tabulated by dosing regimen.

Intermenstrual bleeding was defined as bleeding between the end of the baseline menses and before the start of the next menses, excluding bleeding attributed to the endometrial biopsy. Mean and median number of days of intermenstrual bleeding was calculated for each dosing regimen and pairwise comparisons to placebo were made using a Kruskal–Wallis test, as the data were not normally distributed.

Changes from baseline in hormone concentrations were calculated for each dosing regimen and analyzed for each treatment visit using separate one-way ANOVAs with dosing regimen as the factor. The seven pairwise comparisons at each visit (pairwise comparisons to placebo and a comparison of 10 mg QD to 5 mg BID) were assessed using Hochberg’s multiple comparison procedure (Hochberg, 1988).

**Results**

**Demographic information**

A total of 60 women participated in the study; the mean (range) age was 34.3 (21–45) years and their body mass index ranged from 19 to 31 kg/m². Forty-two (70%) of the women were Hispanic, 13 (22%) Caucasian and the

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**Table I. Demographic data for the 60 volunteers enrolled in the study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n = 12)</th>
<th>5 mg QD (n = 8)</th>
<th>5 mg BID (n = 8)</th>
<th>10 mg QD (n = 8)</th>
<th>25 mg QD (n = 8)</th>
<th>25 mg BID (n = 8)</th>
<th>50 mg BID (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>32.3 ± 6.3</td>
<td>37.9 ± 4.1</td>
<td>34.5 ± 6.2</td>
<td>34.9 ± 5.6</td>
<td>35.6 ± 3.7</td>
<td>29.9 ± 9.0</td>
<td>36.0 ± 7.1</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>2 (16.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (37.5%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (16.7%)</td>
<td>3 (37.5%)</td>
<td>1 (12.5%)</td>
<td>4 (50%)</td>
<td>0</td>
<td>3 (37.5%)</td>
<td>0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>24.8 ± 3.5</td>
<td>23.9 ± 2.4</td>
<td>25.2 ± 3.8</td>
<td>25.1 ± 2.0</td>
<td>25.2 ± 2.1</td>
<td>23.3 ± 2.7</td>
<td>25.3 ± 3.0</td>
</tr>
</tbody>
</table>

BMI: body mass index.
QD: once a day.
BID: twice a day.
remaining five (8%) were African-American. The placebo and six treatment groups were similar with respect to BMI, age and racial distribution (Table I).

Effects on the menstrual cycle and luteal phase progesterone levels indicative of luteinization

Asoprisnil produced a dose-dependent delay in the onset of menstruation with an increase in cycle length (linear contrast \( P < 0.001 \)). The mean cycle lengths for the 10 mg QD and higher doses were significantly greater than with placebo (Figure 1).

With all doses of asoprisnil above 5 mg QD, progesterone levels indicative of luteinization were measured in fewer subjects than in the pooled placebo group (Figure 2). Low progesterone levels indicative of the absence of luteinization were observed in most subjects at the highest dose (seven of the eight women at 50 mg BID). Asoprisnil suppressed progesterone concentrations to non-luteal levels inconsistently among the subjects in other dose groups. An increase in cycle length did not necessarily correlate with presumably anovulatory cycles based on low luteal phase progesterone levels. As is evident in Table II, some women with a cycle length exceeding 40 or 50 days showed progesterone levels indicative of luteinization during the 28 days of dosing. Table III provides additional information about the proportion with cycles with non-luteal (≤2.0 ng/ml), subnormal (2.0–3.5 ng/ml), and normal luteal phase (>3.5 ng/ml) progesterone levels in subjects with cycle length shorter and longer than 40 days. There was no apparent association between cycle length and serum progesterone concentrations.

Effects on hormones

LH: Asoprisnil at low-doses (5 mg/day and 10 mg/day) had little effect on mean LH concentrations that showed typical cycle-dependent changes with maximum values on Day 14 in most groups (Figure 3A). At higher doses (25–100 mg/day) (Figure 3B) larger increases from baseline were observed with increasing doses of asoprisnil, so a dose-dependent effect of asoprisnil was observed at Day 23 (linear contrast \( P < 0.01 \)). This effect seemed to persist on Day 28. The effect of asoprisnil on the LH surge could not be determined due to limited sampling.

FSH: The interpretation of FSH data is somewhat limited since this hormone was measured only on Days 1, 14 and 28. In all groups there were slight decreases from baseline at Days 14 and 28. No apparent association between cycle length and serum progesterone concentrations.

Table II. Number of subjects exhibiting luteal phase progesterone concentrations indicative of luteinization during the 28 day treatment period with menstrual cycle lengths of ≥40 days (or ≥50 days)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>5 mg QD</th>
<th>5 mg BID</th>
<th>10 mg QD</th>
<th>25 mg QD</th>
<th>25 mg BID</th>
<th>50 mg BID</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥40 days</td>
<td>1/1</td>
<td>0/0</td>
<td>1/4</td>
<td>3/7</td>
<td>4/7</td>
<td>4/7</td>
<td>0/7</td>
</tr>
<tr>
<td>≥50 days</td>
<td>1/1</td>
<td>0/0</td>
<td>1/2</td>
<td>3/5</td>
<td>3/6</td>
<td>3/6</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Numerator: the number of subjects exhibiting luteal phase progesterone concentrations; denominators: the number of subjects with cycle lengths ≥40 days or ≥50 days respectively. Luteal phase progesterone concentrations indicative of luteinization are defined based on at least one progesterone measurement >3.5 ng/ml during the treatment period.
doses were significantly different from placebo (data not shown).

E2: Typical, cycle-dependent increases in E2 were observed for all doses of asoprisnil as well as placebo (Figure 4A and B). Although no statistically significant pairwise differences were evident, the highest asoprisnil dose (50 mg BID) was associated with lower E2 concentrations. There was a trend towards lower mean E2 concentrations mid-cycle (Day 17) (linear contrast \( P = 0.01 \)).

E1: Cycle-dependent increases in E1 concentration were similar to E2. None of the asoprisnil groups were significantly different from placebo (data not shown).

Progesterone: The patterns of progesterone concentrations at 5 mg QD (Figure 5A) were consistent with normal occurrence of corpus luteum formation and luteolysis. At higher asoprisnil doses (Figure 5B), the mean luteal phase progesterone increases were smaller as the asoprisnil dose increased (linear contrast at Day 17 \( P < 0.05 \)) reflecting the lower rate of subjects with progesterone levels indicative of luteinization (Figure 5B). In those asoprisnil-treated women who showed luteal phase progesterone levels, the duration of the luteal phase seemed to be normal.

C: There were no statistically significant differences between any asoprisnil dose and placebo with respect to changes in baseline morning C levels (Figure 6A and B). Moreover, the diurnal rhythm of C was maintained in both treatment and placebo groups, as mean C concentrations were lower in the evening than in the morning (data not shown).

DHEA-S: There were no meaningful changes in DHEA-S during the treatment cycle. A significant trend on Days 28 and 29 (linear contrast \( P < 0.01 \) on both days) was due primarily to increases in the 50 mg BID group, but the actual increases in mean concentration were not clinically meaningful (97.6 mcg/dl at baseline to 117.5 and 120.6 mcg/dl at Days 28 and 29, respectively).

fT: There was a dose-dependent increase in fT on Days 14 and 28 (linear contrast \( P \leq 0.01 \) at both time points). This data is not presented because of potential cross-reactivity of antibodies used to measure testosterone.

SHBG: A slight mean increase was observed at Day 14 in the placebo group. In contrast, decreases from baseline at Days 14 and 28 were observed for all dose regimens of asoprisnil, and were significantly different from placebo (Figure 7) except with the doses of 5 mg BID and 10 mg QD (Day 28). A dose effect was apparent on Days 14 and 28 (linear contrast \( P \leq 0.01 \) at both time points).

Prolactin: Mean increases from baseline were observed in all dose groups, including placebo at Days 14 and 28. Other than a spurious high response on Day 28 which caused the 5 mg QD group to be significantly different from placebo, there were no significant differences between the various doses of asoprisnil and placebo (data not shown).

![Figure 3. Mean ± SEM response of LH. (A) Placebo and low dose treatment groups (5 mg QD, 5 mg BID and 10 mg QD). (B) Placebo and high dose treatment groups (25 mg QD, 25 mg BID and 50 mg BID).](https://academic.oup.com/humrep/article-abstract/20/4/1090/701316)
Other effects

Intermenstrual bleeding: With the exception of the 25 mg BID dose group, little intermenstrual bleeding was reported. However, in the 25 mg BID dose group, five women reported bleeding and in one, this persisted intermittently for 15 days.

Endometrial thickness: Although slight mean decreases in endometrial thickness were observed with all asoprisnil doses, particularly in those women who received the highest doses, none of the changes were significantly different from placebo (data not shown).

Ovarian cysts: None of the women developed cysts >5 cm in diameter. One woman in the 10 mg QD group, one in the 25 mg QD group, and two women who received placebo had cysts measuring from 3.1 to 4.4 cm during the treatment phase. All of these were asymptomatic, and either disappeared or regressed to <3 cm at the follow up visit.

Endometrial biopsy

In the placebo group, seven of the 12 women showed normal secretory phase endometrium, three were classified as proliferative, and the remaining two showed ‘non-physiologic secretory effects’. A total of 47 biopsies were available for histological analysis in women treated with asoprisnil. The endometrial biopsies were consistent with partial (mixed) progesterone agonist/antagonist activity of asoprisnil. Twelve women (25%) demonstrated ‘non-physiologic secretory effects’. This was characterized by weak to moderate secretory activity in the glands with no mitotic figures. Another 12 (25%) women had endometrial histology classified as ‘secretory pattern, mixed type’. This was characterized by weakly proliferative and secretory glands with infrequent mitotic figures. In both categories, the endometrial stroma was either compact or showed non-uniform edema. Clusters of unusual ‘thick-walled’ arterial vessels were occasionally observed. Another eight women showed a normal luteal phase endometrium. Five women demonstrated weak proliferative endometrium and another four an active proliferative endometrium. Finally, inactive or atrophic endometrium was observed in six women. No woman showed endometrial hyperplasia. In the asoprisnil groups, the non-physiologic secretory patterns (‘non-physiologic secretory effects’ and ‘secretory pattern, mixed type’) were evident at doses equal to and higher than 5 mg BID and 10 mg QD. There was no clear correlation between the frequency of these effects and asoprisnil dose. In asoprisnil
groups, there were also no clear differences in endometrial morphology between biopsies obtained during anovulatory cycles and those indicative of luteinization. Figure 8 presents representative endometrial biopsy appearances observed after asoprisnil treatment for 28 days.

Adverse events

The majority of the women reported at least one adverse event (Table IV) and the overall incidence was similar in those who received placebo (67%) or asoprisnil (38–100%). The most commonly reported adverse events were headache, abdominal pain, nausea, dizziness and metrorrhagia. Metrorrhagia was reported as an adverse event in eight subjects: four of these were in the 25 mg BID group with intermenstrual bleeding mentioned previously, two were in the 25 mg QD group and one each was in the placebo and 50 mg BID groups. In three of these eight subjects (two in the 25 mg QD group and one in the 25 mg BID group), uterine bleeding was attributable to a weak menstruation as it occurred near the end of or after 28 days of treatment. The remaining five subjects experienced 1 or 2 days of intermenstrual bleeding during treatment. All adverse events were considered mild in intensity except for one woman in the 25 mg BID group who developed a urinary tract infection that was considered to be unrelated to asoprisnil. No woman experienced a serious adverse event or was prematurely discontinued from treatment due to the occurrence of an adverse event.

Hematology, blood chemistry values (including liver and renal function, TSH, T4), and urinalysis results showed no significant trend across all asoprisnil dose groups over the treatment and post-treatment observation period. Also, there were no changes in vital signs, physical examination, Pap smear and ECG that suggest a safety concern with asoprisnil.

Discussion

This study demonstrates that the administration of asoprisnil for 28 days prolongs the menstrual cycles in a dose-dependent manner primarily by targeting the endometrium. Asoprisnil did inhibit corpus luteum formation in some subjects, as determined by measuring luteal phase progesterone levels; however, this effect was inconsistent and lacked dose dependency. It was only at the highest dose of asoprisnil (50 mg BID) that non-ovulatory luteal progesterone concentrations were observed in seven out of eight women. The analysis of progesterone patterns in those subjects who showed a suppression of menstruation after asoprisnil treatment did not reveal any association with serum progesterone levels. Thus, prolongation of the menstrual cycle with asoprisnil was evident even in the presence of a normal luteal phase and luteolysis, and implies that asoprisnil directly targets the endometrium to eliminate or reduce menstrual bleeding. Intermenstrual bleeding, such as spotting or breakthrough bleeding, was rare in asoprisnil groups, with the exception of the 25 mg BID dose group. This is in contrast to continuous progesterin treatment, which is associated with high frequency of bleeding abnormalities during the initial months of treatment (Fraser and Hickey, 2000).
Since treatment was commenced at the beginning of the cycle, the changes in hormonal patterns in the placebo-treated cycle characterized by a fall in FSH, mid-cycle increase in LH, increase in E2 with a less prominent increase in E1, as well as the elevation of progesterone during the luteal phase, were expected. Asoprisnil did not appear to have a meaningful effect on LH, FSH, prolactin, E2 and E1 concentrations, with the exception of LH patterns in high-dose (25 mg daily or greater) asoprisnil groups which showed an increase versus placebo during the late luteal phase. The interpretation of LH increase at high asoprisnil doses is difficult due to low frequency of blood sampling. Two possibilities should be taken into consideration: (i) an increase in basal LH concentrations during the luteal phase, and (ii) a delay in LH peak. Further studies will be needed to determine the mechanism of LH increase observed in high-dose asoprisnil groups.

There was also a suggestion that with the highest asoprisnil dose, E2 levels were slightly lower. This is compatible with the absence of corpus luteum formation, most likely due to anovulation, observed in this group of women. Thus, the cycle prolongation with asoprisnil occurs in the presence of early follicular phase levels of E2. In most of the asoprisnil-treated groups, mean progesterone levels were below that of the placebo, reflecting the absence of luteinization in some women, especially those who received the higher doses. It should be stressed that luteinization was defined in this study based on progesterone concentrations typical for the normal luteal phase. Hence, the presence of luteal phase progesterone may be indicative of either ovulation or luteinized unruptured follicle. Serial ultrasound examinations of the dominant follicle and frequent measurement of ovarian and pituitary hormones will be needed to determine the effects of asoprisnil on ovulation.

Suppression of menstruation irrespective of the effects on luteinization and progesterone withdrawal is a surprising finding that, to our knowledge, has not been previously described with any known pharmacological agent. Continuous administration of oral contraceptives or high-dose progestins is accompanied by amenorrhea, but both regimens consistently produce anovulation (Lobo and Stanczyk, 1994). Moreover, these treatments are associated with breakthrough bleeding and spotting. Similarly, amenorrhea induced by GnRH agonists or antagonists is due to the complete inhibition of ovarian hormonal activity and to anovulation (Conn and Crowley, 1991). Continuous administration of the progesterone antagonist mifepristone also suppresses menstrual cyclicity at daily doses of 2 mg and 5 mg (Brown et al., 2002) and more consistently at higher doses of 50 or 100 mg per day (Kettel et al., 1994). Similarly, the progesterone antagonist onapristone administered in doses of 15 or 50 mg daily for 7 days in the follicular phase also prolongs the cycle, although a dose of 5 mg produced inconsistent effects (Croxatto et al., 1994). However, there are fundamental differences between the effect of asoprisnil and both mifepristone and onapristone on the events of the menstrual cycle, since the progesterone antagonists prolong the menstrual cycle by delaying or blocking the LH surge. As a consequence, there is delayed or even absent ovulation, depending on the duration of treatment (Kettel et al., 1994; Croxatto et al., 1998; Brown et al., 2002).

The ability to control endometrial bleeding by targeting the endometrium appears to be characteristic of 11β-benzaldoxime substituted SPRMs with partial progesterone agonist–antagonist activities since other compounds belonging to this class, including asoprisnil ecomate (J956;
induced by 11β-benzaldoxime substituted SPRMs is still unknown. In cynomolgus monkeys, treatment with J1042 for 21 days significantly reduced the formation of spiral arterioles and were accompanied by endometrial atrophy, stromal compaction and the presence of weakly secretory glands (Chwalisz et al., 2000). Endometrial atrophy was also observed in toxicological studies with asoprisnil in monkeys (Chwalisz et al., 2002). Based on these studies we hypothesized that 11β-benzaldoxime substituted SPRMs may control both endometrial bleeding and proliferation via a vascular effect (Chwalisz et al., 2000). This study revealed the formation of unusual, thick-walled arterioles in the endometrium of women exposed to asoprisnil (Figure 8B). In subjects treated with asoprisnil for 3 months or longer, thick-walled vessels are consistently found in Pipelle biopsies (unpublished data). These vessels clearly differ from those typically observed in the endometrium from women treated with continuous progestins. Such treatment results in the patchy appearance of abnormally small and abnormally large, thin-walled, fragile vessels in the superficial regions of exposed endometrium (Hickey et al., 2000; Hickey and Fraser, 2000; Simbar et al., 2004). There is growing evidence to confirm that thin-walled microvessels are in some way linked to the troublesome symptoms of breakthrough bleeding and spotting in many women using long-acting progestins (Fraser and Hickey, 2000). Hence, asoprisnil-induced morphological changes in endometrial vessels and perivascular stroma might be, at least in part, responsible for amenorrhea. The molecular mechanisms underlying these effects are still unclear.

In asoprisnil groups, there was no evidence of endometrial hyperplasia or other appearances indicative of ‘unopposed’ estrogen effects on the endometrium in spite of follicular phase estrogen concentrations and non-luteal progesterone levels in many subjects. On the contrary, the endometrial biopsies conducted during the treatment period showed unique effects characterized by the presence of weakly secretory glands with minimal or absent proliferation, as evidenced by absent or rare mitotic figures, and variable effects in the stroma ranging from stromal compaction to focal predecidual reaction. These effects, described in the new classification system developed by TAP Pharmaceutical Products inc, Diagnostic Cytology Laboratories, and the expert panel of gynecological pathologists as ‘non-physiologic secretory effects’ and ‘secretory patterns, mixed type’, have not been reported before with any other drugs. They are consistent with mixed progesterone agonist/antagonist effects on the glandular epithelium and stroma. Although secretory appearances of endometrial glands were weak, they were consistently observed at asoprisnil doses equal to and higher than 10 mg/day (Figure 8). In addition, no clear dose effect was evident. Longer studies with larger numbers of subjects are needed to fully assess the effects of asoprisnil on endometrial morphology. These studies are currently being conducted and will be published separately. The endometrial effects of asoprisnil seem to be different from those induced by progestins and progesterone antagonists such as mifepristone and onapristone. Although low doses of mifepristone (2 or 5 mg) decreased endometrial proliferation (Baird et al., 2003), proliferative patterns, rather than secretory effects, have been reported in premenopausal women treated with higher doses of mifepristone (Murphy and Castellano, 1994). Indeed ‘unopposed’ estrogenic effects with mifepristone may be seen with doses as low as 10 mg daily (Eisinger et al., 2003; Steinauer et al., 2004).

This study is consistent with animal studies indicating high PR selectivity of asoprisnil. With asoprisnil, there was no increase in C with any of the dose regimens, and the diurnal rhythm of C was maintained. Mean changes in DHEA-S concentrations were also inconsistent. This is all evidence that asoprisnil, unlike mifepristone, lacks antiglucocorticoid activity at the doses tested. This response was consistent with animal studies, which showed that asoprisnil has significantly less antiglucocorticoid activity than mifepristone (DeManno et al., 2003).

SHBG levels were suppressed by asoprisnil in a dose-dependent manner. SHBG is a liver protein that is increased by estrogens, decreased by androgens, but uninfluenced by progesterone (Rosner, 1990; Saarikoski et al., 1990). The decrease in SHBG suggests an androgenic effect in the liver. A decrease in SHBG can potentially lead to an increase in fT, but uncertainties about the validity of the fT assay used in this study do not allow conclusions to be made about fT at this time. However, there were no significant changes in plasma lipids; neither were clinical androgenic effects (e.g. acne) apparent in these women. It must be stressed that this was only a 28-day study, and longer treatments are ongoing to determine if there is any interaction of asoprisnil with androgen receptors in humans.
Asoprisnil showed a favorable safety and tolerability profile in this study. The most commonly reported adverse events were headache, abdominal pain, nausea, dizziness and metrorrhagia. Minimal breakthrough bleeding occurred with asoprisnil with the exception of one woman who reported 15 days of intermittent spotting. Thus, unlike progestins, asoprisnil does not seem to induce breakthrough bleeding. Adverse events typical for progestins such as mood changes, bloating, etc., were not observed. There were also no concerns regarding ovarian cysts. Although longer studies will be needed to evaluate safety and tolerability of asoprisnil, the existing results are encouraging and support further clinical investigation.

In conclusion, the results of this study show that the SPRM asoprisnil in a total daily dose of up to 100 mg for 28 days was safe and well tolerated by premenopausal women. Asoprisnil reversibly prolonged the menstrual cycle and suppressed menstruation in a dose-dependent manner at doses >10 mg QD, irrespective of an effect on luteal phase progesterone concentrations indicative of luteinization. Asoprisnil induced these menstrual effects without compromising ovarian estrogen production, and induced amenorrhea primarily by targeting the endometrium and its blood vessels. These unique characteristics make asoprisnil a strong candidate for further development in the treatment of disorders such as uterine leiomyoma, endometriosis and abnormal uterine bleeding.

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1-month asoprisnil administration in healthy women