Effect of delayed albumin hemodilution on infarction volume and brain edema after transient middle cerebral artery occlusion in rats

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The authors examined the effect of delayed high-concentration albumin therapy on ischemic injury in a highly reproducible model of middle cerebral artery (MCA) occlusion in rats. Male Sprague–Dawley rats weighing 270 to 320 g were anesthetized with halothane and subjected to 120 minutes of temporary MCA occlusion induced by means of a poly-L-lysine–coated intraluminal nylon suture inserted retrograde via the external carotid artery into the internal carotid artery and MCA. The agent (20% human serum albumin [HSA]) or control solution (sodium chloride 0.9%) was administered intravenously at a dosage of 1% of body weight immediately after suture removal following a 2-hour period of MCA occlusion. The animals’ neurological status was evaluated during MCA occlusion (at 60 minutes) and daily for 3 days thereafter. The brains were perfusion-fixed, and infarct volumes and brain edema were determined. The HSA significantly improved the neurological score compared with saline at 24 hours after MCA occlusion. The rats treated with HSA also had significantly reduced total infarct volume (by 34%) and brain edema (by 81%) compared with saline-treated rats. There was a strong correlation between hematocrit level and brain edema (p < 0.01), and between total infarct volume or brain edema and neurological score at 24, 48, and 72 hours postinjury (p < 0.0002).

These results strongly support the beneficial effect of delayed albumin therapy in transient focal ischemia and indicate its possible usefulness in treating patients with acute ischemic stroke.

KEY WORDS • albumin • focal cerebral ischemia • hemodilution • neuroprotection • stroke • rat

HERAPEUTIC hemodilution to treat acute ischemic stroke has been the subject of many studies since the 1960s in both animal experiments and clinical trials, but its use remains controversial. Much of this controversy can be ascribed to differences in hemodilution protocols, particularly with regard to the type of hemodiluting agent and the timing of its use, the hematocrit level achieved, and the degree to which normovolemia, hypervolemia, or hypovolemia develop. Previous investigations using hyperosmolar plasma volume-expanding agents (dextran, mannitol, and glycerol) in the treatment of experimental focal cerebral ischemia have yielded encouraging results.20,23 The beneficial effects of these agents have been variously ascribed to increased cardiac output, improved collateral circulation, hemodilution, decreased platelet aggregation, and hyperosmolarity. A protective effect of a concentrated albumin solution in the treatment of focal cerebral ischemia was reported by Matsui and Asano26 and by Cole and coworkers,4 whereas other researchers were unable to detect a positive effect.21,30

Two large trials were conducted in Scandinavia28 and Italy16 to test the clinical application of hemodilution as a potential therapy for cerebral ischemia, but no positive results were obtained. However, both studies have been criticized because of the moderate degree of hemodilution achieved and the delay in entering patients in the study.

Most previous studies have used hemodiluting agents before4,5 and immediately, or within 1 hour, after the onset of ischemia.21,25,32,33 In a clinical setting, however, it is highly unlikely that hemodilution can be instituted immediately after the onset of cerebral ischemia. Only a few studies have investigated delayed hemodilution after cerebral ischemia.25,37 Thus, the purpose of this study was to examine the effect of delayed high-concentration albumin therapy on ischemic injury in a highly reproducible model of middle cerebral artery (MCA) occlusion in rats.2

Materials and Methods

Animal Preparation

Eighteen adult male Sprague–Dawley rats weighing 270 to 320 g were denied food overnight but had free access to water. Protocols for these studies were approved by the University of Miami Animal Care and Use Committee. Following intraperitoneal administration of atropine sulfate (0.5 mg/kg), anesthesia was induced with 3.5%
halothane in a mixture of 70% nitrous oxide and 30% oxygen. The rats were orally intubated, immobilized by intravenously administered pancuronium bromide (0.6 mg/kg), and mechanically ventilated. Temperature probes were inserted into the rectum and the left temporalis muscle, and separate heating lamps were used to maintain rectal and cranial temperatures at 37 to 37.5°C. The right femoral artery and vein were catheterized for continuous blood pressure monitoring and periodic blood sampling for arterial gas levels, pH, hematocrit, and plasma glucose (15 minutes before MCA occlusion, at 15, 90, and 110 minutes after MCA occlusion and 15 minutes after MCA suture removal). Rectal temperature and body weight were monitored before MCA occlusion and periodically for 3 days thereafter. The mean arterial blood pressure (MABP) was measured via an indwelling femoral artery catheter connected to a precalibrated pressure transducer and was recorded continuously via polygraph. Serial measurements were made of arterial blood gas levels and pH and plasma glucose.

**Experimental Model**

The right MCA was occluded for 2 hours by using the intraluminal suture method as described by Zea Longa, et al., and modified by us. Briefly, the right common carotid artery was exposed via a midline neck incision and dissected free of surrounding nerves, the occipital branches of the external carotid artery were coagulated, and the pterygopalatine artery was ligated. A 4-cm length of 3-0 monofilament nylon suture was inserted via the proximal external carotid artery into the internal carotid artery and MCA, a distance of 19 to 20 mm from the common carotid artery bifurcation according to the animal’s weight. Prior to use, the tip of the suture was heat-blunted, and a 20-mm distal segment was coated with poly-L-lysine solution (0.1% w/vol) and dried at 60°C for 1 hour; we have shown that this coating procedure enhances the reproducibility of the resulting infarct. Following suture placement, the neck incision was closed, the animals were allowed to awaken from anesthesia and were tested using a battery of standardized neurobehavioral tests.

**Behavioral Testing**

Behavioral tests were conducted in all 18 rats before, during (at 60 minutes), and daily for 3 days after MCA occlusion. Thebattery consisted of two tests that have been used previously to evaluate various aspects of neurological function: 1) the postural reflex test, developed by Bederson and colleagues to examine sensorimotor integration in forelimb placing responses to visual, tactile, and proprioceptive stimuli. Neurological function was graded on a scale of 0 to 12 (normal = 0, maximum = 12). The rats that did not demonstrate an initial right upper-extremity paresis were excluded from further study. After 2 hours of MCA occlusion, rats were

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**TABLE 1**

Physiological variables tested in an animal model before and after MCA occlusion*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saline (9 rats)</th>
<th>HSA (9 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preischemia</td>
<td>Postischemia</td>
</tr>
<tr>
<td>cranial temp (°C)</td>
<td>37.2 ± 0.03</td>
<td>37.1 ± 0.01</td>
</tr>
<tr>
<td>rectal temp (°C)</td>
<td>37.4 ± 0.04</td>
<td>37.3 ± 0.03</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>102.2 ± 4.3</td>
<td>110.0 ± 4.4</td>
</tr>
<tr>
<td>hematocrit (%)</td>
<td>38.8 ± 2.2</td>
<td>39.8 ± 3.2</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>123.0 ± 4.3</td>
<td>109.3 ± 2.1†</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.02</td>
<td>7.40 ± 0.01</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>38.0 ± 0.7</td>
<td>39.6 ± 0.3</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>106.7 ± 4.9</td>
<td>115.9 ± 3.9</td>
</tr>
</tbody>
</table>

* Values are presented as the mean ± the SEM. Preischemia = 15 minutes before MCA occlusion; postischemia = 15 minutes after MCA occlusion.
† Significantly different from preischemia, p < 0.05.
Delayed albumin therapy of cerebral ischemia

quantitate the outlined areas and compute integrated volumes and infarction frequency distribution.45

The volume of infarction was calculated as the product of the cross-sectional area for all sections and the distance between the sections. To compensate for brain swelling in the ischemic hemisphere,3 the infarct volume in each rat was corrected by first computing the volume of the left and right hemispheres and applying the following formula: corrected infarct volume = left hemisphere volume — (right hemisphere volume — measured infarct volume). The degree of associated brain edema was determined as the difference in brain volume between the two hemispheres.

Statistical Analysis

Infarcts were analyzed by repeated-measures analysis of variance (ANOVA) and by post hoc Bonferroni testing, which accounted for multiple comparisons. Total (corrected) infarct volumes, overall brain edema, neurological score, and physiological variables were compared by Student’s t-test and were further analyzed by linear regression analysis. The Fisher exact test was used to compare the frequency of infarction between groups. A probability value of less than 0.05 was regarded as significant. Values are presented as the mean ± the standard error of the mean (SEM).

Sources of Supplies and Equipment

The Sprague–Dawley rats were obtained from Charles River Laboratories, Wilmington, MA. Temperature readings were obtained by means of a Mon-a-therm probe (model 7000) purchased from Malinckrodt, Inc., St. Louis, MO. The Statham pressure transducer (model P23XL) was acquired from Viggo-Spectramed, Inc., Oxnard, CA, and the polygraph (model RS3400) from Gould, Inc., Valley View, OH. The blood gas and pH analyzer (model ABL 330) was manufactured by Radiometer America, Inc., Westlake, OH, and the glucose monitor (model 2300 Stat) by Yellow Springs Instrument Co., Inc., Yellow Springs, OH. The HSA was purchased from Baxter Travenol Laboratory, Deerfield, IL. The Xillix CCD-based camera system, Nikon macro lens and filter, MCID image analysis system, and DEC-Alpha workstation were purchased from Xillix Technologies Corp., Vancouver, BC, Canada; Nikon, Tokyo, Japan; Imaging Research, St. Catherine’s, ON, Canada; and Digital Equipment Corp., Maynard, MA, respectively.

Results

General Physiological Parameters

Rectal and cranial (temporalis muscle) temperatures, MABP, and plasma glucose and blood gas levels in the 18 animals studied showed no significant differences between groups (Table 1). The hematocrit level in the control group was 38.8 ± 2.2% (baseline) and 39.8 ± 3.2% (15 minutes after saline hemodilution). The hematocrit level in the albumin-treated group was 40.1 ± 1.1% at baseline and was reduced to 25.3 ± 0.3% by HSA treatment (p < 0.05, Student’s t-test).

Neurological Assessment

Contralateral forelimb placing deficits were clearly present at 60 minutes after MCA occlusion in both groups (9 ± 0). The HSA significantly improved the neurological score compared with saline at 24 hours after MCA occlusion (6.4 ± 0.7 and 8.5 ± 0.3, respectively; p < 0.01) and there was a nonsignificant trend toward better recovery in HSA-treated rats than in the saline-treated control group at 48 and 72 hours.

Infarct Volume and Edema

Treatment with HSA significantly reduced the total (cortical + subcortical) infarct volume (Fig. 1A) compared with treatment with saline (88.9 ± 17.9 mm³ vs. 133.8 ± 7.6 mm³, respectively; p < 0.04). Figure 1B demonstrates the rostrocaudal distribution of total infarct areas in both groups. Infarct areas were significantly smaller in HSA-treated rats than in the saline-treated control group at coronal levels five (bregma −1.3 mm), six (bregma −1.8 mm), seven (bregma −3.8 mm), and eight (bregma −5 mm). When considered separately, cortical infarct volume was significantly reduced by treatment with HSA compared with saline (56.1 ± 24 mm³ and 128.9 ± 8.9 mm³, respectively; p < 0.01). Figure 2A demonstrates the rostrocaudal distribution of cortical infarct areas in both groups. Infarct areas were significantly smaller in HSA-treated rats than in the saline-treated group at coronal levels five (bregma −1.3 mm), seven (bregma −3.8 mm), and eight (bregma −5 mm). Although striatal infarct volume did not differ significantly between groups, striatal infarct areas were significantly smaller in HSA-treated rats than in the saline-treated group (Fig. 2B) at coronal level six (bregma −1.8 mm). Figure 3 demonstrates the frequency distribution of cerebral infarction at nine stereotactic levels in rat brains treated with saline and HSA, as well as the results of Fisher’s exact test comparing the two groups.

![Graph A: Cortex](image)

![Graph B: Caudoputamen](image)

**Fig. 2.** Bar graphs showing rostrocaudal distribution of areas of infarction at nine cortical levels in HSA- and saline-treated rats. A: Areas of cortical infarction. B: Areas of subcortical infarction. Data are presented as the mean ± the SEM for eight animals in each group. *p < 0.03, HSA- compared with saline-treated groups (repeated-measures ANOVA followed by Bonferroni test).
Treatment with HSA also significantly reduced brain edema (Fig. 4A) compared with that seen in saline-treated rats (2.49 ± 1.98% vs. 13.05 ± 2.06%, respectively; p < 0.002). Figure 4B demonstrates the rostrocaudal distribution of brain edema in both groups. Administration of HSA greatly reduced brain edema at almost every coronal level studied.

Linear regression analyses of the pooled data for the 16 surviving rats revealed highly significant correlations between neurological scores at 24, 48, and 72 hours and brain edema (r = 0.73–0.76, p = 0.001–0.002), total infarct volume (r = 0.82–0.85, p = 0.0001–0.0002), and cortical infarct volume (r = 0.81–0.88, p = 0.0001–0.0003). In contrast, neurological scores were not significantly correlated to striatal infarct volume, and hematocrit level and brain edema showed a significant inverse correlation (r = 0.69, p = 0.01).

Two animals died during the experiment (one from each group, both at 24 hours). Autopsies revealed a large ipsilateral hemispheric infarct and extensive brain edema in both animals.

Discussion

The goal of our study was to determine whether acute but delayed HSA administration was efficacious in protecting the brain after a temporary focal ischemic insult. Our results clearly demonstrate that this form of therapy improves outcome as measured both by neurological score and by final pathological estimation of the size of infarction and the extent of edema. Albumin treatment initiated after 2 hours of MCA occlusion significantly reduced brain infarction (by 34%) and brain edema (by 81%).

Experimental Model and Method of Hemodilution

Experimental MCA occlusion in rats may produce varying volumes of infarction, depending on the strain of rat used, the method of MCA occlusion, and the anatomical location of the occlusion site. In the model of MCA suture occlusion, an additional source of variation in infarct size appears to be related to the extent of insertion of the suture, its size, and other characteristics of the suture itself. In the present study and in recently published observations, we have used a poly-L-lysine–coated suture and have found that this method yields reliable and highly consistent results (coefficient of variation of infarct volume, 9%).

Therapy with high-concentration HSA has led to striking hemodilution (fall in hematocrit levels from 32 to 28%),21,23 In addition to producing hemodilution, concentrated albumin solutions have important oncostic effects, acting as a dehydrating agent on extravascular cerebral tissues and producing a net movement of water from tissue to blood. Cerebral edema may be prevented or significantly reduced by means of albumin solutions.7 In the current study, administration of HSA greatly reduced brain edema at almost every brain level examined. Furthermore, there was a highly significant correlation between total infarct volume or brain edema and neurological score at 24, 48, and 72 hours (p < 0.0002) and between cortical infarction and neurological score at the same intervals.
effect. Among the roles of albumin are: 1) the binding and inactivation of toxic products; 2) regulation of plasma and interstitial fluid concentrations of endogenous and exogenous substances and drugs; 3) participation in anticoagulation; 4) maintenance of microvascular permeability to proteins; and 5) scavenging of free radicals and prevention of lipid peroxidation.6

Neurological Evaluation

Administration of HSA significantly improved the neurological score compared with saline at 24 hours after MCA occlusion, and there was a more prominent trend toward better recovery in the HSA- than in the saline-treated group at 48 and 72 hours. These data are in close agreement with the findings of Wood and Fleischer,24 who noted significant neurological improvement at 24 hours after hypervolemia induced with the hemodiluting solutions of dextran and/or HSA in alert patients with acute focal cerebral ischemia. A beneficial effect of concentrated albumin solutions in the treatment of cerebral ischemia was found by Matsui and Asano25 and Cole, et al.,9 whereas other authors were unable to detect a positive effect.21,30

Rationale for Hemodilution in Ischemia

The concept of therapeutic hemodilution has been extensively studied30 and is based on the close correlation of hematocrit level and whole blood viscosity. Lowering hematocrit levels results in reduced viscosity and less resistance to blood flow, especially in regions of relative stasis. Arguments favoring hemodilution therapy for cerebral ischemia are based on observations that hemodilution improves cerebral blood flow (CBF) and final clinical outcome in animal models of cerebral ischemia as well as human studies.14,20 Experimental studies have demonstrated significant elevations in regional CBF in focally ischemic brain following the acute reduction of hematocrit via hypervolemic or isovolemic infusion of autologous plasma or low-molecular-weight dextran.25,52,53 The demonstration of significant elevations in regional CBF in ischemic but not in normal brain implies that the effect of hypervolemic hemodilution on brain perfusion is greater in regions of low blood flow.34 The effects of viscosity and oxygen content on CBF have been studied in normal and ischemic rats.6 The data support the hypothesis that in normal brain both viscosity and oxygen content affect CBF, whereas in ischemic brain a decrease in viscosity, but not in oxygen content, increases CBF.6 Arguments against hemodilution are based on the poor correlation of CBF and outcome after stroke. Physiological manipulations such as hemodilution, hypervolemia, and hypertension, all of which improve CBF, have nonetheless shown variable degrees of success in affecting outcome following focal cerebral ischemia.7,15,21 In addition, several experimental and clinical reports have drawn attention to the potential risks of raised intracranial pressure and cardiac complications following intravascular volume expansion.8,30

Although hemodilution is believed to improve the rheological properties of the microcirculation, the issue of "optimum hematocrit" is far from settled and considerable controversy continues.32 Wood and Kee59 concluded that a hematocrit level of 33% is ideal for improving blood flow and maximizing oxygen delivery, although Kusunoki, et al.,19 suggested that the optimum level is closer to 40 to 42%. Other experimental and clinical studies have variously suggested that oxygen transport to the brain is optimum at hematocrit levels of 28%,23 29 to 32%,5,20 and 34 to 35%.10,25 In the current study, when a hematocrit level of 25% was achieved by using HSA therapy, brain damage was reduced and a highly significant correlation was found between hematocrit level and brain edema (p < 0.01).

The timing of hemodilution therapy is another important variable. Previous investigations have demonstrated a protective effect when hemodilution is used before6,7 or shortly after cerebral artery occlusion.10,23,32,33 A neuroprotective effect of hemodilution was evident when treatment...
was initiated 1 to 3 hours after the onset of MCA occlusion. By contrast, when administered 6 hours posts ischemia, hemodilution is not helpful and may be harmful. In our study, hemodilution was started 2 hours after MCA occlusion. This time frame is clinically relevant in that it is logistically difficult to institute therapy any earlier in most patients with acute stroke. It is interesting to note that the duration of hemodilution therapy in experimental stroke studies ranged from 20 minutes to 7 days. It has been suggested that if hemodilution therapy is to be effective, it should be completed during the first few hours after the onset of ischemia.

**Clinical Application**

The benefits of hemodilution in patients with cerebral infarction continue to be debated. Although some recent clinical studies have shown that hemodilution is beneficial, two large, multicenter trials undertaken to study the effect of hemodilution in patients with acute stroke failed to show benefit. Although hemodilution alone is unlikely to be a panacea in the treatment of stroke, it is likely that this therapy may be of some benefit, particularly when used in conjunction with other treatments, such as thrombolysis, therapeutic hypothermia, excitatory amino acid antagonism, free radical scavengers, or calcium channel blockers. These therapeutic combinations remain to be tested experimentally and clinically.

**Conclusions**

The current study strongly supports the beneficial effect of delayed high-concentration albumin therapy in a reproducible model of MCA occlusion in rats. Our results show that hemodilution with albumin decreases focal cerebral ischemic injury as judged by neurological score, infarct size, and brain edema. This neuroprotection was achieved when therapy was initiated 2 hours after the onset of temporary MCA occlusion. These data underscore the possible efficacy of delayed, high-concentration therapy with albumin in patients with acute ischemic stroke.

**Acknowledgment**

The authors thank Susan Kraydieh for expert technical assistance.

**References**

28. Scandinavian Stroke Study Group: Multicenter trial of hemodil-
Delayed albumin therapy of cerebral ischemia


Manuscript received March 17, 1997.

This work was supported by a grant from Lilly Research Laboratories, Indianapolis, Indiana, and by United States Public Health Service Grant No. NS 05820.

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