Expression of cyclo-oxygenase in human endometrium during the implantation period

L. Marions and K. Gemzell Danielsson

Department of Woman and Child Health, Division for Obstetrics and Gynecology, Karolinska Hospital, S-171 76 Stockholm, Sweden

1To whom correspondence should be addressed

Prostaglandins (PG) are known to be involved in the process of human implantation. In several animal species, treatment with prostaglandin synthesis inhibitors will prevent implantation. Cyclo-oxygenase (COX) is the rate-limiting enzyme in the synthesis of PGs and exists in two isoforms, COX-1 and COX-2. Defective implantation in COX-2-deficient mice has been demonstrated recently. In the present study, the expression of COX-1 and COX-2 was studied during the implantation period in healthy fertile women before and after treatment with the antiprogestrone, mifepristone. The study consisted of one control cycle and one treatment cycle. The subjects served as their own control. During the treatment cycle the subjects received 200 mg of mifepristone 2 days after the luteinizing hormone (LH) surge. Endometrial biopsies were obtained in the mid-luteal phase (LH + 6 to LH + 8) in both cycles. Using polyclonal antibodies against COX-1 and COX-2, immunostaining for COX-1 was found mainly in the glandular and luminal epithelium and for COX-2 mainly in the luminal epithelium and the perivascular cells. After treatment with mifepristone, expression of COX-1 in glandular epithelium and COX-2 in luminal epithelium significantly decreased whilst the immunostaining for COX-2 in the perivascular cells remained strong. This study shows the expression of both COX-1 and COX-2 during the implantation period and also indicates that treatment with mifepristone in early luteal phase impairs glandular epithelial function and endometrial receptivity.

Key words: antiprogestrone/cyclo-oxygenase/endometrial receptivity/implantation

Introduction

Progesterone is essential for inducing the changes in the reproductive tract necessary for embryo development and implantation. A single dose administration of 200 mg of the potent antiprogestrone mifepristone (RU 486) in the early luteal phase results in inhibition of the implantation with no notable changes in menstrual cyclicity and ovarian steroid profile. Thus the use and development of antiprogestins has emerged as a new possibility for an oestrogen-free contraceptive method. Antiprogestins, e.g. mifepristone, also provide a tool for investigating the mechanism of action of progesterone in the cyclic development of endometrial receptivity. Early luteal phase administration of mifepristone induced desynchronization of endometrial development, repressed glandular secretory differentiation and vascular maturation (Li et al., 1988; Swahn et al., 1988; Johannisson et al., 1989; Gemzell Danielsson and Hamberg, 1994). Endometrial expression of leukaemia inhibitory factor (LIF) is decreased following treatment with mifepristone (Cameron et al., 1997; Gemzell Danielsson et al., 1997). LIF and several other cytokines have been shown to be involved in early embryo development and implantation in rodents (Stewart et al., 1992). The expression of integrin subunits β3 and α4 that are considered to be necessary for implantation also decreased after administration of mifepristone (Marions et al., 1998).

During pregnancy, uterine prostaglandin synthesis is regulated by cytokines which are produced locally in uterine tissues (Wen et al., 1993). Prostaglandin production from endometrial cells can also be influenced by various cytokines in vitro (Habenicht et al., 1985; Raz et al., 1988). In several species, treatment with prostaglandin biosynthesis inhibitors prevents or delays implantation (Smith, 1991).

Treatment with mifepristone during luteal phase has previously been shown to affect levels of prostaglandin F2α (PGF2α) in uterine fluid and PGF2α, PGE2, cyclo-oxygenase (COX) and prostaglandin dehydrogenase (PGDH) in human endometrium. (Gemzell Danielsson and Hamberg, 1994; Nayak et al., 1998).

Prostaglandin synthesis is controlled by two rate-limiting enzymes, phospholipase A2 (PLA2) and cyclo-oxygenase (COX) (Lapetina, 1982). Two distinct forms of COX, COX-1 and -2, have been identified which are encoded by two different genes (Fletcher et al., 1992). Animal studies have suggested different regulation of these enzymes; COX-1 is thought to be regulated by steroid hormones and constitutively expressed, while COX-2 seems to be induced by a variety of stimuli and might even require the presence of an active blastocyst (Chakraborty, 1996). In mouse uterus, the COX-1 gene was expressed in the uterine epithelium at the time for the generalized uterine oedema required for luminal closure. In contrast, the COX-2 gene was expressed in the luminal epithelial and subepithelial stromal cells at the anti-mesometrial pole exclusively surrounding the blastocyst at the time of the attachment reaction, suggesting that PGs generated by COX-1
are involved in decidualization and PGs generated by COX-2 are involved in angiogenesis for the establishment of the placenta and are regulated by the implanting blastocyst.

COX-2-deficient mice have been previously shown to be infertile (Dinchuk et al., 1995). Ovulation failure was considered as the cause of this infertility. However, when a blastocyst transfer was performed from wild-type mice into COX-2-deficient mice uterus, none of the transferred blastocysts implanted (Lim et al., 1997). This result suggests an important role for COX-2 in the implantation process.

The aim of the present study was to investigate the localization and evaluate the expression of COX-1 and COX-2 in human endometrium during the implantation phase and to investigate the response of COX to exposure to anti-progesterone.

**Materials and methods**

A total of 14 healthy women, aged 21–40 years with regular menstrual cycles (25–30 days), volunteered for the study that included one control and one treatment cycle. The subjects served as their own controls. Throughout the study the women were advised to use barrier methods for contraception. The luteinizing hormone (LH) surge in urine was detected using a self-test (Clearplan; Searle, Unipath, Bedford, UK) twice daily from cycle day 10. An endometrial biopsy was obtained from the uterine fundus, using a Randall curette (Dimeda, Tuttlingen, Germany) in the mid-luteal phase (LH 6–8). During the treatment cycles the subjects received 200 mg mifepristone 2 days after the LH surge.

The study was approved by the ethics committee at the Karolinska Hospital. Informed consent was obtained from each subject before she was included in the study.

**Immunohistochemistry**

Polyclonal antibodies against COX-1 and COX-2 were used (Scandinavian Diagnostic Services, Falkenberg, Sweden) The specificity of these antibodies has been demonstrated by the manufacturer using Western blot analysis and there is no cross-reactivity between COX-1 and COX-2. Immunostaining was performed on cryostat sections of endometrial biopsies. Cryostat sections (~10 μm) were placed on slides and fixed in acetone for 10 min. After rinsing with phosphate-buffered saline (PBS), endogenous peroxidases were quenched with incubation in 0.3% H₂O₂ in MeOH for 30 min. Repeated rinses with 0.05% bovine serum albumin (BSA) in PBS were performed. The slides were then incubated at room temperature for 30 min after blocking with 1.5% normal goat serum. The primary antibody, against COX-1 (1:25), COX-2 (1:20), was placed onto the
Antiprogestin and cyclo-oxygenase

Figure 3. Distribution and regulation of cyclo-oxygenase-1 (COX-1) in human endometrium during implantation period, before and after treatment with mifepristone. Means ± SEM. **Significantly different from control (P < 0.01).

Figure 4. Distribution and regulation of cyclo-oxygenase-2 (COX-2) in human endometrium during implantation period, before and after treatment with mifepristone. Means ± SEM. *Significantly different from control (P < 0.05).

cryosections and allowed to bind for 90 min at room temperature. The secondary antibody, consisting of biotinylated goat anti-mouse antibody, was then placed on the cryosections and incubated for 30 min. Avidin–biotin–peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories Inc, Burlingame, CA, USA) was added to the sections for 30 min before adding 3-amino-9-ethyl-carbazole (AEC) for 3 min to complete the reaction. Samples were washed in water and counterstained with 5% haematoxylin for 3 min. The endometrial samples were mounted after washing with water.

Negative controls were incubated similarly, but the primary antibody was replaced by PBS. To check for primary antibody specificity, the primary antibody was replaced with pre-immune serum from the same species (i.e. goat immunoglobulin G).

Quantification
The staining was evaluated by two independent persons using a semiquantitative subjective scoring system, using a Zeiss light microscope at ×200 magnification. Observations were made in 10 different fields of the section to avoid errors due to uneven staining. Cells were assigned a score of 0–3 based on the number of cells specifically stained as follows: 0 (0% stained cells) = weak (<25% positive cells), 1 = moderate (25–75% positive cells), or 3 = strong (>75% positive cells.)

Statistical analysis
The two-tailed Wilcoxon signed rank test was used to evaluate the differences in immunostaining.

Results
Immunostaining for COX-1 was localized to the luminal and glandular epithelium. There was also some staining in the endothelial cells whilst the stromal cells showed no staining. After treatment with mifepristone there were significantly decreased immunoreactivity in the glands (P < 0.01) but the staining of the luminal epithelium was unaffected (Figure 1). Immunostaining for COX-2 was mainly localized to the luminal epithelium and also to the perivascular cells. The most superficial glands showed some staining, but otherwise the glandular epithelium was almost immunonegative for COX-2, as were the stromal cells. After treatment with mifepristone the immunoreactivity in luminal epithelium significantly decreased (P < 0.05). The remaining staining was predominantly localized to the apical part of the luminal cells (Figure 2). Perivascular staining remained strong after treatment with mifepristone (Figures 3 and 4).

Discussion
We have previously shown that a single dose of 200 mg of mifepristone on cycle day LH + 2 is an effective contraceptive method (Gemzell Danielsson et al., 1993). The mode of action of mifepristone is not fully understood. However, there is evidence that early luteal phase treatment with mifepristone results in desynchronization between embryo and endometrium with inhibition of progesterone-induced secretory transformation (Li et al., 1988; Swahn et al., 1988; Gemzell Danielsson et al., 1994).

An adverse effect on endometrial receptivity may involve an effect on endometrial prostaglandin synthesis and/or metabolism. The concentration of endogenous prostaglandin rises
in the endometrium during the luteal phase when progesterone concentrations are high (Downie et al., 1974). PGF2α concentrations in human uterine fluid at the time of implantation have been shown to decrease following treatment with mifepristone during the early luteal phase. The decrease was of the same magnitude as following treatment with a prostaglandin biosynthesis inhibitor (Gemzell Danielsson and Hamberg, 1994). A mifepristone-induced anti-implantation effect in the rhesus monkey could not be reversed by either prostaglandin synthesis inhibitor or a prostaglandin analogue (Nayak et al., 1997). In contrast, antiprogestin has been shown to stimulate PGF production by human endometrial stromal and glandular cells in vitro and by decidual cells in vivo (Kelly et al., 1986). Thus, the role and regulation of prostaglandins in peri-implantation stage endometrium in humans is not clear.

In the present study, COX-1 and COX-2 were localized to epithelial and perivascular cells while stromal cells were immunonegative. Results from various non-primate species also suggest that prostaglandins play a critical role in the development of endometrial vascular permeability, implantation and decidualization. In several animal models, it has been shown that prostaglandin synthesis inhibitors prevent or delay implantation. This effect has been shown to depend on the inhibition of uterine-derived prostaglandin production and not on an effect on prostaglandins from the ovary or the embryo (for reviews see Smith, 1991; Weitlauf, 1994).

COX-2 staining was found to be strong in perivascular cells and remained so following treatment with antiprogestrone. Previously PGDH, which is the principal enzyme for prostaglandin catabolism, has been reported to decrease after antiprogestrone treatment (Cheng et al., 1993). This would result in locally increased prostaglandin concentrations in endometrial vessels. In the rhesus monkey, an increased ratio of PGF2α to PGE2 has been observed in mifepristone-exposed endometrium (Ghosh and Sengupta, 1998). PGF2α is known to be a potent vasoconstrictor. Changes in endometrial vascularization with constriction and regression of endometrial small vessels following treatment with mifepristone have been described earlier (Johannissen et al., 1989).

Increased vascular permeability is one of the first signs of implantation in many species (Psychoyos, 1973). Through a series of events involving cytokines such as interleukin-1 (IL-1), epithelial growth factor (EGF) and LIF (Cameron et al., 1991; Edwards, 1994; Bany and Kennedy, 1995a,b) there is an increase in the ratio of endometrial PGE2:PGF which may mediate vascular dilatation and decidualization (Ghosh et al., 1996), providing growth factors and nutrition for the invading embryo before the establishment of the placenta. It may also facilitate the local immune response by the invasion of immunocompetent cells. PGE2 has been shown to have both chemotactic and immunosuppressive effects (Ford-Hutchinson et al., 1977; Goto et al., 1983).

Decidual reactions, i.e. oedema and increased vascular permeability, are dependent on an intact luminal epithelium. Factors that induce the decidual reaction, e.g. oil injection, trauma or blastocysts, can do this only through an effect on the luminal epithelium. If stroma is exposed to the same stimulus or if luminal epithelium is removed, there is no reaction (Finn, 1977; Lejeune and Leory, 1980; Lejeune et al., 1981).

COX-1 and COX-2 are expressed in the uterus during the peri-implantation phase, which was confirmed in the present study. COX-1 is induced by oestrogen and progesterone while COX-2 is induced transiently in response to inflammatory stimuli (Zwiefel et al., 1995). Expression of COX-2 has also been shown in bovine embryos during the peri-implantation period (Chargipny et al., 1997).

Our present results show an inhibition of COX-1 in the glandular epithelium after treatment with mifepristone as well as a significant decrease in COX-2 expression in the luminal epithelium. This observation is in agreement with the effect of mifepristone on COX expression in the rhesus monkey (Gosh et al., 19986). In that study expression of COX was decreased in the endometrial epithelial cells. However, a polyclonal antibody, which can detect both COX-1 and 2, was used.

The different effect of mifepristone on COX-1 expression in luminal and glandular epithelium could possibly be explained by different local regulation of the enzyme. The antinidatory action of mifepristone seems to be mediated through a multifactorial mechanism where changes in local prostaglandin milieu could also be a part.

The observed changes in COX-1 and 2 expression are suggestive of impaired glandular epithelial function and endometrial receptivity as well as vascular changes following early luteal phase treatment with mifepristone.

Acknowledgements

This study was supported by the Swedish Medical Research Council, No. 5696. Mifepristone was kindly supplied by Roussel Uclaf, Paris, France. We are grateful to Berit Stäby for excellent assistance with the immunohistochemistry.

References


