Hemodilution With Low-Molecular-Weight Hydroxyethyl Starch After Experimental Focal Cerebral Ischemia in Rabbits

P.D. Lyden, MD, L.I. Alving, BS, J.A. Zivin, MD, PhD, and J.F. Rothrock, MD

Newly developed colloid volume-expanding agents with mean molecular weights lower than currently available agents may improve outcome after stroke with fewer allergic and coagulation system side effects. The smaller molecule, however, may exacerbate ischemic cerebral edema if it accumulates in areas where ischemia has damaged the blood–brain barrier. We administered low-molecular-weight hydroxyethyl starch to rabbits after embolic infarction and measured specific gravity and total water content. We found evidence of ischemic edema in the hemisphere ipsilateral to the embolic arterial occlusion, but the measures of edema were not different in treated and control groups. Of those rabbits suffering severe neurologic deficit, mortality was 2 of 13 in the treated compared with 7 of 12 in the control groups (p < 0.01). Thus, hemodilution with low-molecular-weight hydroxyethyl starch did not exacerbate cerebral edema and may have improved survival in this model. (Stroke 1988; 19:223–227)

Materials and Methods

The animal model of focal cerebral ischemia used in this study has been described in detail. In brief, female New Zealand White rabbits, 2.5–3.0 kg, were anesthetized with ketamine and xylazine. Through a right lateral neck incision, the two branches of the external carotid artery were ligated. A Silastic catheter (0.025 in., Dow Corning, Chicago, Illinois) was inserted into the lateral branch and advanced retrograde so that the tip lay adjacent to the ostium of the internal carotid artery. The wound was closed after securing the catheter in place, and the catheter was filled with heparinized saline. The rabbits were allowed 24 hours to recover; any that exhibited neurologic deficit after surgery were not used further. To induce a stroke in the remaining 67 unanesthetized rabbits, 0.10 ml of autologous blood incubated 24 hours at 37°C was injected into the catheter, followed by 0.5 ml saline. The rabbit was observed immediately for evidence of hemiparesis, nystagmus, circling, seizure, and obtundation. Arterial blood was sampled for osmolality by freezing point depression (Osmette A, Precision Systems Incorporated, Natick, Massachusetts), hematocrit (microcrit centrifuge), and arterial blood gases (ABL2, Radiometer, Copenhagen, Denmark). One day after stroke all rabbits received 1 ml/kg 3% Evans blue i.v. and were killed 30 minutes later with thiopental. The brains were quickly removed and cooled in kerosene at 0°C for exactly 2 minutes. The brains were sectioned, and the area of maximal Evans blue staining was used to identify the region of edema. One-cubic-millimeter sections were removed from gray and white matter in the area of maximal edema; homologous control sections were removed from the contralateral hemisphere. Sections were also taken from the hippocampus and basal ganglia, regardless of whether these areas were stained with Evans blue. All sections were immediately dropped into a bromobenzene/kerosene column for measurement of specific gravity. The

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Supported in part by a Veterans Administration Career Development Award (to P.D.L.), by a grant from American Critical Care Corporation, and by National Institutes of Health Grant 5 R01 NS 23373-02.

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Received May 18, 1987; accepted August 10, 1987.
The remainder of each hemisphere was weighed, dried to constant weight (≥ 48 hours), and reweighed for wet: dry weight ratios.

**Experimental Design**

After embolization the rabbits were examined and divided into mild and severe stroke classes. A severe stroke was defined as the immediate presence of hemiparesis plus two of the following: nystagmus, circling, or obtundation; in addition, any convulsions implied severe stroke. Any rabbit with findings less severe than the above was classified as mild; this group included those rabbits with a normal examination. We have previously observed in this model that this differentiation by clinical criteria reliably separates rabbits that will exhibit large lesions from those that will exhibit small lesions on histopathologic examination.7 After classification as mild or severe, the rabbits were randomized to treatment groups. Hemodilution rabbits received hetastarch intravenously beginning 40-50 minutes after stroke. In a preliminary study (acute group) seven rabbits were given 40 ± 5 ml low-molecular-weight hetastarch over 5 minutes to lower hematocrit to 80% of baseline. These acute group rabbits and a control group of seven embolized rabbits that received <10 ml saline were killed 6 hours after stroke. 

In hemodiluted rabbits the hematocrit was reduced from 38 ± 2.4% (mean ± SD) to 32 ± 3.3%, or 84% of baseline (p < 0.0001, n = 7, paired t test), whereas hematocrit did not change over 6 hours in untreated rabbits (baseline 41.9 ± 2.3%, 6-hour 39.2 ± 1.3%; n = 7). Serum osmolality was not changed in hemodiluted rabbits (289 ± 5 mosm). The resulting specific gravity and water content data are presented in Table 1. There was no significant difference in these measures of edema between untreated and hemodiluted rabbits.

The remainder of the rabbits were observed for 24 hours. There were 28 with mild strokes, of which 10 were randomized to hemodilution, and 25 with severe strokes, of which 13 were randomized to hemodilution. The control groups received no treatment. Table 2 lists the hematocrit, osmolality, and brain water content data for the mild and severe classes and the treatment groups. As shown, hematocrit was reduced to 80% of baseline within 4 hours in both hemodiluted groups and remained near that level over the entire 24 hours. The total amount of low-molecular-weight hetastarch required over 24 hours to achieve this degree of hemodilution was 55 ± 11 ml. This volume was usually administered in two or three boluses over the period of observation, based on serial hematocrits, although some rabbits received a continuous infusion. Osmo-

### Table 1. Effect of Hemodilution on Specific Gravity and Water Content 6 Hours After Ischemia in 7 Rabbits in Preliminary Trial

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Specific gravity</th>
<th>Water content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White matter</td>
<td>Gray matter</td>
</tr>
<tr>
<td>Untreated ipsilateral</td>
<td>1.0400 ± 0.0008</td>
<td>1.0433 ± 0.0015</td>
</tr>
<tr>
<td>Untreated contralateral</td>
<td>1.0408 ± 0.0014</td>
<td>1.0428 ± 0.0014</td>
</tr>
<tr>
<td>Hemodiluted ipsilateral</td>
<td>1.0398 ± 0.0023</td>
<td>1.0423 ± 0.0014</td>
</tr>
<tr>
<td>Hemodiluted contralateral</td>
<td>1.0419 ± 0.0016</td>
<td>1.0426 ± 0.0017</td>
</tr>
</tbody>
</table>

Hemisphere ipsilateral or contralateral to occluded artery; water content as percent wet wt. Data are mean ± SD.

### Results

To look for immediate effects of hemodilution on cerebral edema and other physiologic variables, we conducted a preliminary trial by killing a group of seven rabbits 6 hours after stroke and measuring specific gravity and total water content. There was no significant change in respiration or arterial blood gases. In hemodiluted rabbits the hematocrit was reduced from 38 ± 2.4% (mean ± SD) to 32 ± 3.3%, or 84% of baseline (p < 0.0001, n = 7, paired t test), whereas hematocrit did not change over 6 hours in untreated rabbits (baseline 41.9 ± 2.3%, 6-hour 39.2 ± 1.3%; n = 7). Serum osmolality was not changed in hemodiluted rabbits (289 ± 5 mosm). The resulting specific gravity and water content data are presented in Table 1. There was no significant difference in these measures of edema between untreated and hemodiluted rabbits.

### Table 2. Effect of 24 Hours Hemodilution on Hematocrit, Osmolality, and Brain Water Content in Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0</th>
<th>4</th>
<th>24</th>
<th>1</th>
<th>4</th>
<th>24</th>
<th>Brain water content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild, hemodilated</td>
<td>10</td>
<td>39 ± 0.3</td>
<td>30 ± 0.8*</td>
<td>34 ± 1.5*</td>
<td>287 ± 2</td>
<td>286 ± 2</td>
<td>293 ± 2</td>
<td>80.1 ± 1</td>
</tr>
<tr>
<td>Mild, control</td>
<td>18</td>
<td>39 ± 0.7</td>
<td>37 ± 0.6</td>
<td>36 ± 1.4</td>
<td>283 ± 2</td>
<td>282 ± 1</td>
<td>292 ± 2</td>
<td>79.8 ± 2</td>
</tr>
<tr>
<td>Severe, hemodilated</td>
<td>13</td>
<td>41 ± 0.7</td>
<td>34 ± 0.8*</td>
<td>36 ± 1.5*</td>
<td>290 ± 1</td>
<td>293 ± 8</td>
<td>289 ± 3</td>
<td>80.5 ± 1</td>
</tr>
<tr>
<td>Severe, control</td>
<td>12</td>
<td>39 ± 0.8</td>
<td>37 ± 1.7</td>
<td>38 ± 1.9</td>
<td>284 ± 1</td>
<td>284 ± 2</td>
<td>294 ± 2</td>
<td>79.3 ± 0.3</td>
</tr>
</tbody>
</table>

Mild and severe stroke defined in text. Hematocrit 0, 4, and 24 hours after stroke as mean ± SD percent; osmolality 1, 4, and 24 hours after stroke as mean ± SD milliosmoles; brain water content as mean ± SD percent wet weight. I, ipsilateral hemisphere; C, contralateral hemisphere.

*Significantly different from baseline by analysis of variance, Newman-Keuls, p<0.05.
lality did not change significantly during the experiment.

At sacrifice, all brains in the severe class (hemodiluted and control groups) exhibited staining with Evans blue, facilitating the selection of ischemic tissue. Lesions were invariably found in the ipsilateral parietal and temporal lobes, usually involving subcortical structures to a greater extent than cortex. Lesion size was variable and was not quantified because of the necessity to rapidly remove sections of tissue for microgravimetry. In the mild class (hemodiluted and control groups), approximately one third of the rabbits showed no staining, and sections were taken from areas of palpable softening. Lesions were found in the same locations as described for the severe class. Figure 1 presents the specific gravity data for the hemodiluted and control groups for the hemisphere contralateral to the arterial occlusion; Figure 2 presents the data for the lesioned (ipsilateral) hemisphere. The ipsilateral hemisphere showed increased edema in all groups compared with the contralateral hemisphere, suggesting the development of ischemic edema in and around the infarcts. There were no significant differences between the hemodiluted and control groups, although the severe control group did show a trend toward lower specific gravity in the ipsilateral hemisphere. This group was small because of high mortality after stroke; of 13 hemodiluted rabbits with severe strokes, only two died compared with seven deaths among 12 controls with severe strokes. This difference in mortality was
Mild, hemodiluted

Severe, hemodiluted

Mild, control

Severe, control

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4 of 18 in the control group. Hemodilution did not affect mortality in the mild class; 2 of survivors in the severe control group. The increased rabbits with severe strokes because of the small number hemodilution itself ameliorated cerebral edema in the modilution with low-molecular-weight hydroxyethyl starch exacerbates experimental ischemic edema. To the contrary, in control rabbits suffering severe strokes we detected evidence of increased cerebral edema in the ipsilateral hemispheres. We cannot conclude that the hemodilution itself ameliorated cerebral edema in the rabbits with severe strokes because of the small number of survivors in the severe control group. The increased survival in the severe hemodiluted group was significant, however. We did not measure arterial blood pressure or cardiac output in this experiment because the degree of hemodilution we used has been shown by previous authors to reliably increase cardiac output without changing blood pressure in animals and humans.1-6

Our failure to demonstrate a harmful effect of hemodilution may be explained by several possibilities. First, there may in fact be no harmful effect of low-molecular-weight hetastarch on ischemic edema. Second, variability in the size and severity of the observed lesions may have precluded the detection of an increase in edema in the hemodiluted groups. We selected the most involved tissue, by sampling from the area of maximal Evans blue staining, and thus should have minimized this possibility.

A final possibility is that we killed the rabbits before the full appearance of edema. Ischemic edema fully develops in rats, rabbits, and cats within 24–36 hours of vascular occlusion6,14 and within 24–48 hours in humans.12 Consequently, one would expect to find an adverse effect of hemodilution on cerebral edema at 24 hours if such an effect does exist. The decrease in specific gravity observed in the ipsilateral hemispheres of all our groups (Table 3) confirms the development of ischemic edema by 24 hours. Thus, it is very likely that we would have observed any deleterious effect of hemodilution within 24 hours. The production of ischemic edema within this time period is thought to reflect the movement of water, sodium, and proteins across the damaged blood–brain barrier.13 Mannitol (molecular weight 182) has been shown to exacerbate ischemic edema, presumably by accumulating in ischemic brain tissue and exerting an osmotic gradient effect.12 The mean molecular weight of low-molecular-weight hydroxyethyl starch (246,000 average) is 3–4 times that of albumin, and it is not known whether a molecule of this size would cross a damaged osmotic barrier that had become permeable to albumin-Evans blue complexes. Our data suggest that a molecule of this size does not accumulate in ischemic brain tissue to a degree sufficient to cause a change in brain water or specific gravity.

Hemodilution (using dextran 40) has been shown in a canine model to increase intracranial pressure following cerebral ischemia, but the effect on cerebral water content was not measured.3 Wood et al2 implied that the cause of the elevation of intracranial pressure was an increase in cerebral blood volume, not an increase in cerebral water. Our results support this explanation. Hemodilution with dextran 40 or perfluorocarbons has been shown to elevate cerebral blood flow, to improve survival, and to restore ischemic biochemical parameters in experimental models of ischemic stroke.1,2,13-15 Low-molecular-weight hydroxyethyl starch appeared to improve survival after severe but not mild stroke in our model. It remains to be proven that this agent successfully protects brain from infarction after focal ischemia.

Acknowledgment

The authors gratefully acknowledge the expert editorial assistance of Ms. Barbara Reader.

References

5. Wood JH, Fleischer AS: Observations during hypervolemic
TABLE 3. (Continued)

<table>
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<th></th>
<th>Gray matter</th>
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<td>1.0428 ± 0.0004</td>
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<td>1.0443 ± 0.001</td>
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<td>11</td>
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<td>1.0408 ± 0.001</td>
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<td>4</td>
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</table>

hemodilution of patients with acute focal cerebral ischemia. JAMA 1982;248:2999–3004


KEY WORDS

- brain edema
- cerebral ischemia
- hemodilution
- hydroxyethyl starch
- rabbits
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Stroke. 1988;19:223-227
doi: 10.1161/01.STR.19.2.223

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