Cyclooxygenase-2 inhibitors constrict the fetal lamb ductus arteriosus both in vitro and in vivo

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Cyclooxygenase-2 inhibitors constrict the fetal lamb ductus arteriosus both in vitro and in vivo. Am J Physiol Regulatory Integrative Comp Physiol 278: R1496–R1505, 2000.—Nonselective cyclooxygenase (COX) inhibitors are potent tocolytic agents; however, they also have adverse fetal effects such as constriction of the fetal ductus arteriosus. Recently, selective COX-2 inhibitors have been used in the management of preterm labor in the hope of avoiding fetal complications. However, both COX-1 and -2 are expressed by cells of the ductus arteriosus. We used fetal lambs (0.88 ± 0.08 gestation) to assess the ability of selective COX-2 inhibitors celecoxib and NS398 to affect the ductus arteriosus. Both selective COX-2 inhibitors decreased PGE2 and 6-ketoPGF1α production in vitro; both inhibitors constricted the isolated ductus in vivo. The nonselective COX-1/COX-2 inhibitor indomethacin produced a further reduction in PG release and an additional increase in ductus tension in vitro. We used a prodrug of celecoxib to achieve 1.4 ± 0.6 µg/ml, mean ± standard deviation, of the active drug in vivo. This concentration of celecoxib produced both an increase in pressure gradient and resistance across the ductus; celecoxib also decreased fetal plasma concentrations of PGE2 and 6-ketoPGF1α. Indomethacin (0.7 ± 0.2 µg/ml) produced a significantly greater fall in ductus blood flow than celecoxib and tended to have a greater effect on ductus resistance in vivo. We conclude that caution should be used when recommending COX-2 inhibitors for use in pregnant women, because COX-2 appears to play a significant role in maintaining patency of the fetal ductus arteriosus.

cyclooxygenase; cyclooxygenase-1; cyclooxygenase-2; prostaglandin E2; prostaglandin I2; indomethacin; celecoxib

A PATENT DUCTUS ARTERIOSUS is essential for fetal wellbeing, because it allows the right ventricular output to bypass the high-resistance pulmonary vascular bed (17). The fetal ductus arteriosus synthesizes two important vasodilatory PGs [PGE2 and prostacyclin (PGI2)] that play a major role in maintaining its patency in utero (4, 8, 29). The enzyme cyclooxygenase (COX) converts arachidonic acid to PGH2, which is then further metabolized to various PGs and thromboxanes.

Inhibition of COX inhibits PG production and produces constriction of the ductus arteriosus (27, 25) in utero. With advancing gestation, the fetal ductus becomes more reactive to COX inhibition (10, 37). In some cases, this can lead to right ventricular failure.

COX exists in two isoforms: COX-1 and -2. COX-1 is constitutively expressed by most tissues and seems to be responsible for the majority of PG production in the adult (28). COX-2 is an inducible form of the COX enzyme, which is stimulated by proinflammatory agents (21, 22). In contrast to COX-1, selective inhibition of COX-2 does not seem to be associated with adverse effects on the gastrointestinal tract or blood platelets (2, 14, 15, 33, 34). It has been hypothesized that selective COX-2 inhibitors may have the same anti-inflammatory effects as nonselective COX inhibitors, without their unwanted side effects (36). Celecoxib, a selective COX-2 inhibitor, has been approved recently by the Food and Drug Administration for the treatment of arthritis in humans.

Indomethacin, a nonselective inhibitor of both COX-1 and -2, has been used as a tocolytic agent since the mid-1970’s. Unfortunately, it crosses the placenta and causes constriction of the fetal ductus arteriosus (11, 25). Recently, COX-2 has been found to play a significant role in the process of parturition (18). This finding has led some investigators to use selective COX-2 inhibitors in the management of preterm labor (32) in the hope of avoiding fetal complications. Unfortunately, there is limited information about the effects of COX-2 inhibition on the fetus. Although COX-2 is induced by cytokines, it is also constitutively expressed by certain organs during fetal development (16, 18, 31). We have recently shown that both COX-1 and -2 are expressed by cells of the fetal lamb ductus arteriosus; in addition, both COX-1 and -2 contribute to ductus arteriosus PG production in vitro (3). The relative roles of COX-1 and -2 in vivo remain to be addressed. Although nonselective inhibitors of both COX-1 and -2, like indomethacin, constrict the ductus in vivo, it may be that the activity of either isoenzyme is sufficient to maintain ductus patency. For example, fetal knockout mice lacking either COX-1 (20) or -2 (27a) have not been reported to have ductus-related problems in utero.

In the following study, we assessed the ability of selective COX-2 inhibitors celecoxib and NS398 to...
affect PG production and contractility of the fetal lamb ductus in vitro and in vivo. We compared their effects with the nonselective COX inhibitor indomethacin. We observed that COX-2 plays a significant role in maintaining patency of the fetal ductus arteriosus.

METHODS

In Vivo Studies

Animals and surgical preparation. Thirteen pregnant sheep (mixed Western breed) were studied at 127–131 days gestation (full term is ~145 days). The surgical preparation has been described in detail previously (12). Briefly, intravenous anesthesia with ketamine hydrochloride (0.2–0.4 mg·kg\(^{-1}\)·min\(^{-1}\)) and diazepam (0.001 mg·kg\(^{-1}\)·min\(^{-1}\)), a midline laparotomy was performed on the ewe, and the fetus was exposed through a small uterine incision. A skin incision was made after administering local lidocaine anesthesia in the fetal forelimb, and catheters were advanced into the ascending aorta from the brachial artery and into the superior vena cava from the brachial vein. The incision was closed, and a new incision was made over the left chest of the fetus. After opening the pericardium, catheters were inserted directly into the main pulmonary artery and a 4- to 6-mm Doppler flow transducer (Transonic Systems, Ithaca, NY) was placed around the ductus arteriosus. The thoracotomy and fetal skin were closed. A catheter was placed in the amniotic cavity, and the uterine incision was closed after replacing amniotic fluid losses with warm saline and administering antibiotics into the amniotic cavity (penicillin G and gentamicin sulfate). All vascular catheters were sealed with heparin and exteriorized to the left flank of the ewe along with the transducer cable. The laparotomy was closed in layers, and the ewe was returned to the cage for recovery. Antibiotics (penicillin G and gentamicin sulfate) were administered intravenously daily to the ewe and into the amniotic cavity.

An additional 17 pregnant sheep were used to determine the dosing regimen needed to achieve the desired fetal plasma concentrations of indomethacin and celecoxib. These fetuses had placement of the systemic arterial and venous vascular catheter, but no thoracotomy.

Dosing regimen for indomethacin and celecoxib. During the last 40% of human gestation, indomethacin crosses the placenta easily and the maternal/fetal serum ratio is 0.97 ± 0.07 (mean ± SD) (26, 35). After maternal indomethacin therapy, mean maternal indomethacin concentrations of 0.688 ± 0.139 µg/ml (1.9 ± 0.4 × 10\(^{-6}\) M) have been associated with fetal ductus arteriosus constriction (25). This concentration range has also been reported to produce effective closure of the neonatal ductus arteriosus when preterm infants are treated with 0.2 mg/kg indomethacin (1). Therefore, we planned to achieve basal indomethacin concentrations between 0.5 and 0.8 µg/ml (1.4 and 2.2 × 10\(^{-6}\) M). Indomethacin (Sigma Chemical, St. Louis, MO) was dissolved in 50 mM Tris-HCl (pH 8) and infused into the fetal or maternal vein. Indomethacin was rapidly cleared from the fetal plasma (half-life = 62.5 ± 3.5 min, n = 3) after a bolus dose to the fetus of 1 mg/kg (fetal body weight). Conversely, a 2-mg/kg (maternal body weight) bolus dose to the mother produced low fetal concentrations (maximum concentration < 0.08 µg/ml) over the next 4 h. In contrast, continuous infusions of indomethacin into the fetus (10 ml/h; dose 0.1–0.5 mg·kg\(^{-1}\)·h\(^{-1}\)) produced a rapid and stable concentration in the fetus (Fig. 1). We chose a fetal dosing rate of 0.2 mg·kg\(^{-1}\)·h\(^{-1}\) (fetal body weight) for subsequent studies because this produced stable fetal concentrations of 0.654 ± 0.240 µg/ml (1.8 ± 0.7 × 10\(^{-6}\) M; Fig. 1).

Celecoxib is a highly selective COX-2 inhibitor. The recommended clinical doses of celecoxib (200 and 400 mg/day) produce significant anti-inflammatory effects in osteoarthritis and rheumatoid arthritis (33). The maximal plasma concentrations (0.7 and 1.4 µg/ml, respectively) produced by these doses do not inhibit COX-1 activity in animal studies (34) or in humans (33). Therefore, we planned to study the behavior of the ductus arteriosus when fetal plasma concentrations were between 0.7 and 1.4 µg/ml. In preliminary trials, intravenous infusions failed to achieve the desired plasma concentration range despite the use of several drug-carrier systems (polyethylene glycol-400, β-cyclodextrin sulfobutyl ether). Therefore, we used the water-soluble, biologically inactive prodrug of celecoxib, SC309A, which is converted to active celecoxib by fetal plasma esterases (data not shown). In three preliminary studies, we found that continuous intravenous infusions of SC309A (20 and 30 mg·kg\(^{-1}\)·h\(^{-1}\)) would produce the desired circulating concentrations of celecoxib (see RESULTS). When the infusion of SC309A was discontinued, the half-life of celecoxib in the fetal plasma was 64 ± 10 min.

Experimental protocol. After a 48-h recovery period, the ewe was placed in a study cage and allowed free access to food and water during the experiment. Blood gases and hemodynamic measurements were collected during a 30-min control period. After this, six fetuses were continuously infused with a “low dose” of SC309A [19 ± 5 mg·kg\(^{-1}\)·h\(^{-1}\) (means ± SD), 10 ml/h, after a priming dose of 19 mg/kg] and seven fetuses with a “high dose” [31 ± 8 mg·kg\(^{-1}\)·h\(^{-1}\), 10 ml/h, after a priming dose of 31 mg/kg] for the next 5 h. Within 14 h of discontinuing the infusion, blood gases and hemodynamic parameters had returned to preinfusion values and celecoxib could no longer be detected in the fetal plasma. Nine of these thirteen fetuses (3 low dose/6 high dose) subsequently received an intravenous infusion of indomethacin (0.18 ± 0.02 mg·kg\(^{-1}\)·h\(^{-1}\), 10 ml/h) after a priming dose of 0.18 mg/kg given over 10 min. This was performed 2 days after the initial SC309A study in an attempt to avoid drug interactions. At the end of the infusion studies, the ewe and fetus were given a lethal dose of pentobarbital sodium followed by bilateral thoracotomy. At necropsy, the fetus was weighed, and catheter and flow transducer placement was confirmed. There were no significant differences between the three groups in fetal weights at necropsy (3.75 ± 0.83 kg, n = 13). The estimated fetal weights used during the experiment were based on standardized fetal sheep growth charts established in our laboratory. The final drug dosages used in the infusions were recalculated for the true weights found at necropsy.

Measurements. Arterial and venous pressures were measured by Statham P23 Db pressure transducers (Statham Instruments, Hato Rey, Puerto Rico). Mean pressures were obtained by electrical integration. Ductus arteriosus blood flow was measured with an ultrasonic flowmeter (Transonic Systems). All hemodynamic variables were continuously recorded on a Gould multichannel electrostatic recorder (Gould, Cleveland, OH). Systemic arterial blood gases and pH were measured on a Corning 158 pH/blood gas analyzer (Corning Medical and Scientific, Medfield, MA). Whole blood lactate and glucose concentrations were measured by a 1500 Sport lactate analyzer and 1500 Sidekick glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Ductus arteriosus resistance was calculated as (mean pulmonary arterial pressure minus mean systemic arterial pressure)/ductus arteriosus blood flow per kilogram.
Indomethacin concentrations were determined from 0.1 ml plasma. The samples, after precipitating plasma proteins with 0.5 ml CH₃CN containing an internal standard (Carprofen 0.1 µg/ml), were vortexed and centrifuged, and an aliquot of the supernatant was injected for HPLC analysis into an Altex Ultrasphere Octyl 5-µm (5 cm x 4.6 mm) column using a 40% CH₃CN 0.2% H₃PO₄ (pH 4) mobile phase and detected at 260-nm wavelength with an ultraviolet detector (24). The linear range of detection was 26–4,000 ng/ml.

Celecoxib concentrations were determined from 0.1 ml of plasma; celecoxib prodrug concentrations were determined from 0.1 ml of a dilution of 0.025 ml sample plasma mixed with 0.350 ml control plasma. Samples were treated with 0.25 ml of 0.2 N phosphoric acid. After the addition of 0.05 ml of an internal standard (0.01 mg/ml in a solution of acetonitrile), the sample was extracted with a solid phase extraction column (IST HCX 130 mg sorbent mass) that was previously conditioned with 2 ml of acetonitrile and 2 ml of water. The column was washed with 2 ml of water. The sample was eluted from the column with 1 ml of 1% ammonium hydroxide in methanol. The eluate was evaporated under nitrogen, resolubilized with 0.2 ml of acetonitrile and 0.01 M sodium acetate (50:50, pH 4.1), and analyzed by HPLC using a 0.39 x 15.00-cm Novapak C-18 column with a C-18 guard column (Waters, Milford, MA). The isocratic mobile phase consisted of acetonitrile and 0.01 M sodium acetate (50:50, pH 4.1). The injection volume of the sample was 0.075 ml. The flow rate was 1 ml/min, and the temperature of the column was room temperature. Fluorescence detection was at 240 nm excitation and 380 nm emission. The assay range was 0.05–10.00 µg/ml.

**In Vitro Studies**

Nineteen fetal lambs (between 125 and 137 days) were delivered by cesarean section. The ewe was anesthetized with a constant intravenous infusion of ketamine HCl and diazepam throughout the procedure. The fetus was given ketamine HCl (30 mg/kg im) before rapid exsanguination. The ductus arteriosus was dissected free of loose adventitial tissue and divided into 1-mm-wide rings that were placed in separate 10-ml organ baths and kept in a dark room, as we have described previously (7). Throughout the experiment, the rings were suspended between two stainless steel hooks at 38°C in a modified Krebs solution (in mM: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 0.9 MgSO₄, 1 KH₂PO₄, 11.1 glucose, and 23 NaHCO₃) equilibrated with 5% CO₂ (pH 7.4) balanced with 30% O₂ and 65% N₂. The bath solution was changed every 30 min. Isometric responses of circumferential tension were measured by Grass FT03C force transducers (Quincy, MA). Each
of the rings was stretched initially to a length that results in a maximal contractile response to increases in oxygen tension (6). The rings were stretched during a 15-min interval in medium equilibrated with fetal PO2 (20–34 mmHg, 0.15–0.26 kPa, starting tension). The bath solution was then bubbled with 30% O2-65% N2-5% CO2 (to a PO2 of 175–200 mmHg, 1.31–1.50 kPa) until the tension reached a new plateau (~90–120 min). Inhibitors of COX (celecoxib (30, 33), indomethacin (19, 23), and NS398 (14, 19)) were then added to the bath solution. The specific COX-2 inhibitors (NS398 and celecoxib) were added in concentrations that were specific for their targeted enzymes (14, 19, 30, 33). In all experiments, we allowed the tension in the rings to reach a new steady-state plateau after a drug addition before another experimental agent was added to the bath. After the addition of all contractile drugs, potassium Krebs solution (containing 100 mM KCl substituted for an equimolar amount of NaCl) was used to measure the maximal tension and sodium nitroprusside (10^-4 M) was used to determine the minimal tension that could be developed by the ductus.

The difference in tensions between the maximal and minimal tension was considered the net active tension developed

Fig. 2. Inhibitors of cyclooxygenase (COX) contract fetal lamb ductus arteriosus. Ductus rings were incubated in 30% oxygen. Each ring was exposed to increasing concentrations of either celecoxib or NS398 (COX-2 inhibitors) or nonselective COX inhibitor indomethacin (Indo). Maximal tension was determined with 100 mM KCl-Krebs solution (K+); minimal tension was determined with 10^-4 M sodium nitroprusside (SNP). All tensions were expressed as percent net active tension (means ± SD). Net active tension is measured in grams. Starting tensions: 6 ± 1 (A), 6 ± 1 (B), and 5 ± 2 g (C). Ring weight: 41 ± 9 (A), 55 ± 27 (B), and 57 ± 29 mg (C). *P < 0.01, COX-inhibitor-induced tension vs. O2-induced tension.
by the ring. The difference in tensions between the steady-state tension achieved in 30% oxygen and that at minimal tension was considered the O2 tension. The difference in tensions between the COX inhibitor-induced tension and the steady-state tension achieved in 30% oxygen was considered the COX-inhibitor tension. Changes in tension for each experimental condition were expressed as a percentage of net active tension. The net active tension was always greater than the difference in tension between the maximal tension and the starting tension by 12.69% (P, 0.01, n = 32 rings). This indicates that the ductus rings were actively contracting even at the time of their initial mounting in the organ bath.

In some experiments, PG production by the rings of ductus arteriosus was measured. For these experiments, the bath solution was changed every 40 min. The rings were exposed to two changes of bath solution for each experimental condition.

**Fig. 3.** PGE2 and 6ketoPGF1α production by rings of ductus arteriosus. Height of columns represents means ± SD immunoreactive PGE2 or 6ketoPGF1α released into a 10-ml bath during 40-min incubation period per 100 mg tissue weight. Rings were incubated in 30% oxygen with or without following inhibitors: celecoxib, NS398, and Indo. Ring weight, 48 ± 20 mg; starting tension, 6 ± 1 g. Dashed lines represent limit of detection in assay. *P < 0.05, COX inhibitor vs. control condition.

**Table 1.** Hemodynamic changes in indomethacin-treated lamb fetuses

<table>
<thead>
<tr>
<th>Infusion Time, h</th>
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<tbody>
<tr>
<td><strong>Ductus arteriosus blood flow, ml·kg⁻¹·min⁻¹</strong></td>
<td>235 ± 50</td>
<td>150 ± 74*</td>
<td>218 ± 65</td>
<td>205 ± 82</td>
<td>208 ± 98</td>
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<td><strong>Ductus arteriosus resistance, mmHg·ml⁻¹·min⁻¹·kg⁻¹</strong></td>
<td>0.007 ± 0.006</td>
<td>0.056 ± 0.046*</td>
<td>0.036 ± 0.024*</td>
<td>0.059 ± 0.07*</td>
<td>0.102 ± 0.222*</td>
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<td><strong>Pressure gradient, mmHg</strong></td>
<td>2 ± 1</td>
<td>8 ± 6*</td>
<td>7 ± 4*</td>
<td>9 ± 8*</td>
<td>8 ± 6*</td>
</tr>
<tr>
<td><strong>Pulmonary artery pressure, mmHg</strong></td>
<td>56 ± 7</td>
<td>64 ± 10*</td>
<td>64 ± 7*</td>
<td>63 ± 12*</td>
<td>60 ± 9</td>
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<tr>
<td><strong>Aortic pressure, mmHg</strong></td>
<td>54 ± 7</td>
<td>56 ± 7</td>
<td>57 ± 8</td>
<td>54 ± 9</td>
<td>52 ± 9</td>
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<tr>
<td><strong>pH</strong></td>
<td>7.41 ± 0.02</td>
<td>7.40 ± 0.03</td>
<td>7.39 ± 0.03</td>
<td>7.37 ± 0.05*</td>
<td>7.37 ± 0.08*</td>
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<td><strong>Paco₂, Torr</strong></td>
<td>52.4 ± 3.4</td>
<td>52.6 ± 1.9</td>
<td>53.5 ± 4.5</td>
<td>53.6 ± 5.3</td>
<td>55.1 ± 5.5</td>
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<tr>
<td><strong>Pao₂, Torr</strong></td>
<td>15.7 ± 3.3</td>
<td>16.1 ± 2.4</td>
<td>15.0 ± 2.7</td>
<td>15.7 ± 2.8</td>
<td>14.8 ± 2.7</td>
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<td><strong>Lactate, mM</strong></td>
<td>2.28 ± 0.92</td>
<td>3.06 ± 0.91*</td>
<td>3.19 ± 1.17*</td>
<td>3.52 ± 1.64*</td>
<td>4.22 ± 2.89*</td>
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<td><strong>Glucose, mg/dl</strong></td>
<td>18 ± 6</td>
<td>21 ± 8*</td>
<td>18 ± 8</td>
<td>18 ± 6</td>
<td>23 ± 8</td>
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Data are expressed as means ± SD for n = 9 fetuses. *P < 0.05 comparing baseline value (0 h) with each data point by Wilcoxon's signed rank test; pressure gradient, difference in mean blood pressure between pulmonary artery and aorta. Average concentration of indomethacin was 0.7 ± 0.2 µg/ml.
Table 2. Hemodynamic changes in SC309A/celecoxib-treated lamb fetuses (low concentration range)

<table>
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<tr>
<td>Ductus arteriosus blood flow, ml·kg⁻¹·min⁻¹</td>
<td>206 ± 17</td>
<td>235 ± 19*</td>
<td>187 ± 53</td>
<td>204 ± 26</td>
<td>223 ± 31</td>
<td>223 ± 31</td>
</tr>
<tr>
<td>Ductus arteriosus resistance, mmHg·ml⁻¹·min⁻kg</td>
<td>0.007 ± 0.008</td>
<td>0.024 ± 0.012*</td>
<td>0.030 ± 0.018*</td>
<td>0.030 ± 0.031</td>
<td>0.036 ± 0.041</td>
<td>0.028 ± 0.027*</td>
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<td>Pressure gradient, mmHg</td>
<td>1 ± 2</td>
<td>6 ± 3*</td>
<td>6 ± 3</td>
<td>6 ± 5</td>
<td>7 ± 8</td>
<td>6 ± 5*</td>
</tr>
<tr>
<td>Pulmonary artery pressure, mmHg</td>
<td>57 ± 3</td>
<td>59 ± 7</td>
<td>60 ± 9</td>
<td>58 ± 10</td>
<td>59 ± 11</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>Aortic pressure, mmHg</td>
<td>55 ± 3</td>
<td>53 ± 5</td>
<td>54 ± 6</td>
<td>52 ± 6</td>
<td>52 ± 6</td>
<td>54 ± 2</td>
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<tr>
<td>pH</td>
<td>7.41 ± 0.03</td>
<td>7.41 ± 0.02</td>
<td>7.42 ± 0.03</td>
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<td>7.39 ± 0.06</td>
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<td>(P_{aco_2}), Torr</td>
<td>48.5 ± 3.7</td>
<td>48.8 ± 4.9</td>
<td>47.4 ± 5.9</td>
<td>48.0 ± 7.3</td>
<td>48.5 ± 7.8</td>
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<tr>
<td>(P_{a_2}), Torr</td>
<td>20.6 ± 4.5</td>
<td>19.4 ± 4.1</td>
<td>19.7 ± 3.0</td>
<td>19.5 ± 3.6</td>
<td>20.0 ± 2.9</td>
<td>19.0 ± 3.4</td>
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<tr>
<td>Lactate, mM</td>
<td>1.34 ± 0.43</td>
<td>1.20 ± 0.34*</td>
<td>1.20 ± 0.31</td>
<td>1.16 ± 0.27</td>
<td>1.63 ± 1.14</td>
<td>1.46 ± 0.75</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>19 ± 4</td>
<td>20 ± 4</td>
<td>18 ± 6</td>
<td>15 ± 5</td>
<td>15 ± 4*</td>
<td>15 ± 6</td>
</tr>
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</table>

Data are expressed as mean ± SD for \(n = 6\) fetuses. *P < 0.05 comparing baseline value (0 h) with each data point by Wilcoxon’s signed rank test; pressure gradient, difference in mean blood pressure between pulmonary artery and aorta. Average concentration of celecoxib was 0.5 ± 0.1 µg/ml (see Fig. 4).

The second change in bath solution was collected for PGE₂ or 6ketoPGF₁α (PGI₂ Metabolite) Radioimmunoassay. After the experiment, the rings were removed from the baths, blotted dry, and their wet weights determined.

PGE₂ and 6ketoPGF₁α (PGI₂ Metabolite) Radioimmunoassay

To determine the amount of PGE₂ and 6ketoPGF₁α, the stable metabolite of PGI₂, samples of bath solution or plasma were acidified to pH 3 with glacial acetic acid and applied to octadeacysil silica columns. The columns were washed with 15% aqueous ethanol followed by petroleum ether and then eluted with mthyl formate. Methyl formate was dried (Speed Vac) and PGs reconstituted in PBS for radioimmunoassay (16, 31). The recovery of radioactive PGE₂ and 6ketoPGF₁α was >96%, and the interassay variability was <5%.

Chemicals

The following compounds were used: celecoxib (mol wt 381.4) and SC309A (a prodrug of celecoxib, mol wt 459.4; G. D. Searle, Skokie, IL), indomethacin (Sigma), NS398 (Cayman Chemical, Ann Arbor, MI), and a radioimmunoassay kit for PGE₂ and 6ketoPGF₁α (Advanced Magnetics, Boston, MA).

For the in vitro studies, indomethacin was prepared in ethanol (42 · 10⁻³ M). Both NS398 (51 · 10⁻³ M) and celecoxib (53 · 10⁻³ M) were prepared in DMSO. The final concentration of ethanol or DMSO in the bath solution did not affect tissue contractility. All other compounds were dissolved directly in Krebs solution. Statistics

Statistical analyses were performed by the appropriate Student’s t-test and by analysis of variance. Scheffé’s test was used for post hoc analyses. Nonparametric data were compared with Wilcoxon’s signed-rank test. Values are expressed as means ± SD. Drug doses refer to their final molar concentration in the bath. RESULTS

In Vitro Studies

In the presence of 30% O₂, rings of ductus arteriosus contracted to a tension that was ~30% of the rings' net active tension (Fig. 2). The nonselective COX inhibitor indomethacin caused an additional dose-dependent increase in tension (Fig. 2C). Both COX-2 inhibitors celecoxib and NS398 caused dose-dependent increases in ductus tension at concentrations that were specific for COX-2 (14, 19, 23, 30, 33, 34). At the highest concentrations tested, the contraction caused by celecoxib (5 · 10⁻⁶ M) was 51% of that caused by NS398 (5 · 10⁻⁶ M). After the contractions induced by the selective COX-2 inhibitors, indomethacin produced an additional significant (\(P < 0.01\)) increase in tension (Fig. 2, A and B). SC309A, the prodrug of celecoxib, had no effect on ductus tension at concentrations of 1, 10, or 50 µg/ml (2.2, 22, or 109 · 10⁻⁶ M, respectively) (\(n = 4\)).

Table 3. Hemodynamic changes in SC309A/celecoxib-treated lamb fetuses (high concentration range)

<table>
<thead>
<tr>
<th>Infusion Time, h</th>
<th>0</th>
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</thead>
<tbody>
<tr>
<td>Ductus arteriosus blood flow, ml·kg⁻¹·min⁻¹</td>
<td>250 ± 78</td>
<td>257 ± 89</td>
<td>233 ± 94</td>
<td>231 ± 101</td>
<td>238 ± 104</td>
<td>241 ± 109</td>
</tr>
<tr>
<td>Ductus arteriosus resistance, mmHg·ml⁻¹·min⁻kg</td>
<td>0.006 ± 0.004</td>
<td>0.015 ± 0.005*</td>
<td>0.046 ± 0.061*</td>
<td>0.054 ± 0.066*</td>
<td>0.046 ± 0.057*</td>
<td>0.046 ± 0.057*</td>
</tr>
<tr>
<td>Pressure gradient, mmHg</td>
<td>2 ± 1</td>
<td>4 ± 1*</td>
<td>8 ± 6*</td>
<td>8 ± 5*</td>
<td>7 ± 3*</td>
<td>7 ± 3*</td>
</tr>
<tr>
<td>Pulmonary artery pressure, mmHg</td>
<td>57 ± 5</td>
<td>58 ± 6</td>
<td>60 ± 8*</td>
<td>58 ± 6</td>
<td>56 ± 6</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>Aortic pressure, mmHg</td>
<td>55 ± 6</td>
<td>54 ± 6</td>
<td>52 ± 8</td>
<td>50 ± 8</td>
<td>49 ± 8*</td>
<td>49 ± 8*</td>
</tr>
<tr>
<td>pH</td>
<td>7.42 ± 0.03</td>
<td>7.38 ± 0.04*</td>
<td>7.36 ± 0.05*</td>
<td>7.35 ± 0.06*</td>
<td>7.32 ± 0.09*</td>
<td>7.32 ± 0.09*</td>
</tr>
<tr>
<td>(P_{aco_2}), Torr</td>
<td>50.1 ± 5.9</td>
<td>53.7 ± 3.3</td>
<td>56.1 ± 6.3*</td>
<td>57.6 ± 5.0*</td>
<td>58.2 ± 7.6*</td>
<td>58.3 ± 7.6*</td>
</tr>
<tr>
<td>(P_{a_2}), Torr</td>
<td>16.8 ± 3.4</td>
<td>16.0 ± 2.6</td>
<td>15.2 ± 3.7</td>
<td>14.9 ± 3.5</td>
<td>15.4 ± 2.6*</td>
<td>15.2 ± 3.1*</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>2.25 ± 0.87</td>
<td>2.54 ± 1.47</td>
<td>2.91 ± 1.57</td>
<td>3.63 ± 2.29</td>
<td>3.99 ± 2.38</td>
<td>4.01 ± 1.99*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>15 ± 5</td>
<td>15 ± 5</td>
<td>15 ± 5</td>
<td>16 ± 5</td>
<td>15 ± 4</td>
<td>16 ± 4</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for \(n = 7\) fetuses. *P < 0.05 comparing baseline value (0 h) with each data point by Wilcoxon’s signed rank test; pressure gradient, difference in mean blood pressure between pulmonary artery and aorta. Average concentration of celecoxib was 1.4 ± 0.6 µg/ml (see Fig. 4).
Both the nonselective COX inhibitor indomethacin and the selective COX-2 inhibitors celecoxib and NS398 decreased the rate of release of PGE$_2$ and the stable metabolite of prostacyclin, 6ketoPGF$_{1\alpha}$, into the surrounding bath solution (Fig. 3). After the reduction in PGE$_2$ and 6ketoPGF$_{1\alpha}$ release by the two COX-2 inhibitors, indomethacin produced a further reduction in the release of both PGs ($P < 0.05$; Fig. 3). The changes in PG release paralleled the changes we saw in ductus tension (Figs. 2 and 3).

**In Vivo Studies**

Before starting drug infusions, the baseline fetal hemodynamic variables, systemic arterial blood gases, pH, lactate, and glucose concentrations were similar among the three study groups (indomethacin (Table 1); low-dose SC309A/celecoxib (Table 2); high-dose SC309A/celecoxib (Table 3)). These variables were within the accepted range for our laboratory. Normal saline or 50 mM Tris-HCl (pH 8 buffer) were infused (10 ml/h) into a separate group of nine fetuses for 5 h to study the effects of the infusion protocols on the fetus; there were no significant changes in any of the hemodynamic or metabolic variables during either infusion (data not shown).

In the indomethacin-treated fetuses, ductus arteriosus blood flow decreased during the first hour of the infusion, but returned toward baseline over the next 3 h (Table 1). The pressure gradient and calculated resistance across the ductus increased within 15 min of starting the infusion (data not shown) and persisted throughout the infusion (Table 1). There was a significant increase in lactate and decrease in pH during the indomethacin infusion. These findings are similar to previous reports (13).

Continuous infusions of the prodrug SC309A produced a gradual increase in plasma celecoxib concentrations (Fig. 4); by 1 h of infusion, celecoxib concentrations had only reached 66% of the desired low dose and high dose concentrations. Because of the delay in reaching the desired celecoxib concentrations, the infusion and measurements were continued 1 h longer than the indomethacin experiments. During the infusions, maximal concentrations of the prodrug SC309A were 51 ± 19 (110 ± 41 × 10$^{-6}$ M) and 25 ± 6 µg/ml (54 ± 13 × 10$^{-6}$ M) in the high and low dose groups, respectively.

At high concentrations of celecoxib, there was a small, transient drop in ductus flow, which returned toward baseline by the end of the infusion (Fig. 5). On the other hand, there was a sustained increase in the pressure gradient and resistance across the ductus that persisted throughout the infusion (Table 3). The maximum increase in pressure gradient and resistance at the higher concentrations were not significantly different from those reached during the indomethacin infusions (Fig. 5). The fetuses also developed a progressive drop in blood pH associated with an increase in Pa$_{CO_2}$ and lactate (Table 3). Even at low concentrations, celecoxib produced a significant increase in pressure gradient and resistance across the ductus that persisted throughout the infusion in five of the six fetuses (Table 2; Fig. 5). There were no sustained changes in pH or lactate during the low dose celecoxib infusions. Indomethacin lowered circulating concentrations of PGE$_2$ and 6ketoPGF$_{1\alpha}$ by 50 ± 33% and 66 ± 29%, respectively. The minimum concentration was reached 2.1 ± 0.7 h after starting the infusion. Celecoxib also lowered circulating concentrations of PGE$_2$ and 6ketoPGF$_{1\alpha}$ (Fig. 6). Celecoxib lowered plasma PGE$_2$ concentrations by 37 ± 11% (high concentration) and 46 ± 20% (low concentration); 6ketoPGF$_{1\alpha}$ concentrations were reduced by 60 ± 20% (high concentration) and 60 ± 28% (low concentration). The time to reach the minimum concentration of either PGE$_2$ or 6ketoPGF$_{1\alpha}$ was 3.2 ± 1.3 h in the low concentration group and 2.3 ± 1.2 h in the high concentration group.

**Discussion**

We have shown previously that both COX-1 and -2 are expressed by the fetal lamb ductus arteriosus and play a role in regulating ductus contractility in vitro (3). In the present study, we investigated the actions of two
selective COX-2 inhibitors, celecoxib and NS398, and compared them to the nonselective inhibitor indomethacin. NS398 and celecoxib decreased PG production and produced a significant increase in tension in isolated rings of lamb ductus arteriosus at concentrations that are specific for COX-2 (Figs. 2 and 3) (14, 19, 33, 34). Whether endogenous PGs, made by the ductus arteriosus, are solely responsible for its regulation in vivo is still unclear. Circulating PGs in the fetus are markedly elevated compared with the newborn or adult (5) and may contribute to in vivo patency. We found that celecoxib concentrations that normally are achieved during the treatment of arthritis and that are specific for COX-2 (33) also decreased fetal plasma concentrations of PGs (Fig. 6) and produced significant constriction of the fetal lamb ductus in vivo (Tables 2 and 3; Fig. 5). However, despite a similar reduction in circulating PG levels in both celecoxib- and indomethacin-treated fetuses, indomethacin tended to have a greater effect on ductus closure in vivo (see below); this may suggest a role for locally produced PGs in regulating ductus tone. Whereas these findings do not clarify whether circulating PGs are more or less important than locally produced PGs, they do point out that COX-2 has an important role in vasoregulation of the fetal ductus arteriosus in vivo.

In our in vivo studies, an inactive prodrug of celecoxib (SC309A) was used to achieve the desired circulating concentrations of celecoxib. It is unlikely that SC309A was responsible for the ductus constriction, because SC309A had no effect on ductus tone in vitro. It is possible that the importance of COX-2, in maintaining ductus patency in vivo, might have been overestimated by our experimental model. Injury of the vessel

**Fig. 5. Changes in ductus arteriosus resistance (A), mean pulmonary arterial pressure (mPAP)-to-mean systemic arterial pressure (mSAP) gradient (B), and ductus arteriosus blood flow (C) in fetal lambs infused with SC309A (celecoxib) or Indo. Values are means ± SD. Values reported in A and B are maximum values and in C, minimum values obtained during infusions. These are compared with preinfusion values. Celecoxib (low concentration), n = 6; celecoxib (high concentration), n = 7; Indo, n = 9. *P < 0.05, **P < 0.01 vs. preinfusion values. Duration of infusion to reach maximum ductus resistance (A) were: celecoxib (low) = 125 ± 66 min, celecoxib (high) = 197 ± 75 min, and Indo = 118 ± 80 min; maximum pressure gradient (B) were: celecoxib (low) = 75 ± 41 min, celecoxib (high) = 163 ± 75 min, and Indo = 136 ± 77 min; minimum ductus blood flow (C) were: celecoxib (low) = 135 ± 116 min, celecoxib (high) = 167 ± 91 min, and Indo = 101 ± 77 min.
fetuses (128–2, produced a significantly greater inhibition of endogenous PG production (Fig. 3) and a significantly greater increase in ductus tone in vitro (Fig. 2) than did the selective COX-2 inhibitors. These results are similar to results we previously have published (3). Similarly, indomethacin produced a significantly greater fall in ductus blood flow in vivo. Although the difference did not reach statistical significance, indomethacin also tended to have a greater effect on ductus resistance when compared with celecoxib (Fig. 5). These findings are consistent with both COX-1 and -2 having important functions in ductus arteriosus vasoregulation (3).

During the indomethacin infusions, fetuses frequently developed a lactic acidosis (Table 1). The etiology of this is unclear; it does not appear to be due to ductus constriction, because indomethacin still produces a lactic acidosis even after the ductus wall has been infiltrated with Formalin, making constriction impossible (data not shown). High concentrations of celecoxib also caused a lactic acidosis (Table 3). This was not observed in the low celecoxib concentration group. Whether celecoxib will have less constrictive effects on the ductus and will be less likely to produce lactic acidosis than indomethacin, in clinical practice, will depend on the drug levels needed to achieve equivalent effects. Even at low celecoxib concentrations, fetuses may still develop a lactic acidosis; in preliminary studies, we have seen a marked metabolic acidosis develop if fetuses were hemodynamically compromised before the administration of low concentrations of celecoxib (data not shown).

Perspectives

At this time, it is premature to extrapolate our in vivo results to the human fetus. In rats, celecoxib crosses the placenta; fetal concentrations are similar to maternal concentrations (data not shown). However, celecoxib’s pharmacokinetic profile in the human fetus is unknown. In addition, fetuses in our experimental model were exposed to maximal drug levels for longer durations than would be expected from the dosing regimens used in the clinical trials. The current findings also may represent a species-specific expression of COX-2 that is unique to the fetal lamb ductus. We have previously shown that COX-2 expression is very low in the fetal pig ductus (although a high level of expression was found in the neonatal ductus of the pig). No data are available currently for the human ductus. We have recently found that another fetal primate, the baboon, expresses COX-1 and -2 in its ductus in amounts and distribution that are similar to what we have observed in the fetal lamb (R. I. Clyman and S. Seidner, unpublished results). Until further studies are available, we feel that caution should be used when recommending COX-2 inhibitors for use in pregnant women (32).

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