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Expression of Cyclooxygenase Isoforms in Developing Rat Placenta, Human Term Placenta, and BeWo Human Trophoblast Model

Yan Xu, Gregory T. Knipp, and Thomas J. Cook*

Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854-8020

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Abstract: Cyclooxygenase (COX) catalyzes the rate-limiting step in the conversion of essential fatty acids (EFAs) to bioactive molecules such as prostaglandins (PGs), which play critical roles in many aspects of female reproduction and in fetal development. There are two primary related COX isoforms, the constitutively expressed COX-1 and the inducible COX-2. Although the expression of COX-1 and COX-2 has been demonstrated in the amnion, chorion, and decidua, relatively little information exists with regard to their expression and physiological function in the placenta during gestation. In this study, we have elucidated the spatial and temporal patterns of COX-1 and COX-2 expression in the labyrinthine and junctional zones of the developing rat placenta, in the human term placenta, and in the BeWo human trophoblast model using semiquantitative RT-PCR, Western blot, and immunohistochemical analyses. The mRNA and protein expression of COX-1 and COX-2 were demonstrated in the developing rat placenta with increasing expression observed toward parturition. COX-2 exhibited greater expression than COX-1 after mid-gestation and had a corresponding shift in spatial expression from the labyrinthine to the junctional zone at term. COX-1 and -2 were also expressed in human term placenta, while BeWo cells exhibited moderate expression of COX-1 and weak expression of COX-2. The results demonstrate that COX-1 and COX-2 are expressed in the rat and human placentas. The differential expression patterns in the rat placenta, especially of COX-2, imply that there may be gestational changes in the biosynthesis of PGs and other potential bioactive EFA metabolites. Establishing the expression of the COX isoforms provides a framework for future investigations into the functional and physiological significance of COX-1 and COX-2 in the placenta, particularly with respect to influencing normal pregnancy and fetal development, and to provide insights into therapeutic utilization of COX inhibitors in pregnancy.

Keywords: Cyclooxygenase; placenta; trophoblast; pregnancy; metabolism; rat; human

1. Introduction

Cyclooxygenases (COXs), also known as prostaglandin G/H synthases (PGHs), are key membrane bound enzymes responsible for catalyzing the rate-limiting step in the conversion of arachidonic acid (AA), eicosapentaenoic acid (EPA), and other essential fatty acids (EFAs) to biologically active prostanoids including thromboxane A_2 (TXA_2), prostacyclins, and various prostaglandins (PGs: PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, etc.).[1−4] The metabolites formed by the COX

* Corresponding author. Mailing address: Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 160 Frelinghuysen Road, Piscataway, NJ 08854. Phone: 732-445-3831. Fax: 732-445-3134. E-mail: tjcook@rci.rutgers.edu.

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isoforms, in particular PGs, are well-known as mediators of many cellular processes and also participate in some physiological and pathological conditions including the regulation of several female reproductive events that are associated with pregnancy: ovulation, implantation, decidualization, placental blood flow, and the initiation and progression of labor.[5−8] In addition, these bioactive compounds may have significant effects on fetal development, e.g., high circulating PG levels in the fetus might influence fetal endocrine, respiratory, and cardiovascular functions.[5] Since little or no COX expression was observed in fetal tissue until mid- to late-gestation, PGs derived from the placenta have been suggested to play critical roles in guiding proper fetal organogenesis and development.[9]

The two main COX isoforms that have been widely studied, COX-1 and COX-2, are encoded by two separate genes and exhibit distinct cell-specific expression, regulation, and subcellular localization, yet share similar structural and kinetic properties.[2−4] COX-1 is a constitutively expressed enzyme and is associated with the endoplasmic reticulum (ER) where the resulting metabolites function via G protein coupled cell surface receptors to mediate "housekeeping" functions.[2−4] In contrast, the COX-2 isoform appears to be more actively regulated. COX-2 messenger RNA (mRNA) and protein levels are normally low, but are rapidly induced in a variety of cell types by proinflammatory or mitogenic agents.[2−3]

Although several studies have demonstrated the existence of abundant COX-1 and COX-2 mRNA and protein expression in the human amnion, chorion, and, to a less extent, the decidua,[6,8,10−12] relatively little information exists with regard to their expression in the developing placenta and their subsequent effects on fetal development.[12−15] This situation is becoming increasingly more important given the extensive use of nonsteroidal antiinflammatory drugs (NSAIDs), which are COX-1 and/or COX-2 inhibitors, and the potential effects on the fetus, particularly with recent reports of cardiotoxicity for some COX-2 selective inhibitors.[16] For example, several human case reports documented impaired fertility in females who are administered NSAIDs,[17−19] as well as an increased risk of a number of neonatal complications such as necrotizing enterocolitis, intracranial hemorrhage, persistent pulmonary hypertension, and renal dysfunction.[20,21]

In order to establish a framework for investigating the roles of COX isoforms in the placenta and the potential impact on fetal development and toxicity, we identified the spatial and temporal expression patterns of COX-1 and COX-2 in the developing rat placenta, in the human term placenta, and in the BeWo human trophoblast model utilizing RT-PCR, Western blot, and immunohistochemical analyses. The rat placenta has been widely used as a model to study placental development because of the short estrus cycle and defined gestation period. It has two developing stages, choriovitelline and chorioallantoic placenta, which represent the early and

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middle−late stage of gestation, respectively.[22] Furthermore, the rat chorioallantoic placenta begins differentiating into two distinct zones from mid-gestation to parturition: the maternal-facing junctional zone that is characterized by maternal blood vessels and is involved in the endocrine and invasive functions; and the adjacent, fetal-facing labyrinthine zone that is predominantly responsible for maternal−fetal exchange and functions as the biological barrier for nutrient and xenobiotic transport across the placenta. Of the three common human placental cell lines (i.e., BeWo, JEG, and JAR), we chose BeWo cells for our studies since they have the ability to form confluent monolayers (unlike JEG and JAR cells) and they have been used for xenobiotic transport studies.[23−27]

Interest in specific and nonspecific COX inhibitors on reproductive toxicity and teratogenicity of drug candidates provides an impetus for conducting these studies. To the best of our knowledge, this is the first study to examine the spatial and temporal expression of COX enzymes in the developing placenta. The results of our study provide a foundation for further investigation into the differential roles of COX-1 and COX-2 in the placenta and their physiological significance, particularly with respect to influencing normal pregnancy and fetal development.

2. Materials and Methods

2.1. Reagents. TRIzol reagent and RT-PCR kits were obtained from Life Technologies (Gaithersburg, MD). Reagents for gel electrophoresis and Western blot were purchased from either Bio-Rad (Hercules, CA) or the Pierce Chemical Company (Rockford, IL). Gel extraction and purification kits were obtained from Qiagen (Valencia, CA). DAB immunohistochemical staining kits were obtained from Zymed (South San Francisco, CA). Unless otherwise noted, all other chemicals and reagents were purchased from Fisher Scientific (Atlanta, GA).

2.2. Animal Care and Tissue Collection. Sprague−Dawley rats (Hilltop Laboratory Animals, Scottdale, PA) were housed in an environmentally controlled facility and allowed free access to food and water. Timed pregnancies and tissue dissections were performed as previously described.[28] The presence of a copulatory plug or sperm in the vaginal smear was designated as day 0 of pregnancy. At gestational days 13, 16, 19, and 21, rats were euthanized with an intraperitoneal dose (50 mg/kg) of pentobarbital sodium. Upon sacrificing, the placentas, fetuses, maternal liver, kidney, and other tissues were immediately isolated, and the labyrinthine and junctional zones of the placenta were dissected under a microscope. The tissues were then quickly frozen in liquid nitrogen and then stored at −80 °C until further analysis. All animals were ethically and humanely handled in accordance with protocols approved by the Animal Care and Facilities Committee of Rutgers, The State University of New Jersey.

2.3. Cell Culture. The BeWo human trophoblastic cell line, which was initiated from a malignant gestational choriocarcinoma of a fetal placenta,[29] was obtained from ATCC (American Type Culture Collection, Manassas, VA). BeWo cells were maintained in Kaighn's modification of Ham's F12 medium (F12-K) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin in an atmosphere of 95% air−5% CO_2 at 37 °C. Studies described were performed with cells at ∼90% confluence under normal cell culture. All cells used in these studies were between passages 11 and 13.

2.4. Semiquantitative RT-PCR and PCR Product Sequencing. Total RNA isolation, RT-PCR, and gel electrophoresis were performed as described previously[30] with optimized conditions and normalized to β-actin expression using gene-specific primers (Table 1). PCR reaction products were electrophoretically separated on a 1.5% agarose gel. Ethidium bromide stained bands were visualized and the resulting densitometry analysis was performed using a NucleoTech 920 Image detection system (NucleoTech Corporation, San Mateo, CA). The molecular weight for each band was determined in reference to a 100 bp ladder (New England Biolabs, Beverly, MA). Absence of contaminating DNA in the final RNA preparations was confirmed in the preliminary study by a PCR assay with input RNA as template, omitting the reverse transcription step. RT-PCR utilizing the nontemplate control was also performed to ensure the specificity of the assay (data not shown). Further confirmation of the identity of the amplified PCR sequences was obtained by sequence analyses as previously described[30]

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Table 1. Gene-Specific Primer Sequences

target gene	accession no.[a]	direction	5′−3′ primer sequences	product size (bp)
rCOX-1	NM_017043	forward	CAT TCT GCC CTC TGT ACC CAA AGA	628
		reverse	GAG CTG GAG GAA ATA GCC ACT CAA	
rCOX-2	NM_017232	forward	CCA CTT CAA GGG AGT CTG GAA CAT	665
		reverse	TAT CAC ACA CTC TGT TGT GCT CCC	
rβ-actin[b]	NM_031144	forward	ATC GTG GGC CGC CGC CCT AGG CA	244
		reverse	TGG CCT TAG GGT TCA GAG GGG	
hCOX-1	NM_080691	forward	AGC ATC TGG CTG TCT AGA ATG TGG	430
		reverse	CAA GGA TCC CAG CAG AAA GTC TCA	
hCOX-2	AY_462100	forward	ATG ATC TAC CCT CCT CAA GTC CCT	525
		reverse	TAC TTT CTG TAC TGC GGG TGG AAC	
hβ-actin	BC_002409	forward	GCC AAC CGC GAG AAG ATG ACC	303
		reverse	CTC CTT AAT GTC ACG CAC GAT TTC	

[a] NCBI GenBank accession ID, as confirmed by BLAST sequence analysis. [b] Primer sequences cited from ref 31.

by the DNA Sequencing and Synthesis Core Facility at UMDNJ Robert Wood Johnson Medical School (Piscataway, NJ).

2.5. Western Blot Analyses. Whole cell protein from the rat placental tissue was homogenized and solubilized with protein lysate buffer (50 mM Tris-Cl buffer (pH 7.4), 150 mM NaCl, 0.1% SDS, 1 mM EDTA, 1% Triton X-100, 0.5 mM PMSF, 2 mM DTT, 0.1% protease inhibitor cocktail) (Roche, Nutley, NJ) at 4 °C. After centrifugation at 3500*g* for 20 min at 4 °C, the supernatant lysates were collected and stored at −80 °C until utilized for analysis. The protein levels were measured by the BCA assay (Pierce Chemical Company) according to the manufacturer's protocol. Western blot analyses were performed as previous described.[31] Briefly, 30 μg of protein was loaded per lane and separated in 7.5% SDS polyacrylamide gels under reducing conditions and electrophoretically transferred to PVDF membrane (Bio-Rad). The membranes were blocked with 5% nonfat milk, incubated with the appropriate mouse monoclonal primary antibody to COX-1 or COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:1000 dilution, and then incubated with a 1:15000 dilution of HRP-conjugated secondary antibodies (Pierce Chemical Company). The immunoblots were visualized using the Supersignal Western Femo detection kit (Pierce Chemical Company). The signals were detected and recorded by the NucleoTech 920 Image detection system (NucleoTech Corporation). To confirm equal loading, each PVDF membrane was stripped and analyzed for β-actin protein expression, demonstrating that the band intensities had no significant change among the samples studied.

2.6. Immunohistochemistry. Rat placental tissues were frozen upon dissection, fixed in formaldehyde, and embedded in paraffin wax prior to sectioning. Human term placental slides were obtained from Maxim Biotech (South San Francisco, CA). Immunohistochemical analysis was performed as described previously.[30] Briefly, the paraffin slides were deparaffinized with xylene and rehydrated in a graded series of ethanol. The slides were then immersed in 3% hydrogen peroxide to quench the endogenous peroxidase activity. After being blocked with 10% nonimmune serum for 30 min at room temperature, the slides were incubated with mouse monoclonal antibody raised against COX-1 or COX-2 diluted in PBS (1:100) containing 5% v/v nonimmune serum overnight at 4 °C. Subsequently, the slides were incubated for 30 min at room temperature with a 1:100 dilution of biotinylated anti-goat secondary antibody (Zymed, South San Francisco, CA) and visualized with the DAB chromatogen system. Negative controls were prepared by incubating with nonimmune serum instead of anti-COX-1 or COX-2 antibody. Slides were observed under a Zeiss microscope (Millor Optical, Philadelphia, PA) and recorded with the Spot Insight System (Diagnostic Instruments, Sterling Heights, MI) at 630× magnification.

2.7. Data Analysis. All experiments were repeated a minimum of three times. Data generated from RT-PCR were quantitated by densitometry (Gel Expert software program, NucleoTech Corp., San Mateo, CA) and normalized to β-actin expression. All the data were presented as means ± standard deviation (SD) of at least three replicates. Statistical significance relative to vehicle controls, shown in each figure, was determined using a one-way ANOVA followed by the Student *t*-test, where p values <0.05 were considered to be statistically significant.

3. Results

3.1. mRNA and Protein Expression of COX Isoforms in Rat Developing Chorioallantoic Placenta. Distinct temporal and spatial patterns of mRNA expression were determined in the developing rat placenta for both the COX-1 and COX-2 genes, as illustrated in Figure 1 ($n \geq 3$). Sequencing analyses confirmed the specificity and accuracy of the amplified products (100% identity, data not shown). Both COX isoforms were detected throughout gestation in each region of the rat placenta. COX-1 expression was upregulated in both the labyrinthine and junctional zones from gestational day 13 to day 21 and had statistical

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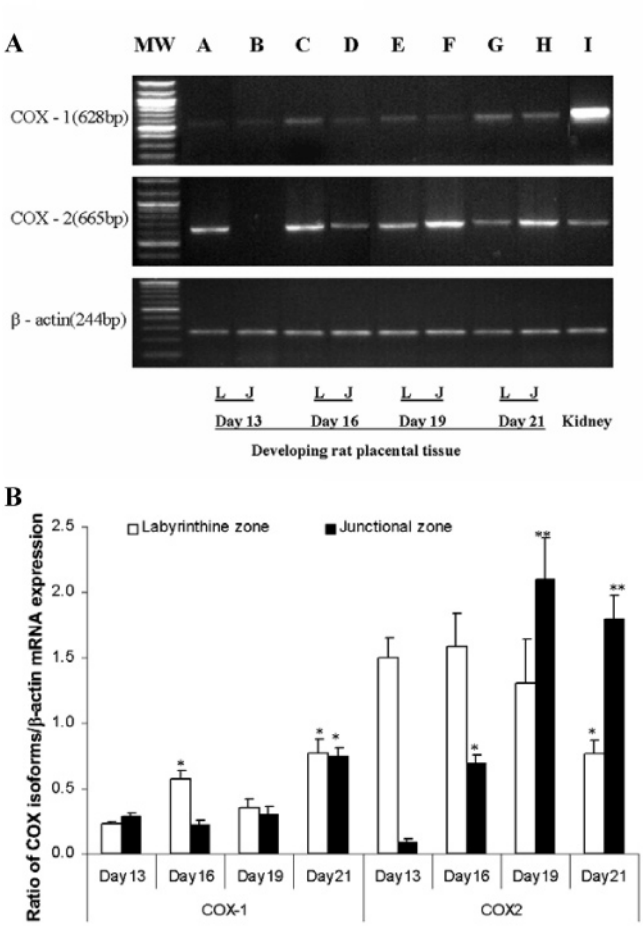

Figure 1. Expression of COX-1 and COX-2 mRNA in rat placenta during gestation. mRNA from developing rat placental tissue was analyzed by RT-PCR to show the spatial and temporal expression patterns of COX-1 and COX-2. (A) Representative RT-PCR gel electrophoresis image. The signals exhibited expected products masses, as compared to a 100 bp molecular ladder. Lanes A−H: RNA from rat placenta of gestational days 13, 16, 19, and 21 (L, labyrinthine zone; J, junctional zone). Lane I: RNA from rat kidney as positive control. Lane MW: 100 bp molecular weight ladder. (B) Densitometric analysis of the RT-PCR gel image. Data shown were relative arbitrary values after normalization with β-actin (means $\pm$ SD, $n \geq 3$). Statistical analysis was performed for COX-1/-2 gene expression at gestational days 16, 19, and 21 compared to day 13 at respective labyrinthine or junctional zone. (*) $p < 0.05$. (**) $p < 0.01$.

significance ($p < 0.05$) except at day 19. In addition, COX-1 was preferentially expressed in the labyrinthine zone at mid-term pregnancy and there was no obvious change in spatial expression. Although normalized COX-2 mRNA expression was higher when compared with COX-1 from mid-gestation ($p < 0.05$), the change in expression levels with gestational advancement was similar to that for COX-1. In the labyrinthine zone, COX-2 expression reached a peak at days 16 and 19 and then slightly decreased at day 21. In the junctional zone, the highest level of COX-2 mRNA was observed close

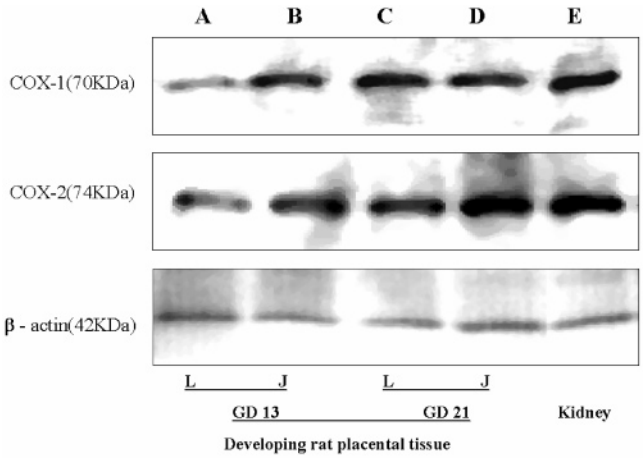

Figure 2. Western blot of COX-1 and COX-2 protein in rat placenta during gestation. Whole protein from developing rat placental tissue was analyzed by Western blot to show the temporal and spatial expression patterns of COX-1 and COX-2. Mouse monoclonal antibodies to COX-1 and COX-2 were used under optimized conditions. β-Actin expression was determined to control for unequal loading. The signals exhibited expected protein molecular masses: COX-1, ~70 kDa; COX-2, ~74 kDa. Lanes A−D: protein from rat placenta of gestational days 13 and 21 (L, labyrinthine zone; J, junctional zone.) Lane E: protein from rat kidney as positive control.

to parturition at days 19 and 21 ($p < 0.01$). In contrast to the observations for COX-1 at the same gestational age, there was an apparent shift in the spatial expression of COX-2 from the labyrinthine zone to the junctional zone as gestation progressed.

Western blot analysis was utilized to ascertain whether the protein expression of the COX isoforms correlated with the mRNA expression data (Figure 2). COX-1 and COX-2 protein levels were determined in the rat developing placental tissue, demonstrating a pattern that was consistent with observations made with the mRNA expression. COX-1 protein expression in the labyrinthine zone near parturition at day 21 was higher than that in mid-gestation at day 13, while its expression in the junctional zone did not exhibit any substantial gestational difference. The protein expression of COX-2 increased at day 21 in both the junctional zone and the labyrinthine zone compared to that at day 13, with the induction levels being slightly higher in the junctional zone.

3.2. Immunohistochemical Localization of COX Isoforms in Rat Developmental Tissue Sections. Paraffin tissue slides from the developing rat placenta at days 13, 16, 19, and 21 were used to determine the spatial patterns of COX-1 and COX-2 expression. Immunohistochemical analyses demonstrated the presence of COX-1 and COX-2 in both the labyrinthine and junctional zones from mid- to late-gestation (Figures 3 and 4), supporting the mRNA and protein expression data.

COX-1 was localized to the syncytiotrophoblasts in the labyrinthine zones, and the trophoblast giant cells in both

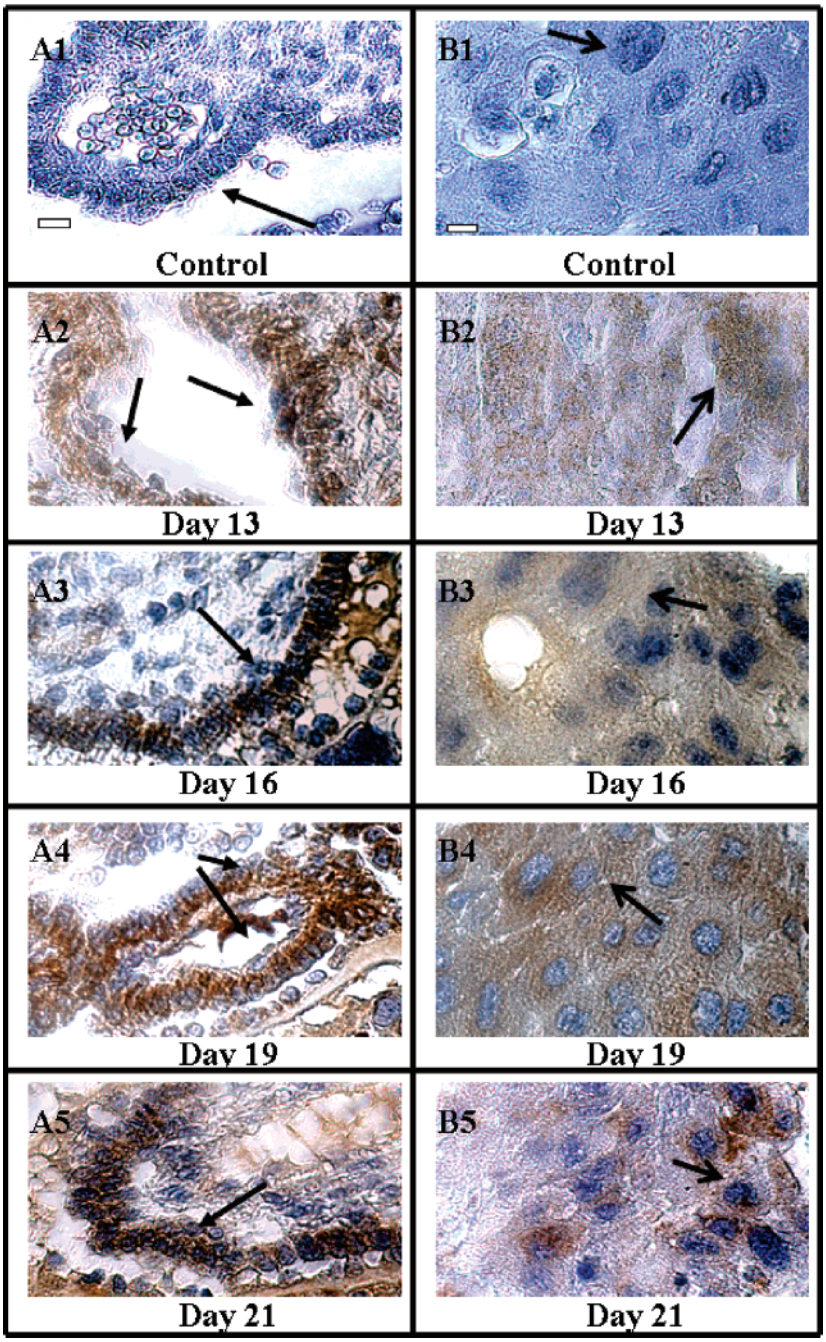

Figure 3. Immunohistochemical staining of COX-1 in the developing rat placenta. The slides were blocked with 10% nonimmune serum, incubated with a mouse monoclonal antibody raised against COX-1 followed by biotinylated anti-mouse secondary antibody, and visualized with the DAB chromatogen system. Negative controls were incubated with nonimmune serum instead of specific anti-COX-1 antibody. The photos were taken with a Zeiss microscope and recorded with the Spot Insight System (630×) (magnification bar, 5 μm). (A) Labyrinthine zone: A1, control; A2, day 13; A3, day 16; A4, day 19; A5, day 21. (B) Junctional zone: B1, control; B2, day 13; B3, day 16; B4, day 19; B5, day 21. Arrows with solid heads: syncytiotrophoblasts. Normal arrows: trophoblastic giant cells.

the labyrinthine and junctional zones (Figure 3). Similar cellular distribution and localization was observed for COX-2 (Figure 4). Consistent with the RT-PCR and Western blot results, immunohistochemical staining for COX-2 at mid-gestational day 13 in both the junctional and labyrinthine zones appeared weaker than that of later gestational ages of day 19 and day 21 (Figure 4). COX-1 and COX-2 were also detected in the smooth muscle cells and endothelial cells of maternal/fetal blood vessels in each region of the rat placenta, with COX-2 intensity increasing slightly at later gestation (data not shown).

3.3. mRNA Expression of COX Isoforms in Human Term Placenta and BeWo Trophoblast Model. Human term placenta tissue and the BeWo human choriocarcinoma

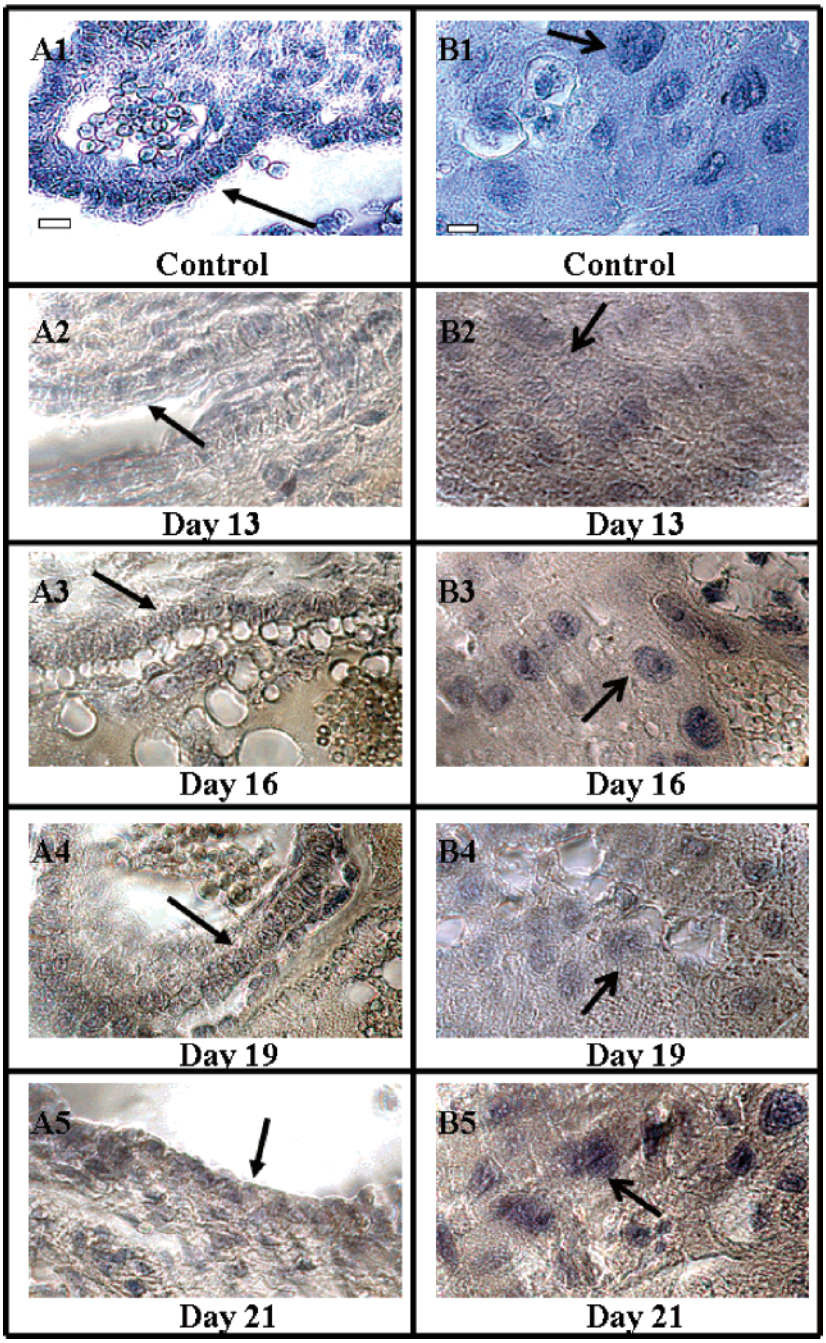

Figure 4. Immunohistochemical staining of COX-2 in the developing rat placenta. The slides were blocked with 10% nonimmune serum, incubated with a mouse monoclonal antibody raised against COX-2 followed by biotinylated anti-mouse secondary antibody, and visualized with the DAB chromatogen system. Negative controls were incubated with nonimmune serum instead of specific anti-COX-2 antibody. The photos were taken with a Zeiss microscope and recorded with the Spot Insight System (630×) (magnification bar, 5 μm). (A) Labyrinthine zone: A1, control; A2, day 13; A3, day 16; A4, day 19; A5, day 21. (B) Junctional zone: B1, control; B2, day 13; B3, day 16; B4, day 19; B5, day 21. Arrows with solid heads: syncytiotrophoblasts. Normal arrows: trophoblastic giant cells.

cells were also evaluated for their expression of COX-1 and COX-2. Sequencing analyses of the amplified products were performed and 100% identity was confirmed for both COX isoforms (data not shown).

COX-1 and COX-2 mRNAs were both detected in the human term placenta, with the normalized intensity of COX-1 appearing to be stronger than that of COX-2 (Figure 5). BeWo cells demonstrated moderate expression of COX-1, whereas COX-2 expression appeared to be significantly weaker (Figure 5), suggesting a lack of induction of COX-2 under our normal culture conditions, where BeWo cells grew with cytotrophoblast-like features without induced differentiation. Further differentiation, which exhibits characteristics similar to the fusion of cytotrophoblasts to syncytial

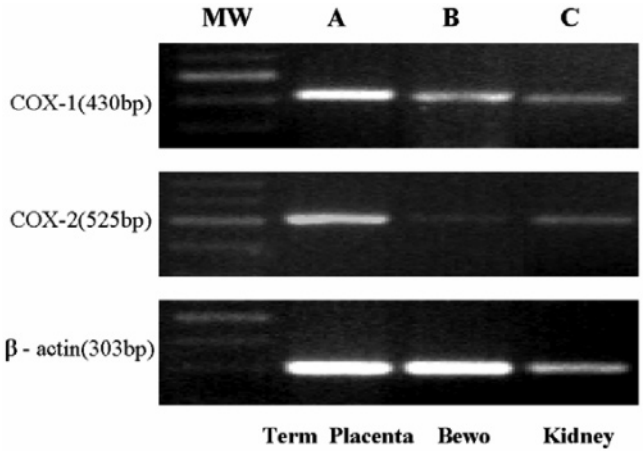

Figure 5. Expression of COX-1 and COX-2 mRNA in human term placenta and BeWo trophoblastic cell line. RT-PCR analyses of COX-1 and COX-2 mRNA in human term placenta tissue and BeWo trophoblast model using isoform-specific primers and β-actin primers were performed. RT-PCR products were separated in 1.5% agarose gels and stained with ethidium bromide. Lane A: human term placenta. Lane B: BeWo cell. Lane C: human kidney cDNA as positive control. Lane MW: 100 bp molecular weight ladder.

trophoblasts in primary culture, has to be induced by specific reagents, such as growth factors, methotrexate, cyclic AMP analogues, or forskolin, an adenylate cyclase stimulator.[23]

3.4. Immunohistochemical Localization of COX Isoforms in the Human Term Placenta. Immunohistochemical analysis with the specific anti-COX-1 and COX-2 monoclonal antibodies in human term placental tissue demonstrated the presence of both COX isoforms. In Figure 6, the staining for COX-1 and COX-2 appeared to be localized primarily to the syncytiotrophoblast layer (syncytial knots) in the villi, although the capillary endothelium in the connective tissue space within the villi also exhibited the immunostaining. In the slides studied, cytotrophoblasts, precursor cells that help to form the syncytium in humans, were inconspicuous and only had faint staining of the COX proteins.

4. Discussion

Considerable information about the differential role of COX-1 and COX-2 on female reproduction and fetal development has been gained from studies in mice deficient in either COX-1 or COX-2.[3,6,32] Overall, it appears that a deficiency of COX-2 has more profound effects on female reproduction than a COX-1 deficiency, as shown by the significant deleterious reproductive effects and increased neonatal mortality in the absence of COX-2. In contrast, COX-1 deficient mice have relatively normal phenotypes in regard to the early and late inducible events of gestation except for delayed parturition, which suggests that COX-1 is required for normal onset and progression of term labor, and cannot be compensated for by unimpaired COX-2 activity.[33]

The present study utilizes RT-PCR and Western blot analysis data to determine the expression of COX-1 and COX-2 in the developing rat placenta from mid- to late-gestation, days 13−21, as illustrated in Figures 1 and 2. In addition, cellular localization was accomplished by immunohistochemical staining of the respective tissue slides (Figures 3 and 4). COX-1 and COX-2 were detected in all of the samples investigated. The level of COX-2 expression increased as gestation progressed, which was consistent with previous studies demonstrating that human COX-2 expression increased significantly toward the end of gestation and may contribute to the mechanisms of pregnancy maintenance and labor initiation.[6,8,11−13] It is well established that PGs play a critical role in regulating labor onset including the induction of myometrial contractility, the regulation of changes in extracellular matrix metabolism associated with cervical ripening during parturition,[7,8] fetal adaptation to the labor process, and maintenance of uterine and placental blood flow.[8] Alterations in these processes might influence pregnancy outcome and, as a result, may provide potential targets for therapeutic intervention.

Our results also demonstrate that the spatial expression of COX-2 intensity shifts from the labyrinthine zone to the maternal-invasive junctional zone with the advance of pregnancy (Figures 1, 2, and 4). This shift corresponds with previous studies that suggest that PG biosynthesis in the decidua at late-gestation regulates parturition,[12] and thus, a shorter transfer to the myometrium enhances the significance of their biological effects. It has been demonstrated that placental-derived PGs reached the site of action through a predominantly passive diffusion process,[34−36] and that there was an increased rate of PG transfer post spontaneous labor induction.[36] It should be noted that the COX-2 mRNA expression in the junctional zone was lower than that observed in the labyrinthine zone at day 13; however, the labyrinthine zone had a lower amount of the COX-2 protein compared to the junctional zone. Such a discrepancy cannot be explained from our present data, and may be due to different transcript variants, posttranslational modifications,

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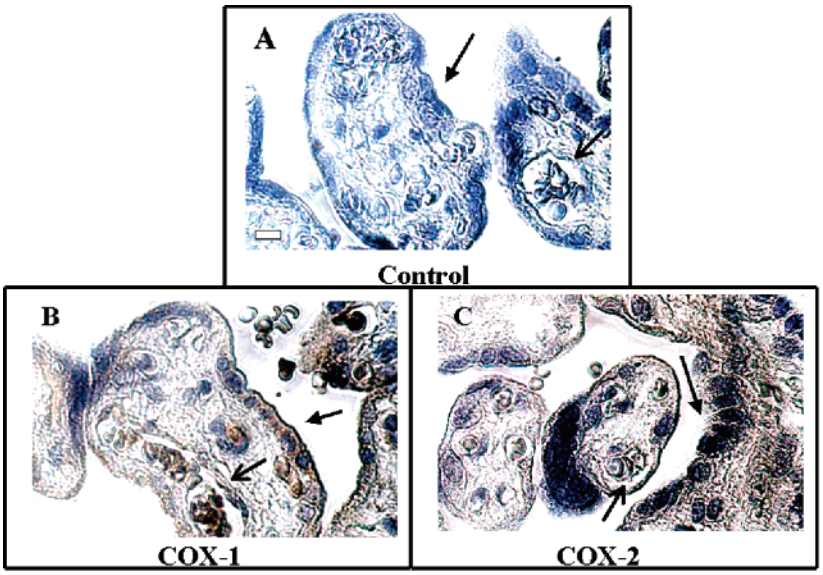

Figure 6. Immunohistochemical staining of COX-1 and COX-2 in the human term placenta. The slides were blocked with 10% nonimmune serum, incubated with a mouse monoclonal antibody raised against COX-1 or COX-2 followed by biotinylated anti-mouse secondary antibody, and visualized with the DAB chromatogen system. Negative controls were incubated with nonimmune serum instead of specific anti-COX-1/-2 antibody. The photos were taken with a Zeiss microscope and recorded with the Spot Insight System (630×) (A) Control. (B) COX-1. (C) COX-2. Arrows with solid heads: syncytiotrophoblasts. Normal arrows: capillaries within the villi. Magnification bar: 5 μm.

and/or different turnover rates for the COX isoforms' mRNAs and proteins.

Although COX-2 exhibited higher gene expression and induction levels than COX-1 (Figure 1) after mid-gestation ($p < 0.05$), the existence of COX-1 was demonstrated throughout gestation with a slight increase nearing parturition. This data suggests a potential role of COX-1 in the parturition cascade, which has been previously implied in COX-1 deficient mice studies.[33] Since COX-1 mRNA was not strongly induced, it is possible that this isoform participates in the very beginning stages of labor onset and functions in "housekeeping" regulation of pregnancy and fetal development. As COX-1 undergoes suicide inactivation,[37] the COX-2 isoform might then be primarily responsible for sustained myometrial contractility and other events that culminate in delivery of the fetus. Further studies need to be conducted to determine the physiological significance of COX-1 and/or COX-2 in the induction of preterm labor.

The mRNA (Figure 5) and protein (Figure 6) levels of COX-1 and COX-2 were determined in human term placenta. Both COX isoforms were observed in the syncytiotrophoblast layer and in the capillary endothelium within the villi. The presence of the COX isoforms in the capillary endothelium suggests their role in regulating placental vascular function. A slight discrepancy did exist between these results (Figure 5) and those results reported by another laboratory,[38] where expression of COX-2 was observed in the BeWo human trophoblast model here and absent in their studies. This inconsistency may be a consequence of differences in cell culture conditions or the sensitivity of the experimental methodology. However, the results of this investigation did suggest substantially lower mRNA expression levels of COX-2 versus COX-1 in BeWo cells.

The normal and adaptive physiological functions of organs require the coordinated expression and regulation of "housekeeping" and inducible genes. In this respect, metabolites generated by COX-1 are considered important for the maintenance of normal homeostasis, while those generated by COX-2 are known to participate in adaptive circumstances, e.g., inflammation. It is important to note that the COX-2 promoter region possesses several putative regulatory sequences, including TATA box, Sp1, nuclear factor κB (NF-κB), cAMP response element (CRE), nuclear factor-interleukin 6 (NF-IL6) sites, etc.,[2−4,8] all of which might alter COX-2 expression by different xenobiotic ligands.[39,40] In contrast to the upregulation of the COX-2 expression,

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suppression of COX-2 expression has also been studied, which might have more significant clinical relevance. It was suggested that the antiinflammatory glucocorticoids (e.g., dexamethasone) inhibit one or more steps of the PG synthesis pathway, including the suppression of COX-2 expression induced by inflammatory stimulus.[41,42] Suppression of COX-2 expression that is mediated by peroxisome proliferator-activated nuclear receptors (PPARs), established regulators of cellular EFAs homeostasis,[43] has also been reported in various tissues and cell lines.[44−46] Although there has been no report of a direct PPAR effect on placental COX-2 expression, a recent study reported that the human PPARγ levels were reduced once active labor commenced, and coincided with a relative increase in COX-2 expression at fetal membrane.[47] Since fatty acids and PGs can serve as PPAR ligands, better elucidation of the regulation of COX isoform expression in the placenta is helpful, especially when maternal nutrient imbalances are present.

NSAIDs are one of the most commonly prescribed groups of drugs worldwide. They are high effective as analgesic, antipyretic, and antiinflammatory agents. In addition, these COX inhibitors are used as tocolytics for the prevention of preterm delivery, which is a major cause of perinatal mortality and morbidity.[48] However, adverse effects have been reported in the mother and fetus as a result of exposure to COX inhibitors,[17−21] which have hindered the wide tocolytic use of NSAIDs. There is a considerable hope that COX-2-selective drugs could potentially spare COX-1-specific reproductive- and fetal-related side effects, and thus produce the protective effect observed in preterm labor prevention. Experience with the preferential COX-2 inhibitor nimesulide demonstrated that it was linked with constriction of the ductus arteriosus and oligohydramnios.[49] It is unclear whether this is due to COX-2-dependent side effects or due to accumulation of drug in the fetal circulation leading to levels that would cause COX-1 inhibition. Further work needs to be performed to decipher the true effect of nimesulide. Another interesting point is the possible link between COX-2 and teratogenicity postulated when thalidomide, a drug known for its teratogenicity, was shown to have the ability to modulate COX-2 expression.[50]

In conclusion, this study demonstrates the spatial and temporal expression patterns of COX-1 and COX-2 in the developing rat placenta, in the human term placenta, and in the BeWo choriocarcinoma trophoblast cell line. The data suggest that COX-1 and COX-2 are both expressed in the rat and human placentas. The differential expression patterns, especially the increased expression of COX-1 and COX-2 isoforms near term, suggests that there may be changes in requirement for COX mediated bioactive EFA metabolites with progressing gestation and parturition. The presence of the COX isoforms in BeWo cells was also detected, providing a potentially valuable in vitro model for further investigations of their functional importance in pregnancy. Taken together, these results provide a framework for investigation of functional and physiological significance of COX-1 and COX-2 in guiding proper pregnancy, labor, and fetal development, which could also provide insights into the potential development of therapeutic strategies aimed toward mitigating abnormal pregnancy outcomes, e.g., advancement of potent selective COX inhibitors for therapeutic intervention of preterm contractions.

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