Intrauterine release of progesterone antagonist ZK230211 is feasible and results in novel endometrial effects: a pilot study

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BACKGROUND: Continuous administration of progesterone antagonists (PAs) results in endometrial suppression and amenorrhoea in several model systems. We compared the effects of intrauterine release of a highly specific PA, ZK230211, to those of a progestin using the levonorgestrel-releasing intrauterine system (LNG-IUS).

METHODS: Forty-two women were randomly fitted with an IUS releasing either ZK230211 at a rate 1, 4 or 8 μg/24 h (ZK-IUS) or LNG (at 20 μg/24 h, LNG-IUS) at 4–8 weeks before hysterectomy. Bleeding patterns, endometrial morphology and content of ZK230211, and various immunohistochemistries (IHCs) were evaluated. RESULTS: Days of bleeding and spotting were unchanged by the use of ZK-IUSs but were increased by LNG-IUS (P<0.01). ZK230211 was measurable in all endometrial specimens. Endometrium was partly suppressed in 9–30% of women following the use of ZK-IUSs, and in 67% after LNG-IUS. IHCs for Ki-67 and phosphorylated histone H3 were not suggestive of proliferative activity in any group. Compared to LNG, progesterone receptor (PR) was increased following ZK230211 in surface epithelium (all three doses P<0.01–P<0.05) and stroma at 4 μg/24 h (P<0.05). Although low, androgen receptor staining was higher in endothelial epithelium following LNG than ZK230211 (P<0.05). Insulin-like growth factor-binding protein-1 (IGFBP-1) was detectable only following LNG (P<0.0001).

CONCLUSIONS: Short-term intrauterine release of ZK230211 did not change bleeding patterns or result in endometrial suppression. Expression of proliferation markers was low following the use of both IUSs. Absence of IGFBP-1 and increase in PR reflect the PA effects of ZK230211.

Keywords: progesterone antagonist; endometrium; insulin-like growth factor-binding protein-1; levonorgestrel; ZK230211

Introduction

Unscheduled breakthrough bleeding (BTB) associated with the use of progestin-only contraception remains a major problem, often resulting in poor compliance and discontinuation of progestin-only contraceptive methods (Dugoff et al., 1995; Kovacs, 1996). Intrauterine release of levonorgestrel (LNG) by means of the LNG-intrauterine system (LNG-IUS) is highly effective for contraception as well as for the treatment of heavy menstrual bleeding, menorrhagia (Andersson et al., 1994; Luukkainen and Toivonen, 1995; Hurskainen et al., 2004). However, initiation of LNG-IUS treatment is associated with a high incidence of BTB, which typically resolves following the first few months of LNG-IUS use (Andersson et al., 1994; Hurskainen et al., 2004).

The effects on the endometrium of continuous administration of progesterone antagonists (PAs) such as mifepristone and ZK137316 have been evaluated both in women (Baird et al., 2003; Narvekar et al., 2004) and in non-human primates (Wolf et al., 1989; Slayden and Brenner, 1994, Slayden et al., 1998, 2001; Chwalisz et al., 2000). In non-human primates, PAs inhibit endometrial proliferation and induce amenorrhoea (Wolf et al., 1989; Slayden et al., 1998, 2001). When administered chronically at relatively low doses, PAs block the mitotic activity of endometrial epithelium and induce stromal compaction in a dose-dependent manner in both spayed and intact monkeys (Wolf et al., 1989; Slayden and Brenner, 1994, Slayden et al., 1998, 2001; Heikinheimo et al., 1996). As follicular development is not suppressed during PA administration (Heikinheimo et al., 1995; Slayden et al., 2001), endometrial suppression is not associated with a decrease in circulating
levels of estradiol. Thus the mechanism of action of endometrial suppression remains somewhat enigmatic; atrophy of the uterine spiral arteries being a likely explanation (Chwalisz et al., 2000).

The PA ZK230211 is highly potent PA with no progestogenic effects (Fuhrmann et al., 2000; Slayden et al., 2001). Systemic daily administration of ZK230211 at doses of ≥16 μg/kg to cynomolgus monkeys suppresses ovulation and menstruation (Slayden et al., 2001). Similarly, intrauterine release of 3–4 μg and 26–30 μg of ZK230211/24 h had an antiproliferative effect on primate endometrium (Nayak et al., 2000). Thus intrauterine release of PAs, especially ZK230211, may make them useful in both contraception and hormone therapy for various gynaecological indications. Specifically, endometrial suppression by means of PA-releasing IUS might be effective in the treatment of heavy or prolonged uterine bleeding. Moreover, a PA-IUS is likely to convert the endometrium into a non-receptive state, which may be utilized in the development of novel contraceptive strategies.

In the present randomized, single-blinded, prospective proof-of-concept trial, we evaluated the bleeding patterns and endometrial effects of intrauterine release of the PA ZK230211 versus progestin LNG in women scheduled for hysterectomy owing to heavy or painful menstruation.

Materials and Methods
The study was performed between August 2002 and June 2003. The study subjects were identified among women on waiting list for hysterectomy at the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital. Women scheduled for hysterectomy owing to idiopathic heavy menstrual bleeding (menorrhagia) or painful menstruation (dysmenorrhoea) participated in the study. Flow of the participants through the study is outlined in Fig. 1. Prior to their participation, each woman signed an informed consent document. The study protocol was approved by the Institutional Review Board of Helsinki University Central Hospital and the Finnish National Agency for Medicines.

The criteria for inclusion in the study were written informed consent, age 30–48 years, regular menstrual periods with cycle length between 21–35 days, depth of the uterine cavity between 6 and 10 cm and good general health. The exclusion criteria were: endometrial polyps or hyperplasia, submucosal myoma or intramural myoma exceeding 4 cm in greatest diameter, or myoma distorting the uterine cavity, an ovarian cyst exceeding 4 cm, epithelial cell atypia in the Pap smear, concomitant use of an intrauterine device, history of climacteric symptoms and systemic use of sex steroids within the last 3 months.

The experimental IUSs releasing ZK230211 (ZK-IUS) at rates of 1, 4 and 8 μg/24 h were provided by Schering Ag (Berlin, Germany). The LNG-releasing IUS (20 μg/24 h; MIRENA®, Schering Oy, Turku, Finland) was used as a comparator. Similarly as for LNG-IUS, the ZK-IUS consisted of a polyethylene body in the shape of T with a mixture of ZK230211 and polydimethyl siloxane mounted around the vertical arm. The dimensions of the ZK-IUS and LNG-IUS were similar (32 × 32 mm).

The women were randomized using SAS/PLAN by Schering Oy to any of the three experimental ZK-IUSs or LNG-IUS. Each investigator (authors O.H., A.T. and P.H.) had a separate stock of identically packaged IUSs and randomization numbers. However, the nature of the IUS became apparent to the study personnel following opening of the packaging. The IUSs were inserted between 4 and 8 weeks prior to scheduled hysterectomy. The duration of the trial was based on previous toxicological data obtained from non-human primates, with the drug regulatory agency permitting the study to last up to 8 weeks. Insertion was performed between days 1 and 7 of the menstrual cycle. Following insertion, fundal location of the IUS was verified by means of pelvic ultrasonography.

The primary outcome measure was assessment of endometrial morphology during the use of ZK-IUS in comparison to that of LNG-IUS. The secondary objectives included assessment of bleeding patterns, determination of uterine and serum concentration of ZK230211 and evaluation of the effect of ZK-IUS versus LNG-IUS on selected endometrial markers.

Figure 1: Flow of the subjects through the study
Table 1: Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Protein of interest</th>
<th>Antigen retrieval</th>
<th>Primary antibody (AB)</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>Microwave buffer—0.01 M Na citrate</td>
<td>Monoclonal mouse anti-PR AB (Novocastra, Newcastle, UK) (1:40)</td>
<td>Mouse immunoglobulin (Ig) G (Sigma, Dorset, UK) (1:800)</td>
</tr>
<tr>
<td>ERα</td>
<td>Microwave buffer—0.01 M Na citrate</td>
<td>Monoclonal mouse anti-ERα AB (Dako, Cambridge, UK) (1:400)</td>
<td>Mouse immunoglobulin IgG (Sigma, Dorset, UK) (1:2400)</td>
</tr>
<tr>
<td>ERβ</td>
<td>Pressurecook buffer—0.05 M glycine/0.01% EDTA</td>
<td>Monoclonal mouse anti-ERβ AB (Serotec, Oxford, UK) (1:40)</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>AR</td>
<td>Pressurecook buffer—0.01 M Na citrate</td>
<td>Monoclonal mouse anti-AR AB (Biogenex, CA, USA) (1:240)</td>
<td>Mouse immunoglobulin IgG (Sigma, Dorset, UK) (1:300)</td>
</tr>
<tr>
<td>Ki67</td>
<td>Microwave buffer—0.01 M Na citrate</td>
<td>Monoclonal mouse anti-Ki67 AB (Novocastra, Newcastle, UK) (1:50)</td>
<td>Mouse immunoglobulin IgG (Sigma, Dorset, UK) (1:500)</td>
</tr>
<tr>
<td>PH3</td>
<td>Pressurecook buffer—0.01 M Na citrate</td>
<td>Rabbit anti-phosphohistone AB (Upstate Biotech., Buckingham, UK) (1:1000)</td>
<td>Rabbit immunoglobulin IgG (Vector Lab., UK) (1:1000)</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Vectastain ABC kit (Vector Lab., CA, USA)</td>
<td>Monoclonal anti-IGFBP-1 AB (Mab 6303 (Medix Biochemica, Kauniainen, Finland) (1:1000)</td>
<td>Mouse immunoglobulin IgG (Vector Lab., CA, USA) (1:1000)</td>
</tr>
</tbody>
</table>

**Table 2: Summary of the antigen retrieval methods, primary antibodies and negative controls used in the various immunohistochemical analyses**

**Serum and uterine tissue measurements of ZK230211**

The samples were pre-prepared and sent for analysis to the analytical laboratory of Schering AG, Berlin, Germany. A liquid chromatography-mass spectrometer/mass spectrometer method was used for the measurement of ZK230211 concentrations. The lowest limit of quantification in human serum samples was 50 pg/ml, and in the uterine tissue samples (endometrial and myometrial), it was 10–25 ng/g.

**Endometrial morphology**

Endometrial morphology was assessed from haematoxylin and eosin-stained tissues taken from the fundus, corpus or isthmus. In addition to routine diagnostic analysis, estrogenic (E) and progestogenic (P) activities were evaluated by means of the following criteria: E0, atrophic glands; E1, small single-layered glands with rare mitoses; E2, moderately sized glands with two or three cell layers and occasional mitoses; E3, tortuous glands, several cell layers, easily detectable mitotic activity; P0, no signs of a progestogenic effect; P1, basal vacuolization in most cells; P2, dilated glands with secretory activity; P3, stromal decidualization. The combined E and P score was assigned to one of the three categories: fully active = E2-P0; partially suppressed = E2-P1-3 or E1P0-3; or fully suppressed = E0. The samples were assessed independently by two pathologists. In cases of disagreement, a third pathologist was included. If the hormonal effect varied between anatomical locations, the overall result from two of the three locations (fundus, corpus or isthmus) was taken as the consensus score.

**Immunohistochemistry**

Tissue expression of androgen receptor (AR), progesterone receptor (PR) and estrogen receptors alpha and beta (ERα and ERβ) was assessed as described previously (Critchley et al., 2003; Slayden et al., 2001; Henderson et al., 2003). The proliferation markers Ki-67 and phosphorylated histone H3 (PH3) were evaluated as described by Brenner et al. (2003) and Narvaker et al. (2004). Finally, insulin-like growth factor-binding protein-1 (IGFBP-1) was evaluated according to Pekonen et al., 1992. The antigen retrieval methods, primary antibodies and negative controls used in the immunohistochemical analyses are summarized in Table 2.

The amounts of the above-mentioned epitopes were assessed by two blinded observers in a semiquantitative manner on a 4-point scale: 0, no staining; 1, mild/minimal staining; 2, moderate immunostaining and 3, intense immunostaining.

**Statistical analysis**

Statistical analyses were performed using the chi-square test, the Fisher’s exact test, the Mann–Whitney U-test or the Kruskal–Wallis test, as appropriate. A two-tailed P-value lower than 0.05 was considered statistically significant.
was considered statistically significant. The calculations were performed with StatView statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Bleeding patterns

Figure 2 shows the numbers of days of bleeding and spotting (mean ± SD) during the 30-day periods immediately preceding insertion of the IUSs, and preceding hysterectomy. The number of days of spotting and bleeding increased significantly in the LNG-IUS group (P < 0.01).

Uterine tissue and serum concentrations of ZK230211

ZK230211 was measurable in all endometrial specimens. The mean (+ SD) endometrial concentrations of ZK230211 (per gram of tissue wet weight) were 83.7 ± 40.6, 83.8 ± 39.5 and 166.9 ± 134.6 ng/g in the groups using ZK-IUSs releasing 1, 4 and 8 μg of ZK230211/24 h, respectively. Only four subjects (one in each group using a ZK-IUS releasing 1 and 4 μg of ZK230211, and two in the group using an IUS releasing 8 μg of ZK230211) had measurable levels of ZK230211 in the myometrium. The individual concentrations varied from 8.4 to 47.7 ng/g of myometrial-wet weight. However, serum concentrations of ZK230211 were below the quantification limit of the assay in all samples analysed (n = 31).

Endometrial morphology

Figure 3 illustrates endometrial morphology after treatment with an IUS releasing 8 μg of ZK230211/24 h, or LNG (20 μg/24 h). Endometrial morphology differed in relation to ZK230211 and LNG treatment. In LNG-treated endometrium, marked stromal decidualization was seen and the morphology of the glands varied from inactive to secretory. On the other hand, stromal decidualization was not observed after ZK230211 treatment. The glands, in contrast, were often dilated and the epithelial cells showed little proliferative activity and a secretory morphology.

Figure 3: Haematoxylin and eosin-stained human endometrium following treatment with an IUS releasing 8 μg of ZK230211/24 h (panels on the left), and treatment with an LNG-IUS (panels on the right). Note the stromal compaction and non-functional secretory morphology in the glands following ZK230211 treatment. In LNG-treated endometrium, marked stromal decidualization was seen and the morphology of the glands varied from inactive to secretory.
Endometrial morphology was judged to reflect partial suppression in ≥2 of the 3 locations (fundus, corpus and isthmus) in 30%, 9% and 10% of women with ZK-IUSs releasing 1, 4 and 8 µg/24 h, respectively, and in 67% of women with an LNG-IUS. The difference in endometrial morphology was statistically significant between the groups of women using the LNG-IUS versus ZK-IUSs releasing 4 and 8 µg/24 h (P < 0.01 and P < 0.02, respectively). The difference between women with an LNG-IUS versus a ZK-IUS releasing 1 µg/24 h approached significance (P = 0.11).

A high degree of morphologically estrogenic effects (grade 3 in 60–82%) were seen following the use of ZK-IUSs, whereas only 11% of the endometrial specimens collected following use of an LNG-IUS displayed grade 3 estrogenic effects. Moreover, the distribution of morphologically estrogenic effects (grade 1–2 versus 3) differed significantly between subjects using an LNG-IUS versus IUSs releasing 1, 4 and 8 µg of ZK230211/24 h (P < 0.05, P < 0.002 and P < 0.005, respectively). However, morphologically progestogenic effects (grade 0–2 versus 3, or grade 0–1 versus 2–3) were not significantly different between the endometrial specimens exposed to an LNG-IUS or any of the ZK-IUSs.

**Immunohistochemistry**

Figure 4 shows a summary of the various immunohistochemical analyses performed. Staining was analysed separately for endometrial glands and stroma. In addition, staining for AR, ERβ and PR was analysed separately in endometrial surface epithelium.

**Proliferation markers**

Tissue expression of the proliferation markers PH3 (Fig. 4A and B) and Ki-67 (data not shown) was weak in endometrial glands and stroma exposed to either ZK230211 or LNG. Staining for Ki-67 and PH3 was similar irrespective of the dose of ZK230211. When analysed semiquantitatively, the median values varied between 0 and 1, and did not differ between the different groups.

**Sex steroid receptors**

Immunolocalization ERβ in endometria following intrauterine treatment with ZK230211 and LNG is shown in Fig. 4C and D, respectively. Staining for ERβ was intense in the surface epithelium, glands and stroma following exposure to both LNG and all three doses of ZK230211. No statistically significant differences in ERβ staining emerged between the endometria exposed to LNG and ZK230211.

In general, immunostaining for ERβ was more intense than for ERα (data not shown). Staining for ERα in both endometrial glands and stroma was similar following intrauterine exposure to LNG and all the three doses of ZK230211.

Immunostaining for PR in endometria exposed to intrauterine delivery of ZK230211 and LNG is illustrated in Fig. 4E and F, respectively. PR was not detectable in the surface epithelium following exposure to intrauterine LNG. It was, however, detectable in the surface epithelium following exposure to ZK230211; the difference was significant between the groups exposed to LNG and all three doses of ZK230211 (P < 0.01–<0.05). In endometrial glands and stroma, intense immunoreactivity for PR was observed after intrauterine administration of ZK230211. Immunostaining for PR in endometrial stroma differed significantly (P < 0.05) between the groups exposed to LNG and to ZK230211 at 4 µg/24 h.

Minimal staining for AR (data not shown) was detectable in the endometrial epithelium following exposure to intrauterine LNG. No immunoreactivity for AR was detectable in endometrial epithelium following ZK230211 (P < 0.05 between groups exposed to LNG versus ZK230211). The endometrial stroma displayed moderate immunoreactivity for AR (NS between different subject groups).

**IGFBP-1**

Figures 4G and H depict the results of IGFBP-1 IHC in endometria following treatment with intrauterine ZK230211 and LNG, respectively. No immunostaining for IGFBP-1 was observed in endometria exposed to intrauterine ZK230211. However, IGFBP-1 was detectable in all but one of the endometrial specimens following intrauterine LNG administration (P < 0.0001).

**Discussion**

In the present study, we report that intrauterine release of the PA ZK230211 by means of an IUS is feasible and that it results in significant endometrial levels of ZK230211. Use of a ZK-IUS had no effect on the number of days of bleeding or spotting, whereas an increase in uterine bleeding was noted during the short period of exposure of the endometrium to LNG. When compared with the LNG-IUS, clear signs of PA effects, such as maintenance of endometrial PR expression in the glandular epithelium and lack of IGFBP-1 protein expression, were seen during the short period of exposure to a ZK-IUS.

The present study was randomized and blinded in design. In addition, in contrast to the majority of studies in which the endometrial effects of PA have been assessed, the present work was performed in human subjects. A proof of concept study such as this, with obvious restrictions as regards clinical work, can only be performed among women presenting electively for hysterectomy. Thus a concern about interpretation of the data is that the IUSs were tested in women presenting with menstrual complaints and abnormal uteri. Furthermore, the study was of a relatively short duration and some of the eventual endometrial effects associated with both the LNG-IUS and ZK-IUSs may not be apparent until after a longer period of exposure. Analysis of the uterine blood vessels was not included in the present study. This is an interesting research question that should also be addressed in future studies.

As expected, the number of days of spotting and bleeding increased following insertion of an LNG-IUS. However, the ZK-IUSs failed to have an effect on uterine bleeding patterns reported by the women. Since administration of PA to nonhuman primates results in a rapid induction of amenorrhoea (Wolf et al., 1989; Slayden and Brenner, 1994; Slayden et al., 1998, 2001), the lack of an immediate effect of the
ZK-IUSs on uterine bleeding was an unexpected observation. In previous studies in which ZK230211 has been evaluated in non-human primates, ovulation and menstruation were suppressed in a dose-dependent fashion (Slayden et al., 2001). The doses of ZK230211 used in the present work may have been insufficient for induction of amenorrhoea.
However, in a preliminary study on non-human primates, intrauterine release of ZK230211 at 3–4 µg/24 h resulted in marked endometrial suppression (Nayak et al., 2000). The difference in bleeding patterns during the use of ZK-IUSs may be dose-related or the result of subtle differences between human and non-human primate endometrium.

Several investigators have reported profound atrophy of the endometrial glands and decidualization of stroma in women following use of an LNG-IUS for 3 months or longer (Silverberg et al., 1986; Critchley et al., 1998a; Phillips et al., 2003). In the present study, the endometrium was categorized as suppressed in two-thirds of the LNG-IUS users. More profound suppression of the endometrium may have been observed had the duration of intrauterine LNG administration been for a longer period of time. Endometrial suppression, as evaluated morphologically, was weak following administration of ZK230211. In contrast, clear morphological signs of an estrogenic effect were evident in endometrial specimens collected following the use of intrauterine ZK230211. This is in agreement with the results of a recent study carried out by Baird et al. (2003), in which prolonged administration of low doses of oral mifepristone resulted in either a proliferative condition or cystic dilatation in the majority of women.

Data concerning the morphological effects of PA, and more broadly those of selective PR modulators, on human endometrium have only recently been reported (Chwalisz et al., 2005; Williams et al., 2007). Categorization of the effect on the endometrium of ZK230211 is problematic, and the nomenclature associated with morphological events, such as estrogen-or progestin-like, seen during the normal menstrual cycle, may not be justified. Indeed, it has been recognized that use of PR modulators leads to non-classical endometrial morphology, and new descriptions have been developed (Williams et al., 2007).

In the present study, endometrial proliferation was assessed using IHC for both Ki-67 and PH3. PH3 antibody identifies chromosomes during mitosis in cultured cells or whole mounts, thus providing accurate information about cellular proliferation (Brenner et al., 2003). The immunoreactivity of these was negligible in both endometrial epithelial and stromal cell compartments following intrauterine delivery of LNG and ZK230211. Similarly, Hurksainen et al. (2000) reported that endometrial staining for Ki-67 was weak during the use of an LNG-IUS. Previous studies concerning the effects of administration of PAs on endometrial expression of Ki-67 have also been reported. Low levels of Ki-67 immunostaining were seen during administration of mifepristone at doses of 2 or 5 mg for 120 days in female volunteers (Baird et al., 2003). In non-human primate endometrium, however, Ki-67 levels comparable to those seen in proliferative phase endometrium were detected following systemic administration of ZK230211 in cynomolgous monkeys (Slayden et al., 2001). As regards PH3 immunolabelling, a significant decrease in the expression of PH3 has been reported in human endometrium following chronic low dose administration of mifepristone (Narvekar et al., 2004).

Down-regulation of endometrial ERs and PRs is a well-characterized progestin effect, and is observed with intrauterine administration of LNG (Critchley et al., 1998b; Hurksainen et al., 2000). In the present study, PRs were down regulated both in the surface epithelium and in the stroma of the endometria exposed to LNG when compared with endometria exposed to ZK230211. As observed in non-human primate endometrium, endometrial PR levels were maintained during PA administration (Slayden et al., 1993). The present data on PR expression in endometrium exposed to intrauterine PA are consistent with the PA nature of ZK230211. Endometrial expression of both ERs and ERβ was similar to the use of either an LNG-IUS or a ZK-IUS. This contrasts with the effects of ZK230211 on primate endometrium, in which systemic administration of ZK230211 counteracted the effects of progesterone, resulting in up-regulation of ERβ (Slayden et al., 2001).

Expression of AR is down regulated in secretory phase human endometrium and in endometria collected from women using an LNG-IUS (Burton et al., 2003). In contrast, PA-induced suppression of endometrial growth is associated with up-regulation of endometrial ARs in non-human primates (Brenner and Slayden, 2005) and in women (Narvekar et al., 2004). Furthermore, antagonism of androgen action by co-administration of flutamide to PA-treated cynomolgous monkeys has been reported to result in loss of endometrial suppression (Brenner and Slayden, 2003). These data suggest that androgen action in essential for the suppressive effects of PAs on the endometrium. In the present study, expression of ARs in the glandular epithelium was minimal among the subjects exposed to intrauterine ZK230211 administration. This lack of up-regulation of ARs may explain in part the lack of endometrial suppression following intrauterine delivery of ZK230211.

The endometrial content of IGFBP-1 is increased in human secretory phase and decidualized endometrium (Rutanen et al., 1998a, b). Similarly, addition of progestins to cultures of human endometrial tissue explants has been reported to increase the synthesis of IGFBP-1 (Bell et al., 1991; Gao et al., 1994). Endometrial exposure to LNG results in strong expression of endometrial IGFBP-1 (Pekonen et al., 1992). Thus, sequestration of IGF as a result of elevated tissue content of IGFBP-1 has been proposed as one of the mechanisms explaining endometrial suppression during use of an LNG-IUS (Rutanen et al., 1997). In the present study, strong expression of IGFBP-1 protein was evident in the specimens collected from women treated with an LNG-IUS. However, IGFBP-1 was not detectable in any of the endometrial specimens collected from women using ZK-IUSs. It is possible that in the absence of IGFBP-1, IGF actions on the endometrium are maintained, which may contribute to the endometrial morphology observed during use of a ZK-IUS. Such data further confirm the PA action of ZK230211 on the human endometrium.

In conclusion, the effects on the endometrium of intrauterine release of the PA ZK230211 and the progestin LNG differ. Endometrial suppression following administration of ZK230211 was negligible. However, lack of proliferative activity was evident in endometria from women exposed to either LNG or ZK230211. A high level of endometrial PR expression as well as lack of IGFBP-1 protein expression following intrauterine...
administration of ZK230211 confirms the PA nature of ZK230211 action on the human endometrium. The potential clinical effects of intrauterine delivery of ZK230211 and LNG on the human endometrium are probably mediated via different molecular and cellular mechanisms. The clinical potential of intrauterine delivery of PA should be evaluated in further clinical trials.

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