Inhibition of cyclooxygenase-2 prevents inflammation-mediated preterm labor in the mouse

GIL GROSS,1 TAKUJI IMAMURA,2 SHERRI K. VOGT,2 DAVID F. WOZNIAK,3 D. MICHAEL NELSON,1 YOEL SADOVSKY,1 AND LOUIS J. MUGLIA1,2,4

Departments of 1Obstetrics and Gynecology, 2Pediatrics, 3Psychiatry, and 4Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

The molecular mechanisms determining the initiation and progression of human parturition, despite intense study, have yet to be elucidated. Preterm labor (PTL) and delivery remain the leading causes of infant mortality worldwide (8, 27), and the molecular events controlling labor must be better understood so that therapies to retard PTL can be optimally designed. Many molecules have been implicated as important for human parturition and central among these are the prostaglandins (PGs). PGE2 and PGF2α parturition and delivery remain the leading causes of infant mortality worldwide (8, 27), and the molecular events controlling labor must be better understood so that therapies to retard PTL can be optimally designed. Many molecules have been implicated as important for human parturition and central among these are the prostaglandins (PGs). PGE2 and PGF2α are produced by maternal and fetal tissues during pregnancy, and amniotic fluid concentrations of both types of PGs increase during labor (3, 15). PGs stimulate uterine contractions in vitro and in vivo and promote the controlled inflammatory response that results in dilation and thinning of the cervix (16). In rodents, PGs induce luteolysis and are essential for parturition because mice with targeted disruption of the PGF2α receptor do not progress into labor (38).

PG synthesis requires the initial conversion of arachidonic acid to PGH2. Cyclooxygenases catalyze this first committed step. The two known isoforms of cyclooxygenase (COX) are COX-1 and -2. Although these two isoforms share similar structure, they differ markedly in their pattern of regulation and physiological functions (33, 37). COX-1 is readily detected in many tissue types and is thought responsible for “housekeeping” activities, such as gastrointestinal cytoprotection, renal blood flow regulation, and platelet aggregation. COX-2, in contrast, is considered the inducible isoenzyme. It is generally not detected in most tissues but can be found in large amounts in macrophages and other inflammatory cell types following exposure to cytokines, growth factors, and mitogens. Both COX-1 and -2 are expressed in the uterus and extraembryonic membranes (35, 42), and COX-2 promoter activity increases in human trophoblasts after lipopolysaccharide (LPS) exposure (1).

Nonsteroidal anti-inflammatory drugs (NSAIDs), which block PG production, have proven to be effective agents in attenuating the progression of term labor and PTL in animal model systems (10, 34), as well as in human studies (2, 17). The current NSAIDs in clinical use, such as indomethacin, inhibit both COX-1 and -2. Despite the efficacy of indomethacin in attenuating PTL, fetal and maternal side effects have limited its use during human pregnancy. Maternal side effects are for the most part limited to gastritis and the potentiation of peptic ulcer disease (2, 41). Significant fetal side effects include premature closure of the ductus arteriosus, reduction in renal perfusion that results in decreased amniotic fluid volume, and increased incidences of both intraventricular hemorrhage and necrotizing enterocolitis (25, 28). The definition of the consequences of selective inhibition of COX-1 or -2 for labor or toxicity by administration of isoform-selective NSAIDs may permit efficacious therapy without significant morbidity.

Our laboratory has demonstrated recently an essential role for COX-1 in normal term murine labor (13). It is not known whether the initiation of PTL, either idiopathic or inflammation mediated, involves mechanisms similar to those in normal term labor. In this...
current study, we tested the hypothesis that selective inhibition of COX-1 or -2 could stop inflammation-mediated PTL in the mouse by exploiting both pharmacological and genetic systems that specifically alter COX-1 or -2 activity. These trials in an animal model system provide a requisite step before considering selective COX inhibition during human pregnancy.

MATERIALS AND METHODS

Animal housing. Wild type (WT) and COX-1-deficient (KO) mice (19) were housed on a 12:12-h light-dark cycle with ad libitum access to rodent chow. All mouse protocols were in accordance with National Institutes of Health Guidelines and approved by the Animal Care and Use Committee of Washington University School of Medicine. Matings of estrous females to stud males were confirmed via copulation plug detection, and the fertilized females were isolated from males after overnight mating to ensure adequate gestation timing. Females were then isolated from males after overnight mating to ensure adequate gestation timing.

LPS model of PTL. Timed pregnancies were established with 6- to 10-wk-old C3H/HeN (Harlan Sprague Dawley; Indianapolis, IN) female mice mated to C57BL/6 male mice (Jackson Laboratory; Bar Harbor, ME). This allogeneic mating type has been utilized previously in gestational studies because it is not prone to spontaneous abortion (32). Pregnant females had PTL induced with 100 µg of LPS (Escherichia coli 0111:B4; Sigma; St. Louis, MO) in phosphate-buffered saline via subcutaneous injection at day 14.5 of gestation (~75% of term 19.5 days gestation). In our initial dose-response series 100 µg was the lowest dose of LPS that reproducibly induced PTL without significant morbidity or mortality, in the gravid C3H/HeN females. To determine the effect of COX inhibition, mice were pretreated with either vehicle (phosphate-buffered saline plus 1% Tween 80) or vehicle plus agent 30 min before LPS injection and redosed at 8 and 24 h after LPS. Agents were administered via gavage using divetted needles. Parturition phenotypes were studied in mice treated with the nonspecific COX inhibitor indomethacin at 75 and 150 µg/dose (~2.5 and 5 mg/kg, respectively), the COX-1-specific inhibitor SC-560 (Monsanto; St. Louis, MO) at 300 µg/dose (10 mg/kg), and the COX-2-specific inhibitor SC-236 (Monsanto; St. Louis, MO) at 100 µg/dose (10 mg/kg). Doses for the specific inhibitors were determined based on their previously reported IC₅₀ values for inhibition of COX-1 (29, 36) or COX-2 (29) relative to indomethacin (22). COX-1 KO mice were generously provided by Dr. S. Marham. Production of the COX-1 KO mice has been described previously (19). The COX-1 KO mice used for our experiments were maintained on an outbred 129/Sv × C57BL/6 genetic background, with COX-1-intact littermates of the same outbred background used as controls. Mice were 2- to 4-mo-old at the time of testing. Dose-response analysis on this outbred background demonstrated that 400 µg of LPS were required for reproducible PTL (in contrast to C3H/HeN matings) and did not result in maternal mortality (data not shown). Differences in the proportions of mice exhibiting PTL as a function of drug treatment in the C3H/HeN group and genotype in the COX-1 KO studies were assessed using a series of 2 × 2 contingency tables to calculate probabilities through use of the Fisher’s exact test (2-tailed).

Progesterone supplementation. Pregnant C3H/HeN females on day 10.5 (n = 4) of gestation were anesthetized by isoflurane inhalation and then had 15-mg progesterone pellets (Innovative Research of America; Sarasota, FL) inserted into the subcutaneous space at the nape. On day 14.5, 100 µg of LPS were administered, and the subsequent delivery phenotype was recorded. Serum progesterone levels achieved in ovarietomized, nongravid females supplemented with these pellets were 42.5 ± 3.5 ng/ml (n = 3) as determined by radioimmunoassay (Diagnostic Products; Los Angeles, CA).

RNA analyses. Tissues harvested from euthanized mice were immediately frozen in liquid nitrogen and stored at ~80°C. RNA was prepared by the guanidine thiocyanate-cesium chloride method (6). Five (ovary) or 10 µg (uterus) of total RNA from three to six mice per treatment group were subjected to electrophoresis through 1.2% agarose-formaldehyde gels and transferred to nitrocellulose membranes. [α-³²P]UTP-labeled RNA probes specific for mouse COX-1 (1,298 bp Sca I fragment in pBluescript SK II+) or COX-2 (462 bp EcoR I fragment in pBluescript SK II+) mRNAs were generated by transcription with T7 polymerase and then hybridized at 60°C in 50% formamide-containing buffer. After being washed, hybridized probes were quantitated on a Molecular Dynamics PhosphorImager (Sunnyvale, CA). Specificity of our probes for each of the isoforms was confirmed by detection of a 2.8-kb transcript for COX-1 (19) and a 4.2-kb transcript for COX-2 without evidence of cross-hybridization to the other isoform. Each mRNA hybridization signal was corrected for loading and recovery by normalization to cyclophilin A hybridization intensity. Statistical analyses of normalized hybridization signal intensities were by ANOVA, with significance defined as P < 0.05.

PG measurement. Uterine (n = 3–7) and ovarian (n = 4–7) PGF₂α, was measured on tissue immediately frozen in liquid nitrogen and stored at ~80°C. Tissues were weighed while frozen and then homogenized in 100% ethanol for extraction of PGs. Debris was removed by centrifugation, and each supernatant was assayed in duplicate for PGF₂α by ELISA kit per the manufacturer’s instructions (Oxford Biomedical; Oxford, MI). Data are presented as means ± SE. Differences in uterine and ovarian PG concentrations as a function of drug treatment were evaluated using one-way ANOVAs with subsequent pairwise comparisons being conducted after a significant overall F. Alpha levels were adjusted for multiple comparisons using Bonferroni’s correction. Serum PGE₂, as vehicle (n = 6) and SC-560-treated (n = 3) COX-1 KO mice after LPS administration were measured by ELISA kit per the manufacturer’s instructions (Oxford Biomedical). Data are presented as means ± SE, with statistical significance assessed by ANOVA.

Calcium ionophore-stimulated thromboxane B₂ production in whole blood. Inhibition of COX-1 in vivo was determined by ex vivo analysis of whole blood stimulated with the calcium ionophore A-23187 (Sigma; St. Louis, MO) as previously described (36), except that retroorbital phlebotomy was performed 2.5 h after treatment with vehicle, indomethacin (5 mg/kg), SC-236 (10 mg/kg), or SC-560 (10 mg/kg) to correspond to the time point in our LPS PTL paradigm. Adult female mice (n = 3 or 4) were evaluated. Thromboxane B₂ (TXB₂) was quantitated by ELISA kit according to the manufacturer’s specifications (Oxford Biomedical). Statistical significance was determined by ANOVA.

RESULTS

Attenuation of PTL in the mouse by inhibition of COX-1 and -2 activity. To differentiate the relative importance of COX-1 and -2 during inflammation-induced PTL in the mouse, we initially confirmed the efficacy of indomethacin, a potent inhibitor of both isoforms, in attenuating premature delivery (34). C3H/HeN females, which had been mated with C57BL/6J...
males, uniformly delivered their litters within 24 h of administration of LPS at 14.5 days of gestation (Fig. 1). In contrast, a dose-dependent reduction in PTL after LPS was seen with increasing indomethacin administration, so that at a dose of 150 µg twice daily (≈10 mg·kg⁻¹·day⁻¹) only 20% of females delivered their litters within 24 h of LPS administration (P = 0.01 vs. LPS alone). Despite maintaining the pregnancies, most of the gravid females experienced in utero fetal deaths, with only 25% of maintained pregnancies harboring viable fetuses. Doses of indomethacin exceeding 10 mg·kg⁻¹·day⁻¹ resulted in maternal death, with necrotic bowel visualized at autopsy.

Term labor in mice has been shown to result from PG-induced luteolysis and progesterone withdrawal (13, 38). To determine whether the mechanism of indomethacin in attenuating LPS-induced PTL was to block progesterone withdrawal, we supplemented gravid females with sustained-release progesterone pellets to maintain serum progesterone at levels approximating those of midpregnancy. Similar to indomethacin, all progesterone-supplemented females maintained their pregnancies for more than 24 h after LPS administration (n = 4). These progesterone-supplemented females uniformly demonstrated piloerection, lethargy, diarrhea, and in utero fetal demise, suggesting that induction of PG synthesis had not been altered as a consequence of sustained serum progesterone concentrations.

COX-1 and -2 mRNA induction in uterus and ovary after LPS. Evidence for the relative contribution of each of the COX isoforms in promoting PTL was obtained by examination of induction of gene expression following LPS administration. Because luteolysis, induced by uterine- or ovarian-derived PG, is an essential component of term labor in mice (38, 40), we performed Northern blot analyses of total RNA from uterus and ovary of the LPS-treated mice. The uterus demonstrated a statistically significant twofold induction of COX-2 mRNA 2 h after administration of LPS compared with untreated controls (Fig. 2, A and B). Ovarian COX-2 mRNA in LPS-treated mice increased to a lesser extent than COX-2 mRNA in the uterus and did not achieve statistical significance (Fig. 2, A and B). In contrast, a statistically insignificant decrease in COX-1 mRNA levels was observed after LPS administration in both uterus and ovary at this time point in comparison to control mice (Fig. 2, A and C). Eight hours after LPS, COX-2 mRNA had returned to its basal level in the uterus, and COX-1 mRNA levels were unchanged compared with control mice (data not shown). As expected, administration of indomethacin with LPS did not alter the pattern of COX-1 or -2 gene expression in comparison to LPS alone (Fig. 2).

Attenuation of PTL in mouse by isoform-selective COX inhibition. Recently, pharmacological agents capable of specifically inhibiting either COX-1 or -2 in vitro and/or in vivo have been characterized. To determine the efficacy of isoform-specific inhibition in attenuating LPS-induced PTL, we analyzed the effects of specific COX-1 inhibition (SC-560) (36) and specific COX-2 inhibition (SC-236) (29) in our model system. To confirm the efficacy of SC-560 for in vivo inhibition of COX-1, we measured calcium ionophore-stimulated platelet TxB₂ production, an assay that selectively evaluates COX-1 activity. WT mice treated with SC-560 showed a greater than 90% reduction in ionophore-stimulated TxB₂ production (Table 1). Furthermore, administration of SC-560 did not reduce serum PGE₂ concentration or uterine PGF₂α concentration after LPS administration in COX-1 KO mice, demonstrating COX-1 selectivity [serum PGE₂ (n = 3–6 per group), SC-560 3.6 ± 0.3 ng/ml vs. vehicle 3.6 ± 0.3 ng/ml; uterine PGF₂α (n = 3 per group), SC-560 4.0 ± 1.2 pg/mg tissue vs. vehicle 3.5 ± 0.2 pg/mg tissue]. Selective inhibition of COX-1 with SC-560 resulted in a moderate, statistically significant, decrease in PTL after LPS, with 58% of females delivering their litters within 24 h (P = 0.04). Of the maintained pregnancies, 40% had viable fetuses. Pregnant mice treated with SC-236, a selective COX-2 inhibitor, demonstrated a marked reduction in preterm deliveries in comparison to both mice treated with LPS alone and those treated with LPS and SC-560, such that only 8% of animals delivered litters within 24 h (Fig. 1, P < 0.001 vs. LPS, P = 0.03 vs. SC-560). Additionally, a trend toward increased fetal viability in SC-236-treated females was noted, with 73% of the maintained pregnancies harboring viable fetuses. SC-236 at the doses used to inhibit PTL did not alter platelet TxB₂ production, verifying COX-2 selectivity (Table 1).

We evaluated the pattern of PG production following LPS treatment together with indomethacin, SC-560, or SC-236 administration to determine how effectively COX function had been abrogated. The results of a
one-way ANOVA indicated that the drug-treatment groups differed significantly with regard to uterine PG concentration 2 h after LPS exposure \( F(4,23) = 8.40, P < 0.0005 \) (Fig. 3A). Subsequent pairwise comparisons of the uterine PGF\(_{2\alpha}\) concentrations showed the LPS alone group to be significantly higher than the control group \( F(1,23) = 9.16, P = 0.006 \). Indomethacin resulted in near complete suppression of uterine PGF\(_{2\alpha}\) production after LPS administration \( F(1,23) = 22.43, P < 0.0005 \), whereas LPS together with SC-236 or SC-560 resulted in PG levels similar to control mice not treated with LPS and significantly reduced in comparison to mice treated with LPS alone \( F(1,23) = 22.74, P < 0.0005 \) and \( F(1,23) = 13.32, P = 0.001 \) for SC-236 and SC-560 vs. LPS alone, respectively (Fig. 3A). After 8 h, uterine PGF\(_{2\alpha}\) concentrations had returned to their basal levels in mice treated with LPS alone (data not shown). The results of the data analysis pertaining to ovarian PG concentrations were similar to those found for the uterus (Fig. 3B). One-way ANOVA revealed that drug treatment groups differed significantly with respect to ovarian PGF\(_{2\alpha}\) concentration \( F(4,19) = 9.48, P < 0.0005 \). Subsequent pairwise comparisons showed that the LPS alone group was significantly elevated.
Table 1. Calcium ionophore-stimulated TxB₂ production in whole blood ex vivo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TxB₂, ng/ml</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>Vehicle + ionophore</td>
<td>543.3 ± 126.0</td>
</tr>
<tr>
<td>SC-560 + ionophore</td>
<td>46.6 ± 20.0*</td>
</tr>
<tr>
<td>SC-236 + ionophore</td>
<td>470.0 ± 23.8</td>
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<tr>
<td>Indomethacin + ionophore</td>
<td>10.2 ± 1.4†</td>
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Values are means ± SE; n = 3–4 mice/group. Compounds were administered via gavage 2.5 h before sample collection. Whole blood was obtained by retroorbital phlebotomy and stimulated with calcium ionophore A-23187. Thromboxane B₂ (TxB₂) was measured by ELISA. *P < 0.05 vs. vehicle plus ionophore. †P < 0.01 vs. vehicle plus ionophore.

relative to control [F(1,19) = 17.32, P = 0.001], and indomethacin, SC-236, and SC-560 all significantly reduced ovarian PGF₂α production after LPS [F(1,19) = 33.40, P < 0.0005], [F(1,19) = 18.01, P < 0.0005], and [F(1,19) = 22.10, P < 0.0005] vs. LPS alone for indomethacin, SC-236, and SC-560, respectively. All P values exceed the Bonferroni-adjusted alpha level set to equal 0.01.

PTL in COX-1 KO mice. We have recently shown that COX-1 is essential for normal term murine labor (13). To further define the role of COX-1-generated PG in inflammation-mediated PTL, LPS was administered to COX-1 KO mice (19). Similar to COX-1-intact control mice of the same genetic background, 88% of COX-1 KO mice delivered litters within 24 h of LPS administration (Fig. 4), further implicating PG generated by COX-2 as important for inflammation-induced PTL. Consistent with this hypothesis, Northern blot analysis demonstrated increased COX-2 mRNA in the uterus of COX-1 KO mice after LPS (Fig. 5A). However, the magnitude of the induction was more variable than WT controls of the same genetic background and thus statistical significance was not achieved (P = 0.10). Baseline COX-2 mRNA concentration in gravid COX-1 KO mice did not differ from WT controls. This finding is consistent with the lack of compensation at the transcriptional level of COX-2 for COX-1 in the setting of chronic COX-1 deficiency. Evaluation of PG production after LPS in the COX-1 KO mice revealed markedly lower baseline and LPS-stimulated PG concentrations in comparison to WT mice (Figs. 3 and 5B). However, the increase in PG in the KO mice after LPS was similar to WT mice. No significant change in uterine or ovarian PGE₂ occurred in the KO mice after LPS, suggesting that increases in other PG did not compensate for the lower PGF₂α level (data not shown).

**DISCUSSION**

The data presented in this study demonstrate that inhibition of PG synthesis is effective in attenuating PTL in the mouse. Animals supplemented with exogenous progesterone failed to deliver within 24 h after LPS, lending further evidence to the notion that progesterone withdrawal secondary to luteolysis is the initiating event for PG-induced parturition in mice. COX-2-dependent PG production contributes significantly to the increase in PG observed following LPS administration. Although the mechanism for term labor in mice and humans significantly differs (4, 5), our results suggest that effective inhibition of COX-2 may have a central role in attenuation of PTL due to inflammation in humans.

COX-1 activity is required for the appropriate timing of term labor in mice, and COX-1 mRNA increases markedly in abundance in late gestation mouse uterus (13). In contrast, this study showed that during LPS-induced PTL, a prominent induction of COX-2 mRNA occurs in the uterus of WT mice, and this timing
coincides with the peak in PGF$_{2\alpha}$ in both the uterus and ovary. The timing for the onset of PTL in general occurred between 8 and 24\,h after administration of LPS. This delay in onset of PTL after elevation in PGF$_{2\alpha}$ is consistent with previous studies demonstrating a lag of up to 24\,h for induction of labor after acute injection of PGF$_{2\alpha}$ (13) or surgical luteolysis by ovariotomy (38). The rapid induction of COX-2 is in accord with its initial characterization as an immediate early response gene in cell culture (11, 31) and has been found after LPS administration in vivo (32) as well. This increase in COX-2, taken together with the lack of significant change in COX-1 mRNA in both uterus and ovary, supports a central role for COX-2 in inflammation-induced PTL in mice. Recent studies in humans have demonstrated induction of both COX-1 and -2 (23, 24) in the extraembryonic membranes in association with PTL. In general, these studies of PTL were not limited to women with infection or other inflammatory precipitants and thus could involve either pregnancies with early onset of “term” labor (e.g., idiopathic PTL) or inflammation-mediated PTL. A variable contribution to PG production by COX-1 and -2 might depend on the underlying etiology of labor. Alternatively, COX-1 expression is very high in mouse decidua during pregnancy without LPS stimulation (13) and may have little capacity for further increases, whereas in human amnion this may not be the case. Lastly, the LPS model of PTL we utilized induces a systemic inflammatory response. Inflammation-associated PTL in humans in many cases results from initially localized inflammation due to clinical or subclinical infection within the uterus. The relative contributions of COX-1 and -2 to PG production may vary under these different circumstances.

Poor ovulation and impaired blastocyst implantation in COX-2 KO female mice precludes analysis of the pattern of delivery of gravid COX-2 KO mice after LPS-induced PTL (20). Instead, we employed selective pharmacological blockade of COX-1 or -2. We confirmed the ability of indomethacin to attenuate preterm delivery after LPS, again supporting a crucial role for PG in inflammation-related premature labor. Despite the efficacy of indomethacin in maintaining pregnancy during LPS-induced PTL, we found a high rate of fetal loss in the indomethacin-treated group. This finding suggests that the maintenance of fetal viability is not the critical determinant for attenuation of PTL by NSAIDs in mice. In WT mice, administration of the selective COX-2 inhibitor SC-236 with LPS markedly reduced the rate of preterm delivery to a level comparable to that of nonspecific COX blockade by indomethacin. SC-236

![Fig. 4. PTL after LPS administration in COX-1-deficient (KO) mice. Pregnant outbred wild-type (WT), COX-1 heterozyote (Het), and COX-1 KO females at 14.5 days of gestation were given LPS and then monitored for delivery of fetuses. Number of pregnancies evaluated per treatment group is shown above the corresponding bar. Differences among groups were not statistically significant.](image)

![Fig. 5. Prostaglandin production after LPS administration in COX-1 KO mice. A: quantitation of COX-2 gene expression following LPS in COX-1 KO mice. Northern blot analysis of total RNA from uterus of WT and COX-1 KO mice at 14.5 days of gestation 2 h after vehicle control or LPS administration (n = 3 WT, n = 6 KO). COX-2 mRNA hybridization was normalized to cyclophilin mRNA intensity in same lane. *P < 0.01 vs. WT control. B: prostaglandin production in COX-1 KO 2 h after administration of LPS. Uterus (n = 3) and ovary (n = 5) tissue extracts from mice treated at 14.5 days of gestation were assayed for PGF$_{2\alpha}$. Data are means ± SE. *P < 0.001 vs. control COX-1 KO uterus. **P < 0.0001 vs. control COX-1 KO ovary.](image)
dramatically blunted the increase in PG found 2 h after LPS, a time corresponding to the elevation seen in COX-2 mRNA. The residual uterine PGF$_{2\alpha}$ production during SC-236 treatment of WT mice is consistent with basal COX-1-generated PG because PGF$_{2\alpha}$ is nearly undetectable in COX-1 KO mice at this time in gestation (13). Specific COX-1 blockade by SC-560 proved as effective as COX-2 blockade in decreasing LPS-stimulated uterine and ovarian PG production. Inasmuch as basal PG production in the mouse uterus is predominantly attributable to COX-1, and LPS causes a twofold rise in uterine PG, we propose that specific COX-1 inhibition may reduce PTL by inhibiting basal PG synthesis and maintaining overall LPS-stimulated uterine and ovarian PG concentrations in the range of unmanipulated gravid mice.

Our results indicate that COX-1 inhibition yields an equivalent reduction in uterine and ovarian PG production in WT mice to that seen with SC-236 and yet is not as effective in attenuating PTL as specific COX-2 inhibition. As evidenced by our platelet TxB$_2$ assay and evaluation of PG production after LPS in COX-1 KO mice, SC-236 and SC-560 are indeed COX-2 and -1 selective, respectively. Thus the attenuation of uterine and ovarian PG production and subsequent PTL is not due to crossover effects of these agents on the other, nontargeted isoform. Although the primary role of PG is to directly induce luteolysis, the difference we observe in PTL with specific COX-1 vs. COX-2 inhibition may be attributable to the role of COX-2-generated PG in tissues outside the uterus and ovary. For instance, in the placenta (12, 21, 39) or central nervous system (18, 30), where COX-2 predominates, increased PG production could lead to decreased synthesis of luteotrophic factors such as prolactin, gonadotropins, or placental lactogen and facilitate pregnancy termination. Alternatively, different cell populations within the uterus or ovary may differentially express the two COX isoforms. In this case, the luteolytic consequences of COX-2-generated PG may result from specific paracrine actions within the uterus or ovary important in the evolution of PTL. The ability of COX-2, but not COX-1, to generate PG other than PGF$_{2\alpha}$, capable of promoting luteolysis or uterine contractions is an additional plausible mechanism for the greater efficacy of COX-2 inhibition in blocking PTL. Our data suggest that this other PG would not be PGE$_2$ because uterine and ovarian PGE$_2$ levels do not increase in COX-1 KO mice during LPS-stimulated PTL.

In contrast to term labor in COX-1 KO mice, the timing for the onset of labor after LPS administration was indistinguishable in COX-1 KO females compared with WT mice. Although this finding may be viewed as discordant with the partial attenuation of PTL demonstrated by acute pharmacological COX-1 inhibition, at least two factors require consideration. First, acute COX-1 inhibition with LPS results in no net change in uterine PG concentration in comparison to naive mice, and LPS results in an overall increase in uterine and ovarian PG production in the chronically deficient COX-1 KO mice. Interestingly, whereas the percent increase in PG is similar in COX-1 KO and WT mice, the absolute increment in PG after LPS is markedly reduced in the COX-1 KO mice despite a similar, although somewhat variable, increase in COX-2 mRNA. This implicates a role for COX-1-generated PGs in augmenting COX-2 activity or altered modulation of COX-2 function in the context of chronic COX-1 deficiency. Second, the ability of low levels of PGF$_{2\alpha}$ to induce labor in COX-1 KO females suggests increased sensitivity of the COX-1 KO mice to PG due to chronic deficiency. One mechanism that could lead to increased PGF$_{2\alpha}$ receptor sensitivity is a decrease in ligand-induced receptor desensitization by G protein-coupled receptor kinases and subsequent arrestin binding (9).

Selective inhibition of COX-2 efficiently attenuates inflammation-mediated PTL in mice, supporting the role of COX-2 in PTL. These studies are necessary before posing the use of COX-2-selective inhibitors in human PTL trials. However, a concern remains whether specific COX-2 vs. combined COX, or selective COX-1, inhibition results in decreased fetal morbidity. Our studies were designed to determine the roles of COX-1 and -2 in PTL and the ability of inhibitors to attenuate PTL, thus pregnancies were terminated at most 24 h after LPS to confirm pregnancy in treated females if no fetuses were expelled, as frequent resorptions occur after LPS treatment. Aborted fetuses were often cannibalized by their mothers, prohibiting the calculation of the ratio of viable to total fetuses in this paradigm. However, the number of pregnancies with viable fetuses at the time of death suggested that COX-2-specific blockade may result in less toxicity in comparison to combined COX or COX-1-specific inhibition. Future studies designed to assess long-term viability of pups treated in utero with COX-2-specific blockade are indicated. For example, both isoforms have been found in the ductus arteriosus, with variable contributions from COX-1 or -2 depending on the gestational age at analysis and species studied (7, 14). In addition, the diminished survival capacity of COX-2 KO mice (26) may indicate that COX-2 inhibition, especially on a chronic basis, could impart toxicity. Such studies of safety should be conducted before extrapolating our encouraging results on the inhibition of inflammation-mediated mouse PTL to therapeutic use of COX-2 inhibitors in human PTL.

Perspectives

The studies described in this report distinguishing the role of COX-1 in term labor with that of COX-2 in PTL in the mouse highlight the differences in the molecular events involved in normal and abnormal parturition. Recent studies in the mouse demonstrate that luteolysis and a fall in plasma progesterone, precipitated by activation of PGF$_{2\alpha}$ receptors on the ovarian corpus luteum, is the essential initiating event for term labor (13, 38). We now show that PG-induced luteolysis is also critical for inflammation-induced PTL in the mouse. In humans, the maintenance of late-gestation pregnancy does not depend on continued luteal function, and term labor is not heralded by a fall
in plasma progesterone (5). Determining the efficacy of COX-2-selective inhibition for treating the cascade of events culminating in human PTL poses the next challenge.

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Address for reprint requests and other correspondence: L. J. Muglia, Washington Univ. School of Medicine, One Children’s Place, St. Louis, MO 63110 (E-mail: muglia_l@ids.wustl.edu).

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REFERENCES


