Prostaglandin E₂ Is a Product of Induced Prostaglandin-endoperoxide Synthase 2 and Microsomal-type Prostaglandin E Synthase at the Implantation Site of the Hamster*

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Certain uterine prostaglandins (PGs) are elevated at implantation sites and are needed to trigger the events of blastocyst implantation that include blastocyst-uterine attachment and stromal decidualization with vascular permeability changes. Several decades of investigations showed that treatment with PG synthase inhibitors, prior to or during the time of implantation, resulted in either complete inhibition or a delay in implantation or reduction in the number of implantation sites with diminished decidual tissue. Consistent with these findings, we observed that whereas a selective PG endoperoxide synthase (Ptgs) 1 inhibitor SC-560 failed to inhibit implantation, a selective Ptgs2 inhibitor SC-236 showed significantly reduced number and size of implantation sites in progesterone-treated ovarioctomized pregnant hamsters. It is known that Ptgs2 expression and Ptgs2-derived prostacyclin (PGI₂) synthesis at implantation sites are needed for implantation in the mouse (a rodent that needs ovarian estrogen for implantation). However, it is unknown which Ptgs and PG synthases produce which PGs at implantation sites of the hamster (a rodent that does not need ovarian estrogen for implantation). Here we demonstrate that as blastocyst implantation proceeds, a reduction in Ptgs1 expression from uterine luminal epithelial cells and a gradual induction in Ptgs2 expression exclusively in luminal epithelial and adjacent decidual cells occurred at implantation sites of hamsters. Results also reveal that PGE₂, but not PGL₂, is the major PG at implantation sites where Ptgs2 and microsomal type PGE synthases but not PGI synthases are co-expressed. This elevated uterine PGE₂ at implantation sites may serve to initiate or amplify physiological signals required for specific aspects of the implantation process required in hamsters.

Blastocyst implantation is a required event of pregnancy, and its failure is a leading cause of infertility in females. Since their discovery, prostaglandins (PGs)¹ have been implicated as critical regulators of important reproductive events including implantation-associated changes during early pregnancy. This was demonstrated by treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) prior to or during the time of implantation that resulted in either complete or a partial delay in implantation, depending on the species. NSAIDs inhibit implantation in mice (1, 2) and rats (3, 4) but not rabbits (5). However in rabbits, indomethacin did reduce the following: 1) the intensity of the uterine blue dye reaction at the implantation site, 2) the size of the decidual swellings, and 3) the number of implantation sites. When indomethacin is given to progestrone (P₄)-treated ovarioctomized pregnant hamsters, it caused a delay in implantation (6). These adverse effects of NSAIDs are attributed to their actions on either the uterus, the embryo, or both (5, 6–8).

NSAIDs target PG synthesis by inhibiting the cyclooxygenase (COX) activity of the PG G/H synthase (9). PG G/H synthase, now known as PG-endoperoxide synthase (Ptgs) or COX because of its COX activity, exists in two isoforms Ptgs1 (COX1) and Ptgs2 (COX2). These two enzymes convert arachidonic acid into PGH₂, a common precursor of all PGs (9). Ptgs1 is considered a constitutive enzyme. Ptgs2 is an inducible enzyme and is mainly elevated at the sites of inflammation after a plethora of insults. More recently, however, it has been demonstrated that both Ptgs isoforms are inducible in certain tissues. Consistent with this idea, it has been demonstrated in the mouse uterus that although Ptgs1 is inducible by treatment with estrogen and P₄ together, Ptgs2 is only inducible by implanting blastocysts at the site of implantation in mice (10). Creation of Ptgs2-mutated mice showed distinct functions of Ptgs1 and Ptgs2 in reproductive processes. Mice devoid of Ptgs1 exhibit parturition defects (11). Ptgs2-depleted mice showed implantation and decidualization defects during early pregnancy (12, 13), suggesting the involvement of Ptgs2-directed PG synthesis in the process of implantation. Expression of Ptgs1 is observed in the uterine epithelium prior to implantation in mice. It is then down-regulated at the implantation site. However, expression of Ptgs2 mRNA and protein is observed exclusively in the uterine epithelial and sub-epithelial stromal cells at the implantation site (10, 13). This implantation-related expression of Ptgs2 is consistent with the defects in implantation and decidualization observed in Ptgs2 mutant mice (19).

¹ The abbreviations used are: PGs, prostaglandins; Ptgs1, prostaglandin-endoperoxide synthase 1; Ptgs2, prostaglandin-endoperoxide synthase 2; COX, cyclooxygenase; Ptgs, G/H synthase; mPtgs, microsomal-type Ptgs; Ptgs, G/H synthase; P₄, progestrone; E₂, estradiol; NSAIDs, nonsteroidal anti-inflammatory drugs; PDZ, primary decidual zone; PBS, phosphate-buffered saline.
Studies before the creation of Ptgs-mutated mice suggested various functions of uterine PGs during early pregnancy. Although PGE2 is responsible for luteolysis, PGE2 exerts actions opposite to PGE2α to favor establishment of pregnancy with luteo-protective (either luteotrophic or anti-luteolytic) actions (14). PGE2 may also have immunomodulatory roles at the implantation site (15). Recently, it has been demonstrated in the mouse that uterine PGF2α and PGE2 are important for ovulation and initiation of implantation, respectively (16, 17). In contrast, Kennedy (18) showed that in the rat PGE2 but not PGF2α is essential to initiate the process of blastocyst implantation in a P4-primed uterus (23).

The earliest contact between the implantation-competent blastocyst and the receptive uterus in any mammal designates the onset of blastocyst implantation. In mice and rats, maternal estrogen is essential to initiate the process of blastocyst implantation in a P4-primed uterus (23–25). In contrast, maternal estrogen is not required to initiate the process of implantation in the P2-primed uterus in hamsters, rabbits, pigs, guinea pigs, monkeys, and perhaps in humans (26–36). Although the importance of PGs during early pregnancy has been recognized in several species that showed P2-dependent implantation, the process of PG synthesis in the uterus of these species has not been studied in detail. As part of our continuing effort to understand the contribution of Ptgs-derived PGs to the process of implantation in various species, we have studied the expression, regulation, and function of Ptgs and Ptgs products during early pregnancy in hamsters that showed P2-dependent implantation. Our results reveal that Ptgs2-derived PGE2 is involved in the successful establishment of implantation in hamsters.

EXPERIMENTAL PROCEDURES

Animals—Adult virgin male and female golden hamsters (Mesocricetus auratus) 8–12 weeks old were purchased from either Sasco, Omaha, NE, or Charles River Laboratories, Wilmington, MA. They were maintained in a 14-h light, 10-h dark cycle in the Animal Facility Laboratory of the Kansas Medical Center (Kansas City, KS) and Vanderbilt University (Nashville, TN) with an unlimited access to water and food according to the institutional guidelines on the care and use of laboratory animals.

Preparation of Pregnant Hamster and Uterine Tissue Collection—Only hamsters with three consecutive 4-day estrous cycles were used in this study. One female was housed with two fertile males overnight on the evening of proestrus. Finding of sperm in the vaginal smear the next morning (estrus) indicated the 1st day (day 1) of pregnancy. Hamsters on days 1–3 of pregnancy were killed at 0900 h, and whole uteri were collected after confirmation of pregnancy by flushing and recovering embryos from oviducts and/or uteri (37). Whereas whole uteri were collected on the morning of day 4 (0900 h), implantation and interimplantation sites were collected on the afternoon (1600 h) of day 4 and the morning (0900 h) of days 5 and 6 after an intravenous injection of Chicago Blue B dye solution (Sigma; 0.25 ml of 1% dye in saline). Implantation sites on these days were visualized by intermittent blue bands along the horns. On days 7–8, implantation sites were distinct and were identified visually without blue dye injection (37). Uterine tissues were immediately frozen in cold Super Friendly Freeze-it (Curtin Matheson Scientific, Houston, TX) and stored at -70°C until extraction of RNA, in situ hybridization, or immunohistochemistry/immunoﬂuorescence.

Implantation occurs without delay in hamsters ovariectomized or hypophysectomized on day 2 of pregnancy and given P2 daily (29, 33, 35). This suggests that implantation in hamsters is P2-dependent. Thus, it is possible that implantation occurred with correct expression of implantation-specific genes because of the regulation of these genes by either P2 or blastocysts. To address this issue, a group of pregnant hamsters was ovariectomized on day 2 (0900 h) and given a subcutaneous injection of P2 (Sigma; 1 mg in 0.1 ml of sesame seed oil/hamster) on either days 2 and 3 or days 2–4. Control hamsters underwent sham operation and were injected with 0.1 ml of vehicle, sesame seed oil/hamster. Whole uteri were collected on the morning of day 2 (0900 h) for in situ hybridization.

Analysis of Blastocyst Implantation after Treatment of Ptgs Inhibitors in Ovariectomized P4-Treated Pregnant Hamsters—The potential of isomorph-selective Ptgs inhibitors to inhibit or delay the initiation of implantation was determined in ovariectomized P4-treated pregnant hamsters. Pregnant hamsters were ovariectomized on day 2 (0900 h) and maintained on daily P4 (1 mg/hamster) injections. One group of hamsters was used as control and received vehicle (0.2 ml of 0.5% (w/v) methylcellulose (Sigma) and 0.1% (v/v) polysorbate 80 ( Sigma)) through oral gavage. A second group of animals received SC-560 (20 mg/kg oral gavage twice per day (0900 and 1700 h) from day 0 until 0900 h on day 5) in a cocktail of (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole; Cayman Chemical, Ann Arbor, MI) is a selective inhibitor of Ptgs1. The remaining hamsters received SC-236 (20 mg/kg oral gavage) once per day (0900 h) on days 2–4. SC-236 (4-(5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide; Calbiochem) is a selective inhibitor of Ptgs2 and has a longer half-life in vivo, which allowed for dosing once/day (38). Hamsters were killed on the morning of day 5 (0900 h) 15 min after blue dye injection. The uteri were examined for evidence of local accumulation of the blue dye at the blastocyst implantation sites. The number of blue implantation sites and the intensity of their blue color were visually recorded. Wet weight of the entire implantation site was also recorded. Significant differences in number of implantation sites and their wet weights between vehicle and Ptgs inhibitors were determined by Student’s t test.

Measurement of PG Levels at Implantation Site—To determine and compare the PG levels between implantation and interimplantation sites, normal pregnant hamsters were killed on day 5 (0900 h) after blue dye injection. Blue bands (implantation sites) and areas between two blue bands (interimplantation sites) were carefully separated, weighed, and snap-frozen for PG assay. The PG content (PGE2, PGF2α, PGD2, thromboxane B2 (a stable metabolite of thromboxane A2), and 6-keto-PGF1α, a stable metabolite of PGFL) of implant and interimplantation sites was then quantified by utilizing gas chromatography/negative ion chemical ionization mass spectrometric assays as described previously (39). Differences in PG levels between implantation and interimplantation sites were compared by Student’s t test.

Hormonal Effects on Uterine Ptgs2 mRNA Expression—To determine the effect of steroid hormones on uterine Ptgs expression, hamsters were ovariectomized without regard to their stage of estrous cycle and rested for 12 days. These hamsters were treated with a subcutaneous injection of either P2 (500 μg/hamster) or estradiol-17β (E2, Sigma; 1.0 μg/hamster) or E2 plus P4 or the vehicle sesame seed oil. All steroids were dissolved in sesame oil. Hamsters were killed at 2, 6, 12, and 24 h after injection of hormones, and uteri were collected for in situ hybridization. The dosages of P2 and E2 were chosen depending on the previously reported sensitivity of hamster uterine Ptgs (33, 37). The hamster uterus is very sensitive to P2, and treatment of 500 μg of P2 daily is reported to be sufficient to maintain pregnancy in ovariectomized pregnant hamsters (33). In contrast, sensitivity of the hamster uterus to E2 is poor (40), and single injection of 1 μg of E2 is required to induce genes in the ovariectomized hamster uteruses (37).

Cloning of the Hamster Ptgs1, Ptgs2, mPtges, and Ptgis Partial cDNAs—Reverse transcription-PCR was employed to generate the hamster-specific Ptgs1, Ptgs2, mPtges, and Ptgis partial cDNA clones. The mouse complete coding sequences for all four genes were compared for homology with the human and the rat coding sequences using the ClustalW Multiple Sequence Alignment program. The mouse coding region sequence homology between the three species was used to design sense and antisense PCR primers for Ptgs1, Ptgs2, mPtges, and Ptgis.

The primers were Ptgs1 (GenBank™ accession number NM008969; spanning nucleotides 1427–1797; size, 371; 5′-CTC TCA ATG AAC AGA AGG-3′ (sense) and 5′-GTA ATC TGG CGC AGG-3′ (antisense)), Ptgs2 (GenBank™ accession number NM008968; spanning nucleotides 435–514; size, 480; 5′-GCT GCT GGT CAC AGA TGG-3′ (antisense)), mPtges (GenBank™ accession number AB041997; spanning nucleotides 514–751; size, 410; 5′-ACA CTG CAT GCA TGG-3′ (antisense)), and Ptgis (GenBank™ accession number NM008967; spanning nucleotides 309–1118; size, 588; 5′-GCT GCT CTA CTA CAC C-3′ (sense) and 5′-CTA CCT GAG TGT CTT TGA CTG-3′ (antisense)), respectively. Total RNAs (1 μg) from day 4 pregnant hamster uterus and kidney were reverse-transcribed with oligo(dT) at 42 °C for 50 min using the
Ptgs2-derived PGE2 Synthesis at Hamster Implantation Sites

Effects of Ptgs inhibitors on implantation in ovariectomized progesterone (P4)-treated hamsters

Pregnant hamsters were ovariectomized on day 2 (0800 h) and maintained with daily injections of P4 (1 mg/hamster). Drugs were given from days 2 to 4 of pregnancy. Implantation sites were determined by blue dye injection on day 5 at 0900 h. Wet weights of the implantation sites were recorded. Numbers in parentheses indicate number of observations.

SuperScript First Strand Synthesis System (Invitrogen). PCR was carried out with an initial denaturation of 95 °C for 10 min followed by 40 cycles consisting of denaturation at 94 °C for 1 min, annealing at 64 °C for 30 s, and elongation at 72 °C for 30 s. This was followed by a final extension at 72 °C for 8 min. Reverse transcription-PCR generated uterine Ptgs1, Ptgs2, and Ptgs, and kidney mPtges products were subcloned into a pCR®-II-TOPO vector (Invitrogen) using TOPO TA Cloning kit, version 2.1 (Invitrogen), and nucleotide sequences of the clone were determined on both strands to verify the identity of the cDNA clones. Nucleotide sequences of these partial cDNA clones showed more than 85% sequence similarity with that of the GenBank nucleotide data base for rats, mice, and humans. The GenBank™ accession numbers for hamster Ptgs1 and Ptgs2 are AF414605 and AF435433, respectively.

RNA Probe Preparation—For in situ hybridization, plasmids bearing hamster cDNAs were extracted, purified, and linearized to generate antisense and sense riboprobes, which were transcribed using appropriate RNA polymerases (Ptgs1, SP6/NotI for antisense and T7/HindIII for sense; Ptgs2, SP6/Xhol for antisense and T7/HindIII for sense; mPtges, T7/BamHI for antisense and SP6/NotI for sense; Ptgs, SP6/NotI for antisense and T7/HindIII for sense) and labeled with 35S (41).

All labeled sense and antisense CRNA probes used for hybridizations had specific activities of ~2 × 106 dpm/μl. In situ Hybridization—The protocol was followed as described by Das et al. (41). Briefly, uterine cryosections were mounted onto poly-L-lysine-coated glass slides and fixed in 2% paraformaldehyde for Ptgs2 for 15 min and in Bouin's solution for Ptgs1. After removal of the primary antibody, the sections were washed with PBS and incubated with biotin-goat anti-rabbit IgG (Zymed Laboratories Inc., San Francisco, CA) for 10 min at room temperature (sc-236 (15 mg/kg) significantly (p < 0.05) from vehicle and SC-560-treated groups.

TABLE I

<table>
<thead>
<tr>
<th>Treatment (days 2–4 of pregnancy)</th>
<th>No. pregnant hamsters</th>
<th>No. of hamsters with implantation sites</th>
<th>No. implantation sites, mean ± S.E.</th>
<th>Wet weight of implantation sites, mean ± S.E.</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>6/6</td>
<td>10.83 ± 0.8 (6)</td>
<td>31.18 ± 0.34 (48)</td>
<td></td>
</tr>
<tr>
<td>SC-560 (Ptgs1 inhibitor)</td>
<td>5</td>
<td>5/5</td>
<td>9.25 ± 1.2 (5)</td>
<td>30.78 ± 0.72 (36)</td>
<td></td>
</tr>
<tr>
<td>SC-236 (Ptgs2 inhibitor)</td>
<td>7</td>
<td>6/7</td>
<td>6.2 ± 0.9 (6)</td>
<td>20.23 ± 0.33 (30)</td>
<td></td>
</tr>
</tbody>
</table>

* Values indicate that 14 of 37 implantation sites in 6 animals showed less accumulation of blue (visual observation) at implantation sites.

** Values were significantly different (Student’s t test; p < 0.05) from vehicle and SC-560-treated groups.

RESULTS

Isoform-specific Ptgs Inhibition Alters Embryo Implantation in Ovariectomized P4-treated Pregnant Hamsters—Studies in various species demonstrated that pharmacological inhibition of Ptgs by NSAIDs leads to either complete or partial delay in implantation (1–5, 43). These observations were supported by an infertility phenotype of Ptgs2 null mice (13). The most commonly studied NSAID is indomethacin, which inhibits activity of both Ptgs1 and Ptgs2 (44). Recently, compounds that selectively inhibit the activity of each Ptgs isoform have been produced. Thus, we used a pharmaceutical approach to perturb Ptgs1 or Ptgs2 functions during early pregnancy, and we examined their separate effects on implantation process in hamsters. Compared with the vehicle treatment, hamsters that received the Ptgs1 inhibitor SC-560 (15 or 30 mg/kg) showed no implantation failure on day 5 (Table I). We observed no or little differences in the number of implantation sites as well as their wet weight between the vehicle-treated control group and the SC-560-treated group. Treatment with the Ptgs2 inhibitor SC-236 (15 mg/kg) significantly (p < 0.05) reduced the number of normal implantation sites when compared with controls. One of seven SC-260-treated animals showed no implantation. Three degenerated blastocysts were recovered upon flushing the uteri from this animal. The remaining SC-236-treated hamsters showed less blue dye accumulation (visual observation) and reduced wet weight (p < 0.05) of implantation sites on day 5. The dosages of these drugs were reported not to be toxic to rodents (45). We also observed no maternal deaths, although the outcome of pregnancy on later days was unknown. The results of this experiment suggest that whereas inhibition of Ptgs1 activity has no effect on normal implantation process, inhibition of Ptgs2 activity might lead to impairment of the embryo implantation process. These observations lead to an investigation of Ptgs1 and Ptgs2 expression patterns during early pregnancy in hamsters.

Ptgs1 and Ptgs2 mRNAs Are Differentially Expressed in Uterine Cells during the Peri-implantation Period—Ptgs1 and -2 are rate-limiting enzymes for PG synthesis from arachidonic acid (9). Uterine expression patterns and functions of Ptgs1 and -2 in the mouse (10) suggest that PG synthesis requires both ovarian P4 and estrogen for the initiation of the implantation process. However, there are no studies in species such as...
the hamster, which requires only ovarian P₄ for implantation. The uterus is composed of heterogeneous cell types that undergo rapid and dynamic changes during early pregnancy under the control of ovarian steroids (46). Thus to define the expression of Ptgs1 and Ptgs2 in uterine cells during normal pregnancy, we first investigated Ptgs1 and -2 mRNA expressions by in situ hybridization analysis using hamster-specific Ptgs1 and Ptgs2 cDNA probes, respectively. Figs. 1 and 2 showed autoradiographic images of the distribution of Ptgs1 and Ptgs2, respectively. On day 1 of pregnancy, Ptgs1 and Ptgs2 mRNA expressions were observed only in luminal epithelial cells but not in glandular epithelial, stromal, myometrial, or vascular endothelial cells. The autoradiographic signals of both Ptgs1 and Ptgs2 in the luminal epithelium were reduced on day 2 of pregnancy. On day 3 of pregnancy, both signals increased in the entire luminal epithelium. Although autoradiographic signals for Ptgs1 persist in the entire luminal epithelium, Ptgs2 signals were localized mainly in uterine luminal epithelial cells surrounding the blastocyst as the uterus prepares to initiate the process of implantation on the morning of day 4 (0800–0900 h). During the time of implantation on the afternoon of day 4, Ptgs1 mRNA expression was reduced in the luminal epithelial cells surrounding the implanting blastocyst as compared with its expression away from the blastocyst. In contrast, Ptgs2 mRNA signal was observed in high intensity in luminal epithelial cells as well as in several layers of stromal cells only surrounding, but not away from, the implanted blastocyst. These patterns of Ptgs1 and Ptgs2 mRNA signals were also maintained in implantation sites on days 5 and 6. Whereas Ptgs1 expression (Fig. 1) was reduced in luminal epithelial cells surrounding the implanted blastocyst, increased expression of Ptgs2 mRNAs (Fig. 2) was noticed both in the luminal and stromal cells in the immediate vicinity of implanting blastocyst on day 5 of pregnancy. On day 6 of pregnancy, Ptgs1 was expressed in low levels only in the remaining mesometrial epithelial cells. Ptgs1 was not expressed in the stromal or decidual cells. However, cells of the entire primary decidual zone (PDZ), but not the secondary decidual zone, expressed Ptgs2 mRNA on day 6. No Ptgs1 and Ptgs2 mRNA expressions were observed in embryonic cells. On days 7 and 8 of pregnancy, Ptgs1 expression was not observed in any cell types at the implantation site (Fig. 1). However, whereas Ptgs2 signal was reduced in the PDZ on day 7, it started appearing in the trophoblast cells (Fig. 2). Trophoblast cells of the day 8 embryo showed prominent expression of Ptgs2. No Ptgs2 mRNA expression was observed in other cells beside trophoblast cells. Sections of day 5 implantation sites showed no signals when hybridized with sense cRNA probes (data not shown).

Because of the restricted Ptgs2 expression pattern at the implantation site and because Ptgs2 is an early inducible gene in response to cytokines, growth factors, and blastocysts (47), we next compared the expression of Ptgs2 in the uterus of ovariecutomized P₄-treated pregnant and normal pregnant hamsters only on days 4 and 5.

Uterine Ptgs2 mRNA Expression Is Similar in Ovariectomized P₄-Treated Pregnant and Normal Pregnant Hamsters on Days 4 and 5—Because hamsters do not need ovarian estrogen for implantation, delayed implantation does not occur in ovariecutomized P₄-treated pregnant hamsters (33). The observed expression patterns of Ptgs2 (Fig. 3) in the ovariecutomized P₄-treated pregnant uterus were similar to its expression during normal pregnancy on days 4 and 5. As the time of implantation approached on day 4, Ptgs2 mRNAs were tending to accumulate in the presumptive implantation site. The Ptgs2 mRNA expression pattern in day 5 implantation sites of ovariecutomized P₄-treated pregnant hamsters showed very similar expression patterns as observed during normal day 5 of pregnancy. These results indicate that in the absence of ovarian estrogen, the influence of P₄ is enough to bring about implantation-related uterine changes in Ptgs2 expression in the presence of a blastocyst. To clarify further whether ovarian steroids influence Ptgs2 in uterine cells, we examined Ptgs2 expression in ovariecutomized hamsters treated with P₄ and/or E₂ or the vehicle (oil).

Ptgs2 Is Regulated by Steroid Hormones in the Ovariectomized Hamster Uterus—Control animals treated with oil show no expression of Ptgs2 mRNAs. Ovariectomized hamsters treated with P₄ and E₂ alone showed little increase in the levels of Ptgs2 in uterine luminal epithelial cells as compared with
the oil-injected control (Fig. 4). However, a substantial amount of Ptgs2 mRNA accumulation was observed in the epithelial cells of ovariectomized hamsters treated with both P4 and E2 by 6 h followed by a gradual decrease by 24 h. The expression of Hgfl mRNA expression was studied as a positive control (data not shown) in these ovariectomized uterine tissues after various hormone treatments and matched previously reported patterns (37). The day 1 pregnant uterus is used as a positive control for Ptgs2.

Expression of Ptgs2 Protein in the Peri-implantation Uterus

**Fig. 3.** Cell-specific Ptgs2 mRNA expression as detected by in situ hybridization in ovariectomized progesterone-treated (OVX + P4) and sham-operated pregnant (days 4–5) hamsters. Photographs were captured under dark field at ×20. Uterine tissues were collected on the morning (0900 h) of days 4 and 5 (D4 and D5). am, antimesometrial side; bl, blastocyst; le, luminal epithelium; m, mesometrial side; myo, myometrium; s, stroma.

**Fig. 4.** In situ hybridization of Ptgs2 mRNAs in steroid-treated adult ovariectomized hamsters. Hamsters were given a single injection of sesame seed oil (vehicle for steroids), P4 (500 µg/hamster), or E2 (1 µg/hamster) or a co-injection of P4 with E2 and killed 2, 6, 12, and 24 h later. Dark field photographs were shown at ×20 magnification. A section from a day 1 pregnant hamster uterus was used as a positive control. Because oil-treated uterus did not show positive hybridization at any time point, a representative oil-treated uterine section at 6 h was presented. le, luminal epithelium; myo, myometrium; s, stroma.

**Fig. 5.** Peri-implantation (days 1–8) hamster uterus showed cell-specific localization of Ptgs2 protein as detected by immunohistochemistry. Photographs were captured under bright field. Magnification is indicated in each panel. Uterine tissues were collected on the morning (0900 h) of each day from days 1 to 8 (D1–8). However, on day 4 implantation sites were also collected at 1600 h (D4 pm). bl, blastocyst; em, embryo; ge, glandular epithelium; le, luminal epithelium.

**Expression of Ptgs2 Protein in the Peri-implantation Uterus**

*In Cell- and Implantation-specific—*The above-described expression patterns of Ptgs2 mRNAs in the peri-implantation uterus and hormonal regulation of this gene in ovariectomized hamsters suggest that Ptgs2 is most likely an inducible gene in the hamster uterus. Thus, we examined the cellular distribution of Ptgs2 gene product Ptgs2 protein in the peri-implantation uterus. Uterine Ptgs2 expression as detected by immunohistochemistry follows the similar pattern of Ptgs2 mRNA expression observed during the first 8 days of pregnancy. This protein is mainly found in uterine epithelial cells on day 1 and the morning of day 4 of pregnancy (Fig. 5). Immunoreactive Ptgs2 protein was detected in epithelial and stromal cells adjacent to implanting the blastocyst on the afternoon of day 4 and the morning of day 5. Whereas only a few stromal cells showed Ptgs2 expression on the afternoon of day 4, it is expressed in several layers of subepithelial stromal cells on day 5 of pregnancy. At the implantation site on day 5, several stromal cells also showed perinuclear localization of Ptgs2 protein. There were no such signals observed when the day 5 implantation sites were incubated with only IgG (data not shown). A set of decidual cells that forms the PDZ around the implanted embryo was positive for Ptgs2 on day 6. Expression of Ptgs2 protein overlapped with Ptgs2 mRNA expression in the implan-
Ptgs2-derived PGE$_2$ Synthesis at Hamster Implantation Sites

Fig. 6. Comparison of level of PGs at implantation versus interimplantation sites on day 5 of pregnancy in the hamster. Numbers in parentheses indicate number of observations. The error bars indicate the S.E. Statistical analysis was performed using Student’s t test (*, p < 0.05).

Implantation Where Ptgs-2 Is Expressed—We investigated the expression mPtges and Ptgis in implantation sites on days 5 and 6 of pregnancy. Close similarity was observed in the expression Ptgs2 mRNA and mPtges mRNA, but not Ptgis mRNA, at the implantation site on days 5 and 6 of pregnancy. mPtges expression occurred in luminal epithelial and stromal cells around the implanting blastocyst on day 5 (Fig. 7). With the progression of implantation to day 6, mPtges was mainly expressed in the cells of the PDZ (Fig. 7). Ptgis mRNA expression was not observed in uterine epithelial, stromal, and decidual cells at the implantation sites on days 5 and 6. However, we noticed Ptgis mRNA expression in the outer longitudinal muscle layer of the uteruses on both days 5 and 6 and in the developing embryo on day 6 but not on day 5 (Fig. 7). Immunocytochemistry confirmed the presence of mPtges protein at the implantation site on days 5 and 6 of pregnancy. mPtges protein was co-localized with mPtges mRNA. In a subset of cells, mPtges was most densely expressed in the perinuclear region.

Because both Ptgs2 and mPtges, but not Ptgis, are expressed in the same location of the implantation site, we investigated the possible co-expression of Ptgs2 and mPtges proteins by double immunostaining on day 5 implantation sections. As shown in Fig. 8, these two enzymes were co-expressed in a subset of stromal cells at the implantation site. The distinct staining pattern and more abundant expression of Ptgs2 exclude the possibility that there was cross-reaction between these two antibodies.

These results suggest that elevation of PGE$_2$ at the implantation site involves the Ptgs2/mPtges system in hamsters.  

DISCUSSION

Prostaglandins trigger a variety of blastocyst-uterus interactions associated with implantation. These responses include the blastocyst-uterine attachment reaction, uterine vascular permeability changes, and stromal decidualization (23). Evidence for these PG responses was further supported by mutation of Ptgs2 in the mouse (12, 13), where implantation occurs in response to ovarian estrogen (24, 25). Ovarian estrogen is not a requirement for initiation of implantation in certain species including hamsters, rabbits, pigs, monkeys, and perhaps humans (26–36). However, it is unknown which PGs are...
Autoradiographic (in situ hybridization) photographs were captured under dark field at ×100 for day 5 and ×200 for day 6. Immunohistochemical photographs were captured under bright field at ×40, antimesometrial side; bl, blastocyst; em, embryo; le, luminal epithelium; m, mesometrial side; myo, myometrium; pdz, primary decidual zone; sdx, secondary decidual zone.

At the beginning of this study we sought to determine whether uterine PG synthesis has any role in implantation in hamsters. Ovariectomized P4-treated pregnant animals were used to avoid any effects ovarian estrogen might have in initiation of implantation in hamsters. Our results show that the Ptgs-selective inhibitor SC-560 has no effect on implantation compared with the vehicle treatment group. This result is similar to the results obtained in mice treated with either SC-560 or aspirin that preferentially blocks Ptgs1 (13, 43), indicating that selective Ptgs1 inhibition alone has little or no influence on implantation. However, inhibition of PG synthesis with indomethacin at or around the time of implantation either partially or completely prevents implantation in rats, mice, hamsters, and rabbits (1, 4, 6, 44, 50, 51). Evidence has also accumulated that PGs may be required for decidualization (52). Overall, it appears that Ptgs2-derived PGs play an important role during the process of implantation in hamsters. However, the uterine site of PG production has not been established previously in this species.

One of the ways of studying PG synthesis is to analyze the uterine expression patterns of both Ptgs1 and Ptgs2. It was observed that before implantation (days 1–4) uterine luminal epithelial cells are the major source of Ptgs1 and Ptgs2. The reason for the expression of both enzymes in the same cells is unknown. It is possible that both of these enzymes play the same role in the uterine epithelium on these preimplantation days. Most interesting, however, when blastocysts undergo implantation on days 5 and 6, Ptgs1 is gradually decreased from epithelial cells around the implantation sites. It only persists in luminal epithelial cells away from the implantation site. The reverse is true for Ptgs2 expression on these days. Ptgs2 gradually disappears from the epithelial cells away from the blastocysts but concentrates exclusively in both epithelial and stromal or PDZ cells surrounding the implanting embryos. Ptgs2 expression at day 5 implantation sites is in agreement with the Ptgs2 expression in mice (10). However, unlike mice that showed only mesometrial stromal expression of Ptgs2 on the day 6 implantation site, hamsters showed Ptgs2 expression in decidual cells (PDZ cells) immediately surrounding the implanting embryos. Expression of Ptgs2 in cells of the PDZ of days 5 and 6 and its expression in trophoblast cells of the growing embryo on later days of pregnancy suggest that Ptgs2 could not only be associated with inflammatory aspects of implantation


day 5 implantation sites of pregnant hamsters. Photographs were captured under bright field at ×40, antimesometrial side; bl, blastocyst; le, luminal epithelium; m, mesometrial side.

be the result of reduced uterine capillary permeability. Overall

day 5 implantation process has been reported in mice with
uterine expression of enzymes responsible for PG synthesis was
studied in an animal model that does not require ovarian

produced and how they are synthesized at the implantation site
under such hormonal regulation of implantation. This informa-
tion would be of value in understanding some defective implantation
processes. To address some of these questions, the uterine
expression of enzymes responsible for PG synthesis was
studied in an animal model that does not require ovarian

(PG synthesis (54). A delay in implantation and
decidualization could result from the effect of this drug either
on the blastocyst, the uterus, or both. However, our results suggest that Ptgs2 is not expressed in the hamster blastocyst. Thus, it is assumed that SC-236 inhibits uterine Ptgs2 activity in hamsters. Our observations that SC-236 adversely affects the implantation process in hamsters are similar to the previous reports in mice treated with Ptgs2 inhibitor celecoxib or DuP97 (13, 43). Overall, it appears that Ptgs2-derived PGs play an important role during the process of implantation in hamsters. However, the uterine site of PG production has not been established previously in this species.
but also be involved in protection of the embryo proper by its immunomodulatory and cell-cell barrier formation properties (55, 56). In rodents, implantation is followed by loss of uterine luminal epithelial cells and their barrier functions just around the implanted embryo (42). This loss of epithelial cells together with local inflammation at the implantation site may pose a threat to the semi-allogenic embryo from maternal toxic materials including immunoglobulins. In this situation, the ability of the decidual cells to act as a barrier is critically important in terms of embryonic loss. Ptg2-derived PGs have been suggested to be important in restoring epithelial barrier functions with healing of colonic ulcers (57). Thus, the induction of Ptg2 in the decidual cells immediately surrounding the embryo demonstrates a potential role of Ptg2-derived PGs in induction of temporary barrier functions in these cells. In this regard we have already demonstrated that the cells of the PDZ surrounding the implanted embryo express tight junctional molecules (42, 58).

The induction of Ptg2 at the implantation site is not surprising because similar induction of Ptg2 occurs at the implantation site of mice (10). What is surprising is that in the absence of ovarian estrogen, implantation sites of P4-treated pregnant hamsters showed a similar pattern of Ptg2 expression suggesting that either P4 or blastocysts are involved in the induction of Ptg2. By using ovariectomized hamsters, it was noted that neither P4 nor E2 is a strong inducer of Ptg2 in the uterus. However, simultaneous treatment with both hormones to ovariectomized hamsters strongly induced Ptg2. The need for estrogen stimulation of Ptg2 in the hamster uterus is somewhat paradoxical. One intriguing possibility is that preimplantation embryos of hamsters may have the capacity to produce estrogen that acts locally on the uterus. In this context, we have preliminary evidence that preimplantation embryos of hamsters express the Cyp19 gene that encodes aromatase protein.2

The differential expression of Ptg1 and Ptg2 at hamster implantation sites suggests that PGs produced at the implantation site are mainly the contributed products of Ptg2. We reported previously (17) that PGL2 rather than PGE2 is the major prostanooid in the mouse implantation site. In contrast, Kennedy (3, 18) showed that in the rat and hamster PGE2 is a key mediator at the implantation site. The data presented here support the later observations and show increased PGE2 content in the implantation site as compared with its content in the interimplantation site of hamsters. It is unknown, however, which Ptges is involved in the synthesis of PGE2. There are two isoforms of Ptges (mPtges and cPtges) that catalyze the conversion of PGH2 to PGE2. In 1999, Jakobsson et al. (59) identified a human mPtges that was inducible by interleukin-1β, an inflammatory cytokine, suggesting the involvement of mPtges in inflammation and in the acute-phase response. Because inflammatory changes occur at the implantation site with reduced expression of Ptg2 mRNA, it is likely that mPtges is involved in the Ptg2-derived PGE2 synthesis. Indeed, we observed that Ptg2 and mPtges were co-expressed in the uterus in a temporally and spatially similar manner, suggesting that they are functionally linked. Preferential coupling of Ptg2 and mPtges was demonstrated recently in a cell line that was transfected with mPtges together with either Ptg1And Ptg2 (60). In this experiment cells co-transfected with mPtges and Ptg2 produced PGE2 from much lower concentrations of arachidonic acid compared with those transfected with mPtges and Ptg1. This group also demonstrated that cPtges is functionally linked to Ptg2 (48). It has been demonstrated previously (61) that preferential coupling of Ptg2 with mPtges is needed to generate PGE2 in brain endothelial cell. mPtges expression in stromal and epithelial cells around the implanting embryo has also been reported in implantation sites of mice (52). Our observation of Ptg2 and mPtges co-expression around the implantation site suggests that Ptg2 may be preferentially coupled with mPtges for PGE2 production at the implantation site of hamsters (Fig. 9).

The specific role of PGs in human implantation is not clear. Previous studies investigating expression of the components of the PGE2 biosynthetic and signaling pathways in the human endometrium have outlined temporal variation across the menstrual cycle. Expression of COX/PGE synthase enzymes and synthesis of PGE2 are highest in the perimenstrual and proliferative phases of the menstrual cycle, and the sites of expression/synthesis are localized to multiple cellular compartments within the endometrium including epithelial, stromal, and perivascular cells (62). Both Ptg1 and Ptg2 are expressed in the uterine luminal epithelial, but not in stromal, cells during peri-implantation phases in the human endometrium (63). Thus, in the uterus, Ptg1 and Ptg2 functions are not also mutually exclusive because one isoform can also compensate for the lack of another isoform (64, 65). In the current study, we demonstrated that PGE2 is the preferential product of Ptg2 and mPtges at the implantation site, and inhibition of Ptg2 activity by using Ptg2-selective inhibitor causes both the reduction in the number and the size of the implantation sites on day 5 of pregnancy in hamsters. Implantation together with stromal decidualization, i.e. cell proliferation, edema, and increased vascular permeability, has been recognized as a proinflammatory event, and identification of its actual mechanism is still an important issue in modern research. Lipid mediator PGs have been implicated in triggering inflammatory processes. Because PGE2 is a potent inflammatory PG, our finding suggests that this lipid mediator is involved in local implantation associated changes in the uterus at the site of implantation. As discussed above PGE2 could be also involved in the protection of embryo proper from the harmful materials originating from the mother. In general, complete success of the blastocyst implantation process involves sequential maternal uterine changes to nurture further growth and protection of the embryo. Ptg2-derived PGE2 at the implantation site helps to complete the implantation process without any adverse effects.
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