Persistent responsiveness of the neonatal ductus arteriosus in immature lambs: a possible cause for reopening of patent ductus arteriosus after indomethacin-induced closure

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ABSTRACT Reopening of the ductus arteriosus after successful indomethacin-induced closure has become a major problem with indomethacin treatment. In full-term human newborns and lambs, the ductus behaves like ischemic tissue after its initial constriction. Its ability to continue to relax or contract depends on the amount of left-to-right shunt through the ductus lumen. To see if ductus constriction in preterm lambs would produce the same loss of ductus responsiveness, we delivered 42 lambs by cesarean section and ventilated them for 6.6 ± 0.5(SE) hr. We measured ductus arteriosus resistance and left-to-right shunt with the use of radionuclide-labeled microspheres. After the hemodynamic measurements were obtained, the ductus was studied in vitro. Immature lambs were more likely to have reactive ductus (after their initial ductus constriction) than were more mature lambs. This was due to a diminished degree of ductus constriction as well as persistence of ductus responsiveness in immature lambs when compared with more mature lambs. This persistence of ductus responsiveness in immature lambs after ductus constriction may account for the high reopening rate in preterm infants after successful indomethacin-induced closure.


SEVERAL recent controlled trials have demonstrated that indomethacin is effective in treating the patent ductus arteriosus (PDA) in preterm infants. However, it has become apparent that once the ductus arteriosus has been closed by indomethacin, it may reopen at a later date, with recurrence of the left-to-right shunt. The reported incidence of reopening of the ductus arteriosus after successful indomethacin treatment has varied from 20% to 100%. These studies are difficult to compare since indomethacin was given at different postnatal ages to infants of different gestational ages and in different amounts per dose. However, reopening of the ductus arteriosus did not appear to be associated with excessive fluid therapy before or after treatment or with low plasma concentrations of indomethacin during the initial therapy.

The mechanisms regulating patency and closure of the ductus arteriosus are only partially understood. There appears to be a balance between the constricting effect of oxygen and the vasodilating effect of prostaglandins. The marked sensitivity of the lamb ductus to prostaglandin E₂ (PGE₂) suggests that this is the most important prostanoid for regulation of vessel patency in this species. However, within a few days after birth the relaxing effects of PGE₂ and hypoxia are lost and the ductus remains "irreversibly" closed thereafter. Fay and Cooke have proposed that irreversibility reflects a mechanical restraint imposed by cellular necrosis, loss of an intact endothelium, and intermingling of cells from opposing walls such that there was no longer an anatomic lumen. Because necrosis and cell dislocation are limited to the central regions of the ductus, several authors have suggested that these processes result from interruption of luminal blood flow.

We have previously observed a decrease in the ability of the ductus arteriosus to dilate and contract that occurs within the first hours after postnatal ductus constriction and before the loss of an anatomically patent lumen in human newborns and in full-term lambs.
This generalized loss of ductus responsiveness was directly related to the degree of prior ductus constriction and the subsequent reduction in luminal blood flow. The loss of ductus responsiveness was independent of arterial Pao2, pH, and PGE2 concentrations. In addition, this loss of responsiveness prevented the ductus arteriosus from reopening once it had constricted.

The purpose of the study reported here was to determine if premature lambs developed the same loss of ductus responsiveness after postnatal constriction as did late-gestation lambs.

**Methods**

**Neonatal lambs — in vivo.** Forty-two neonatal lambs were delivered by cesarean section at between 120 and 147 days gestation (147 days was full term in our lambs) from time-dated pregnant ewes. In each neonate polyvinyl catheters were placed, via peripheral vessels, in the ascending aorta proximal to the ductus arteriosus and in the descending aorta, inferior vena cava, left ventricle, pulmonary artery, and superior vena cava as previously described. Each fetus was intubated before separation from the umbilical circulation. Fetuses were treated with 50 mg/kg surfactant lipid diluted in 0.1N NaCl by instillation into the endotracheal tube. The surfactant was isolated from lungs of healthy adult ewes. We have previously shown that surfactant treatment of lambs has no effect on the behavior of the ductus arteriosus in vivo or in vitro.

After separation from the umbilical circulation the newborn lambs were paralyzed with 0.4 mg iv pancuronium and ventilated with a timed-cycle pressure–limited infant ventilator. The initial ventilator settings were 28 cm H2O peak inspiratory pressure and 3 cm H2O positive end-expiratory pressure at a respiratory rate of 40/min. The initial inspired gas was 100% oxygen. The ventilator settings and fraction of inspiratory oxygen were altered to maintain a PaO2 between 80 and 150 torr, Paco2 less than 40 torr, and arterial pH between 7.35 and 7.45. Rectal temperature was monitored and the temperature was maintained at 38° to 39°C by a radiant warmer. Lambs were kept alive for at least 3 hr after delivery (the mean survival time, 6.6 ± 0.5 hr [± SE, n = 42]). We measured cardiac output and its distribution by injecting radionuclide-labeled microspheres, 15 μm diameter, into the left ventricle of each lamb. Reference samples were drawn from the ascending and descending aorta. Heparinized maternal blood was used to replace blood loss caused by sampling. Right-to-left ductal shunting was evaluated by microsphere injections into the superior vena cava; there was no right-to-left ductal shunting in any of these animals. After the experiment each animal was autopsied to check catheter placement. The lungs were dissected, weighed, and processed for isotope counting as previously described. Left ventricular output and blood flow through the ductus arteriosus were calculated with use of the concentration of microspheres in the reference sample, the total number of microspheres recovered from the whole animal, and the total number of microspheres in the lungs. An assumed bronchial blood flow of 2% of left ventricular output was subtracted from the measured lung blood flows. The microsphere measurements were used to estimate left-to-right ductal shunts. Microsphere measurements were made at 1 hr after delivery and were repeated at one or two hourly intervals as long as the animal survived. Up to nine different microsphere injections were made in each animal.

Since no right-to-left ductal shunts were observed, systemic blood flow equalled left ventricular output minus the left-to-right shunt through the ductus arteriosus. We estimated the resistance to flow across the ductus by dividing the mean descending aortic–pulmonary arterial pressure difference (torr) by ductus arteriosus flow (liters/kg-min). Systemic vascular resistance was estimated by dividing the mean descending aortic–inferior vena caval pressure difference by systemic blood flow. Hemodynamic measurements made during the last 2 to 3 hr before the animals were killed were averaged to determine the degree of ductus constriction and reduction in luminal blood flow in each animal.

**Lamb ductus — in vitro.** After completion of the study in vivo, the lambs were killed rapidly by exsanguination. The ductus was dissected free from the adventitial tissue and divided into 1 mm thick rings that were placed in separate 150 ml organ baths kept in the dark within an enclosed box. The rings were suspended between two stainless steel hooks in a modified Krebs Tris solution at 38°C. Isometric responses of circumferential tension were measured by Grass FT03C force transducers.

Each of the rings was stretched to an initial length that would result in a maximal contractile response to increases in oxygen tension. Initially the O2 of the bath solution was maintained at 20 to 25 torr and the rings were allowed to equilibrate for about 1 hr until a steady tension developed. The bath solution was then bubbled with 100% O2 (to a PO2 of 680 to 700 torr) until the tension achieved a new plateau. Indomethacin was added to the bath in a final concentration of 2 μg/ml (5.6 × 10^-6M) and the rings were allowed to achieve a new increase in tension over the next hour. A cumulative dose response to PGE2 was obtained in the oxygen- and indomethacin-contracted rings; we allowed the tension to achieve a new plateau before higher concentrations of PGE2 were added. After the experiment the tissues were removed from the baths and blotted dry and their wet weights were determined. The tension developed in the ring was expressed as force per unit cross-sectional area (g/mm²).

To allow comparison with the activity in vitro of the 42 ductus that were isolated from newborn lambs after several hours of ventilation, 38 fetuses (120 to 146 days gestation) were delivered by cesarean section and killed before they took a breath. Their ductus were divided into rings and suspended in the organ baths by the same method as described for the newborn ductus.

**Data analysis.** All values are given as mean ± SE. Significance was tested by a two-tailed paired t test. Fisher exact test, or chi-squared analysis, when appropriate.

**Results**

We studied neonatal lambs of different gestational ages to see how prematurity might alter the normal events that occur in the ductus arteriosus after birth. Table 1 shows the degree of ductus arteriosus constriction that occurred in vivo in the 27 immature lambs (120 to 134 days gestation) and the 15 near-term lambs (135 to 147 days). We defined the degree of ductal constriction as either ‘tight’ or ‘moderate.’ In tightly constricted ductus there was both left-to-right shunting that was less than 10% of the systemic blood flow and calculated ductus resistance greater than 1000 torr/liter-min·kg. In moderately constricted ductus left-to-right shunting was greater than 10% of the systemic flow and resistance was less than 300 torr/liter-min·kg.
Immature lambs produced significantly weaker degrees of ductus constriction than the more mature lambs: Only 26% (7/27) of the immature lambs developed a tightly constricted ductus, whereas 67% (10/15) of the more mature animals were able to do so.

After the series of hemodynamic measurements, the animals were killed to see how the effects of ductus constriction in vivo altered the responsiveness of the ductus arteriosus in vitro. With increasing degrees of ductus constriction in vivo, there was a significant limitation of the ability of the more mature (≥135 days) ductus to actively contract with oxygen and indomethacin or to relax with PGE$_2$ (table 2). Ductus isolated from near-term lambs (≥135 days), which were tightly constricted in vivo after several hours of ventilation, could generate only one-seventh the active tension in vitro (when exposed to oxygen and indomethacin) of that generated by ductus isolated from near-term fetuses that were never ventilated and that had patent ductus in vivo. In addition, PGE$_2$ was only 58% as effective in relaxing ductus isolated from near-term newborn lambs (in which ductus were tightly constricted several hours after delivery) as it was in relaxing those isolated from near-term fetal lambs (table 2).

In contrast, both the ability to actively contract as well as to actively relax was significantly greater in the ductus arteriosus of immature lambs (120 to 134 days) when compared with the ductus of more mature lambs (135 to 147 days) after similar degrees of constriction.

### TABLE 1

Characteristics of neonatal lambs with moderate and tight ductus constriction

<table>
<thead>
<tr>
<th>Degree of ductus constriction in vivo</th>
<th>$Q_{ductus}/Q_{systemic}$ (%)</th>
<th>$R_{ductus}$ (torr/l·min·kg)</th>
<th>$R_{ductus}/R_{sys}$ (%)</th>
<th>Survival (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambs 120–134 days gestation (n = 20)</td>
<td>45 ± 5</td>
<td>115 ± 26</td>
<td>32 ± 7</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>Lambs 135–147 days gestation (n = 5)</td>
<td>36 ± 7</td>
<td>127 ± 50</td>
<td>36 ± 15</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td>Tight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambs 120–134 days gestation (n = 7)</td>
<td>7 ± 2</td>
<td>3508 ± 1158</td>
<td>1439 ± 579</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>Lambs 135–147 days gestation (n = 10)</td>
<td>7 ± 1</td>
<td>3944 ± 836</td>
<td>1227 ± 308</td>
<td>6.0 ± 0.9</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Hemodynamic values for each lamb were obtained by averaging the measurements made during the last 2 to 3 hr before they were killed. See text for definitions of tight and moderate constriction.

$Q_{ductus}$ = left-to-right shunt through the ductus; $Q_{systemic}$ = systemic blood flow; $R_{ductus}$ = resistance to flow across the ductus; $R_{sys}$ = systemic vascular resistance.

### TABLE 2

Ductus arteriosus contractility: response in vitro vs that in vivo

<table>
<thead>
<tr>
<th>Degree of ductus constriction in vivo</th>
<th>Active tension (O$_2$ + indomethacin, g/mm$^2$)</th>
<th>Maximum PGE$_2$ inhibition of active tension (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120–134 days</td>
<td>135–147 days</td>
</tr>
<tr>
<td></td>
<td>135–147 days</td>
<td></td>
</tr>
<tr>
<td>Fetal</td>
<td>4.71 ± 0.24</td>
<td>4.4 ± 0.32</td>
</tr>
<tr>
<td>(n)</td>
<td>(23)</td>
<td>(15)</td>
</tr>
<tr>
<td>Neonatal: moderate</td>
<td>3.07 ± 0.28</td>
<td>1.79 ± 0.42$^a$</td>
</tr>
<tr>
<td>(n)</td>
<td>(20)</td>
<td>(5)</td>
</tr>
<tr>
<td>Neonatal: tight</td>
<td>1.11 ± 0.20</td>
<td>0.60 ± 0.10$^a$</td>
</tr>
<tr>
<td>(n)</td>
<td>(7)</td>
<td>(10)</td>
</tr>
</tbody>
</table>

Values are mean ± SE. See text for definition of moderate and tight constriction. Fetal ductus came from animals that were never ventilated. All rings first were allowed to equilibrate in a low-P$_O_2$ environment (20 to 25 torr); steady-state low P$_O_2$ tensions were: fetal, 120–134 days = 3.14 ± 0.37 g/mm$^2$; fetal, 135–147 days = 4.38 ± 0.29 g/mm$^2$; moderate constriction, 120–134 days = 3.00 ± 0.23 g/mm$^2$; moderate constriction, 135–147 days = 2.86 ± 0.45 g/mm$^2$; tight constriction, 120–134 days = 2.34 ± 0.33 g/mm$^2$; tight constriction, 135–147 days = 2.70 ± 0.33 g/mm$^2$. The oxygen + indomethacin tension is the difference between the steady-state tensions at low P$_O_2$ and the tensions after the rings had been contracted with high P$_O_2$ (680 to 700 torr) and indomethacin.

$^ap < .05$ when value for 135–147 day animals is compared with 120–134 day animals with a similar degree of ductus constriction (fetal, moderate, or tight).

$^a$Rings from two lambs with tightly constricted ductus had such a minimal (0 and 0.2 g/mm$^2$) active tension that PGE$_2$ inhibition could not be performed.
in vivo (table 2). Isolated ductus from both immature and mature fetuses that had never breathed had similar abilities to contract with oxygen and indomethacin and maximally relax with PGE₂ (table 2). However, there was no loss of the ability of PGE₂ to maximally relax the isolated ductus (studied in vitro) after increasing degrees of postnatal constriction in vivo in the immature animals as there was in the more mature lambs. Similarly, after each degree of postnatal constriction in vivo, ductus from immature lambs retained twice the contractile activity (to oxygen and indomethacin) of ductus from older lambs (table 2).

As we observed previously, 11 ductus from the most immature fetuses were more sensitive to PGE₂ than were ductus from near-term fetuses (table 3). Although the number of animals in each category was small, postnatal ductus constriction did not appear to alter this developmental shift in sensitivity.

Discussion

We have previously observed a generalized loss of ductus responsiveness and this occurred in the first hours after ductus constriction and before the loss of an anatomically patent lumen. 12 This functional change probably reflects early ischemic damage to the inner muscle wall. As demonstrated in the present study, immature lambs are more likely to have reactive ductus (after their postnatal constriction) than are more mature lambs: (1) Immature lambs do not constrict their ductus as tightly as more mature lambs (table 1). This probably results from an increased sensitivity of the immature ductus to PGE₂ and higher circulating concentrations of PGE₂ in immature lambs. 11, 22, 23 (2) For the same degree of ductus constriction in vivo, there is an increased persistence of ductus responsiveness in immature lambs when compared with more mature lambs (table 2). The reason for this is unknown, but this persistence of ductus responsiveness in immature lambs after ductus constriction may account for the high reopening rate in preterm infants after successful indomethacin-induced closure.

From January 1, 1979 to December 31, 1983, 123 premature infants weighing less than 2500 g at birth who were admitted before 24 hr of age to the Newborn Intensive Care Unit at Mount Zion Hospital and Medical Center were treated with indomethacin for symptomatic patent ductus arteriosus. The evidence of significant left-to-right shunting in these infants has been previously described 1 and includes increases in at least three of the following signs: precordial activity, the intensity and duration of the murmur, pulse pressure, cardiothoracic ratio, pulmonary vascular engagement, and ratio of the left atrial diameter to the aortic root diameter, and two-dimensional echocardiographic and Doppler results. All infants received three oral doses of indomethacin: the first was 0.2 mg/kg body weight, the second (given 12 hr later) was 0.1 mg/kg, and the third (given 36 hr after the first) was 0.1 mg/kg.

TABLE 4
Characteristics of infants treated with indomethacin

<table>
<thead>
<tr>
<th>Birthweight (g)</th>
<th>570–1000 (n = 36)</th>
<th>1001–1500 (n = 56)</th>
<th>1501–2500 (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk ± SE)</td>
<td>27.2 ± 0.2</td>
<td>29.0 ± 0.2</td>
<td>32.8 ± 0.4</td>
</tr>
<tr>
<td>Respiratory distress syndrome (%)</td>
<td>89</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>44/56</td>
<td>44/56</td>
<td>61/39</td>
</tr>
<tr>
<td>Age when initial indomethacin treatment given (days)</td>
<td>6.4 ± 0.8</td>
<td>6.2 ± 0.7</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>Initial PDA closure rate with indomethacin treatment</td>
<td>30/36 (83%)</td>
<td>51/56 (91%)</td>
<td>26/31 (84%)</td>
</tr>
<tr>
<td>Incidence of PDA recurrence after initial closure</td>
<td>10/30 (33%)</td>
<td>13/51 (25%)</td>
<td>2/26 (8%)</td>
</tr>
<tr>
<td>Interval between starting initial treatment and PDA reopening (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 13)</td>
<td>(n = 2)</td>
<td></td>
</tr>
<tr>
<td>PDA closure rate with second indomethacin treatment</td>
<td>8/10 (80%)</td>
<td>9/13 (69%)</td>
<td>2/2 (100%)</td>
</tr>
</tbody>
</table>

PDA = patent ductus arteriosus.
Eighty-seven percent of the infants treated with oral indomethacin had a positive initial response and no longer had symptoms of hemodynamically significant patent ductus arteriosus (table 4). There was no difference in the initial response rate by birthweight or gestational age. However, in 23% of those in whom the ductus was initially closed by indomethacin, the ductus reopened and they again developed a symptomatic left-to-right shunt requiring further treatment. The incidence of reopening was inversely related to the birthweight: 33% of the infants with birthweights of less than 1000 g reopened their ductus after initial closure, while only 8% of infants with birthweights greater than 1500 g reopened their ductus. Although indomethacin has been found to be less effective in closing the ductus arteriosus when given later in the neonatal course, this would not explain why infants weighing less than 1000 g had such a high reopening rate.

Infants whose ductus reopened after initial closure still appeared responsive to indomethacin. In 76% of the infants who were treated with indomethacin a second time (according to the same three-dose protocol) the ductus again closed (table 4). This is consistent with our finding that the ductus arteriosus in immature lambs maintains its ability to relax and contract after postnatal constriction.

We thank Dr. John Pike (Upjohn Co., Kalamazoo, MI) for providing us with PGE\sub{2} standards, and Mr. Carl McWatters and Bruce Payne for their help in the preparation and analysis of the microsphere data. We also thank Mr. Richard Truelove for his skillful preparation of this manuscript and Dr. A. M. Rudolph for his support during this project.

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