Human Albumin Therapy of Acute Ischemic Stroke
Marked Neuroprotective Efficacy at Moderate Doses and With a Broad Therapeutic Window

Ludmila Belayev, MD; Yitao Liu, MD; Weizhao Zhao, PhD; Raul Busto, BS; Myron D. Ginsberg, MD

Background and Purpose—We examined the neuroprotective efficacy of moderate-dose human albumin therapy in acute focal ischemic stroke and defined the therapeutic window after stroke onset, within which this therapy would confer neurobehavioral and histopathological neuroprotection.

Methods—Sprague-Dawley rats were anesthetized with halothane/nitrous oxide and received 2-hour middle cerebral artery occlusion (MCAo) by a poly-l-lysine–coated intraluminal suture. Neurological status was evaluated during occlusion (60 minutes) and daily for 3 days after MCAo. In the dose-response study, human albumin doses of either of 0.63 or 1.25 g/kg or saline vehicle (5 mL/kg) were given intravenously immediately after suture removal. In the therapeutic window study, a human albumin dose of 1.25 g/kg was administered intravenously at 2 hours, 3 hours, 4 hours, or 5 hours after onset of MCAo. Three days after MCAo, brains were perfusion-fixed, and infarct volumes and brain swelling were determined.

Results—Moderate-dose albumin therapy significantly improved the neurological score at 24 hours, 48 hours, and 72 hours and significantly reduced total infarct volume (by 67% and 58%, respectively, at the 1.25- and 0.63-g/kg doses). Cortical and striatal infarct volumes were also significantly reduced by both doses. Brain swelling was virtually eliminated by albumin treatment. Even when albumin therapy (1.25 g/kg) was initiated as late as 4 hours after onset of MCAo, it improved the neurological score and markedly reduced infarct volumes in cortex (by 68%), subcortical regions (by 52%), and total infarct (by 61%).

Conclusions—Moderate-dose albumin therapy markedly improves neurological function and reduces infarction volume and brain swelling, even when treatment is delayed up to 4 hours after onset of ischemia. (Stroke. 2001;32:553-560.)

Key Words: brain edema ■ cerebral ischemia, focal ■ hemodilution ■ middle cerebral artery occlusion ■ neuroprotection

Ischemic cerebrovascular disease ranks among the leading causes of death and long-term disability throughout developed countries. Preventive measures targeted at major risk factors have reduced stroke incidence and mortality rates.1 Until recently, however, no specific drug therapies have been developed, which, if administered after the onset of acute stroke, would succeed in diminishing the extent of tissue damage and improving functional outcome. The sole exception in current clinical practice is the thrombolytic agent recombinant tissue-type plasminogen activator (tPA), shown in a randomized North American clinical trial to improve functional outcome in ischemic stroke if administered within 3 hours of stroke onset.2 Although some prospective, multicenter surveys of tPA use have subsequently confirmed favorable clinical outcomes and low symptomatic intracerebral hemorrhage rates in clinical practice,3 other community practice surveys have called attention to frequent deviations from national treatment guidelines and high rates of intracerebral hemorrhage and in-hospital death.4 In actual clinical practice, only a very small proportion of patients with acute ischemic stroke actually receive tPA therapy.

Rigorous laboratory investigations of cerebral ischemia conducted over the past 2 decades have identified key biochemical and molecular mechanisms that contribute to the death of brain tissue—factors whose prompt antagonism might result in tissue salvage.5 Critical thresholds of ischemic brain injury have been defined,6 and the central roles of excitotoxicity,7 tissue calcium overload,8 oxygen radicals,9 inflammatory mediators,10 and other factors have been established. These insights have stimulated major commercial efforts to develop and test a variety of pharmaceutical antagonists of these processes.11,12 The emergence of vexatious adverse effects in early-phase clinical trials, however, has in many cases thwarted these efforts. To date, no pharmaceutical neuroprotectant has been proven to be efficacious in randomized clinical trials of ischemic stroke.12

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Thus, in the therapy of acute ischemic stroke, there is a clear and urgent need for a neuroprotective agent that has proven efficacy, carries minimal risk of side effects or toxicity, is acceptable both to medical personnel and to patients and their families, and can be administered without the need for complicated laboratory studies or sophisticated delivery systems. Human serum albumin (Alb) is a unique multifunctional protein with neuroprotective properties. In recent experimental studies of focal cerebral ischemia, we have shown that high-dose human Alb therapy (2.0 to 2.5 g/kg), if administered promptly (2 hours) after stroke onset, is highly effective in improving neurological status and in reducing infarction volume and extent of brain swelling.13–15 These studies were conducted in a widely used, minimally invasive model of middle cerebral artery occlusion (MCAo) in the albino rat that gives rise to a consistent behavioral deficit and a large, highly reproducible cortical and subcortical infarct resembling the lesion of thromboembolic stroke in patients.16 In our previous studies, however, we had not defined the therapeutic window of neuroprotective efficacy for human Alb therapy and we had not assessed the efficacy of lower (clinically more achievable) Alb doses. These goals constituted the objectives of the present study, in which we now demonstrate that human Alb remains highly neuroprotective when administered at moderate doses and that the therapeutic window extends to 4 hours after stroke onset.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats weighing 270 to 360 g were studied after an overnight fast. Animal protocols were approved by the University of Miami Animal Care and Use Committee. After administration of atropine sulfate (0.5 mg/kg IP), animals were anesthetized with halothane (3.5% for induction, 1% for maintenance), 70% nitrous oxide, and a balance of oxygen; immobilized with pancuronium bromide (0.6 mg/kg IV); and mechanically ventilated. Both rectal and cranial (left temporalis muscle) temperatures were monitored with temperature probes (Omega model CN/76000) and regulated at 37.0° to 37.5°C by separate heating lamps. Femoral catheters were passed through the proximal ECA into the internal carotid artery and thence into the MCA, a distance of 19 to 20 mm from the carotid bifurcation according to the weight of the animal.16 Before use, the suture was coated with poly-L-lysine solution as previously described16 to enhance its adhesion to the surrounding endothelium and increase the reproducibility of the resulting infarct. The neck incision was then closed. Animals were allowed to awake from anesthesia and, at 60 minutes of MCAo, were tested on a standardized neurobehavioral battery (described below) to confirm the presence of a high-grade neurological deficit. Rats that did not demonstrate an initial left upper extremity paresis were excluded from further study. Animals were then reanesthetized for removal of the intraluminal filament after 2 hours of MCAo. They were then transferred to a temperature-controlled incubator at 37°C for 24 hours, where they received supplemental oxygen and were observed carefully for signs of discomfort; no such signs were observed.

Middle Cerebral Artery Occlusion

To occlude the MCA, the right common carotid artery was first exposed and the occipital branches of the external carotid artery (ECA) were coagulated. A 3-0 monofilament nylon suture was then passed through the proximal ECA into the internal carotid artery and thence into the MCA, a distance of 19 to 20 mm from the carotid bifurcation according to the weight of the animal.16 Before use, the suture was coated with poly-L-lysine solution as previously described16 to enhance its adhesion to the surrounding endothelium and increase the reproducibility of the resulting infarct. The neck incision was then closed. Animals were allowed to awaken from anesthesia and, at 60 minutes of MCAo, were tested on a standardized neurobehavioral battery (described below) to confirm the presence of a high-grade neurological deficit. Rats that did not demonstrate an initial left upper extremity paresis were excluded from further study. Animals were then reanesthetized for removal of the intraluminal filament after 2 hours of MCAo. They were then transferred to a temperature-controlled incubator at 37°C for 24 hours, where they received supplemental oxygen and were observed carefully for signs of discomfort; no such signs were observed.

Behavioral Evaluation

A standardized battery of behavioral tests16 was used to quantify sensorimotor neurological function at 60 minutes of MCAo (see above) and daily for 3 days thereafter. The battery, which incorporates postural reflex and forelimb-placing tests, yields a 12-point score (normal=0, maximal=12). Tests were conducted by an observer blinded to the treatment group.

Experimental Groups

Dose-Response Study

Animals were randomly assigned to 1 of 3 treatment groups (n=5 each): (1) human serum Alb (Alpha Therapeutic Corp, 25% solution), 0.5% of body weight, for example, 1.25 g/kg; (2) Alb, 0.25% of body weight, for example, 0.63 g/kg; or (3) a similar volume of sodium chloride (0.9%, 5 mL/kg). The respective agent was infused intravenously at a constant rate over a period of 3 minutes, commencing just after reversal of MCAo.

Therapeutic Window Study

The therapeutic window for Alb was investigated with a dose of 1.25 g/kg, which was administered at 2 hours (n=9), 3 hours (n=10), 4 hours (n=10), or 5 hours (n=9) after the onset of MCAo. Vehicle (0.9% sodium chloride, 5 mL/kg) was administered at 1 hour after onset of MCAo (n=9).

Histopathological Evaluation

Animals were allowed to survive for 3 days. Brains were then perfusion-fixed as previously described16 with a mixture of 40% formaldehyde, glacial acetic acid, and methanol (1:1:8 by volume), and brain blocks were embedded in paraffin. Ten-micron-thick sections were cut in the coronal plane and stained with hematoxylin and eosin. To quantify infarct volume and depict infarct frequency distribution, histological sections were digitized at 9 standardized coronal levels (MCID image-analysis system, Imaging Research Corp), from which data were exported to a UNIX-based workstation for further processing. An investigator blinded to the experimental groups outlined the zones of infarction (which were clearly demarcated) as well as the left- and right-hemisphere contours at each level. Software developed by us was then used to quantify infarct size and brain swelling. Infarct volume was corrected for brain swelling as previously described, and swelling was expressed as the percentage difference in brain volume between the two hemispheres.15,16 To facilitate rigorous image-based comparisons of infarction among treatment groups, we mapped the digitized infarct maps of individual animals at each coronal level into a common image template derived from a brain atlas17 and summed these data to generate computer maps depicting infarct frequency at each pixel location; these methods have been previously reported.18

Statistical Analysis

ANOVA with post hoc comparisons was used to compare infarct areas and brain swelling among treatment groups (repeated-measures design) and to compare infarct volumes, brain swelling, neurological score, and physiological variables among groups. Nonparametric ANOVA on ranks was used to compare total neurological scores among groups. Pixel-based intergroup comparison of infarct frequency maps was achieved by Fisher’s exact test. A probability level of P<0.05 was regarded as significant.

Results

Physiological Variables

All animals of this study showed similar values for rectal and cranial temperatures, MABP, arterial blood gases, and plasma glucose (Table 1). Alb therapy led to the expected moderate...
TABLE 1. Physiological Variables

<table>
<thead>
<tr>
<th></th>
<th>Dose-Response Study</th>
<th>Therapeutic Window Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n = 5)</td>
<td>Alb, 0.63 g/kg (n = 5)</td>
</tr>
<tr>
<td>Before MCAo (15 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial temperature, °C</td>
<td>37.2±0.07</td>
<td>37.2±0.05</td>
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<tr>
<td>Rectal temperature, °C</td>
<td>37.3±0.09</td>
<td>37.4±0.10</td>
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<tr>
<td>pH</td>
<td>7.44±0.01</td>
<td>7.38±0.01</td>
</tr>
<tr>
<td>Po2, mm Hg</td>
<td>114±8</td>
<td>102±4</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>38.2±0.6</td>
<td>40.1±0.9</td>
</tr>
<tr>
<td>MAPB, mm Hg</td>
<td>106±4</td>
<td>97±7</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>123±6</td>
<td>121±6</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41.8±0.7</td>
<td>41.6±0.4</td>
</tr>
<tr>
<td>15 min after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial temperature, °C</td>
<td>37.2±0.04</td>
<td>37.1±0.07</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>37.4±0.05</td>
<td>37.3±0.07</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.01</td>
<td>7.37±0.01</td>
</tr>
<tr>
<td>Po2, mm Hg</td>
<td>112±4</td>
<td>102±5</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>38.2±0.5</td>
<td>39.8±0.9</td>
</tr>
<tr>
<td>MAPB, mm Hg</td>
<td>113±8</td>
<td>112±4</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>121±2</td>
<td>113±4</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>43.4±1.6</td>
<td>42.6±0.6</td>
</tr>
<tr>
<td>Plasma colloid oncotic pressure, mm Hg</td>
<td>16.4±0.9</td>
<td>17.7±0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*Different from saline group (P < 0.05, ANOVA followed by Dunnett’s test).

Reduction in hematocrit and increase in plasma colloid oncotic pressure (Table 1).

**Neurobehavioral Assessment**

Before MCAo, neurological score was normal (score = 0) in all animals. High-grade behavioral deficits (score = 10 to 11) were present in all animals when tested at 60 minutes of MCAo (Figure 1); thus, no animals required exclusion on the basis of an inadequate degree of cerebral ischemia. Saline-treated animals continued to exhibit severe behavioral impairments throughout the 3-day survival period. In the dose-response study, the 1.25-g/kg Alb dose significantly improved the neurological score compared with vehicle rats at 24 hours, 48 hours, and 72 hours, and the 0.63-g/kg dose was effective at 72 hours (Figure 1A). The therapeutic window study revealed that treatment with moderate-dose Alb (1.25 g/kg), even when initiated as late as 4 hours after onset of MCAo, significantly improved the neurological score at 24 hours and 48 hours (Figure 1B).

**Histopathology**

The brains of saline-treated animals with MCAo exhibited a consistent pan necrotic lesion involving both cortical and subcortical (mainly striatal) regions of the right hemisphere, characterized microscopically by destruction of neuronal, glial, and vascular elements. By contrast, infarct size was dramatically reduced by Alb therapy in both treatment groups.

The dose-response study revealed that the extent of neuroprotection was profound in neocortex (mean tissue salvage, 66% and 96%, respectively, in the 0.63-g/kg and 1.25-g/kg Alb groups) and extended across multiple coronal levels (Figure 2 and Table 2). The striatum was also significantly protected (by 54% and 52%, respectively; Figure 2 and Table 2). Total infarct volume corrected for brain swelling was reduced by a mean of 58% and 67%, respectively, by treatment with 0.63 g/kg and 1.25 g/kg Alb (Figure 2). Percentage brain swelling was diminished by 75% by treatment with 0.63 g/kg Alb and was essentially eliminated in the 1.25-g/kg Alb group (Figure 2). In the cortical epicenter of
the ischemic lesion—a region protected from panneerosis in animals treated with 1.25 g/kg Alb—microscopic examination revealed sporadic ischemic (shrunken, eosinophilic) neurons in several cases.

In the therapeutic window study, treatment with Alb (1.25 g/kg) reduced total infarct volume (corrected for brain swelling) by a mean of 58% when administered at 2 hours; by 66% at 3 hours; and by 61% at 4 hours (Figures 3 and 4). Cortical infarct areas were significantly reduced by the administration of Alb compared with vehicle (mean tissue salvage, 62%, 83%, and 68%, respectively, in the 2-hour, 3-hour, and 4-hour Alb-treated groups) and extended across multiple coronal levels (Figures 3 and 4). In addition, the subcortical region was also significantly protected (by 52%, 44%, and 52%, respectively, in the 2-hour, 3-hour, and 4-hour Alb-treated groups), with the neuroprotection again extending across multiple coronal levels (Figures 3 and 4). When Alb therapy was delayed to 5 hours after the onset of MCAo, the neuroprotective effect was lost.

Treatment with Alb also significantly reduced brain swelling compared with vehicle-treated rats, even when initiated as late as 4 hours after onset of MCAo (Figure 5).

Discussion

In the first part of this study, we have shown that human Alb therapy substantially improves neurological function, markedly reduces the volume of cerebral infarction, and nearly eliminates brain swelling in animals with acute ischemic stroke when administered promptly at doses of 0.63 or 1.25 g/kg—a dose range potentially amenable to clinical application in patients. In the second portion of the study, we have demonstrated the existence of a broad therapeutic window of neuroprotective efficacy with moderate-dose (1.25 g/kg) human Alb therapy, such that treatment initiated even 4 hours after stroke onset of ischemia is highly effective.

The present study was prompted by our earlier findings demonstrating the neuroprotective efficacy of considerably higher doses of human Alb (2.0 to 2.5 g/kg) administered at earlier times after ischemic or traumatic injury. In focal ischemic stroke, high-dose Alb therapy diminished the volumes of brain infarction and swelling,13,14 ameliorated behavioral function, and improved local perfusion to zones of critical blood flow reduction.15 Focal ischemia-induced blood-brain barrier dysfunction permitted Alb to penetrate into the brain parenchyma, where it was taken up by cortical neurons with normal morphological features, suggesting that Alb may have protected these neurons from ischemic injury.19 Alb also mitigated pannectic histopathology in tissue zones of residual ischemic injury by fostering the partial preserva-
tion of glial and endothelial elements, and it normalized the apparent diffusion coefficient of water on diffusion-weighted magnetic resonance images, even in zones of residual histological injury.\(^{14}\) Taken together, these observations bespeak a direct protective effect exerted by Alb on both parenchymal and vascular elements of the brain.

High-dose Alb therapy was also neuroprotective in experimental models of both transient global ischemia\(^{20}\) and fluid-percussion traumatic brain injury.\(^{21}\) In the former, Alb partially protected vulnerable neurons of the hippocampus from injury; in the latter, it diminished contusion volume. Alb molecules have a prolonged circulating half-life (\(\approx\)20 days), and, because they do not easily leave the intravascular space, they are capable of increasing plasma oncotic pressure over prolonged periods of time.\(^{22}\) Indeed, Alb is responsible for 80% of the plasma colloid oncotic pressure.\(^{23}\) In the present study, Alb therapy (1.25 g/kg dose) increased plasma oncotic pressure acutely by \(\approx\)20% (Table 1), consistent with

### Table 2. Cortical and Striatal Infarct Areas (mm\(^2\))

<table>
<thead>
<tr>
<th>Region Group</th>
<th>Bregma Level, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+5.2</td>
</tr>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.9±0.6</td>
</tr>
<tr>
<td>Alb, 0.63 g/kg</td>
<td>0±0</td>
</tr>
<tr>
<td>Alb, 1.25 g/kg</td>
<td>0±0</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0±0</td>
</tr>
<tr>
<td>Alb, 0.63 g/kg</td>
<td>0±0</td>
</tr>
<tr>
<td>Alb, 1.25 g/kg</td>
<td>0±0</td>
</tr>
</tbody>
</table>

Values are mean±SEM; \(n=5\) in each group.

*Different from saline group (\(P<0.05\), repeated-measures ANOVA followed by Dunnett’s test).

Figure 3. Therapeutic window study. Large panels show histological infarct frequency maps of 5 treatment groups, depicting percentages of brains with infarction at each pixel location. Five coronal levels shown correspond to atlas levels +1.2, −0.3, −1.3, −1.8, and −3.8 mm with respect to bregma.\(^{17}\) Small panels at lower right are statistical maps comparing infarct frequency in saline-treated vs Alb-treated groups on pixel-by-pixel basis by Fisher’s exact test. Maps display 1.0 to \(P\), where \(P\) is level of intergroup statistical significance, and are thresholded at 0.95 to highlight zones of significant difference at \(P<0.05\) level. A, Saline vs Alb–2-hour groups; B, saline vs Alb–3-hour groups; C, saline vs Alb–4-hour groups; D, saline vs Alb–5-hour groups.
its effect in our previous reports. By contrast, plasma osmolality is not affected by Alb administration. This is not surprising in view of the high molecular weight of Alb (69,000); exogenous Alb (1.25 g/kg dose) would therefore contribute <0.001 mOsm/mL to plasma osmolality.

Hemodilution Effects
In this study, Alb therapy reduced the hematocrit acutely by 25% to 30%. This is similar to our previous results, in which Alb therapy produced an acute reduction in hematocrit from 40% to 42% to 23% to 28% that recovered to normal levels by 1 day. A traditional view of therapeutically administered Alb is that it acts solely by its hemodiluting action. We consider it very unlikely, however, that the marked neuroprotective efficacy of moderate- to high-dose human Alb in our studies is mediated solely by hemodilution because both experimental and clinical studies that used other hemodiluents have, in general, been disappointing. Interpretation of the experimental hemodilution literature is difficult, however, because of the large variety of hemodiluents used (human Alb, low-molecular-weight dextran, hydroxyethyl starch, di-aspirin cross-linked hemoglobin, and hetastarch) that were obtained from various sources and administered at differing times and in diverse concentrations to differing species (dog, cat, rat) and ischemia models (permanent versus temporary MCAo, embolism) and resulting in differing degrees of hematocrit reduction. Another confounding factor is the use of both isovolemic protocols (in which the animal’s blood is partially exchanged for the hemodiluent) and hypervolemic protocols (in which a net excess of colloid is administered, with or without partial exchange).

Some hemodilution studies have supported a beneficial effect, particularly in temporary rather than permanent vascular occlusion models and with colloid agents administered in high concentrations close to the onset of the ischemic event (for example, see References 24 through 27). Alb has been specifically assessed as a hemodiluent in occasional experimental ischemia reports, which have varied considerably in both the dose and timing of Alb administration, concurrent therapies, and outcome assessment. In general, these studies have not rigorously tested Alb when administered at higher doses and at early times after stroke. In one study, however, Alb administered at a 2-g/kg dose to rats shortly after permanent MCAo significantly reduced infarction size and attenuated brain swelling.

Other Mechanisms of Alb Action
Several other mechanisms by which Alb therapy may have induced neuroprotection must also be considered. As the major protein of blood plasma, Alb is in fact a unique and complex molecule with a variety of physiochemical properties. It is a principal transporter of plasma fatty acids: Most circulating long-chain fatty acids exist as Alb complexes. Alb also accounts for the majority of drug binding in the plasma.

Antioxidant Effects
Importantly, Alb constitutes the plasma’s primary oxygen radical trapping and antioxidant defense, exceeding that of vitamin E, for example, by 10- to 20-fold. As such, it functions as a major plasma antioxidant defense, both against oxidizing agents generated endogenously (eg, neutrophil myeloperoxidase) as well as against exogenous substances (eg, phenolic dietary compounds). By avidly binding to copper ions, Alb inhibits copper ion–dependent lipid peroxidation and retards the formation of the highly reactive hydroxyl radical species.
Endothelial Effects
Alb also exerts direct effects on vascular endothelium by binding to the endothelial glycocalyx, functioning to maintain normal microvascular permeability, and serving, through its transcytosis across endothelium, as a carrier for various small molecules.32 Perfusion of vessels with protein-free solution dramatically increases hydraulic permeability in various vascular beds—an effect that is prevented by adding serum Alb to the perfusate at low concentrations.33 Microvascular endothelial cells express specific Alb binding sites on their surface that mediate its transcytosis or endocytosis.34 Alb influences erythrocyte aggregation in a complex manner, increasing low-shear viscosity but decreasing erythrocyte sedimentation under no-flow conditions.35 In addition, Alb is a specific inhibitor of endothelial cell apoptosis.36

Anti-Edema Effects
Alb has several characteristics that suggest its theoretical usefulness for cerebral dehydration: It does not equilibrate across the normal blood-brain barrier (BBB) into the interstitial space; it has a long half-life; and it is not excreted in the urine. A large body of literature suggests that Alb would cross the damaged BBB in ischemic regions, bringing with it a certain amount of fluid, and might hold the fluid within the ischemic area.37,38 Evans blue dye, bound to Alb, certainly crosses the damaged BBB, but evidence is lacking that increasing the serum Alb concentration and oncotic pressure would significantly increase the oncotic pressure of edema fluid or worsen edema.21 The oncotic pressure of the edema fluid does not equilibrate with that of circulating plasma.29 In previous studies, Alb administration either has not changed or has decreased dramatically23,28,40 the brain water content, as estimated by the wet weight/dry weight method after cerebral injury. In the present experiment and in our previous studies of Alb therapy of focal ischemia,13,14 treatment with Alb significantly reduced brain swelling compared with vehicle-treated rats as measured by volumetric comparison of ipsilateral versus contralateral hemispheres.

Metabolic Effects
Alb exerts major effects on brain astrocytes, eliciting intercellular calcium waves and functioning as a mitogen.41 As such, it may stimulate glial scar formation in pathological states in which it is able to cross a permeable BBB into the brain. Alb also appears to be a major regulator of the enzyme pyruvate dehydrogenase in astrocytes, capable of more than doubling the flux of glucose and lactate.42 This property takes on relevance in that pyruvate dehydrogenase is inhibited by cerebral ischemia, and this leads to substrate limitation and decreased electron flow into the mitochondrial electron-transport chain.43 As plasma Alb enters the brain under pathological conditions, it may help to sustain neuronal metabolism under pathological conditions by increasing the export of pyruvate to neurons.

Clinical Applications
Several large, randomized, multicenter trials of non-Alb hemodilution have either failed to show improved neurological outcome,44,45 were inconclusive,46 or were terminated prematurely.47 Alb therapy for stroke has, to date, been assessed in only a single, small, prospective clinical study in which it was administered in an individually customized fashion48 (the study was methodologically flawed but suggested a treatment-associated reduction in mortality rate of \( \pm 10\%\)).

In current clinical practice, human Alb is being commonly prescribed for a variety of indications. Thus, Alb is administered in large quantities over prolonged time periods to patients with aneurysmal subarachnoid hemorrhage after surgical clipping of the aneurysm, in an effort to prevent delayed ischemia secondary to vasospasm.49 In a recent report describing 82 patients with subarachnoid hemorrhage who were receiving crystalloids plus Alb infusions under normovolemic or hypervolemic conditions,50 the daily Alb dose amounted to \( \approx 2 \text{ g/kg body wt.}\). In that study, Alb infusions produced no elevation of pulmonary artery diastolic pressure or central venous pressure, and congestive heart failure occurred in only 1 patient. In another study, 25% Alb was administered in moderate to high doses over a 2-week period to elevate plasma oncotic pressure in patients with brain contusion; this therapy safely and effectively reduced contusional edema.51 Similarly, patients with putaminal hemorrhage treated with 12.5 to 25 g per day of Alb for 2 weeks showed reductions of cerebral edema and improved outcome.52 In another study, patients with cirrhosis and spontaneous bacterial peritonitis were treated with intravenous Alb at an initial dose of 1.5 g/kg, followed by 1 g/kg on day 3; this treatment was tolerated and led to reduced renal impairment and lowered mortality rates.53 It is of relevance that the per-kilogram Alb dose ranges administered in the above-cited studies overlaps with the per-kilogram doses shown in our experimental studies to be highly neuroprotective when administered within the first 4 hours after stroke onset. Taken together, these findings support the potential clinical feasibility of administering human Alb in moderate doses to patients with acute ischemic stroke.

In summary, the present study has shown that human Alb therapy, in moderate doses, is strongly neuroprotective in focal ischemia; has a broad therapeutic window extending to 4 hours after stroke onset; and produces no observable adverse effects in young adult experimental animals. This 4-hour time frame is clinically relevant in that it is logistically difficult to institute therapy in many patients with acute stroke at earlier times. We therefore suggest that this agent offers great promise in the therapy of cerebral ischemia and we propose that it may now be appropriate to consider the initiation of early-phase clinical trials in patients with acute ischemic stroke.

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