Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study

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BACKGROUND: The aims of the present study were to examine whether periovulatory administration of a cyclo-oxygenase (COX)-2 inhibitor affects human ovulation and endocrine parameters. METHODS: Thirteen healthy women, 30–40 years of age, without hormonal treatment and with regular menstrual cycles (27–34 days), were given the selective COX-2 inhibitor rofecoxib (n = 6) or placebo (n = 7) in a random double-blind fashion. In an initial control cycle, serial hormonal analyses, detection of a measurable mid-cycle urine LH peak and transvaginal ultrasound scans were performed to confirm normal ovulatory and endocrinological cyclic patterns, in all participating women. During the subsequent treatment cycle, serial ultrasound scans were performed. When the dominant follicle reached 14–16 mm in diameter, 25 mg rofecoxib or placebo was taken orally, once daily for 9 consecutive days, during which follicle size was monitored daily by ultrasound scans and serial hormone analyses were performed. RESULTS: Four of the six women who received rofecoxib demonstrated delayed follicle rupture, >48 h after the LH peak, when compared with the placebo group, who all had follicular rupture >36 h after the detected LH peak. No differences in peripheral serum concentrations of progesterone, oestradiol, LH and FSH were observed between placebo and rofecoxib groups, when analysed at specified time intervals. CONCLUSIONS: This study suggests that selective COX-2 inhibition has a negative, local effect on human ovulation, resulting in delayed follicular rupture, without affecting peripheral hormonal cyclicity.

Key words: cyclooxygenase/ovary/ovulation/prostaglandin/rofecoxib

Introduction

In the preovulatory follicle of mammalian species, the LH surge induces gene expression of a large number of proteins (Richards, 1994) resulting in changes in concentrations of many ovulatory mediators within the follicle, including prosta-glandins (PGs), subsequently leading to follicle wall rupture. In humans, several studies have shown that PGs are important ovulatory mediators (Smith et al., 1986; Killick and Elstein, 1987); however, the precise mechanisms of action on ovulation are still unclear. Effects on follicular blood flow (Kranzfelder et al., 1992) and collagen degradation (Reich et al., 1991) have been suggested.

The enzyme catalysing the rate-limiting step in PG synthesis exists in two isoforms, cyclo-oxygenase (COX)-1 and COX-2 (Kujubu et al., 1991; Wong and Richards, 1991; Xie et al., 1991; O’Banion et al., 1992). COX-1 is normally constitutively expressed and COX-2 is generally inducible in response to inflammatory stimuli or in response to LH in the rat ovary (Richards, 1994). COX-2 knockout mice demonstrate normal follicle development but are infertile due to multiple reproductive failures including defect ovulation, fertilization, implantation and decidualization (Lim et al., 1997). In humans, COX-2 has been demonstrated to be expressed in granulosa-lutein cells isolated from women undergoing IVF (Narko et al., 1997).

Non-steroidal anti-inflammatory drugs (NSAIDs) in women with different autoimmune diseases exert non-selective COX inhibition resulting in ovulatory failure (Akil et al., 1996; Smith et al., 1996). Daily consumption of 150–200 mg indomethacin (Killick and Elstein, 1987; Akil et al., 1996) during the periovulatory period resulted in 100% ovulation inhibition, which was seen as luteinized unruptured follicle (LUF), a condition with delayed and abnormal follicular rupture. Indomethacin affects several different intra-ovarian mediators apart from PGs, such as calcium, kallikrein and 15-hydroxyeicosatetraenoic acid methyl esters (HETEs) (Northover, 1977; Tanaka et al., 1991; Tanaka et al., 1992) and therefore the results from the latter studies may not solely be due to PG inhibition.

The introduction of new selective COX-2 inhibitors in clinical practice has made it possible to study the importance and mechanisms of action of PGs more specifically. The purpose of this study was to examine possible effects of the selective COX-2 inhibitor, rofecoxib (RX), on ovulation in a group of healthy women with regular menstrual patterns.
Materials and methods

Subjects
Nineteen healthy women with no prior history of gynaecological or reproductive disorders volunteered for the study. None of the women were using hormonal contraception or any other medication for at least 2 months prior to the study and all had a history of regular menstrual cycles (27–34 days). All subjects gave informed consent and the study was approved by Göteborg University Human Ethics Committee and the Swedish medical products agency.

Six women were excluded from the study due either to the control cycles being >35 days or to an inability to identify a readily visible dominant follicle, up to cycle day 16, during the control or treatment cycles.

Thirteen women were included in the study. In the placebo group, one woman was para 3, two were para 2, one was para 1 and three were para 0. In the treatment group, four women were para 2, one was para 1 and one was para 0.

Experimental design
This prospective, double-blind, randomized RX-placebo study was performed at the Department of Obstetrics and Gynaecology at Sahlgrenska University Hospital (Göteborg, Sweden). Each woman was monitored for 2–4 consecutive menstrual cycles. Cycle 1 was the control cycle; cycle 2 was the treatment cycle (RX or placebo); cycles 3 and 4 were follow-up cycles in certain cases. Menstrual cycle day 1 (CD 1) corresponded to the first day of the bleeding period.

During cycle 1, blood samples for LH, FSH, oestradiol and progesterone measurement were taken every 3–4 days. Ultrasonic scans were performed with a 5 MHz transvaginal transducer. All scans were performed by two of the authors (M.P. and B.E.F.) and each subject was examined by one investigator. All subjects were examined with ultrasound scans, beginning on CD 8, to confirm the presence of a dominant follicle (14–16 mm) after which Urine Clear-Plan® (Unipath Ltd, Bedford, UK) was used daily, in the morning, to verify the mid-cycle peak. Only women with a demonstrable ovulatory pattern, in concordance with predefined inclusion parameters, were treated with RX or placebo in a second cycle which followed immediately.

During the treatment cycle, blood samples were taken initially every 3–4 days. Beginning at CD 8–10, transvaginal scans were performed daily until a dominant follicle (14–16 mm) was visible. The women were then randomly and double-blindly allotted placebo or RX (Vioxx® produced by Merck, Sharp and Dohme B.V., Haarlem, The Netherlands) 25 mg once daily for 9 consecutive days. The investigators were not aware of which substance the participants received until all treatment cycles were completed. During the 9 treatment days, the women were monitored daily with transvaginal ultrasound scans (24–30 h between scans), serum hormone analysis and Clear-Plan® urine test (until evidence of LH peak).

Follicle rupture was pre-defined as a decrease in mean follicular diameter of at least 3 mm (Coetsier and Dhont, 1996) and appearance of intrafollicular echos. Delayed follicle rupture was defined as the absence of visible rupture within 48 h of the LH peak, measured in serum, but also confirmed with the Clear Plan® urine test. Follicle rupture was pre-defined as a decrease in mean follicular diameter of at least 3 mm (Coetsier and Dhont, 1996). Women with abnormal findings during the treatment cycle were monitored in subsequent cycles until resumption of normal ovulatory pattern was seen.

Assays
All hormone samples from the treatment cycles were analysed after completion of the study. All analyses were performed in duplicates.
Ultrasound images of follicles under placebo/COX-2 inhibitor treatment cycle. (a) 19 mm follicle at the day of the LH peak (day 0) in a woman from the placebo group. (b) Same subject as (a) but at day 1 after the LH peak; a corpus luteum is seen. (c) 22 mm follicle from a woman in the COX-2 inhibitor group on the day of the LH surge (day 0). (d) Maximum follicular size (50 mm) on day +10 after the LH surge. Same subject as (e).

Figure 3. Mean day of follicular rupture in relation to day of LH peak for placebo and COX-2 inhibitor groups (n = 7 for placebo group, n = 6 for COX-2 inhibitor group). Data are presented as mean ± SEM. P < 0.02 for placebo versus COX-2 inhibitor groups.

Figure 4. Mean day of menstrual cycle when LH peak was detected in treatment cycle as measured by LH in serum and LH urine dip sticks (n = 7 in placebo group, n = 6 in COX-2 inhibitor group). Data are presented as mean ± SEM.

Preovulatory follicles demonstrated a considerable size decrease, became more irregular in shape and intrafollicular echoes typical of luteinization appeared <36 h after the LH peak was detected (Figure 2). In the treatment group, the maximum mean follicle size, prior to rupture, was 30 ± 4.3 mm (range 20–50 mm). The mean day of follicular rupture (Figure 3), but not the day of the LH peak (Figure 4), differed significantly between the placebo and RX groups. Follicular rupture, in the placebo group, was seen within 36 h of the LH peak while mean rupture day in the RX group was day 4 (P < 0.03). Two subjects in the RX group had follicular rupture 36–48 h after the LH peak and were therefore classified as having normal ovulatory patterns. The maximum mean follicle size of the four women who demonstrated delayed peak was detected (Figure 2). In the treatment group, the maximum mean follicle size, prior to rupture, was 34 mm (range 27–50 mm) and the follicular size decrease was delayed and occurred at CD LH +3, +4, +9 and +10 respectively. One of these women developed a cystic structure with maximum diameter of 50 mm on CD LH +10 (Figure 2). This cystic structure collapsed during the periovulatory phase during cycle 3. The subject
Figure 5. Mean serum concentrations of progesterone (a), oestradiol (b), FSH (c) and LH (d) in treatment cycle in relation to day of LH peak (LH peak = day 0) for placebo and COX-2 inhibitor groups ($n = 7$ in placebo group, $n = 6$ in COX-2 inhibitor group). Data are presented as means ± SEM.

experienced intense abdominal pain at this time and the collapsed cyst was visible until CD 7 of cycle 4. This case was omitted in statistical analyses concerning follicle diameter and rupture. No other side-effects were experienced in either placebo or RX groups.

**Endocrinological parameters and bleeding patterns**

The mean LH peak day was CD 14 for both placebo and RX groups (Figure 4).

No differences in progesterone, oestradiol, FSH and LH serum concentrations were observed between the placebo and RX groups (Figure 5). No significant differences in the analysed intra-individual hormone parameters were seen. Thus, no delay in decreased progesterone concentration during the luteal phase (Figure 5), or in oestradiol rise in the follicular phase (data not shown), was seen. The regularity of the subsequent menstrual cycle (cycle 3) was unchanged in all women.

**Discussion**

The present study examined the effects of a novel specific COX-2 inhibitor, RX, on ovulation in a group of healthy women. This inhibitor, RX, exerts anti-inflammatory, antipyretic and analgesic effects but with lower levels of gastrointestinal (GI) side effects as compared with non-specific COX inhibitors (Hawkey et al., 2000). Rofecoxib was administered in a double-blind fashion, thereby eliminating possible subject and investigator bias. We demonstrated a delay in ovulation...
In this type of study, in the human female, it is critical to use acceptable methods when monitoring follicular development. Invasive methods such as laparoscopy have been used to monitor changes in the ovulatory cycle (Marik and Hulka, 1978). The dominant follicle is first discernible by transvaginal ultrasound after CD 6 and there seems to be no difference in dominant follicle growth rate and appearance between imminent ovulatory and non-ovulatory cycles prior to expected ovulation (Gore et al., 1995). Daily ultrasound examinations together with serial hormonal analyses, however, are required in order to minimize misinterpretations of follicle development during the periovulatory period (Petisos et al., 1985). The mean time from the LH surge onset to follicle rupture has been demonstrated to be 38 ± 4 h in normal cycling women (Fritz et al., 1992), which is why delayed follicular rupture was defined as the absence of rupture within 48 h, in the present study.

Earlier studies have examined the effects of non-selective PG synthesis inhibition, affecting both COX-1 and COX-2, using traditional NSAIDs on human ovulation (Killick and Elstein, 1987). The two COX enzymes are located in different intracellular areas and use different pools of arachidonate in distinct metabolic pathways (Morita et al., 1995). Non-steroidal anti-inflammatory drugs assert their non-specific inhibition with varying potency and selectivity (Cryer and Feldman, 1998). Pervading characteristics of the influence of PG synthesis inhibition on ovulation include evidence of ovulation without conception capability and LUF; a clinical phenomenon involving ovulatory failure despite a preceding LH peak, rise in luteal phase progesterone and secretory endometrium. The definition and prevalence of LUF, as well as luteal phase follow-up in earlier studies, have varied (Zaidi et al., 1995; Smith et al., 1996). Varying effects on luteal progesterone concentrations have been reported (Hamilton et al., 1985; Killick and Elstein, 1987; Scheenjes et al., 1990). LUF has been described in spontaneous menstrual cycles (Killick and Elstein, 1987; Kugu et al., 1991) but infertile women demonstrate a higher incidence of LUF as compared with fertile women (Summara et al., 1998). Varying effects on luteal progesterone concentrations have been reported (Hamilton et al., 1985; Killick and Elstein, 1987; Scheenjes et al., 1990).

The effect of the non-selective PG synthesis inhibitor indomethacin, when administered concomitantly with HCG as an ovulatory stimulus, was investigated (Killick and Elstein, 1987) and a 10.7% spontaneous LUF rate was found in the control group. The indomethacin treatment group demonstrated 100% LUF and a decrease in early luteal phase serum progesterone. Reports on women taking continuous NSAIDs, such as diclofenac, have described infertility and a high prevalence of LUF, some with subovulatory progesterone values in the mid-luteal phase (Smith et al., 1996). The negative effects of NSAIDs on ovulation appear to be reversible as studies of women taking this type of medication, on a long-term basis, demonstrate signs of normal ovulation within one menstrual cycle after medication interruption (Akil et al., 1996; Smith et al., 1996). Women using Norplant® implants (Alvarez et al., 1996), RU 486 (Kekkonen et al., 1996) and a combination of RU 486 and medroxyprogesterone acetate (Croxatto et al., 1996) have also demonstrated LUF-like disturbances.

In common with an earlier study (Killick and Elstein, 1987), we examined a group of women with no prior history of infertility or any other somatic illness. In the present study, a moderate dose (25 mg/day) of the specific COX-2 inhibitor (RX) was used. Contrary to Killick and Elstein (Killick and Elstein, 1987), we used no artificial ovulatory stimulus and hormone levels in serum were followed regularly during the entire study; daily during the treatment period. Despite the delay in follicular rupture, no alterations in serum progesterone concentrations were demonstrated.

Rofecoxib has demonstrated similar mechanisms of inhibition as another selective COX-2 inhibitor, NS-398, in vitro (Ouellet and Percival, 1995; Chan et al., 1998). We have recently demonstrated that NS-398 reduced ovulation frequency in vivo and in vitro in the rat (Mikuni et al., 1998). A dose-dependent decrease in PGE2 synthesis was seen in vivo but the ovulation rate was only affected by the highest dose tested. This suggested that prostanoids must be reduced below a certain threshold level in order to inhibit ovulation. It is possible that there may exist a dose-dependent response to PG inhibition, also in humans. A complete ovulation inhibition by 150–200 mg indomethacin daily, in women with no prior history of gynaecological problems, has previously been mentioned (Killick and Elstein, 1987; Akil et al., 1996). A case report has described ovulatory delay with subsequent conception following administration of 50 mg indomethacin daily (Nargund and Cheng, 1996). It is possible that there may be dose-dependent effects of specific COX inhibitors as well and that a dose increase of RX could possibly result in a higher incidence of delayed ovulation or possible LUF/conception inhibition.

In this study, we found no measurable difference in oestradiol, progesterone, LH or FSH profiles between the RX and placebo groups or between the initial control and treatment cycles. This is in line with our earlier study using NS-398, in a rat model, where no effect on ovarian production of progesterone or oestradiol was seen, suggesting that inhibition of the inducible PG pathway is enough to cause inhibition of ovulation in spite of undisturbed steroidogenesis. In previous studies with non-selective PG inhibitors in humans, hormonal changes such as decreases in progesterone (Killick and Elstein, 1987) and oestradiol (Hamilton et al., 1985) have been reported. It cannot be excluded that changes seen in hormone patterns during PG inhibition, with indomethacin for example, may be due to the higher dosage used or due to effects on other factors, which subsequently affect the hormone production from the preovulatory follicle.

The results of the present study demonstrate a prolongation of the time from onset of the LH surge until follicular rupture, evident by transvaginal ultrasound, by the COX-2 inhibitor RX. This indicates that the process of ovulation can proceed
in spite of reduced intrafollicular PG concentrations but that the efficiency of the process is impaired. Delayed rupture in the mono-ovulatory human species may be comparable with the reduced ovulation rate seen in the polyovulatory rat after COX-2 inhibition (Mikuni et al., 1998). It is likely that the normal LH surge, seen in RX treated women, triggers resumption of oocyte meiosis, and therefore delayed follicular rupture may result in an asynchrony between oocyte maturation and the expulsion of the oocyte from the interior of the follicle. Since the survival time of the matured and fertilizable oocyte in the female reproductive tract is about 24 h (Langman, 1969), it may well be that the expelled oocyte in RX treated women may have started to degenerate and is not fertilizable. Non-steroidal anti-inflammatory drugs are commonly used among younger women and therefore knowledge regarding effects of these drugs on ovulation is important.

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References


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