The role of intracranial hypotension in neonatal intraventricular hemorrhage

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Most preterm infants develop transient intracranial hypotension, which reaches its lowest level on the 2nd day of life. This corresponds to the time when most neonatal intraventricular hemorrhage (IVH) occurs. In order to test the hypothesis that intracranial hypotension may have an etiological role in the development of IVH in premature infants, the authors induced intracranial hypotension in the preterm rabbit by the intraperitoneal injection of glycerol. The rabbit model is well suited for this study because this animal is at risk of developing spontaneous germinal matrix and ventricular hemorrhage. Compared to control littermates, the glycerol-treated animals exhibited a greater than 3.5-fold incidence of germinal matrix and intraventricular hemorrhage.

KEY WORDS • intraventricular hemorrhage • intracranial hypotension • premature rabbit • germinal matrix hemorrhage

Intraventricular hemorrhage (IVH) occurs in 40% to 60% of preterm infants of less than 35 weeks gestation, requiring their management in intensive care units.1,6,23 These hemorrhages generally arise in the germinal matrix, but other sites of origin are recognized,8,16,24,35 including cerebellar hematomas.12,22 Approximately one-third of the infants with IVH die, and many of the survivors develop obstructive hydrocephalus with or without developmental retardation.1,16

The etiology of IVH remains unknown, although hypoxia,10 hypercarbia,14 arterial hypotension and hypotension,13 loss of autoregulation of cerebral blood vessels,19 alkali therapy,25 hyperosmolarity,26 alveolar rupture,17 and volume expansion have been cited as possible causative factors. Unfortunately, most of these conditions cannot presently be avoided, since they reflect the dire physiological state of these preterm infants, even when maximum clinical support is provided.

As early as 1977, Welch31 noted that the intracranial pressure (ICP) in preterm infants fell rapidly during the first days of life. This fall in ICP is coincident and possibly causally related to a generalized tissue dehydration that is known to occur.34 Subsequently, in 1978,32 Welch formulated the hypothesis that the negative ICP resulting from an inordinate loss of fluid from the brain was the force responsible for spontaneous intracranial bleeding in the premature infant, a suggestion supported later by Coulter (unpublished data, 1980) and more recently by de Courten and Rabinowicz.4

To assess the possibility that intracranial hypotension may be the underlying force in neonatal IVH, we induced such hypotension in preterm rabbits and evaluated the brains for evidence of posttreatment hemorrhage. The rabbit has been shown to be an appropriate model for the study of IVH,18 not only because development of the brain and the germinal matrix relative to time of birth in the preterm rabbit pup parallels that of the infant, but also because these animals are at risk of developing spontaneous germinal matrix and intraventricular hemorrhage.

Materials and Methods

Rabbit pups were delivered by Caesarian section on Day 28 of gestation (conception = Day 0, term = Day 31 to 32). The animals were cared for during the first 24 hours as described previously.18

Intracranial hypotension was induced by the intraperitoneal injection of glycerol. In order to determine the dose of glycerol that would produce a maximum
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fall in ICP, seven preterm rabbits, aged 0 to 4 days and delivered at 28 days gestation, were anesthetized with tribromoethanol (90 mg/kg intraperitoneally) and placed in a stereotaxic frame. The arterial, venous, and cisternal pressures were measured with respect to the heart as described previously. 47 Glycerol was then injected intraperitoneally in various doses to produce a drop in ICP.

Once the optimum dose of glycerol was established, 65 preterm rabbit pups from 10 litters were assigned at the age of 24 hours to two treatment groups. These animals were not anesthetized and did not undergo ICP monitoring. Animals in the first group received 0.3 to 0.5 ml of a 50% glycerol-50% distilled water solution, equivalent to a total dose of 6 gm/kg. Nineteen of these animals were decapitated at 1 hour, and 17 at 6 hours after the injection. The second or control group consisted of littermates that received 6 gm/kg of distilled water only. Nineteen animals were killed at 1 hour and 10 at 6 hours after the injection.

Blood was collected from the severed neck of approximately 50% of these animals, and was used for determination of serum osmolality by freezing-point depression.* The whole brain was removed from the cranium, and approximately a 0.1-gm specimen of the occipital cortex and underlying white matter was weighed and dessicated to constant weight. Percent water content was calculated as: %H2O = (wet weight - dry weight) x 100/wet weight. Sodium and potassium content was determined in the same specimen by flame photometry. The remainder of the brains and the brains from the other 50% of the animals not processed for water or electrolyte determination were fixed by immersion in 10% buffered formalin and processed for histological study. Three coronal sections, 10-μ thick, were cut at three different levels, for a total of nine in each brain, and stained with hematoxylin and eosin. Sections were evaluated independently by two of the investigators without knowledge of treatment. Disagreements in observation were settled by a third investigator.

Results of Glycerol-Induced Hypotension

Intracranial, Arterial, and Venous Pressures

Before injection of glycerol, the cisternal pressure was measured in seven animals at the level of the manubrium, and averaged 2.9 ± 0.3 cm H2O. Following an intraperitoneal injection of glycerol (6 mg/kg), the cisternal pressure fell an average of 5.3 ± 0.4 cm H2O, to a subatmospheric level of -2.7 ± 0.7 cm H2O. The nadir occurred between 30 and 40 minutes after injection, and the pressure rose slowly thereafter. In the longest-surviving animal, the ICP remained subatmospheric for 2 hours following injection. Larger doses of glycerol (8 gm/kg intraperitoneally) produced no greater drop in pressure in two preterm rabbits, while smaller doses (1 gm/kg in two and 3 gm/kg in three, intravenously) led to a submaximal pressure drop. The central venous pressure averaged 1.3 ± 0.5 cm H2O, and after transients associated with the injection, remained at this level. It was not possible to determine whether these transients in venous pressure were associated with an increased volume in the intraperitoneal cavity, with stimulation of the animal, or with both, or were merely due to movement or electrical artifact. The mean arterial blood pressure recorded from seven animals averaged 16.1 ± 1.5 mm Hg and was largely unaffected by the injection. A representative continuous trace is shown in Fig. 1.

Serum Osmolality, Brain Water, and Electrolytes

Little or no difference was found between the average body weights of treated and control pups (Table 1). The increase in serum osmolality observed in pups 1 hour after glycerol administration was accompanied by a marked water and sodium loss from the brain. By 6 hours, when serum osmolality had almost returned to normal, brain water and sodium content had also been restored. In contrast to the level of sodium, the potassium content in the brain remained unchanged at 1 hour but was insignificantly elevated by 6 hours. Thus, the response of the preterm rabbit brain to an osmotic load was in the same direction with respect to water as observed in the adult brain. Sodium movement, however, was in the opposite direction,326 suggesting that the preterm rabbit brain is less capable of the ionic readjustment necessary to protect brain volume.

* Levels were determined using Osmette A, obtained from Precision System, Sudbury, Massachusetts.
Production of Intracranial Hemorrhage

None of the animals that received glycerol or water injections died or exhibited symptoms of hypertonicity before decapitation. Even animals that were subsequently shown to have developed large intracranial hemorrhages remained vigorous. Gross bilateral hemorrhages over the hemispheres were present in a majority of the glycerol-treated animals, while only a few of the control animals had such gross hemorrhages.

Isolated or single intracranial petechial hemorrhages were more common in this series than in a previous series of preterm rabbits studied by us. This was presumably a result of reviewing a greater number of histological sections (nine in this study rather than three). For instance, in the 29 control animals in this study, isolated petechial hemorrhages were observed in the germinal matrix in four, the white matter in five, and the cortex in two. Subarachnoid hemorrhages were observed in 16 of the 29 animals, but in our opinion many of these hemorrhages were attributable to the unavoidable tearing of the meninges during removal of the overlying cranial bone. In the preterm rabbit, the dura adheres tightly to the overlying bone anteriorly along the coronal suture and posteriorly along the transverse sinuses.

Multiple germinal matrix hemorrhages were found in the germinal matrix in four, the white matter in five, and the cortex in two. Subarachnoid hemorrhages were observed in 16 of the 29 animals, but in our opinion many of these hemorrhages were attributable to the unavoidable tearing of the meninges during removal of the overlying cranial bone. In the preterm rabbit, the dura adheres tightly to the overlying bone anteriorly along the coronal suture and posteriorly along the transverse sinuses.

### Table 1

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Body Weight (gm)</th>
<th>Serum Osmolality (mOsM/liter)</th>
<th>Brain H2O %Wet</th>
<th>gm/gm Dry</th>
<th>Na⁺ (mM/kg) Wet</th>
<th>Na⁺ (mM/kg) Dry</th>
<th>K⁺ (mM/kg) Wet</th>
<th>K⁺ (mM/kg) Dry</th>
</tr>
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<tbody>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69.6 ± 2.7</td>
<td>598 ± 2.1</td>
<td>73.1 ± 3.8</td>
<td>632 ± 3.5</td>
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<td>no. of animals</td>
<td>11</td>
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<td>11</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>glycerol-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70.5 ± 2.7</td>
<td>514 ± 2.3</td>
<td>84.5 ± 4.7</td>
<td>615 ± 30</td>
</tr>
<tr>
<td>evaluated at 1 hour</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td>10</td>
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<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>evaluated at 6 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.1 ± 2.3</td>
<td>633 ± 31</td>
<td>88.2 ± 2.8</td>
<td>731 ± 26</td>
</tr>
<tr>
<td>no. of animals</td>
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<td></td>
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</tbody>
</table>

* Brain water content is expressed as a percentage of total wet tissue weight, and as gm of H₂O/gm of total dry tissue weight. Electrolyte concentrations are expressed as millimoles/kg of wet tissue weight and as millimoles/kg of dry tissue weight. Student's t-test was used to determine the significance of the difference between average values for the glycerol-treated and the water-treated control animals († = p ≤ 0.05; ‡ = p ≤ 0.005) and between average values for the 1- and 6-hour glycerol-treated groups († = p ≤ 0.05; ‡ = p ≤ 0.005). No significant difference was found between the average values of the glycerol group at 6 hours and the control group.
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FIG. 4. Left: Photomicrograph of a section through the head of the caudate nucleus and the septal nuclei from a preterm rabbit, 6 hours after glycerol administration. Intraventricular (IVH), multiple subependymal, germinal matrix, septal nuclei (large arrows), and white matter (small arrows) hemorrhages are apparent. H & E, × 25. Right: Photomicrograph of a section through the head of the caudate nucleus and the septal nuclei from a non-glycerol-treated (control) rabbit 6 hours after the intraperitoneal injection of water. H & E, × 25.

In four of the 19 control animals killed at 1 hour and in two of the 10 control animals killed at 6 hours, yielding a combined incidence of spontaneous hemorrhage of 21% (Fig. 2 and Table 2). With the administration of glycerol, the number of animals with intracranial hemorrhages increased, as did the number and size of the hemorrhages in most brain areas examined (Table 2). Compared to control animals, the incidence of germinal matrix hemorrhages in treated animals was 32% in animals killed at 1 hour and 65% in those killed at 6 hours. The latter rate was increased to 77% (13 of 17 animals) when two rabbits with massive IVH but no discernible germinal matrix hemorrhages were added to the group (Fig. 2).

Even though intracranial hypotension reached its lowest point between 30 and 40 minutes after glycerol administration, animals killed 1 hour after the injection had a much lower incidence of multiple hemorrhages (six of 19 animals) than those killed at 6 hours (13 of 17 animals). This would indicate that germinal matrix and ventricular hemorrhages in the preterm rabbit represent a progressive lesion, confirming the hypothesis relating to IVH in the premature infant. Interestingly, of the 13 animals in the 6-hour group with hemorrhages, five (38%) had gross bilateral IVH's (Figs. 3 left and 4 left), while the other eight animals (62%) harbored germinal matrix hemorrhages that had not yet ruptured into the ventricle (Fig. 5). This distribution approximates that reported for 27 premature infants with major (26% of cases) and minor (74% of cases) intracranial hemorrhages detected by ultrasonography (Horbar JD, et al: unpublished data, 1980). Many of the glycerol-treated animals exhibited scattered intracerebral hemorrhages apart from those in the germinal matrix, but those in the germinal matrix were larger and more numerous (Fig. 4 left).

Discussion

The results show that intracranial hypotension induced by intraperitoneal injection of a hypertonic solution of glycerol leads to hemorrhage into the germinal matrix and ventricles of preterm rabbits. As shown in Figs. 3 and 4, the pathological appearance of these hemorrhages is similar to that found in the human preterm infant. In this animal model, germinal

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>No. of Rabbits</th>
<th>Site of Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subarachnoid</td>
<td>Germinal Matrix</td>
</tr>
<tr>
<td>evaluated at 1 hour control</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>glycerol-treated evaluated at 6 hours control</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>glycerol-treated</td>
<td>17</td>
<td>9</td>
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</tbody>
</table>

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FIG. 5. Photomicrograph of a brain section from a glycerol-treated animal showing a hemorrhage (arrows) within the germinal matrix (GM), without rupture into the ventricle. H & E, × 125.

matrix and intraventricular hemorrhages are the predominant part of a diffuse hemorrhagic encephalopathy. This is not, however, inconsistent with the human pathological condition, since a high incidence of subarachnoid and intracerebral hemorrhage has been found in association with IVH in preterm infants.

It is not surprising that intracranial hypotension due to the administration of hypertonic glycerol should result in a diffuse hemorrhagic encephalopathy in the preterm rabbit. Intracranial hypotension has been known to cause subdural, subarachnoid, and intracerebral hemorrhages in full-term infants and in adults. Previous work by Luttrell, et al., has shown that intracranial hemorrhages could be induced in the kitten and adult cat by lowering the ICP with hyperosmolar agents. They also showed that this was an effect of the hypotension and not of the osmotic agent per se, since no hemorrhages were seen when the ICP drop was prevented by the cisternal infusion of saline or mineral oil. What is of interest is the peculiar susceptibility of the germinal matrix to intracranial hypotension. The mechanism by which intracranial hemorrhage is produced by intracranial hypotension is presumably related to the increased transmural pressure across the blood vessels of the brain. It is presently unknown why the vascular bed of the germinal matrix is the area most prone to hemorrhage under this stress, resulting in rupture into the ventricles. The susceptibility of this region may be related to the vascularity, immaturity of the vessel wall and supporting structures, and/or the presence of fibrinolytic activity in this tissue.

The degree of intracranial hypotension induced in this model (−2.7 cm H2O) is similar to that found in the preterm infant (−2.5 cm H2O) at the point of lowest pressure on the 2nd day of life. The correlation between the time of the highest incidence of hemorrhages in premature infants and the nadir of ICP, as determined by Welch and Tsiantos, et al., is illustrated in Fig. 6.

The question arises as to what role, if any, intracranial hypotension plays in IVH in the preterm infant. First, most preterm infants appear to develop neonatal intracranial hypotension. That it does not invariably lead to hemorrhage may be a reflection of the degree of intracranial hypotension and the structural integrity of affected blood vessels. Second, the pathogenesis may be multifactorial. Some of the clinical factors suggested to be associated with IVH may increase the transmural pressure difference, either by their effect on venous pressure (volume loading, pneumothorax) or by further lowering of the ICP (hyperosmolarity). Other conditions, such as hypoxia, hypercarbia, and systemic hypotension, are not as clearly related to the transmural pressure difference. However, it is our hypothesis that neonatal intracranial hypotension can directly produce IVH as shown in this model, but it would also be expected to lower the hemorrhage threshold upon which other factors could act.

References

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