Mannitol Therapy in Perinatal Hypoxic-Ischemic Brain Damage in Rats

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To study the efficacy of mannitol in reducing cerebral edema and improving the ultimate neuropathologic outcome in perinatal cerebral hypoxia-ischemia, 67 7-day postnatal rats were subjected to unilateral common carotid artery ligation followed by exposure to 8% oxygen at 37° C for 3 hours. Twenty-seven rat pups received a subcutaneous injection of 0.1 ml mannitol in a dosage of 4 mg/kg body wt immediately following cerebral hypoxia-ischemia and every 12 hours thereafter for a total of four doses. Control animals received either no therapy (n=16) or an equivalent volume of normal saline (n=24). Mannitol injections in six rat pups not subjected to hypoxia-ischemia produced no mortality but significantly increased serum osmolality from 287 to 361 mos/l (p<0.01). Preliminary studies indicated that substantial mortality occurred when greater doses of mannitol were administered to rats. After 48 hours of recovery from hypoxia-ischemia, the animals were killed and their brains were examined for either tissue water content (33 rat pups) or the presence of neuropathologic alterations (34 rat pups). Mannitol significantly reduced (p<0.001) brain water content, as a reflection of cerebral edema, in both the ipsilateral (88.5% compared with 90.6% in controls) and the contralateral (85.0% compared with 87.2% in controls) cerebral hemispheres. Mannitol therapy did not ameliorate the incidence, distribution, or severity of tissue injury in the cerebral cortex, subcortical white matter, hippocampus, striatum, or thalamus of the ipsilateral cerebral hemisphere compared with the controls. Thus, while mannitol substantially reduces the extent of cerebral edema following hypoxia-ischemia, no beneficial effect on ultimate brain damage occurs. Mannitol therapy affords little or no protection for the perinatal brain from hypoxic-ischemic damage. (Stroke 1990;21:1210-1214)

The role of cerebral edema in aggravating hypoxic-ischemic encephalopathy of newborn infants is unresolved.1,2 Published reports indicate that edema is a prominent feature of human perinatal cerebral hypoxia-ischemia3-5 and that edema is associated with hypoxic-ischemic brain damage in perinatal animals.6,7 However, the question remains as to whether cerebral edema per se causes or contributes to brain tissue injury. Investigations in adult animals suggest that edema accentuates tissue necrosis8-11 and that therapy with hyperosmolar agents, specifically mannitol, reduces the extent of hypoxic-ischemic brain damage.12-14 Until now, parallel studies in perinatal animals have not been accomplished. Furthermore, there have been no controlled clinical trials indicating that the use of therapeutic agents known to reduce cerebral edema in any way ameliorates the extent of ultimate brain damage in asphyxiated newborn infants.1 To study the efficacy of the hyperosmolar agent mannitol in reducing cerebral edema and improving neuropathologic outcome in perinatal cerebral hypoxia-ischemia we subjected 7-day postnatal rats to unilateral common carotid artery ligation combined with hypoxia by the inhalation of 8% oxygen.

Materials and Methods

Dated pregnant Wistar rats were purchased from a commercial breeder (Charles River Laboratories, Inc., Wilmington, Mass.) and housed in individual cages. Offspring, delivered vaginally, were reared with their dams until the time of experimentation at 7 days of postnatal age.

Cerebral hypoxia-ischemia was induced in 67 7-day postnatal rats by a previously described technique.6 Specifically, individual rat pups were lightly anesthetized with halothane (4% induction, 1.0-1.5% main-
tenance) and the right common carotid artery was permanently ligated with 4-0 surgical silk. Upon recovery from anesthesia, the rat pups were returned to their dams for 4 hours, after which they were placed in 500-ml air-tight jars partially submerged in a 37° C water bath. A humidified gas mixture of 8% oxygen-92% nitrogen was delivered through the jars via inlet and outlet portals. This insult produces irreversible brain damage in the form of selective neuronal necrosis and/or infarction predominantly in the cerebral hemisphere ipsilateral to the arterial occlusion in 92% of rats. The rat pups underwent cerebral hypoxia-ischemia for 3 hours, following which they were returned to their dams. During recovery from hypoxia-ischemia, the rat pups were randomly assigned to one of three groups. Each animal in group I (n=27) received a 4 mg/kg subcutaneous dose of 25% mannitol immediately following hypoxia-ischemia; the dose was repeated after 12, 24, and 36 hours of recovery. Preliminary studies had shown that higher doses of mannitol were associated with substantial mortality. Rat pups in group II (n=24) received an equivalent volume (0.1 ml) of normal saline at the same intervals as their mannitol-treated littermates. The 16 animals in group III received neither mannitol nor saline during recovery. Groups II and III comprised the controls. All rat pups were sacrificed 48 hours after the hypoxic-ischemic insult, and the brain of each animal was examined for either the presence of cerebral edema or neuropathologic alterations (see below).

A preliminary study was conducted to ascertain changes in the hematocrit and serum osmolality of animals undergoing mannitol therapy. Six 7-day postnatal rats not subjected to cerebral hypoxia-ischemia received a subcutaneous injection of 4 mg/kg mannitol 1 hour after which they were decapitated and blood was collected from their severed neck vessels. Hematocrit and serum osmolality were measured in these six rat pups and in six animals not receiving mannitol.

Forty-eight hours after hypoxia-ischemia, 33 rat pups (13 from group I, 14 from group II, and six from group III) were decapitated, and 75–100-mg samples of each cerebral hemisphere within the distribution of the middle cerebral artery (MCA) were immediately dissected, placed in tared 5-ml glass vials, and weighed on a microanalytical balance. Subsequently, each tissue sample was desiccated at 70° C for 48 hours. Reweighing of the vial ascertained the hemispheric dry weight, and by subtraction from the total hemispheric weight the wet weight of the tissue sample was obtained. Water content was determined as a percentage of the total hemispheric weight as

\[
\text{(total wt-dry wt)} \times 100\% \text{.}
\]

Also 48 hours after hypoxia-ischemia, 34 other rat pups (14 from group I, 10 from group II, and 10 from group III) were anesthetized with 50 mg/kg body wt i.p. pentobarbital and their brains were perfusion-fixed via the ascending aorta with a 1:1:8 mixture of formaldehyde:glacial acetic acid: absolute methanol (FAM). Brains were left in situ for 2 hours, after which they were removed from the skulls and stored in FAM for later microscopic examination by one of us without knowledge of the animal’s group. The FAM-fixed forebrain was cut coronally and embedded in paraffin. Subserial sections 7 µm thick were stained with hematoxylin and eosin. In each rat pup two sections, one just anterior to the body of the anterior commissure and the other immediately posterior to the infundibulum, were selected for neuropathologic study. Ischemic neuronal alterations in the cerebral cortex, subcortical white matter, hippocampus, striatum, and thalamus of each brain were graded as 0, no neurons damaged; 1, few (<5%) neurons damaged; 2, moderate number (5–50%) of neurons involved; 3, majority (>50%) of neurons involved; 4, infarction localized predominantly to the distribution of the MCA; or 5, infarction widespread within the cerebral hemisphere.

Data were analyzed where appropriate using one-way analysis of variance and the Mann-Whitney U test.

Results

In the preliminary study, for the six mannitol-treated rat pups not subjected to hypoxia-ischemia mean±SEM hematocrit was 45.5±1.7%, with a concomitant serum osmolality of 361±19.6 mos/l. This hematocrit was significantly higher than those of 39.3±1.2% and 39.1±1.6% for six rat pups (p<0.05), and serum osmolality was 24–28% higher than those of 283±6.2 and 291±9.8 mos/l in groups II and III, respectively (p<0.01).

As shown in Figure 1, water content of the contralateral hemisphere in groups II and III did not differ from a reference value of 87.7% derived from 30 age-equivalent (9-day postnatal) rats not subjected to hypoxia-ischemia. In contrast, water content of the ipsilateral cerebral hemisphere increased to 90.7% and 90.5% in groups II and III, respectively (p<0.01). Mannitol therapy lowered the water content of both cerebral hemispheres compared with groups II and III (p<0.01). Indeed, water content of the ipsilateral cerebral hemisphere did not differ significantly from the reference value.

Histologic analysis indicated that major tissue injury had occurred in all brains examined (Table 1). Using the scoring system described, no differences in pathologic outcome were noted among the three groups. To assess further the damaged cerebral hemispheres for intergroup differences, all brains were ranked from the least to the most damaged histologically (Figure 2). The evaluation revealed that no group was more or less damaged than the others. Within each group, as many brains ranked in the less damaged 50th percentile as in the more damaged 50th percentile.

Discussion

Our results indicate that mannitol therapy of immature rats leads to hyperosmolality, which in turn reduces the cerebral edema that precedes or accom-
panies hypoxic-ischemic brain damage. Not only did mannitol decrease the extent of swelling in the cerebral hemisphere undergoing tissue injury, but the drug also decreased the water content of the nonischemic, contralateral hemisphere to a similar extent. Despite the reduction in cerebral edema, mannitol therapy was not associated with any amelioration in either the distribution or the extent of brain damage seen histologically.

Although the increases in brain water content appear modest, the changes equate to substantial increases in brain volume, as a reflection of cerebral edema. Mean water content of the cerebral hemisphere ipsilateral to the carotid artery occlusion in groups II and III increased by 3.3% compared with the contralateral hemisphere and with the reference value. According to the equation 

\[
\% \text{ increase in brain volume} = \left( \frac{\% \text{ dry wt}_{\text{ref}} - \% \text{ dry wt}_{\text{ctrl}}}{\% \text{ dry wt}_{\text{ctrl}}} \right) \times 100\% = 31%.
\]

Thus, when cerebral edema was maximal, the hypoxic-ischemic cerebral hemisphere was 31% larger than the contralateral hemisphere or either hemisphere of the reference animals. Mannitol therapy nearly completely reversed this extent of cerebral swelling.

<table>
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<tr>
<th>Table 1. Neuropathologic Damage Scores Following Cerebral Hypoxia-Ischemia in Mannitol-Treated and Control Immature Rats</th>
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<tbody>
<tr>
<td>Structure</td>
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<tr>
<td>Cerebral cortex</td>
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<tr>
<td>Hippocampus</td>
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<tr>
<td>Striatum</td>
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<td>Thalamus</td>
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<td>Subcortical white matter</td>
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Values are mean±SD. Damage scores were determined for individual rat pups as 0, no; 1, few; 2, moderate number; 3, majority of neurons damaged; 4, infarction localized to middle cerebral artery distribution; or 5, widespread infarction. No significant differences in damage scores were noted among the groups.
The inability of mannitol to influence favorably the severity of hypoxic-ischemic brain damage in immature rats contrasts with its presumed effectiveness in adult animals. Little\textsuperscript{13} subjected adult cats to unilateral MCA occlusion, during which the animals received an intravenous infusion of 1.2 g/kg mannitol. Neuropathologic examination after 0.5–6 hours of recovery revealed considerable preservation of neurons in the brains of the mannitol-treated cats compared with the untreated animals. Little\textsuperscript{13} attributed the protective effect of mannitol to a suppression of ischemic cerebral edema and its consequent disruption of the microcirculation (delayed hypoperfusion). See also References 12 and 13.

Why is mannitol not effective in immature rats in which hypoxic-ischemic brain damage is equal to or greater than that of mature animals?\textsuperscript{8} In adult rats recovering from cerebral hypoxia-ischemia or ischemia alone sufficient to produce brain damage, there is a brief phase (1–2 hours) of cerebral hyperemia followed by secondary hypoperfusion that lasts until 24 hours after the insult.\textsuperscript{17-19} This secondary hypoperfusion occurs coincident with cerebral edema and may be of a degree sufficient to render the adult brain vulnerable to additional ischemic damage. Accordingly, management of brain swelling with a hyperosmolar agent should improve cerebral perfusion and reduce the extent of brain damage. Such is not the case in immature rats, in which an early post-hypoxic-ischemic hypoperfusion does not take place for ≥24 hours of recovery.\textsuperscript{20} Rather, a late hypoperfusion occurs, which results from rather than causes tissue necrosis, seen histologically after 15–50 hours of recovery. Thus, any therapy to improve cerebral blood flow by reducing cerebral edema following hypoxia-ischemia in immature animals should not alter the ultimate neuropathologic outcome.

To explain the age-specific difference in the protective effect of mannitol on hypoxic-ischemic brain damage, mechanical factors must be considered. In immature rats, the brain swelling that occurs following hypoxia-ischemia is accommodated, at least in part, by a widening of the animal’s elastic sutures, a depression of the tentorium cerebelli, and moderate herniation of the vermis cerebelli.\textsuperscript{8} These forms of internal decompression serve to minimize increases in intracranial pressure and its consequent effect on cerebral perfusion. Such internal decompression cannot be accomplished in adult animals with their bony calvariae and rigid dural membranes.

Our findings in immature rats are relevant to newborn human infants in whom cerebral edema arises as a consequence of an asphyxial insult at birth. Recent investigations have shown that asphyxiated newborn infants who exhibit evidence of cerebral edema on computed tomography manifest only modest or no elevation in intracranial pressure and no decrease in cerebral perfusion pressure (mean arterial blood pressure–intracranial pressure).\textsuperscript{21,22} Thus, as in immature rats,\textsuperscript{20} hypoxic-ischemic brain swelling in newborn human infants is not associated with any major disruption of the cerebral circulation so long as systemic blood pressure is maintained.\textsuperscript{23} Presumably, the edematous human infant brain is capable of an internal decompression comparable to that of immature rats, as evidenced by full fontanels and splayed sutures. Accordingly, mannitol therapy should afford little or no protection for the perinatal brain from hypoxic-ischemic brain damage.

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