Characterization of anastrozole effects, delivered by an intravaginal ring in cynomolgus monkeys

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STUDY QUESTION: Is it feasible to deliver anastrozole (ATZ), an aromatase inhibitor (AI), by a vaginal polymer-based drug delivery system in the cynomolgus monkey (Macaca fascicularis) to describe the pharmacokinetic profile?

SUMMARY ANSWER: The present study showed the effective release of ATZ into the systemic circulation from intravaginal rings in cynomolgus monkeys.

WHAT IS KNOWN ALREADY: ATZ is a marketed drug with well documented pharmacological and safety profiles for oral administration. Aromatase is the key enzyme catalyzing estrogen biosynthesis and is overexpressed in endometriotic lesions. AIs show therapeutic efficacy in endometriosis in exploratory clinical trials.

STUDY DESIGN, SIZE, DURATION: The pharmacokinetics of the in vivo release and the pharmacodynamic activity of ATZ released by intravaginal rings (IVR) were investigated in healthy cycling female cynomolgus monkeys in three different dose groups (n = 5) for one menstrual cycle.

PARTICIPANTS/MATERIALS, SETTING, METHODS: IVRs for the cynomolgus monkey, releasing three different doses of ATZ were designed and tested for in vitro/in vivo release for up to 42 days. For pharmacokinetic and pharmacodynamic evaluation, plasma samples were taken once daily from Day 1 to 3 and then every third day until menses occurred (17–42 days).

MAIN RESULTS AND THE ROLE OF CHANCE: ATZ was shown to be compatible with the IVR drug delivery system. An average in vivo release of 277 μg/day/animal of ATZ for one menstrual cycle was effective in causing a decrease of systemic estradiol (E2) levels by ~30% without inducing counter regulation such as the elevation of FSH or the formation of ovarian cysts.

LIMITATIONS, REASONS FOR CAUTION: The study was limited to three dose groups in which only the highest dose decreased the E2 level. Hence, additional research with IVRs releasing higher amounts of ATZ is required to define the threshold for an ATZ-dependent ovarian stimulation in cynomolgus monkeys.

WIDER IMPLICATIONS OF THE FINDINGS: The release rate administered from IVRs is sufficient and in a range that supports feasibility of IVR administration of ATZ as a new approach for long-term therapy of estrogen-dependent diseases such as endometriosis in human.

STUDY FUNDING/COMPETING INTEREST(S): No research funding was received and none of the authors have any conflict of interests.

Key words: endometriosis / anastrozole / aromatase inhibitor / intravaginal ring

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Introduction

The vagina is a suitable site of local and systemic drug administration (Mishell et al., 1970). Drugs absorbed from the vagina do not undergo first-pass metabolism because blood leaving the vagina enters the peripheral circulation via a rich venous plexus, which empties primarily into the internal iliac veins (Richardson et al., 1992; Hussain and Ahsan, 2005). Diverse options of intravaginal delivery formulations and systems are used for drug administration such as suppositories, creams and gels and the intravaginal rings (IVR) (Hussain and Ahsan, 2005). The vaginal drug delivery systems are used to provide sustained and controlled drug release most often for contraceptive steroid hormones (Brache and Faundes, 2010) and hormone replacement therapy (Ballagh, 2001). The advantages of intravaginal drug delivery are manifold if compared with oral administration of drugs, such as the prolonged release and the advantage to use less drug to gain equivalent pharmacodynamic efficacy (Mishell et al., 1970) plus the circumvention of the liver first pass. One additional aspect is that the constant release rates minimize the peak to trough ratio of serum drug levels which may be of importance for Cmax-driven effects (wanted and un-wanted). Furthermore, self-administration of IVRs with longer dosing intervals is perceived as more convenient by some patients compared with daily intake of a pill, and therefore increases the compliance of patients (Novak et al., 2003; Alexander et al., 2004).

Drugs in an IVR formulation are well suited to treat gynecological diseases like endometriosis, a chronic inflammatory disease that affects 5–10% of women of reproductive age, often associated with a devastating impact on their quality of life (Giudice and Kao, 2004). Estrogen deprivation is a clinically proven concept for the treatment of endometriosis. However, none of the approved therapies for endometriosis is able to block endometriotic estrogen production. Thus, blocking the aromatase as a key enzyme of estrogen biosynthesis (Noble et al., 1996) may provide an attractive alternative treatment option, and was shown to be effective in treatment of endometriosis in preclinical models (Atintas et al., 2010; Bilotas et al., 2010; Streuli et al., 2012). Furthermore, evidence for therapeutic efficacy of aromatase inhibitors (AI) in endometriosis was demonstrated in several exploratory clinical trials (for a review of the clinical experience, see (Ferrero et al., 2011)).

Anastrozole (ATZ) is an AI that was developed by Zeneca (now AstraZeneca) Pharmaceuticals under the brand name Arimidex™ for the treatment of hormone-sensitive breast cancer in post-menopausal women (Plourde et al., 1994). Clinical evidence for the use of ATZ in endometriosis showed that 1 mg of ATZ in combination with a combined oral contraceptive (levonorgestrel (0.1 mg/day)/ethinylestradiol 0.020 mg/day)) resulted in a significant reduction of endometriosis-associated pelvic pain (Amsterdam et al., 2005). Thus, an IVR releasing continuously ATZ may provide an attractive new pharmaceutical option for the treatment of endometriosis.

The primary objectives of the present study were to show compatibility of ATZ with a polymer-based vaginal drug delivery system, to get first insights into the in vitro and in vivo release and the pharmacodynamic effects of intravaginally administered ATZ in a relevant preclinical endocrine model. Additionally, ATZ was administered intravenously to cynomolgus monkeys in order to provide the necessary pharmacokinetic parameters for the calculation of the in vivo release rates of the IVRs and the in vitro/in vivo correlation.

Materials and Methods

Anastrozole

Anastrozole,
2-[3-(2-cyanopropan-2-yl)-5-(1,2,4-triazol-1-ylmethyl)phenyl]-2-methylpropiononitrile was bought from OChem Incorporation, IL, USA.

IVRs

IVRs for the cynomolgus monkey, with an outer diameter of 14 mm, releasing three different doses of ATZ (doses A-C, Table I) were designed and tested for in vitro/in vivo release for up to 42 days as described in the published patent WO2011/120925.

In vitro release of ATZ from the IVR

The in vitro release rate of ATZ from the IVRs was tested in 75 ml of 1% hydroxy-propyl-beta-cyclo-dextrin-water solution in shakers at 37°C. Sampling was performed daily except for week-ends. The concentration of ATZ was analyzed by high performance liquid chromatography with UV detection (HPLC-UV). Additionally, ex vivo ring residual drug content of the IVRs was measured at the end of the study by extraction of the worn rings with tetrahydrofuran and analyzed by HPLC-UV.

Ethical approval

Cynomolgus monkeys (Macaca fascicularis) (purpose bred animals, Hartelust BV, Tilburg, NL) aged 5–6 years were housed according to the EU guideline 2010/63 EU. The study (study code: A 04511/09) was approved by the German animal welfare authorities (LAGeSo, Berlin).

Animals

The menstrual cycle of the cynomolgus monkeys was monitored for at least 6 months. The mean weight of the animals at study start was 3.8 ± 0.78 kg. Only animals with a regular cycle were included in the study (n = 5 /group).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Mean in vitro and in vivo (in Cynomolgus monkeys, Macaca fascicularis) release rates of anastrozole (ATZ) from intravaginal rings (IVRs).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release rate (µg/day)</td>
<td>Anastrozole</td>
</tr>
<tr>
<td>Dose A</td>
<td>Dose B</td>
</tr>
<tr>
<td>In vitro release rate (days)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>1–4</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>2–30</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>24–28</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td>42</td>
<td>10 ± 0.5</td>
</tr>
<tr>
<td>Mean in vivo release rate based on ex vivo ring residual drug content</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>54</td>
</tr>
<tr>
<td>Mean in vivo release rate as product of Css and the in vivo CL</td>
<td></td>
</tr>
<tr>
<td>16 ± 5</td>
<td>66 ± 24</td>
</tr>
<tr>
<td>Ex vivo ring residual drug content</td>
<td></td>
</tr>
<tr>
<td>Anastrozole (mg)</td>
<td></td>
</tr>
<tr>
<td>1.1 ± 0.1</td>
<td>4.9 ± 0.5</td>
</tr>
</tbody>
</table>

Css, mean steady state plasma concentration; CL, plasma clearance.
Analytical methods to measure plasma levels of ATZ in cynomolgus monkeys

An HPLC method with tandem mass spectrometry was developed to determine ATZ levels in plasma of cynomolgus monkeys. Blood samples containing Li-heparin were collected at least every third day during the study period (see below for more details) and plasma was prepared by centrifugation. Monkey plasma samples containing the analyte and an internal standard were extracted using a liquid-liquid extraction procedure, evaporated to dryness, reconstituted, and analyzed by HPLC equipped with an AB Sciex API 4000 mass spectrometer (LC–MS/MS). Positive ions were monitored and quantification was performed employing the peak area ratio. The lower limit of quantification (LLOQ) of the method was 0.15 µg/l.

Pharmacokinetic evaluation after IVR insertion and intravenous application

For pharmacokinetic evaluation of the IVR in monkeys, plasma samples were taken once daily from Day 1 to 3 and afterwards every third day until menses occurred (17–42 days). The steady state plasma concentration (C_{ss}) was calculated as mean concentration of all time points between 24 h and the last measured time point (ca. 30 days). ATZ was administered by intravenous infusion (15 min) at a dose of 0.2 mg/kg in 50% polyethylene glycol (PEG)400 to female cynomolgus monkeys (n = 3). Plasma samples were taken over 24 h and analyzed for ATZ levels by LC–MS/MS. Pharmacokinetic evaluation of the concentration versus time data was performed by non-compartmental (moment) analysis using an in-house software tool called KinEx. The pharmacokinetic evaluation method is in accordance with the Global Operational Manual ‘BDP-OI-039’ of Bayer HealthCare ‘Non-Compartmental Analysis (NCA) in Pharmacokinetics (PK)’. In vivo release rate from the IVRs was calculated as the product of the mean steady state plasma concentration (C_{ss}) after IVR administration and in vivo plasma clearance (CL) obtained from intravenous administration (Release Rate = C_{ss} × CL).

Pharmacodynamic study of ATZ IVRs

In order to evaluate the pharmacodynamic effect of intravaginally applied ATZ, intact cynomolgus monkeys were used to study the dose-dependent impact of ATZ on hormone levels. ATZ was applied by IVRs at three dose levels, based on their in vitro release rates (n = 5/group). IVRs were fixed with a suture loop within the vagina for one menstrual cycle. During the treatment period, blood samples were collected at least every third day. To assess the pharmacodynamic effect, estradiol (E2), estroge (E1), progesterone, FSH, LH, testosterone and androstenedione-levels were investigated, with E2 as the major surrogate for treatment efficacy. The hormones were measured by radioimmunoassay according to the manufactures guidelines (E2 (J125-DAK/Diagnostic Systems Laboratories, Switzerland), FSH (J125-DAK/Schering, Germany), E1 (J125-DAK/Diagnostic Systems Laboratories, Switzerland), androstenedione (J125-CT/Diagnostic Systems Laboratories, Switzerland), testosterone (J125-CT/Diagnostic Systems Laboratories, Switzerland), progesterone (J125-CT/Siemens, Germany), LH (J125-DAK/Schering, Germany)). Twice weekly monitoring of the ovaries for the development of ovarian cysts was done with ultrasound imaging by using the Philips/ATL HDI 5000 system. Animals (1/group) that removed the IVRs during the study were not included into the analysis. Of the observed hormone levels was taken in order to reduce the influence of skewed data distributions on the analysis of mean values per treatment group. Dose groups were compared with the corresponding vehicle group using a two-sided t-test approach using an alpha-level of 5%. Due to the exploratory nature of the experiment, statistical group comparisons were not adjusted for multiplicity.

Results

In vitro release of ATZ

Different combinations of ATZ-containing vaginal rings were tested providing three different in vitro release rates of ATZ (doses A – C) (Fig. 1 and Table I). The initial (Day 1) release rates from these rings were found to be 26 µg/day (dose A), 85 µg/day (dose B) and 390 µg/day (dose C). The mean release rates from Day 2 to Day 30 were 16, 64 and 306 µg/day for doses A – C, respectively. A sustained release was achieved for the tested period (42d) with a decline in released ATZ (d1–d4 versus d24–d28) from 22 to 12 µg/day (dose A), 79 to 54 µg/day (dose B) and 361 to 271 µg/day (dose C).

In vivo intravenous pharmacokinetics of ATZ

ATZ was administered i.v. at a dose of 0.2 mg/kg (n = 3). A plasma clearance of 0.58 l/h/kg, a steady-state volume of distribution of 1.7 l/kg and a half-life of 1.8 h was calculated (Table II and Fig. 2).

In vivo release and pharmacodynamic effects of ATZ vaginal rings

The in vivo release and the pharmacodynamic activity of vaginally administered ATZ were investigated in healthy female cynomolgus monkeys in three different dose groups for one menstrual cycle (4–6 weeks). Five animals per group received an IVR with in vitro release rates of dose A – C (Table I).

ATZ showed maximum concentrations at a t_{max} between 27 and 48 h (Fig. 3). The steady state plasma concentration (C_{ss}) was calculated as mean concentration of all time points between 24 h and the last measured time point (ca. 30 days). Only animals with data for at least 2/3 of the time points that were above the LLOQ were included in these analyses. For the calculation of the mean value, an individual data point below the LLOQ was substituted by half of the LLOQ (only for dose A).

![Figure 1](image-url) The in vitro release from anastrozole (ATZ) intravaginal rings (IVRs) (1–42 days). Dose A, dose B, dose C. Shown are mean values (n = 3) with ± SD.
Anastrozole effects in cynomolgus monkey

Table II Summary of pharmacokinetic parameters of ATZ after IVR and i.v. dosing to female cynomolgus monkeys.

<table>
<thead>
<tr>
<th>Route</th>
<th>Anastrozole</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>IVR</td>
<td>i.v.</td>
<td>IVR</td>
<td>i.v.</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>No. of animals</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Mean animal weight [kg]</td>
<td>4.3</td>
<td>3.5</td>
<td>3.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Css [µg/l]</td>
<td>0.3</td>
<td>1.4</td>
<td>5.9</td>
<td>–</td>
</tr>
<tr>
<td>Cssnorm [d/l]</td>
<td>0.018</td>
<td>0.022</td>
<td>0.019</td>
<td>–</td>
</tr>
<tr>
<td>CL [l/h/kg]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.58</td>
</tr>
<tr>
<td>Vd [l/kg]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
</tr>
<tr>
<td>$t_\frac{1}{2}$ [h]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.8</td>
</tr>
</tbody>
</table>

$^{1/2}$ terminal half-life; Vd, volume of distribution; Cssnorm, mean steady state plasma concentration normalized by dose.

Discussion

The purpose of the present study was to show the compatibility of ATZ with a polymer-based vaginal drug delivery system, to get first insights into the in vitro and in vivo release, and to investigate the pharmacodynamic effects of intravaginally administered ATZ in a relevant preclinical endocrine model. One additional objective was to investigate whether it is possible to decrease systemic E2 levels without inducing counter regulation by the hypothalamic–pituitary–gonadal axis (HPG axis), a known effect of aromatase inhibitors, if applied to premenopausal women (Tredway et al. 2004).

Compatibility and sustained release of a drug from an IVR are the prerequisites for its further development with this technology. A sustained in vitro release was achieved for the ATZ IVRs with an acceptable decline of the release rate during the first 28 days (Table II, d1–d4 versus d24–d28) of about 25% for the highest dose. The observation of an overall moderate decline of the release rate during the test period was confirmed by the plasma concentration time profiles of ATZ after IVR dosing to female cynomolgus monkeys (N = 3–5). Data are expressed as mean ± SD.

After IVR insertion, Css was achieved after 27–48 h ($t_{\text{max}}$ in Fig. 3). Mean $C_{\text{ex}}$ values were $0.3 ± 0.12$ µg/l (dose A), $1.4 ± 0.55$ µg/l (dose B) and $5.9 ± 0.89$ µg/l (dose C) (Table II). $C_{\text{ex}}$ values normalized by the mean in vitro release rate (dose normalized, see Table II) indicated dose linearity.

In vivo release rates from the IVRs were calculated by two methods. (i) Based on in vivo plasma levels the in vivo release rate is the product of Css and the in vivo CL of ATZ in cynomolgus monkeys (0.58 l/h/kg) and was calculated to be $16 ± 5.4$ µg/day (dose A), $66 ± 24$ µg/day (dose B) and $278 ± 43$ µg/day (dose C) (Tables I and II). (ii) Based on the ex vivo ring residual drug content measured in the rings at the end of the study the mean in vivo release rates for the three treatment groups were calculated to be $15$ µg/day (dose A), $54$ µg/day (dose B) and $277$ µg/day (dose C), respectively, demonstrating a good match of the two methods.

The study also served to monitor pharmacodynamic effects of ATZ applied intravaginally. Effects on plasma hormone levels as well as ovarian histology were assessed. A trend to a decrease in E2 plasma levels was observed in the dose C group during the complete menstrual cycle but statistical significance was reached only in the proliferative phase (Fig. 4B). One animal of the dose B group had elevated E2 plasma level during the whole study compared with the other animals of the group (Fig. 3A–C); however, overall dose B group showed no significant increases in E2 level during the proliferative and secretory phase. No treatment-dependent changes in E1, progesterone, FSH, LH, testosterone and androstenedione-levels have been observed (Table III). In addition, twice weekly monitoring of the ovary with ultrasound confirmed that no treatment-related changes, including ovarian cyst formation, could be observed in any of the dose groups.
drug release from the IVR. Furthermore, the avoidance of a peak in drug levels may result in a reduction of the drug side effect potential like a Cmax-induced counter regulation and, thereby, potentially increases the safety of the administered drug. In this manner it may be possible to apply higher dosages.

The choice of a relevant preclinical endocrine model to study intravaginally administered drugs that influence the HPG axis is mandatory. The model should have a similar reproductive system to women including anatomy of the vagina and the endocrine control mechanism. The cynomolgus monkey fulfills these requirements, in that the morphology of the reproductive tract, the endocrine system, the control of unilateral single-egg ovulation and the reproductive cycle duration of \( \approx 30 \) days are similar to women (Van Esch et al., 2008; Weinbauer et al., 2008).

ATZ released from IVRs of the dose group C decreased the systemic E2 level significantly during the proliferative phase in the cynomolgus monkeys (Fig. 4) without stimulating the HPG axis, as FSH levels were similar to the levels of the control group. Furthermore, unchanged progesterone values indicate ovulation in all groups (Table III), and no follicular cysts were detected during treatment. It has been known for years that in premenopausal women an induced E2 decrease stimulates the HPG axis to raise the FSH level with subsequent ovarian stimulation, follicular growth and enhanced E2 synthesis (Messinis, 2006). The data collected in this study suggest that an E2 decline only stimulates the HPG axis if the hormone level falls below a particular threshold. However, the current study was limited to three dose groups in which only the highest dose decreased the E2 level. Hence, additional research with IVRs releasing higher amounts of ATZ is required to define the threshold for an ATZ-dependent ovarian stimulation in primates. The therapeutic clinical use of AIs is currently restricted to estrogen-dependent diseases in post-menopausal women like breast cancer. AIs have only been used experimentally to date, for example in the treatment of endometriosis. When AIs are administered to premenopausal women, the production of E2 is suppressed, causing an increase in gonadotrophin levels and stimulation of ovarian function. This may lead to the development of

Figure 4 Estradiol (E2) levels during one menstrual cycle illustrated by the AUC (area under the curve) of (A) the complete cycle, (B) the proliferative phase and (C) in the secretory phase (n = 4). Treatment of cynomolgus monkeys with ATZ IVRs decreases E2 levels predominantly within the proliferative phase. CD, cycle day. Data are expressed as mean ± SD. Two-sided t-test dose versus vehicle * = \( P \)-value < 0.05.
lesions (Bulun et al., 2000), possibly explaining treatment failures by drugs such as progestins or combined oral contraceptives (Amsterdam et al., 2005; Ferrero et al., 2009). The data of the present study indicated that ATZ can be applied to premenopausal monkeys without causing ovarian stimulation questioning the requirement of co-dosing ATZ with therapies that down-regulate ovarian function. This finding may disclose new possibilities for the use of ATZ in the treatment of gynecological diseases of premenopausal women.

There are several reasons to administer ATZ in premenopausal women for estrogen-dependent diseases such as endometriosis. First, aromatase has been shown to be locally overexpressed in endometriotic lesions (Bulun et al., 2000), possibly explaining treatment failures by drugs interfering only with ovarian estrogen production. Second, AIs alone are able to interfere with both, ovarian and endometriotic estrogen production. Evidence for therapeutic efficacy of AIs in endometriosis was shown in several exploratory clinical trials (for a review of the clinical experience, see (Ferrero et al., 2011)), particularly in patients refractory to other treatments (Amsterdam et al., 2005). The findings in this study may enhance the repertoire for future drug development by application of ATZ by IVRs for human diseases.

In conclusion, the present study documents the feasibility of IVR administration of the AI ATZ. The release rate from IVRs is sufficient and in a range that supports feasibility of IVR administration of ATZ as a new approach for long-term therapy of estrogen-dependent diseases such as endometriosis. IVR administration of ATZ is expected to allow a constant AI exposure that is sufficient to block aromatase particularly locally in endometriotic lesions and to reduce only moderately plasma to avoid counter regulation by the HPG axis.

Table III Results of the cynomolgus monkey pharmacodynamic model. Estradiol (E2) levels decline significantly within the proliferative phase between drug free IVRs and dose C.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Anastrozole Dose A</th>
<th>Anastrozole Dose B</th>
<th>Anastrozole Dose C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (µg/l) average level/day without pre-ovulatory surge</td>
<td>4.85 ± 2.70</td>
<td>5.52 ± 3.07</td>
<td>4.90 ± 2.58</td>
<td>4.83 ± 2.91</td>
<td>NS</td>
</tr>
<tr>
<td>LH (µg/l) average level/day without pre-ovulatory surge</td>
<td>1.30 ± 0.51</td>
<td>1.11 ± 0.47</td>
<td>1.27 ± 0.69</td>
<td>1.32 ± 0.75</td>
<td>NS</td>
</tr>
<tr>
<td>Androstenedione (nmol/l) average level/day</td>
<td>2.64 ± 0.91</td>
<td>1.67 ± 0.40</td>
<td>2.51 ± 1.06</td>
<td>2.50 ± 1.04</td>
<td>NS</td>
</tr>
<tr>
<td>Testosterone (nmol/l) average level/day</td>
<td>1.66 ± 0.85</td>
<td>1.06 ± 0.44</td>
<td>1.99 ± 1.23</td>
<td>1.21 ± 0.66</td>
<td>NS</td>
</tr>
<tr>
<td>Progesterone (nmol/l) average level/day proliferative phase</td>
<td>5.65 ± 5.99</td>
<td>5.57 ± 5.11</td>
<td>6.58 ± 3.91</td>
<td>4.58 ± 2.64</td>
<td>NS</td>
</tr>
<tr>
<td>Progesterone (nmol/l) average level/day secretory Phase</td>
<td>51.61 ± 37.54</td>
<td>91.92 ± 52.78</td>
<td>60.02 ± 22.65</td>
<td>92.88 ± 55.50</td>
<td>NS</td>
</tr>
<tr>
<td>Estrone (pmol/l) average level/day</td>
<td>532.38 ± 249.67</td>
<td>533.65 ± 399.41</td>
<td>457.02 ± 242.96</td>
<td>465.87 ± 255.61</td>
<td>NS</td>
</tr>
<tr>
<td>E2 pmol/l × day AUC (Complete, CD 1–26)</td>
<td>3768 ± 3854</td>
<td>324.67 ± 324.67</td>
<td>22.65 ± 22.65</td>
<td>403.2 ± 22.65</td>
<td>NS</td>
</tr>
<tr>
<td>E2 pmol/l × day AUC (proliferative, CD 1–17)</td>
<td>3137 ± 295.5</td>
<td>3854 ± 927.5</td>
<td>3235 ± 110.1</td>
<td>1978 ± 350.6</td>
<td>P &lt; 0.0047</td>
</tr>
<tr>
<td>E2 pmol/l × day AUC (secretory, CD 17–26)</td>
<td>404 ± 211.9</td>
<td>403.2 ± 169.7</td>
<td>605.1 ± 264.1</td>
<td>342.9 ± 135.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ovarian cysts (ultrasound evaluation at end of study)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

No treatment-dependent changes in FSH, LH, estrone, testosterone, androstenedione and progesterone level have been observed.

CD cycle day; AUC area under the curve.

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Authors’ roles

A.R. contributed to the pharmacokinetic evaluation, data analysis and interpretation, manuscript writing and final approval of manuscript. H.K. and O.S. contributed to conception of the ring design and corresponding in vitro studies, data analysis and interpretation and reviewing of the manuscript. H.S. contributed to the data interpretation and writing of the manuscript. U.F. contributed to conception of the in vivo pharmacodynamic study and reviewing of manuscript. K.P. contributed to the in vivo pharmacodynamic study. F.S. contributed to conception of in vivo studies, data analysis and interpretation, manuscript writing and final approval of manuscript.

Authors' roles

A.R. contributed to the pharmacokinetic evaluation, data analysis and interpretation, manuscript writing and final approval of manuscript. H.K. and O.S. contributed to conception of the ring design and corresponding in vitro studies, data analysis and interpretation and reviewing of the manuscript. H.S. contributed to the data interpretation and writing of the manuscript. U.F. contributed to conception of the in vivo pharmacodynamic study and reviewing of manuscript. K.P. contributed to the in vivo pharmacodynamic study. F.S. contributed to conception of in vivo studies, data analysis and interpretation, manuscript writing and final approval of manuscript.
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Conflict of interest

All authors are employees of Bayer Pharma AG.

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