Beagle puppy model of intraventricular hemorrhage

Effect of indomethacin on local cerebral glucose utilization

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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 local cerebral glucose utilization (LCGU). By computerized random design, newborn beagle puppies were pretreated with either indomethacin (a known inhibitor of prostaglandin synthetase) or saline, and then assigned either to receive a hemorrhagic hypotension/volume reexpansion insult or to receive no insult. Pretreatment with indomethacin produced a marked drop in the incidence of IVH as well as significant alterations in the blood pressure responses to the hemorrhagic hypotension/volume reexpansion insult. Carbon-14 autoradiography was used to determine LCGU: no alterations were demonstrated in cerebral metabolism in uninjured pups pretreated with indomethacin compared to saline-pretreated animals. In addition, although the hemorrhagic hypotension/volume reexpansion insult produced marked alterations in LCGU in both groups of traumatized pups, indomethacin prevented the changes in LCGU in the germinal matrix and white matter that were found in the saline-pretreated animals.

KEY WORDS • cerebral glucose utilization • indomethacin • beagle puppy model • intraventricular hemorrhage • germinal matrix

INTRAVENTRICULAR hemorrhage (IVH), or hemorrhage into the germinal matrix tissues (GMH) of the developing brain, is a major neurological problem of preterm neonates, and has been found in over 40% of all preterm neonates with a birth weight of 1500 gm or less. Thought to be secondary to alterations in cerebral blood flow (CBF) to the preterm cerebrum, IVH has also been associated with changes in cerebral metabolism, and both the alterations in CBF and the metabolic changes may contribute to the later neurodevelopmental sequelae found in these tiny patients. Using a hemorrhagic hypotension/volume reexpansion model for IVH in the newborn beagle pup, we have produced hemorrhages neuropathologically similar to those found in preterm neonates. We have demonstrated widespread alterations in local cerebral glucose utilization (LCGU) with uncoupling of CBF and metabolism in this model, similar to the findings in adult animals exposed to hemorrhagic hypotension or graded hypoxemia and in newborn beagle pups exposed to asphyxia. In addition, because many investigators believe that CBF is controlled in part by prostaglandins, which are synthesized in the cerebral microvasculature from arachidonic acid, we have examined the effect on IVH of indomethacin, a known inhibitor of prostaglandin synthetase. We have found that indomethacin prevents IVH. In addition, indomethacin blunts the systemic blood pressure changes found in response to the hemorrhagic hypotension/volume reexpansion insult in newborn beagle pups pretreated with saline, and lowers the baseline CBF values.

In this paper we report our studies of LCGU in newborn beagle pups, which by randomized design were assigned to saline or indomethacin pretreatment followed by hemorrhagic hypotension/volume reexpansion insult or no insult.

Materials and Methods

Newborn beagle pups, aged 24 to 72 hours and weighing 160 to 360 gm, were tracheotomized under local anesthesia with 1% Xylocaine (lidocaine), para-
lyzed with Flaxedil (gallamine triethiodide), and artificially ventilated to maintain PO$_2$ at 40 to 60 torr and pCO$_2$ at 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. All personnel directly involved in the experimental procedures were unaware of which solution (indomethacin or saline-vehicle) the pups received. Indomethacin was made up freshly each day by dissolving 20 mg indomethacin in 20 ml saline. Experimental pups received a slow intravenous injection of the drug at a dose of 0.3 mg/kg, and control pups received an equal intravenous volume of the diluent. This dose is similar to that administered to preterm infants for pharmacological effect, as in closure of a patent ductus arteriosus.13,15,22,35

Thirty minutes following the administration of indomethacin or saline diluent, at a time when this dose of indomethacin is known to produce significant serum levels in preterm infants,15,63 the pups again underwent random assignment to one of two groups, either to receive the insult to produce IVH or to receive no manipulation. The hemorrhagic hypotension/volume reexpansion insult consisted of rapid venous withdrawal of 20% to 25% of the pup's estimated blood volume into a syringe washed with heparin, followed by rapid venous reinfusion 5 minutes later. Blood pressure was monitored throughout the procedure and continuously for the next 50 minutes, at which time all pups underwent carbon-14 ($^{14}$C)-2-deoxyglucose (2DG) determinations of LCGU.

For these studies, the animals were given 200 µCi/kg of $^{14}$C-2DG via the femoral venous catheter. They were observed for an additional 45 minutes, following which they were quickly sacrificed with an overdose of sodium pentobarbital. The brains were rapidly removed and frozen in isopentane at -60°C. The brains were then prepared for $^{14}$C-autoradiography. Sections were cut at 32 µ in a cryostat. Every 25th section was saved and placed on a glass slide; these were rapidly dried at 70°C and then placed sequentially in a cassette loaded with Kodak SB-5 film and Amersham $^{14}$C standards for 5 days.

Cut brains were inspected by personnel naive to which solution the pups had received and which protocol (insult or no insult) they had undergone. Findings were recorded by photography, and Nissl-stained sections were made. Hemorrhages were defined according to the preterm neonate GMH/IVH grading system of Papile, et al.43

The $^{14}$C-2DG concentrations were determined densitometrically from calibrated plastic standards. Values were obtained for six regions of the cortex, six corresponding regions of hemispheric white matter, three regions of the caudate nucleus, and three regions of germinal matrix in autoradiographs corresponding to the section at the head of the caudate nucleus. Since in this study blood glucose concentrations were not performed, it is not possible to provide absolute values for LCGU. However, in an additional randomized trial in which seven pups were assigned to one of four categories (indomethacin-insult, indomethacin-no insult, saline-insult, or saline-no insult), there was no difference in the serum blood glucose values during the time of LCGU determination in any of the pups. Pups in the indomethacin-insult group maintained their blood glucose values between 134 and 142 mg/dl, and pups in the saline-insult group had blood glucose values of 145 to 147 mg/dl. In the indomethacin-no insult group, animals' blood glucose values were 147 to 151 mg/dl during the same time interval, and in the saline-no insult group blood glucose values were 134 to 147 mg/dl. Thus, if one assumes nearly identical arterial blood-time curves and rate and diffusion constants among the animals in all of the groups and between groups as predicted by the additional trial, then the concentrations of $^{14}$C-2DG should reflect the relative LCGU for each region.

Results

Data are available for 17 pups, who were randomized into the following four groups based on pretreatment (indomethacin or saline) and insult or no insult manipulation: 1) four indomethacin-insult (I-I) pups; 2) five indomethacin-no insult (I-NI) animals; 3) four saline-insult (S-I) pups; and 4) four saline-no insult (S-NI) animals. There was no incidence of IVH in either of the groups of animals treated with indomethacin (I-I and I-NI). However, one of the four S-NI pups was found to have a GMH and all four of the S-I animals experienced IVH. Thus, in this small series, indomethacin significantly decreased the incidence of IVH (p < 0.02, Fisher's exact test). There were no significant differences in the weights or arterial blood gas values for any of these four groups of animals, as shown in Table 1.

Blood Pressure Data

There was no significant difference between the baseline mean arterial blood pressure (MABP) values for the I-NI and S-NI animals (Table 2). Similarly, the baseline MABP was 73 mm Hg for the I-I pups and 70 mm Hg for the S-I animals; these values also did not differ from each other or from the no-insult groups. However, the MABP of the I-I pups dropped from a baseline of 73 mm Hg to a trough value of 64 mm Hg during the hypotensive phase, compared to a trough value of 55 mm Hg for the S-I group. Both groups of animals demonstrated only minimal changes in MABP immediately before the volume reexpansion phase of
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**TABLE 1**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Insult Groups</th>
<th>No-Insult Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pO₂ (torr)</td>
<td>pCO₂ (torr)</td>
</tr>
<tr>
<td>indomethacin</td>
<td>55 ± 5.9</td>
<td>36 ± 2.1</td>
</tr>
<tr>
<td>saline</td>
<td>60 ± 7.9</td>
<td>36 ± 2.6</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations.

**TABLE 2**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Baseline</th>
<th>Trough</th>
<th>Before</th>
<th>Peak</th>
<th>Insult Groups</th>
<th>No-Insult Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>indomethacin</td>
<td>73 ± 13</td>
<td>64 ± 14</td>
<td>66 ± 13</td>
<td>74 ± 14</td>
<td>69 ± 10</td>
<td></td>
</tr>
<tr>
<td>saline</td>
<td>70 ± 12</td>
<td>55 ± 8</td>
<td>57 ± 8</td>
<td>75 ± 9</td>
<td>74 ± 12</td>
<td></td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations in mm Hg.

**Local Cerebral Glucose Utilization Data**

The 14C-2DG concentrations as representative of LCGU are found in Table 3. Concentrations of 2DG in the cortical regions of the I-I, S-I, I-NI, S-NI pups were 0.318, 0.302, 0.271, and 0.251 μCi/gm, respectively. Employing an analysis of variance, the indomethacin was found to have no effect on the LCGU of the pups treated with this drug; however, as we have previously demonstrated, a main effect of the insult was found in both groups of injured pups when compared to the uninjured animals (f(1,30) = 8.4, p < 0.01). The 2DG concentrations in the caudate of the I-I, S-I, I-NI, and S-NI groups were 0.301, 0.290, 0.234, and 0.199 μCi/gm, respectively, and, in this region as in the cortex, there was no effect of the indomethacin on the LCGU values, but a main effect of the insult (f(1,30) = 31.49, p < 0.01). The 2DG concentrations in the white matter of the I-I, S-I, I-NI, and S-NI groups were 0.199, 0.266, 0.206, 0.162, 0.234, and 0.173 μCi/gm.

**TABLE 3**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Insult Groups</th>
<th>No-Insult Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex</td>
<td>White Matter</td>
</tr>
<tr>
<td>indomethacin</td>
<td>0.318 ± 0.035</td>
<td>0.199 ± 0.035</td>
</tr>
<tr>
<td>saline</td>
<td>0.302 ± 0.062</td>
<td>0.266 ± 0.066</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations in μCi/gm.
jured animals, with attainment of relative homogeneity of LCGU throughout the cerebral hemispheres, as have newborn beagles traumatized with asphyxia. We recently reported an uncoupling of CBF and metabolism in our hemorrhagic hypotension/volume reexpansion insult produced widespread alterations in LCGU in the intact brain as a result of indomethacin pretreatment which had been pretreated with indomethacin, compared to saline-pretreated injured animals.

Thus, one may conclude that indomethacin did not alter the baseline 2DG values for LCGU in the uninjured pups. Both groups of injured pups were found to have significant alterations in 2DG concentrations in all four regions examined when compared to the uninjured animals, with attainment of relative homogeneity of metabolism in the saline-pretreated pups, all of which had had IVH, and a general increase in 2DG concentrations with preservation of the expected regional variations in indomethacin-pretreated injured pups, none of which had suffered IVH.

Discussion

Clinical and neuropathological studies of preterm neonates with IVH have led to the belief that hemorrhage into the germinal matrix tissues (GMH) of the developing brain may be secondary to alterations in CBF. 

Venous plethysmographic and xenon-133 inhalation studies have demonstrated alterations in CBF in these infants, and animal studies on newborn beagle pups have demonstrated changes in CBF in pups with IVH. 

In addition to these findings, infants with IVH are thought to have disturbances of cerebral metabolism. 

Like adult patients with subarachnoid hemorrhage, these patients demonstrated prolonged hypoglycorrhachia and have been found to have elevations in cerebrospinal fluid lactate and lactate/pyruvate ratios. Adult animals with hemorrhagic hypotension and graded hypoxemia have been demonstrated to have widespread changes in LCGU throughout the cerebral hemispheres, as have newborn pups with asphyxia. We recently reported an uncoupling of CBF and metabolism in our hemorrhagic hypotension/volume reexpansion model for IVH in the newborn beagle pup with the attainment of relative homogeneity of LCGU throughout the cerebral hemispheres.

The introduction of the computerized tomography scanner and the more recent development of sophisticated bedside techniques of cranial ultrasonography have permitted the routine scanning for IVH of large numbers of preterm neonates in tertiary-care nurseries. Over 40% of infants weighing less than 1500 gm at birth have been found to have IVH, and neurodevelopmental assessment programs such as ours at Yale University School of Medicine have demonstrated that, when preterm neonates with GMH or IVH are compared to their peers without known hemorrhage with serial neurodevelopmental testing, they fare considerably less well at 30 months corrected age. Such problems with neurodevelopmental outcome may be secondary not only to changes in CBF to the developing brain, but also to presumed prolonged alterations in metabolism.

Because of the improved survival of preterm neonates with IVH and the recognition of the many problems that these patients may experience, both in the newborn period and at the time of follow-up neurodevelopmental evaluations, several different modes of therapy have been explored to prevent IVH. Many investigators believe that prostaglandins represent the final common pathway of control of CBF. Indomethacin, a known inhibitor of prostaglandin synthetase, has been shown to decrease resting CBF and blunt the response of CBF to alterations in CO2 in experimental animals. Indomethacin does this without altering the ability of CBF to respond to changes in systemic blood pressure over a wide range or to changes in pO2. In addition, in adult experimental animals, indomethacin decreases CBF without altering the cerebral metabolic rate.

Indomethacin is widely utilized in preterm neonates for the pharmacological closure of a patent ductus arteriosus. As in studies with adult experimental animals, our investigations have demonstrated no alterations in LCGU in the intact brain as a result of indomethacin administration. As we have previously shown, indomethacin markedly decreases the incidence of IVH in this model and blunts the systemic blood pressure responses of the animals to this insult. In addition, alterations in CBF to the very important germinal matrix region are blunted by this agent. Similar to adult animals exposed to hemorrhagic hypotension and newborn beagles traumatized with asphyxia, our hemorrhagic hypotension/volume reexpansion insult produced widespread alterations in LCGU. In these studies, indomethacin prevented the increases in LCGU in the germinal matrix and white matter of the drug-pretreated injured animals which were found in the saline-pretreated IVH pups.

Acknowledgment

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**References**


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