Beagle puppy model of intraventricular hemorrhage

Effect of indomethacin on cerebral blood flow

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The newborn beagle puppy has been demonstrated to provide a good model for neonatal intraventricular hemorrhage (IVH). A study was designed to determine if indomethacin can prevent IVH and if indomethacin would produce changes in cerebral blood flow (CBF). Newborn beagle puppies were randomized by computer into two groups: one was pretreated with indomethacin, a known inhibitor of prostaglandin synthetase, and the other was saline. The dogs in both groups were then assigned either to undergo hemorrhagic hypotension/volume reexpansion insult or to receive no insult. Twenty percent of all pups receiving indomethacin and undergoing the insult experienced IVH, compared to 71% of the pups undergoing insult that had been pretreated with saline. Significant alterations in the blood pressure responses to the hemorrhagic hypotension/volume reexpansion insult were noted in the former group compared to the saline-pretreated pups subjected to insult. Finally, employing carbon-14 autoradiography for the determination of CBF, it was demonstrated that indomethacin decreases resting CBF of the newborn beagle pups and, in indomethacin-pretreated animals subjected to insult, prevents the increases in CBF seen in the saline-pretreated traumatized pups.

KEY WORDS: beagle puppy, intraventricular hemorrhage, cerebral blood flow, indomethacin prevention trial

INTRAVENTRICULAR hemorrhage (IVH), or hemorrhage originating in the germinal matrix tissues of the developing brain, has been found in over 40% of preterm neonates of less than 1500 gm birth weight and is believed attributable to alterations in cerebral blood flow (CBF). Although such changes in CBF have been demonstrated in preterm neonates with IVH, the study of CBF in this population is difficult. The newborn beagle puppy has provided an excellent model for the investigation of this problem. Like the preterm neonate, the vessels of the germinal matrix of the newborn beagle pup appear to be lacking both collagen and elastin as well as glial supporting structures. Hemorrhages pathologically similar to those found in preterm infants can be produced in this animal by hemorrhagic hypotension with rapid volume reexpansion, hyperventilation, or the acute onset of hypercarbia, all events known to occur clinically in critically ill preterm infants. The germinal matrix has been demonstrated to be a low-flow region in the newborn beagle pup and it is to this area of developing brain that the CBF increases markedly during IVH.

Many investigators hypothesize that prostaglandins, which are synthesized from arachidonic acid in the cerebral microvasculature walls, are primarily responsible for the local control of CBF. Recently, we have demonstrated that indomethacin, known to inhibit prostaglandin synthesis, prevents IVH in the beagle puppy model exposed to a hemorrhagic hypotension/volume reexpansion insult. In addition, indomethacin blunted the blood pressure responses of the experimental animals to this insult compared to the control animals. We have performed carbon-14 (14C)-iodoantipyrine (IAP) studies of CBF in newborn beagle pups that were randomized by computer into two groups: one received an injection of indomethacin and the other of saline. Dogs from...
both groups were assigned either to undergo hemorrhagic hypotension/volume reexpansion injury to create IVH or to receive no insult.

**Materials and Methods**

Newborn beagle puppies, 24 to 72 hours old and weighing 165 to 343 gm each, were tracheotomized under local anesthesia with lidocaine (1% Xylocaine), paralyzed with Flaxedil (gallamine triethiodide), and artificially ventilated to maintain a PO2 of 40 to 60 torr and a PCO2 of 30 to 40 torr. Bilateral femoral arterial and venous catheters were inserted under local anesthesia by cutdown procedures, and arterial blood pressure was monitored by a pressure transducer and polygraph recording. Body temperature was recorded by a thermal probe and was maintained at 36.5° to 37.5°C by means of a warming light.

When the pups were physiologically stabilized, they were randomly assigned to either indomethacin- or saline-diluent pretreatment groups. All personnel directly involved in the experimental procedures were unaware of which solution the pups had received. Indomethacin was made fresh each day by dissolving 20 mg of indomethacin in 20 ml of saline. Experimental pups received a slow intravenous injection of this drug at a dose of 3 mg/kg, and control pups received an equal intravenous volume of the saline diluent.

Thirty minutes following the injection of either indomethacin or saline diluent, the pups were again randomly assigned either to undergo the manipulation to produce IVH or to receive no insult. The insult consisted of rapid venous withdrawal of 20% to 25% of the pup's estimated blood volume into a heparinized syringe, followed by rapid venous reinfusion 5 minutes later. Blood pressure was monitored throughout the procedure and continuously for the next 60 minutes, at which time all pups underwent 14C-IAP determinations of CBF.

Blood flow determinations were made by the slow injection over a 30-second period of 200 μCi of 14C-IAP/kg body weight. The animals were then rapidly sacrificed by the intravenous injection of KCl, and the brains were removed and placed in isopentane chilled to -60°C. Brain sections, 32 μ thick, were prepared with a cryostat maintained at -15° to -10° C, and every 25th section was placed on a glass slide, dried on a hot plate at 60° to 70°C, and placed sequentially in an x-ray cassette with Kodak SB-5 x-ray film for 7 days. Calibrated plastic standards with known concentrations of 14C were placed adjacent to the tissue sections.

The brains were inspected by observers unaware of the treatments administered to the experimental animals, and the neuropathological findings were recorded by photography. In addition, representative serial Nissl-stained sections were prepared from the brains.

Local tissue concentrations were determined by densitometric measurements using a Leitz microden-
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TABLE 2
Mean arterial blood pressure (MABP) for 12 animals undergoing insult *

<table>
<thead>
<tr>
<th>Determination</th>
<th>MABP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>indomethacin-pretreated</td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>63</td>
</tr>
<tr>
<td>trough</td>
<td>52</td>
</tr>
<tr>
<td>before IVH</td>
<td>60</td>
</tr>
<tr>
<td>peak</td>
<td>64</td>
</tr>
<tr>
<td>saline-pretreated pups</td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>64</td>
</tr>
<tr>
<td>trough</td>
<td>49</td>
</tr>
<tr>
<td>before IVH</td>
<td>49</td>
</tr>
<tr>
<td>peak</td>
<td>67</td>
</tr>
</tbody>
</table>

* In nine other animals that did not undergo insult, the MABP for indomethacin-pretreated and saline-pretreated pups was 60 mm Hg and 66 mm Hg, respectively, throughout the experiment. IVH = intraventricular hemorrhage.

MABP of Group I-I pups dropped from a baseline of 63 mm Hg to a trough value of 52 mm Hg, compared to a trough value of 49 mm Hg for Group S-I animals (Fig. 1). The MABP of Group I-I pups rose to 60 mm Hg just prior to the volume reexpansion maneuver, but the MABP of Group S-I pups remained at 49 mm Hg. Finally, following reinfusion of the withdrawn blood, both groups of pups were found to have peak MABP values of 67 mm Hg. When one examines these data using an analysis of variance, there is a significant difference in the fashion in which MABP changed over time for the I-I and S-I groups of animals. There was no effect for the groups receiving either pretreatment solution (F < 1). However, there was a main effect of time (F(3,21) = 46.7; p < 0.001) and there was an interaction between solution and time (F(3,21) = 6.3; p < 0.01). Thus, indomethacin was demonstrated to blunt the changes in MABP seen in the saline-pretreated pups in response to the hemorrhagic hypotension/volume reexpansion insult.

Cerebral Blood Flow Data

Concentrations of 14C-IAP were determined in several regions of the brain, and are representative of CBF (Table 3). The IAP concentrations in the cortical regions of the I-I, S-I, I-NI, and S-NI pups were 0.300, 0.372, 0.276, and 0.356 µCi, respectively. Employing an analysis of variance, we found that indomethacin significantly lowered the 14C-IAP concentration in the cortices of those pups pretreated with this solution (main effect of solution, F(1,38) = 7.99; p < 0.01). The IAP concentrations in the white matter of Group I-I, S-I, I-NI, and S-NI animals were 0.097, 0.120, 0.086, and 0.120 µCi, respectively, and the concentrations of IAP in the caudate nucleus were 0.260, 0.321, 0.218, and 0.286, respectively, for the same groups. An analysis of variance for both groups revealed that the IAP concentrations were lower for both groups of indomethacin-pretreated animals compared with the saline groups in both the white matter (F(1,38) = 3.7; p < 0.10) and the caudate regions (F(1,38) = 3.5; p < 0.10).

The concentrations of 14C-IAP in the germinal matrix regions of Group I-I, S-I, I-NI, and S-NI pups were 0.110, 0.128, 0.062, and 0.099, respectively. As we have recently demonstrated,37 when one employs an analysis of variance for examination of changes of CBF evoked by the hemorrhagic hypotension/volume reexpansion insult, pups pretreated with both indomethacin and saline show a main effect of the insult (F(1,38) = 4.46; p < 0.05) in the germinal matrix only, but no effect of solution (F < 1). However, a non-paired t-test for the 14C-IAP concentrations as indicative of CBF in the germinal matrix regions of Group S-NI and I-NI pups demonstrated a significant decrease in CBF in the I-NI pups compared to their saline-pretreated peers (p < 0.01). Thus, one may conclude that indomethacin pretreatment lowered CBF in all four regions examined in the newborn pups.

![Fig. 1. Mean arterial blood pressure (MABP) values for animals pretreated with either indomethacin or saline. All of these animals underwent intraventricular hemorrhage by the hemorrhagic hypotension/volume reexpansion model.](image-url)
beagle pup. The hemorrhagic hypotension/volume reexpansion insult increased CBF to the germinial matrix but not to any other region examined, and indomethacin decreased the incidence of IVH in pretreated animals subjected to insult compared to saline-pretreated traumatized pups.

### Discussion

Many investigators believe that prostaglandins, which are known to be ubiquitous throughout most organ systems, represent the final common pathway of control of CBF.7,9,53,60 These compounds are synthesized *de novo* from arachidonic acid within the endothelial lining of the cerebral vasculature.14,19,40-42 endoperoxides and free radical compounds, thought to be harmful to local tissues, are also believed to be produced in association with prostaglandin synthesis.34,60 Although prostaglandin (PG) I2 (prostacyclin) has been demonstrated to be the most common of these compounds in the CNS,19 PGE2, PGF2, and thromboxane (TXA2) are also found within the cerebral vasculature.14,41

Prostacyclin is a cerebrovasodilator,11,49 which is known to increase CBF in experimental animals9,52 and reverses the contractions of human basilar arteries induced by 5-hydroxytryptamine (5-HT), norepinephrine, angiotensin, and PGF2 in *vitro*.47 Both PGE2 and PGF2 are vasoconstricting agents9,52 which are reported to decrease the diameter of cerebral arteries56 and to diminish CBF in adult animals.49,60 Goddard-Finogold and Michael18 have recently reported that PGE2 increases CBF in the 24-hour-old beagle pup, and may produce IVH in this animal model.

In addition to their effects on CBF, prostaglandins are known to be released from anoxic and ischemic brain,34 and neuropathological changes in the cat cerebral microvasculature similar to those evoked by acute hypertension have been produced by the administration of arachidonic acid and PGG2 to these animals.34 Based on these data, Wolfe40 has proposed that such insults to the central nervous system (CNS) as ischemia and acute hypertension may lead to the increased synthesis of prostaglandins, which would be accompanied by an increase in tissue-injurious free radical production, and thus may lead to more extensive tissue destruction. In addition, the insult-induced imbalance of prostaglandins which can result may in turn cause alterations in local or overall CBF. Hallenbeck, *et al.*,21 have referred to this phenomenon as the "blood-damaged tissue interaction."

Indomethacin inhibits the cyclo-oxygenase pathways of prostaglandin synthesis41,56 both in the CNS and in numerous other sites to prevent the conversion of arachidonic acid to the cyclic endoperoxides from which PGI2, TXA2, and other prostaglandins are synthesized. Despite conflicting reports concerning the effects of indomethacin on CBF,56 Pickard, *et al.*,51,52 and others5,14,15,17,29,43,44,50 have demonstrated in numerous experimental animals that, while indomethacin evokes no change in the caliber of the major cerebral vessels when studied by arteriography, within 2 minutes of its intravenous administration it produces constriction of the cerebral microvasculature and decreases CBF. Although indomethacin significantly decreases baseline values for CBF (up to 50% in rodents, and as much as 36% in primates) and markedly blunts the responsiveness of CBF to changes in PCO2,4-7,23,51 it has been shown to have no effect on the control of CBF over a wide range of systemic blood pressure28,41,50 and has no effect on the CBF response to hypoxia.56,58 Prostacyclin has been shown to reverse the vasoconstrictor effects of indomethacin, and these changes in the cerebral microvasculature and CBF have been specifically attributed to the ability of indomethacin to inhibit PGI2 synthesis.28,41,43 Of importance to the developing CNS, indomethacin has been demonstrated to produce this decrease in CBF without causing alterations in the cerebral metabolic rate;6,48 this capability is unlike that of PGE2, a naturally occurring prostaglandin, which decreases CBF by decreasing the cerebral metabolic rate.49

Additionally, indomethacin has been demonstrated to prevent those changes in morphology and permeability found in the cerebral arteriolar walls of animals exposed to either acute hypertension or asphyxia or to fluid percussion injury.29-31,33,35 In support of the theory that the morphological and permeability changes induced by acute insult may be secondary to the release of free radicals associated with excessive prostaglandin synthesis, Kontos, *et al.*,34 demonstrated that such changes in animals exposed to fluid percussion injury could be prevented either by pretreatment with indomethacin or by free radical scavengers. Finally, indomethacin pretreatment has also been shown to prevent ischemic changes caused by compression of the brain14 and enhance posts ischemic reperfusion in dogs,13,20 in conjunction with PGI2, or PGI2 and heparin, indomethacin improves recovery from experimental multifocal ischemia21 and enhances posts ischemic reperfusion in other experimental preparations.4,53 Such studies provide data for the microvascular membrane-stabilizing effects of this drug.

The peripheral effects of indomethacin are numerous, and include alterations in systemic blood pressure as mediated by both the sympathetic nervous system and the renin-angiotensin in mechanism.1,3,12,55 The effect of both systems on blood pressure appears to be influenced by prostaglandins, including PGE2 and PGI2, both of which are known to be potent independent peripheral vasodilating agents. In addition, PGE2 has been demonstrated to pre-synaptically inhibit the output of norepinephrine,25,26,32,44,61 a known vasoconstrictor, and to blunt the response of vascular smooth muscle to the renin-angiotensin II system. Similar to our experience, Hallenbeck and Furlow20 reported a diminution in both the hypo- and hyper-

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tensive responses in experimental animals pretreated
with indomethacin and then exposed to a time-limited
interval of cerebrospinal fluid compression ischemia
compared to untreated animals exposed to the same
manipulation.
As we have previously reported, pups pretreated
with indomethacin and then exposed to the hem-
orragic hypotension/volume reexpansion model to
produce IVH appear to show a significantly decreased
incidence of IVH as compared to pups pretreated with
saline before injury. In these studies we have dem-
onstrated that, as in mature animals, indomethacin
causes a decrease in the baseline CBF in the newborn
beagle puppy in all regions examined. In addition,
when one compares the saline-pretreated to the in-
domethacin-pretreated pups, indomethacin does not
prevent those increases in CBF to the germinal matrix
region, but nevertheless protects the indomethacin-
pretreated group of pups from hemorrhage. Indo-
methacin also blunts the changes in MABP, as can
be seen when comparing saline-pretreated pups with
IVH to those with indomethacin and no IVH. These
effects of indomethacin may be attributable to its
ability to act in two sites centrally (to alter both CNS
prostaglandin synthesis and to stabilize the cerebral
microvascular endothelium) as well as in the periph-
ery to blunt the blood pressure changes evoked by the
hemorrhagic hypotension/volume reexpansion insult.

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