Age-dependent cerebral hemodynamic effects of indomethacin in the newborn piglet

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Although used extensively in the clinical treatment of patent ductus arteriosus (PDA), indomethacin is known to cause transient reductions in cerebral blood flow (CBF) in both newborn humans and animals (8, 19, 34-36, 37). These transient reductions in CBF have been well documented. Age-dependent effects on cerebral hemodynamics, including cerebral blood volume (CBV) and cerebral mean transit time (MTT), as well as CBF, however, have not been reported in very young newborns. Existing studies were generally performed in newborn animals ranging from 1 to 10 days of age (10, 19, 36). With recent discussions in the literature regarding prophylactic use of indomethacin to reduce the incidence and severity of intraventricular hemorrhage (IVH) (22, 23, 25, 38), knowledge pertaining to the cerebral hemodynamic effects of indomethacin in this age group is of significant interest.

At birth, and during the immediate postnatal period, both systemic and cerebral circulations undergo substantial changes. These changes, resulting from adaptation to air breathing, higher oxygenation, and increased arterial pressure, are mediated to some extent by changes in circulating and locally produced prostanoids (1, 10, 12, 18, 33). These changes, resulting from adaptation to air breathing, higher oxygenation, and increased arterial pressure, are mediated to some extent by changes in circulating and locally produced prostanoids (1, 10, 12, 18, 33). Leffler and Busija (18) suggested that the endothelial lining of the newborn piglet cerebrovasculature generally promotes vasodilation through preferential production of prostacyclin, a potent vasodilator in the newborn piglet. It has also been suggested that, in the newborn, there exists a relative lack of vasoconstrictor function of prostanoids and that vasorelaxant prostanoids play a predominant role in the maintenance of cerebrovascular tone (1). Likewise, prostanoids have been implicated in the control of patency of the ductus arteriosus (15, 17, 30, 39-42). Smith (39) suggested that patency of the ductus arteriosus in utero is an active state, likely maintained through the vasorelaxant actions of both circulating and locally released prostaglandin E2 and to a lesser extent prostacyclin. And although spontaneous closure of the ductus after birth is likely mediated through a combination of changes in both prostaglandin levels and oxygen tension (40, 41), the exact mechanism or trigger for closure of the ductus remains unknown.

Indomethacin is known to inhibit the production of prostanoids through blockage of both constitutive and inducible isoforms of the cyclooxygenase (COX) enzyme, COX-1 and COX-2, respectively, in the arachidonic acid cascade (13, 28, 29). Because of their importance in the maintenance and control of cerebral hemodynamics, it has previously been suggested that indomethacin may exert its cerebral hemodynamic effects through inhibition of prostanoid production (14, 19). Postnatal cerebral hemodynamic adaptation is mediated by changes in prostanoid production (1, 11, 12, 17). It is therefore possible that the cerebral hemodynamic response to indomethacin in the unstable immediate postnatal period will be dependent on the degree to which cerebral hemodynamics have adapted to postnatal life. In the present study, we have used a recently developed near-infrared spectroscopy (NIRS) technique with indocyanine green (ICG) (8) to investigate the hypothesis that postnatal adaptive changes influence the cerebral hemodynamic response to indomethacin in an age-dependent manner. In addition to NIRS cerebral hemodynamic measurements, we have also used NIRS to assess the cerebral metabolic rate of oxygen (CMRO2) and oxygen extraction fraction (OEF) (7). Because closure of the ductus arteriosus is a normal part of postnatal circulatory changes and because it may also influence cerebral hemodynamics (32), Doppler flow...
ultrasound was used to diagnose and monitor the presence and persistence of PDA in a subgroup of the piglets studied.

METHODS

Subjects and studies. Thirty-nine newborn piglets were studied (mean age of 20.8 h, range 2–60 h; mean weight of 1.6 kg, range of 1.3–2.4 kg). Piglets were grouped by age and received either 0.2 mg/kg indomethacin \[14 \text{ were } < 13 \text{ h of age (mean age of 8.3 h, range 2–12 h; mean weight of 1.47 kg, range of 1.3–1.8 kg) and 12 were } > 13 \text{ h of age (mean age of 36.5 h, range 16–60 h; mean weight of 1.7 kg, range of 1.5–2.4 kg)} \] or an equivalent volume of saline \[8 \text{ were } < 13 \text{ h of age (mean age of 6.9 h, range 2.5–12 h; mean weight of 1.6 kg, range of 1.4–1.8 kg) and 5 were } > 13 \text{ h of age (mean age of 32 h, range 15–53 h; mean weight of 1.5 kg, range of 1.4–1.6 kg)} \] infusion. The study was approved by the Council on Animal Care-Animal Use Subcommittee at the University of Western Ontario. Piglets were anesthetized and paralyzed using 1% isoflurane with vecuronium, intubated, and ventilated. Physiological parameters, including pH, arterial P\(\text{CO}_2\), arterial P\(\text{O}_2\), mean arterial blood pressure, and temperature were monitored throughout the experiment. Hematocrit measurements were made at baseline and at 120 min postinfusion.

CBF, CBV, and MTT measurements were performed before infusion (at 30 and/or 5 min before the start of infusion), directly postinfusion, and at 30-min intervals for 2.5 h thereafter. Two baseline measurements were performed on 29 piglets, whereas a single baseline measurement was performed on the remaining 10 piglets. For the presentation of results, the end of the infusion period was taken as zero time, such that the start of infusion is at -30 min and the baseline measurements are at -60 and/or -35 min.

CBF, CBV, and MTT measurement. A detailed description of the NIRS spectrometer, a discussion of the arterial and tissue ICG concentration measurements by dye densitometry and NIRS, respectively, and the use of NIRS to measure CBF, CBV, and MTT were given by Brown et al. \(7\). Briefly, the technique requires the intravenous administration of a tracer, the NIR chromophore ICG. CBF, CBV, and MTT calculations were based on a deconvolution technique discussed below. If we consider a network of capillaries in a certain mass of brain tissue, CBF into the network \(F\) in \(\text{mL} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}\) carries with it tracer at concentration \(C_a(t)\) (\(\mu\text{mol}/\text{ml}\)). The concentration of tracer in tissue as a function of time after tracer administration is the tissue residue function \(Q(t); \text{in } \mu\text{mol}/\text{ml}\). In the special case when \(F = C_a\), a delta function such that a unit mass of tracer is deposited in the tissue instantaneously at time \(0\), the tissue residue function becomes the impulse residue function or \(R(t)\) \(5\).

When ICG is injected intravenously at a peripheral vein, the rate of delivery of the tracer to the capillary network is \(F \cdot C_a(t)\). If the mass of ICG in the network is linear with respect to the arterial (input) concentration and \(F\) is constant in time, then by linear superimposition it can be shown that

\[
Q(t) = F \cdot C_a(t) * R(t) \quad (1)
\]

where \(*\) is the convolution operator. \(Q(t)\) and \(C_a(t)\) can be measured by NIRS and dye densitometry \(8\), and deconvolution between the two curves then yields \(F \cdot R(t)\) \(9\). The initial height corresponds to CBF, and the area under the curve corresponds to CBV \(45\). From the central volume principle \(21\), MTT can then be calculated as

\[
\text{MTT} = \frac{\text{CBV}}{\text{CBF}} \quad (2)
\]

\(\text{CMRO}_2\) and OEF measurement. Absolute deoxyhemoglobin concentrations (in \(\mu\text{M}\)) measured by NIRS were used in conjunction with arterial blood samples and CBV measurements to calculate an arteriovenous oxygen difference \(7\)

\[
\text{arteriovenous O}_2 \text{ difference} = ([\text{Hb}], - [\text{Hb}_a]) \cdot 1.39 \text{ ml O}_2/\text{gHb} \quad (3)
\]

where \([\text{Hb}]\), and \([\text{Hb}_a]\) are venous and arterial deoxyhemoglobin concentrations, respectively, and 1.39 is a factor that describes the amount of oxygen bound per gram of hemoglobin \(7\). CMRO\(_2\) can then be calculated as

\[
\text{CMRO}_2 = \text{CBF(arteriovenous O}_2 \text{ difference) (4)}
\]

OEF, the percentage of oxygen extracted from arterial blood during its passage through the brain, was calculated using the method described by Meyer \(26\) and Mintun et al. \(27\)

\[
\text{OEF} = \frac{\text{arteriovenous O}_2 \text{ difference}}{C_a} \quad (5)
\]

where \(C_a\) is the arterial concentration of \(O_2\).

Statistical analysis and data presentation. Differences between groups and between baseline and postinfusion CBF, CBV, MTT, CMRO\(_2\), and OEF measurements within groups were assessed using repeated-measures analysis of variance, treating time as a repeated measure. Piglets were grouped by age (less than and greater than 13 h of age) and by treatment (indomethacin or saline). If a significant effect of time was found, Tukey’s test was applied to isolate specific differences \(2\). For piglets in which two baseline measurements were performed, baseline values were averaged before inclusion in Figs. 2–5. In all figures, asterisks denote the times at which the mean CBF, CBV, or MTT value was significantly different from baseline measurements \((P < 0.05)\).

RESULTS

Figure 1 presents means and standard deviations of measured physiological parameters in control and indomethacin-treated piglets \(< 13 \text{ h of age and control and indomethacin-treated piglets } > 13 \text{ h of age}. \text{ No significant differences between groups were observed, and no changes from baseline were observed in any group at all times.}

Figure 2 presents average CBF, CBV, and MTT for control and indomethacin-treated piglets \(< 13 \text{ h of age and control and indomethacin-treated piglets } > 13 \text{ h of age at baseline, immediately postadministration of indomethacin/saline, and at 30-min intervals after administration of indomethacin/saline for a period of 150 min}. \text{ For CBF, no overall effect of treatment or age and no overall interaction between treatment and age were observed. However, the time-dependent response of CBF to treatment with indomethacin was dependent on age \((P < 0.05)\). For CBV, no overall effect of treatment or age and no overall interaction between treatment and age was observed. Again, however, the time-dependent response of CBV to treatment with indomethacin was dependent on age \((P < 0.05)\). For MTT, no overall effect of treatment or age and no overall interaction between treatment and age was observed. The time-dependent response of MTT to treatment with indomethacin was not dependent on age.

No significant changes of CBF, CBV, or MTT from baseline were observed in either age group of control piglets at any of the time points. In indomethacin-treated piglets \(< 13 \text{ h of age (Fig. 2A), CBF was significantly increased 150 min postinfusion, 22 ml-min}^{-1} \cdot 100 \text{ g}^{-1} \text{ (52%) above baseline values } (P < 0.05). \text{ CBV was significantly increased above baseline at 90, 120, and 150 min postinfusion } (P < 0.05)\); a maximum average increase of 0.8 ml/100 g \((25%) \text{ above baseline values occurred at 150 min postinfusion. MTT was significantly increased
above baseline at 0 and 30 min post-indomethacin infusion ($P < 0.05$); a maximum average increase of 1.1 s (29%) above baseline occurred immediately postinfusion. In indomethacin-treated piglets >13 h of age (Fig. 2B), CBF was significantly decreased below baseline at 0, 30, and 60 min postinfusion ($P < 0.05$); a minimum average decrease of 10.6 ml/100 g (25%) below baseline occurred immediately postinfusion. CBV did not change significantly from baseline at all time points. MTT increased significantly above baseline at 0, 30, and 60 min post-indomethacin infusion ($P < 0.05$); a maximum average increase of 1.7 s (42%) above baseline occurred immediately postinfusion.

Figure 3 presents average CMRO$_2$ and OEF for control and indomethacin-treated piglets <13 h of age and control and indomethacin-treated piglets >13 h of age at baseline immediately postadministration of indomethacin/saline, and at 30-min intervals after administration of indomethacin/saline for a period of 150 min. For OEF, no overall effect of treatment or age and no overall interaction between treatment and age was observed. The time-dependent response of OEF to treatment with indomethacin was not dependent on age. For CMRO$_2$, no overall effect of treatment or age and no overall interaction between treatment and age was observed. No significant interaction between time and treat-
ment, time and age, or time, treatment, and age on CMRO\textsubscript{2} was observed.

There were no significant changes in OEF or CMRO\textsubscript{2} from baseline in either age group of control piglets. There were no significant changes in CMRO\textsubscript{2} from baseline in either age group of indomethacin-treated piglets. In indomethacin-treated piglets \textless{}13 h of age (Fig. 3A), OEF increased significantly immediately postinfusion, 0.09 above baseline ($P < 0.05$). In indomethacin-treated piglets \textgreater{}13 h of age (Fig. 3B), OEF increased significantly above baseline at 0 and 30 min postinfusion ($P < 0.05$); a maximum average increase of 0.23 above baseline occurred immediately postinfusion.

Figure 4 shows NIRS-measured CBF, CBV, and MTT for piglets without PDA (2 were less than and 7 were greater than 13 h of age) and piglets diagnosed with PDA (7 were \textless{}13 h of age) on initial echocardiography at baseline, immediately post-indomethacin infusion, and at 30-min intervals for a period of 150 min thereafter. Of nine piglets \textless{}13 h of age, seven had PDA on initial ultrasound diagnosis. Indomethacin administration induced complete closure of the PDA in four piglets and partial or negligible closure in three piglets. A significant effect of PDA was observed for CBF and CBV but not for MTT ($P < 0.05$). In piglets without PDA and treated with indomethacin, CBF and CBV remained unchanged from baseline throughout the study. MTT increased immediately post-indomethacin infusion by an average of 1.9 s (40.7\%) above baseline ($P < 0.05$). In piglets initially diagnosed with PDA and treated with indomethacin, significant increases in CBF from baseline were observed at 120 and 150 min postinfusion ($P < 0.05$). Significant increases in CBV from baseline were observed at 120 and 150 min postinfusion ($P < 0.05$), with a maximum average increase of 0.8 ml/100 g (24\%) above baseline at 150 min postinfusion. MTT was significantly increased above baseline values immediately postinfusion, with an average increase of 1.2 s (31.3\%) above baseline ($P < 0.05$).

Figure 5 shows CMRO\textsubscript{2} and OEF for piglets with and without PDA on initial ultrasound diagnosis treated with indomethacin. No significant effect of PDA was observed on either CMRO\textsubscript{2} or OEF, and no significant changes from baseline were observed.
The most significant finding of the present study is the observed age-dependent cerebral hemodynamic response to indomethacin. Piglets <13 h of age treated with indomethacin exhibited delayed increases in CBF and CBV, a response not observed in piglets >13 h of age. Furthermore, among piglets treated with indomethacin, those >13 h of age displayed a significant decrease in CBF immediately postinfusion, whereas initial decreases in CBF in piglets <13 h of age did not reach statistical significance. Although this initial reduction in CBF with indomethacin administration has been previously observed and documented in both newborn humans and animals (10, 19, 34, 36, 37), the subsequent period of elevated CBF and CBV in piglets <13 h of age has not been reported. This is due in part to the use of older piglets and in part to a lack of data at similarly extended time periods in previous indomethacin studies. Pourcyrous et al. (36) studied the effect of a 5.0 mg/kg dose of indomethacin (infused over a 5-min period) on CBF in awake newborn piglets 3–5 days old. CBF was measured with radioactive microspheres before and at 10, 60, 120, and 240 min after infusion. They observed a reduction in average CBF (CBF values averaged over the entire brain) of 49% at 10 min postinfusion and that CBF had returned to baseline by 240 min. It should be noted that the dose and infusion time used by Pourcyrous et al. were 25 times higher (5 mg/kg compared with 0.2 mg/kg) and 6 times faster (5 min compared with 30 min) than those used in the present study. It is thus expected that initial decreases in CBF would be much larger (49% compared with 25% found in this study). Chemtob et al. (10) reported similar reductions in CBF of 42% below baseline 20 min after rapid infusion of 3 mg/kg indomethacin in 1- to 3-day-old piglets. Louis et al. (19), working with 1-day-old piglets, reported initial decreases in CBF of ~32% with 0.2 mg/kg indomethacin infused over 10 min. Patel et al. (34), using NIRS, showed an initial decrease in CBF of 5.3 ml·min⁻¹·100 g⁻¹ after indomethacin administration (0.2 mg/kg) in newborn infants (34). Pryds et al. (37) also showed an initial decrease in CBF of 24% as a result of a 0.2 mg/kg indomethacin dose using a ¹³³Xe clearance technique in newborn infants. These studies, along with the study presented here, clearly show that indomethacin administration has a direct and profound effect on cerebral hemodynamics in both newborn animals and humans.

The finding of an age-dependent response to indomethacin in newborn piglets is, to our knowledge, a novel observation. It has been suggested that indomethacin-induced closure of the ductus arteriosus may improve venous return (32) and, hence, perfusion pressure, leading to decreased MTT and increased CBF. However, our results show concomitant increases in CBV with no reductions in MTT; thus it is unlikely that improved venous return plays a role in the observed response to indomethacin. Rather, increases in CBF and CBV with unchanged MTT, mean arterial blood pressure, and hematocrit suggest a vasoactive mechanism. Possible responsible mechanisms include age-dependent differences in basal prostanoid concentrations, differences in regulation of prostanoid production, or differences in sensitivity of the cerebrovasculature to prostanoids. Chemtob et al. (11) have suggested that indomethacin-induced changes in CBF may be the result of a direct effect on the cerebral vasomotor tone acting independently of COX inhibition in the newborn (11). Thus the age-dependent response to indomethacin observed in the present study may also result from differences in responsiveness of the cerebrovasculature to indomethacin directly. Future studies investigating the effects of a second indomethacin dose, given 120 min after the initial dose, may prove useful in better identifying the responsible mechanism.

In the subgroup of piglets studied with Doppler flow ultrasound, piglets with PDA on initial diagnosis (all <13 h of age) exhibited similar cerebral hemodynamic responses to indo-

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**Fig. 3.** Cerebral metabolic rate of oxygen (CMRO₂) and oxygen extraction fraction (OEF) in control and indomethacin-treated piglets. Average CMRO₂ and OEF in control and indomethacin-treated piglets <13 h of age (A) and control and indomethacin-treated piglets >13 h of age (B). Values are means and SE. *Significantly different from baseline (P < 0.05).
methacin as piglets <13 h of age in which the presence of PDA was not assessed (Fig. 2A vs. Fig. 4). Because we have collected data from only two piglets <13 h of age without PDA, it is not possible to distinguish whether the effect of indomethacin is PDA or age dependent. We can, however, conclude that piglets <13 h of age with PDA exhibit delayed increases in CBF and CBV with indomethacin administration. Further study is necessary to isolate the effects of age and PDA on observed delayed increases in CBF and CBV after indomethacin administration.

Fig. 4. CBF, CBV, and MTT in piglets with and without patent ductus arteriosus (PDA). Average CBF, CBV, and MTT in piglets are shown without PDA and with PDA treated with indomethacin. Values are means and SE. *Significantly different from baseline \( (P < 0.05) \).

CMRO₂ and OEF measurements presented in the present paper suggest a compensatory increase of OEF to maintain normal CMRO₂ during indomethacin-induced reductions in CBF (Fig. 2B vs. Fig. 3B). CMRO₂ values reported in the present study are in agreement with those previously reported in the literature (6, 16, 36, 43). Because reductions in CBF may compromise oxygen delivery, concern has been raised as to whether indomethacin administration may also alter CMRO₂. However, the observed maintenance of constant CMRO₂ during indomethacin-induced reductions in CBF suggests that indomethacin does not affect cerebral metabolic processes in the newborn piglet. These findings are in line with previous experiments performed in our laboratory (7) and those conducted in other laboratories (14, 44).

Although no significant differences with time were observed in any of the parameters (CBF, CBV, and MTT) in either age group of control piglets, a trend toward delayed increases in CBF and CBV was present in control piglets >13 h of age. These trends suggest a possible effect of prolonged use of anesthetics on cerebral hemodynamics. However, similar trends were not observed in piglets <13 h of age. Thus, if an anesthetic effect does exist, it seems also to be age dependent and, because it did not reach significance, is small by comparison to the effects of indomethacin.

Well recognized for its role in the treatment of PDA, there is still debate in the literature regarding the effectiveness of indomethacin as a means of decreasing the incidence and lessening the severity of IVH in human neonates (3, 4, 25, 38). Results of the present study, specifically the observed delayed increases in CBF and CBV, however, suggest a potentially deleterious effect of indomethacin on cerebral hemodynamics in the newborn piglet. Although IVH is believed to be triggered by changes in blood flow in the microvasculature of the immature germinal matrix (20, 31, 35), the mechanism by which indomethacin may offer protection from IVH remains uncertain. Clinical studies do not support prolonged alterations in CBF as the primary mechanism by which indomethacin is
proposed to prevent IVH (24, 25). Ment et al. (24) showed that, in newborn beagle pups, indomethacin may stimulate basement membrane deposition around the microvessels of the germinal matrix and that this would increase the structural integrity of the germinal matrix, thus helping to prevent hemorrhage. It is possible, then, that the protective effects of indomethacin persist even in the presence of relatively large transient changes in cerebral hemodynamics, as observed in the present study. Furthermore, it is important to note that observed changes in cerebral hemodynamics as a result of indomethacin administration in this study may not be reproducible in the human neonate and that the dose of indomethacin used in IVH prevention studies (0.1 mg/kg) is half that used in the present study (0.2 mg/kg).

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