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European Journal of Pharmacology 428 (2001) 193–201

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Neuroprotective effect of treatment with human albumin in permanent focal cerebral ischemia: histopathology and cortical perfusion studies

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Received 5 April 2001; received in revised form 17 July 2001; accepted 27 July 2001

Abstract

In recent experimental studies, we demonstrated a highly beneficial neuroprotective effect of moderate- to high-dose human albumin treatment of *transient* focal cerebral ischemia, but we did not define the effect of albumin therapy in permanent focal cerebral ischemia. In this study, anesthetized Sprague–Dawley rats were subjected to permanent middle cerebral artery occlusion by retrograde insertion of an intraluminal nylon suture coated with poly-L-lysine. Albumin was administered i.v. at 2 h after onset of middle cerebral artery occlusion, in doses of either 1.25 ($n = 8$) or 2.5 g/kg ($n = 6$). In a separate group of animals, albumin (2.5 g/kg) was given 1 h after middle cerebral artery occlusion ($n = 6$). Vehicle-treated rats ($n = 6$) received 0.9% saline in equivalent volumes. Neurological status was evaluated during and 24 h after middle cerebral artery occlusion. One day after middle cerebral artery occlusion, infarct volumes and brain edema were determined. In a separate group of animals, cortical perfusion was assessed by Laser-Doppler perfusion imaging. Albumin (1.25 g/kg; $n = 3$) or vehicle (sodium chloride 0.9%; $n = 3$) was administered at 2 h after onset of middle cerebral artery occlusion. Higher-dose albumin therapy (2.5 g/kg) significantly improved the neurological score compared to vehicle rats at 24 h, when administered at either 1 or 2 h after middle cerebral artery occlusion. Total infarct volume was reduced by albumin (2.5 g/kg given at 2 h) by 32% compared with vehicle-treated rats. Both albumin doses (1.25 and 2.5 g/kg) significantly reduced cortical and striatal infarct areas at several coronal levels when administered at 2 h after middle cerebral artery occlusion. Brain swelling was not affected by albumin treatment. Cortical perfusion declined during middle cerebral artery occlusion in both groups. Treatment with albumin led to 48% increases in cortical perfusion ($P < 0.002$), but saline caused no change. These results support a beneficial effect of albumin therapy in permanent focal cerebral ischemia.

Keywords: Albumin; Imaging; Middle cerebral artery occlusion; Stroke; Neuroprotection; (Rat)

1. Introduction

Brain injury resulting from stroke remains a major public health problem (Wolf et al., 1998). Of the approximately 700,000 new cases recognized annually in the United States, roughly 30% die, and 20–30% become severely and permanently disabled (Zivin and Choi, 1991). The stroke-therapies market is estimated at US$1 billion with annual health care costs of US$30–50 billion (this includes one-half of all patients hospitalized for acute neurological disease) (Hademenos, 1999). Stroke afflicts individual of all ages, but the incidence doubles with each decade over 45 and reach 1–2% per year in those over 75 years old. The worldwide incidence of stroke is estimated at between 150 and 200 cases per 100,000 population; and contrary to popular belief, the stroke rate in developing countries including those in Asia and Africa is as high as that in developed countries (Viriyavejakul, 1990).

No medical treatment is approved for the treatment of stroke beyond tissue plasminogen activator (tPA), a thrombolytic agent restricted to administration within 3 h after stroke (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). Despite the effect of having tPA available for clinical use, only a few patients (5–10%) qualify for treatment within this short time from stroke onset. Standard therapy is often ineffective at preventing brain infarction and is meant to support cardiovascular and respiratory function, control intracranial

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01255-9

pressure and prevent recurrent stroke. A large number of the neuroprotective agents have been developed and tested, including glutamate receptor antagonists, Ca^{2+} channel antagonists, enzyme inhibitors, antioxidants, and free radical scavengers (Ginsberg, 1995; Koroshetz and Moskowitz, 1996). Although some clinical trials are still ongoing, the results from the many completed trials have been disappointing (De Keyser et al., 1999). Clearly, there is an urgent need for a neuroprotective agent that is safe, readily administrable, and lacking in psychotropic and other major side effects.

Human serum albumin is a unique multifunctional protein possessing neuroprotective properties. In a series of recent experimental studies, we have shown that high-dose human albumin therapy diminishes brain injury in both cerebral ischemia (Belayev et al., 1997a, 1998, 1999b; Huh et al., 1998) and traumatic brain injury (Belayev et al., 1999a; Ginsberg et al., 2001). We have also demonstrated that moderate doses of albumin therapy markedly improve behavioral function and reduces infarction volume and brain edema, even when treatment is delayed up to 4 h after the onset of focal ischemia (Belayev et al., 2001). In our previous studies, however, we had not defined the effect of albumin therapy in *permanent* focal cerebral ischemia.

Only a few previous studies have considered the effect of albumin treatment on cerebral perfusion after ischemia or related insults (Matsui and Asano, 1993; Cole et al., 1994; Ulatowski et al., 1996). Although some studies have shown improved cerebral circulation after albumin treatment (Matsui and Asano, 1993; Cole et al., 1994), this did not necessarily lead to a beneficial effect on cerebral infarction (Little et al., 1981). We have recently shown that albumin treatment leads to increased cortical perfusion after transient middle cerebral artery occlusion in rats (Belayev et al., 2000), but we did not evaluate permanent middle cerebral artery occlusion.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (weight, 265–316 g; Crl:CD (SD)BR strain, Charles River Laboratories, Wilmington, MA) were studied after an overnight fast. Animal protocols were approved by the University of Miami's Animal Care and Use Committee. Following atropine sulfate (0.5 mg/kg, i.p.), 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, i.v.); and mechanically ventilated. Both rectal and cranial (left temporalis muscle) temperatures were monitored with temperature probes (Omega Model CN/76000, Stanford, CT) and regulated at 37.0–37.5 °C via separate heating lamps. Femoral catheters were inserted in order to monitor arterial blood pressure (Model RS3200 polygraph, Gould, Valley View, OH) and to withdraw samples for measurement of arterial blood gases (Model ABL 50, Radiometer America, Westlake, OH), hematocrit and plasma glucose (Model 2300 Stat, Yellow Springs Instrument, Yellow Springs, OH).

2.2. Middle cerebral artery occlusion

To occlude the middle cerebral artery, the right common carotid artery was first exposed and the occipital branches of the external carotid artery were coagulated. A 3–0 monofilament nylon suture was then passed via the proximal external carotid artery into the internal carotid artery and, thence, into the middle cerebral artery, a distance of 19–20 mm from the carotid bifurcation according to the animal's weight (Belayev et al., 1996). Prior to use, the suture was coated with poly-L-lysine solution as previously described (Belayev et al., 1996), in order 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 min of middle cerebral artery occlusion, 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. They were then transferred to a temperature-controlled incubator at 37 °C for 24 h, where they received supplemental oxygen and were observed carefully for signs of discomfort; no such signs were observed.

2.3. Behavioral evaluation

Behavioral tests were performed in all rats before middle cerebral artery occlusion, during occlusion (at 60 min) and 24 h after middle cerebral artery occlusion. The battery consisted of two tests that have been used previously to evaluate various aspects of neurologic function: (1) the postural reflex test to examine upper body posture while the animal is suspended by the tail (Bederson et al., 1986); and (2) the forelimb placing test to examine sensorimotor integration in forelimb placing responses to visual, tactile and proprioceptive stimuli (De Ryck et al., 1989). Neurological function was graded on a scale of 0–12 (normal score = 0, maximal score = 12), as previously described (Belayev et al., 1996). Rats that did not demonstrate an initial right upper extremity paresis were excluded from further study. Tests were conducted by an observer blinded to the treatment group.

2.4. Study protocols and experimental groups

Two protocols were employed. In Series I, only histopathology was assessed. Human serum albumin (Baxter Healthcare, Glendale, CA; 25 % solution) was

Table 1
Physiological variables

	Series 1 (Histopathology)				Series 2 (LCBF)	
	Saline ($n = 6$)	Albumin (1.25 g/kg, 2 h) ($n = 8$)	Albumin (2.5 g/kg, 2 h) ($n = 6$)	Albumin (2.5 g/kg, 1 h) ($n = 6$)	Saline ($n = 3$)	Albumin (1.25 g/kg, 2h) ($n = 3$)
Before MCAo (15 min)						
Rectal temperature (°C)	36.7 ± 0.05	36.6 ± 0.04	36.7 ± 0.07	36.9 ± 0.06	36.9 ± 0.09	36.9 ± 0.04
Cranial temperature (°C)	37.0 ± 0.09	36.9 ± 0.10	36.9 ± 0.08	36.9 ± 0.06	36.6 ± 0.13	36.4 ± 0.16
pH	7.43 ± 0.01	7.45 ± 0.01	7.42 ± 0.01	7.42 ± 0.01	7.43 ± 0.02	7.42 ± 0.01
PO_2 (mm Hg)	119 ± 8	117 ± 5	119 ± 5	122 ± 8	120 ± 11	123 ± 3
PCO_2 (mm Hg)	37.9 ± 0.6	37.7 ± 0.6	37.4 ± 0.3	37.4 ± 0.6	38.9 ± 0.6	38.4 ± 1.2
MABP (mm Hg)	99 ± 2	106 ± 3	99 ± 2	103 ± 4	102 ± 2	90 ± 4
Plasma glucose (mg/dl)	139 ± 5	131 ± 4	123 ± 5	126 ± 6	127 ± 12	123 ± 2
Hematocrit (%)	39.0 ± 1.1	38.5 ± 0.8	40.8 ± 1.4	40.8 ± 1.4	41.0 ± 2.7	41.5 ± 2.9
Body weight (g)	296 ± 7	302 ± 4	292 ± 4	292 ± 7	293 ± 9	320 ± 12
During MCAo (15 min)						
Rectal temperature (°C)	36.9 ± 0.08	36.9 ± 0.05	36.7 ± 0.06	36.7 ± 0.07	36.9 ± 0.05	36.9 ± 0.12
Cranial temperature (°C)	36.9 ± 0.06	37.0 ± 0.16	36.8 ± 0.08	36.8 ± 0.07	36.5 ± 0.08	36.4 ± 0.12
pH	7.41 ± 0.01	7.43 ± 0.01	7.40 ± 0.01	7.41 ± 0.01	7.41 ± 0.02	7.40 ± 0.03
PO_2 (mm Hg)	116 ± 4	120 ± 3	123 ± 5	127 ± 8	110 ± 4	121 ± 5
PCO_2 (mm Hg)	37.7 ± 0.5	39.3 ± 0.8	38.5 ± 1.1	38.1 ± 0.5	40.2 ± 0.6	38.7 ± 1.8
MABP (mm Hg)	106 ± 2	109 ± 2	104 ± 2	104 ± 3	105 ± 5	91 ± 6
Plasma glucose (mg/dl)	136 ± 3	127 ± 4	120 ± 6	120 ± 6	128 ± 8	124 ± 3
After treatment (30 min)						
Rectal temperature (°C)	37.0 ± 0.10	36.9 ± 0.05	36.7 ± 0.06	36.7 ± 0.07	36.9 ± 0.05	36.9 ± 0.12
Cranial temperature (°C)	36.9 ± 0.06	37.0 ± 0.16	36.8 ± 0.08	36.8 ± 0.07	36.5 ± 0.08	36.4 ± 0.12
MABP (mm Hg)	105 ± 4	109 ± 2	104 ± 2	104 ± 3	105 ± 5	91 ± 6
Hematocrit (%)	38.3 ± 0.4	28.0 ± 1.1*	22.8 ± 0.6*	24.1 ± 0.8*	40.3 ± 1.3	27.3 ± 3.9*
After treatment (24 h)						
Rectal temperature (°C)	38.0 ± 0.27	38.1 ± 0.20	38.0 ± 0.15	38.3 ± 0.14		
Body weight (g)	253 ± 6	264 ± 3	256 ± 3	261 ± 5		

Values are mean ± S.E.M.
MCAo, middle cerebral artery occlusion.
LCBF, local cerebral blood flow.
MABP, mean arterial blood pressure.
* Different from saline group, $P < 0.05$.

administered i.v. at 2 h after onset of middle cerebral artery occlusion, in doses of 1.25 ($n = 8$) or 2.5 g/kg ($n = 6$). Vehicle (0.9 % saline; $n = 6$) was administered in equivalent volumes to the above groups (5 ml/kg). In a separate group of animals, albumin (2.5 g/kg) was given 1 h after middle cerebral artery occlusion ($n = 6$).

In Series II, local cerebral blood flow was measured with the Laser Doppler Perfusion Imager. Albumin (1.25 g/kg; $n = 3$) or vehicle (sodium chloride 0.9%; $n = 3$) was administered at 2 h after onset of middle cerebral artery occlusion.

2.5. Histological assessment of infarction and edema volume

Animals were allowed to survive for 1 day. Brains were then perfusion-fixed as previously described with a mixture of 40% formaldehyde, glacial acetic acid, and methanol (1:1:8 by volume) (Belayev et al., 1996), and brain blocks were embedded in paraffin. Ten-micron-thick sections were cut in the coronal plane and stained with hematoxylin and eosin. To quantitate infarct volume and depict infarct frequency distribution, histological sections were digitized

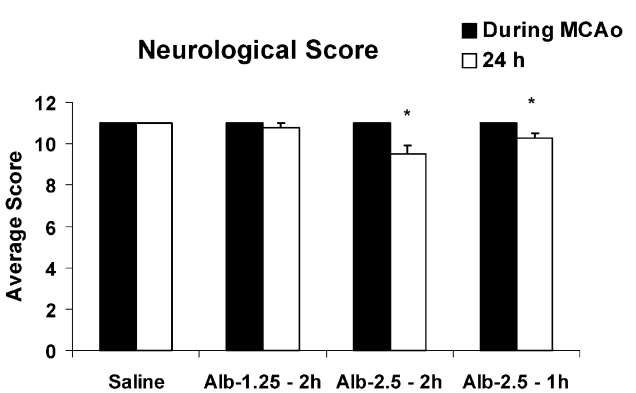

Fig. 1. Total neurological score (normal score = 0; maximal score = 12) in the various treatment groups during and 24 h after middle cerebral artery occlusion. Values are means ± S.E.M. *, significantly different from saline group, $P < 0.05$. MCAo = middle cerebral artery occlusion.

at nine standardized coronal levels (bregma levels: +5.2, +2.7, +1.2, −0.3, −1.3, −1.8, −3.8, −5.0, −7.3 mm) (Paxinos and Watson, 1997), from which data were exported to a UNIX-based workstation for further processing (MCID image-analysis system, Imaging Research, St. Catherines, Canada). 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 quantitate 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 (Swanson et al., 1990; Belayev et al., 1996, 1998).

2.6. Laser Doppler perfusion imaging

Six fasted Sprague–Dawley rats were anesthetized with 3.5% halothane in a mixture of 70% nitrous oxide and a balance of oxygen, then orally intubated, immobilized with pancuronium bromide, and mechanically ventilated. General surgical preparation and middle cerebral artery occlusion were the same as described above for the histological series. The animal was then placed in a stereotactic head

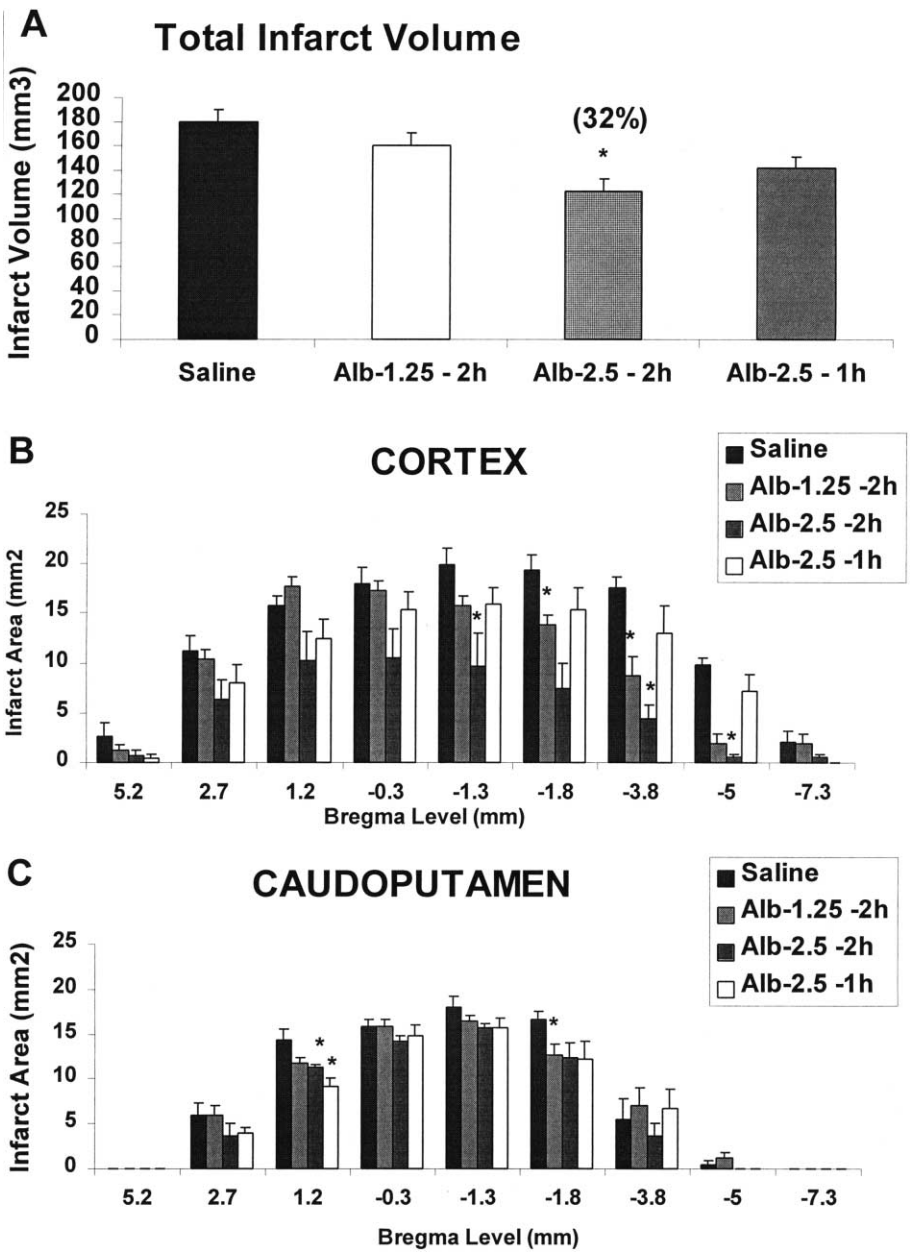

Fig. 2. Bar graphs show effect of albumin on total infarct volume corrected for brain swelling (A) 24 h after permanent middle cerebral artery occlusion; and the rostrocaudal distributions of cortical (B) and subcortical infarct areas (C) at nine coronal levels in albumin- and saline-treated rats. Values are means ± S.E.M. *, significantly different from corresponding saline group by ANOVA followed by Bonferroni tests, $P < 0.05$.

frame in the lateral position (David Kopf Instruments, Tujunga, CA), and a midline skin incision approximately 1.5 cm in length was made parallel to the sagittal suture and extended laterally to the midpoint between the right orbit and the external auditory canal. The temporalis muscle was elevated from the skull and retracted anteriorly. The zygoma was kept intact. To expose the distal middle cerebral artery, a ~4 mm diameter craniectomy was created between the frontal and squamosal-temporal bone, 5 mm anterior to the parieto-squamosal suture, under direct visualization with a Zeiss surgical microscope. The middle cerebral artery and its distal branches were clearly visible through the intact dura.

Cortical perfusion was measured with the Laser-Doppler Perfusion Imager (Moor Instruments, Wilmington, DE). A computer-controlled optical scanner directed a low-power He–Ne laser beam over the exposed cortex. The scanner head was positioned parallel to the cerebral cortex at a distance of 26 cm. The scanning procedure took 1.35 min for measurements of 86×78 pixels covering an area of 1.09×0.99 cm. At each measuring site, the beam illuminated the tissue to a depth of 0.5 mm. An image color-coded to denote specific relative perfusion levels was displayed on a video monitor. After three baseline images were acquired, the middle cerebral artery was permanently occluded. Twelve images were collected during middle cerebral artery occlusion. All images were acquired at 10-min intervals. After treatment, 22 additional images were obtained from each rat at 5-min intervals. All images were stored in computer memory for subsequent analysis. For each animal, a rectangular region-of-interest was defined in the central middle cerebral artery territory and applied to each image of the series. Relative perfusion values for each image were then determined as the average of all pixel values within the region-of-interest divided by the mean of the three baseline measurements for that region-of-interest.

2.7. Statistical analysis

Analysis of variance (ANOVA) with post-hoc comparisons was used to compare infarct size, brain swelling, and cortical perfusion values among groups. Neurological score and physiological variables were compared by Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. General physiological variables

Rectal and cranial temperatures, blood pressure, plasma glucose and blood gases in the 32 animals of this study showed no significant differences between groups (Table 1). Albumin therapy led to the expected moderate reduction in hematocrit (23–28%) compared to the vehicle group (Table 1).

3.2. Neurobehavioral assessment

Prior to middle cerebral artery occlusion, neurological score was normal (= 0) in all animals. High-grade behavioral deficits (score = 10–11) were present in all animals when tested at 60 min of middle cerebral artery occlusion (Fig. 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 1-day survival period. Higher-dose albumin therapy (2.5 g/kg) significantly improved the neurological score compared to vehicle rats at 24 h, when administered at 1 or 2 h after middle cerebral artery occlusion. Lower dose albumin therapy (1.25 g/kg) administered at 2 h after onset of middle cerebral artery occlusion was ineffective (Fig. 1).

3.3. Histopathology

All animals survived uneventfully. The total infarct volume was reduced by albumin (2.5 g/kg given at 2 h) by 32% compared with vehicle-treated rats (Fig. 2A). Fig. 2B demonstrates the rostrocaudal distribution of cortical infarct areas in all groups. Infarct areas were significantly smaller in albumin-treated rats (1.25 g/kg given at 2 h) than the saline group at two coronal levels (Fig. 2B). High dose of albumin (2.5 g/kg given at 2 h) also significantly reduced cortical infarct areas at three coronal levels compared to saline-treated animals. Total cortical infarct volume was significantly reduced only in the group receiving high-dose albumin (2.5 g/kg) at 2 h. The striatal component of the infarct was not consistently affected by albu-

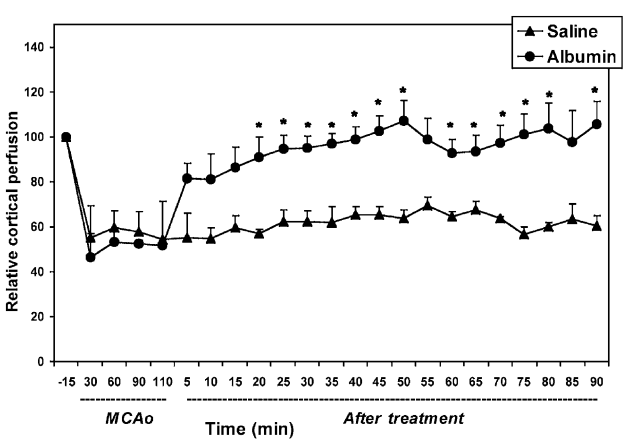

Fig. 3. Average changes of cortical perfusion before and during middle cerebral artery occlusion and 90 min after treatment in albumin- ($n = 3$) and saline-treated rats ($n = 3$). Albumin (1.25 g/kg) was given 2 h after onset of middle cerebral artery occlusion. Values are means ± S.E.M. *, significantly different from corresponding saline group by ANOVA followed by Bonferroni tests, $P < 0.05$.

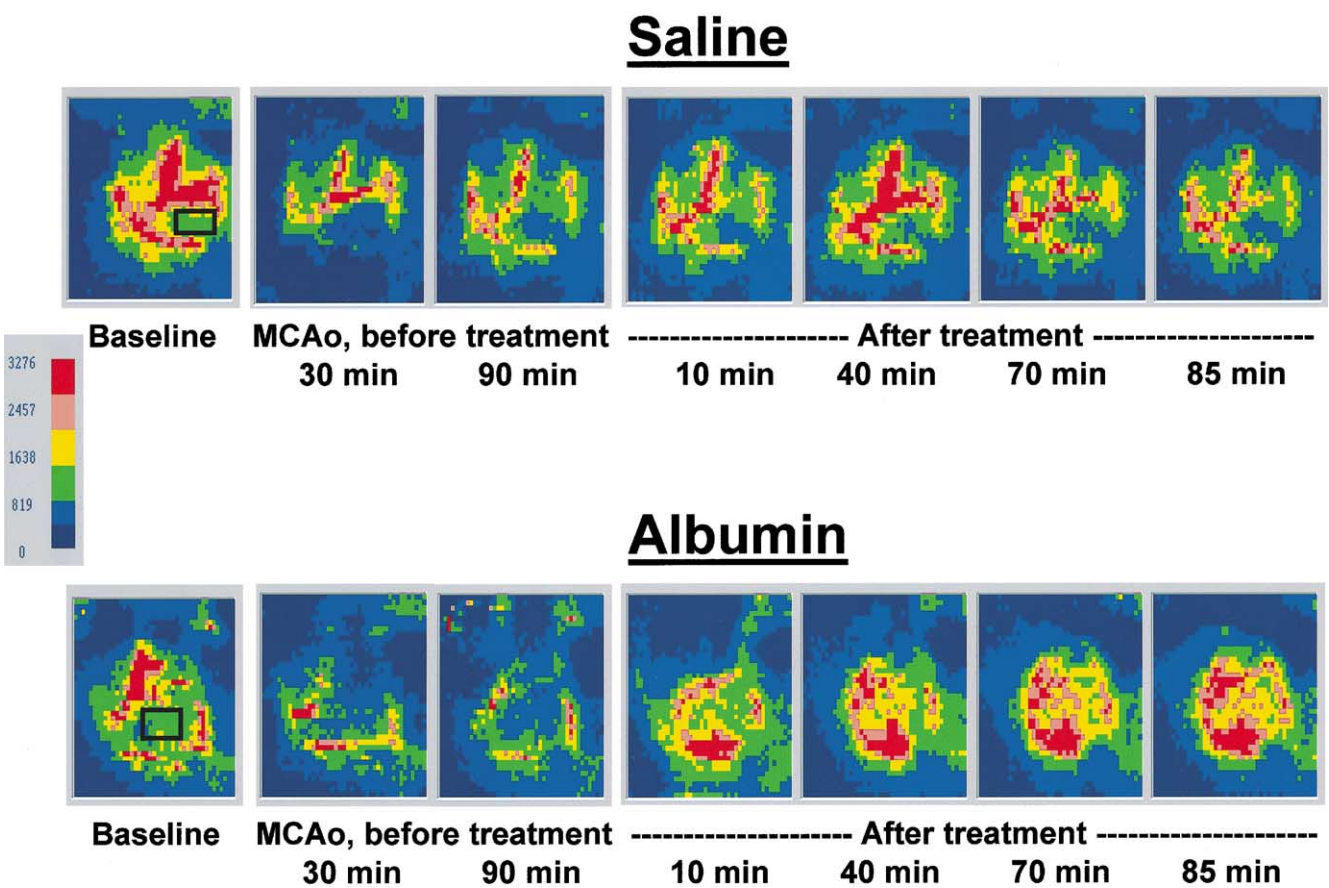

Fig. 4. Representative color-coded Laser-Doppler perfusion images of the middle cerebral artery territory as viewed through cranial window in saline-and albumin-treated rats. The rectangular region-of-interest from which the measurements of Fig. 3 were taken is diagrammed superimposed on the baseline images. (Color bar shows arbitrary linear units). Albumin but not saline treatment is associated with increased cortical perfusion.

min treatment. Brain swelling was also unaffected by albumin treatment (saline: $18.9 \pm 2.8\%$; albumin—1.25 g/kg at 2 h: $18.0 \pm 1.8\%$; albumin—2.5 g/kg at 2 h: $15.8 \pm 3.1\%$; albumin—2.5 g/kg at 1 h: $15.4 \pm 2.3\%$).

3.4. Cortical perfusion

As assessed by Laser-Doppler scanning of the cortical surface overlying the central middle cerebral artery territory, cortical perfusion declined during middle cerebral artery occlusion in both groups (Fig. 3). Treatment with albumin led to a relative perfusion increase of 45% ($P < 0.001$), while saline caused no further change (Fig. 3). Representative sequences of Laser-Doppler perfusion images are shown in Fig. 4.

4. Discussion

The goal of our study was to determine whether acute albumin administration was efficacious in protecting the brain after a *permanent* focal ischemic insult. Our results clearly indicate that human albumin therapy substantially improves neurological function, reduces the volume of cerebral infarction and increases local cerebral perfusion in animals after permanent middle cerebral artery occlusion.

This study was prompted by our earlier findings demonstrating the neuroprotective efficacy of considerably higher doses of human albumin administered at early times after ischemic or traumatic injury. In rats with *temporary* (2 h) middle cerebral artery occlusion, high-dose albumin therapy (2.0 and 2.5 g/kg) given at time of reperfusion significantly improved behavioral function and reduced the total infarct volume by 34% and cortical infarct volume by 57% compared to vehicle-treated rats (Belayev et al., 1997a, 1998). Albumin treatment of temporary middle cerebral artery occlusion also mitigated pan-necrotic histopathology in tissue zones of residual ischemic injury by fostering the partial preservation 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 (Belayev et al., 1998). High-dose albumin therapy was also neuroprotective in experimental models of both transient global ischemia (Belayev et al., 1999b) and fluid-percussion traumatic brain injury (Belayev et al., 1999a). In the temporary middle cerebral artery occlusion model, we have recently shown that a lower albumin dose (1.25

g/kg) is also strongly neuroprotective, with a broad therapeutic window extending to 4 h after stroke onset (Belayev et al., 2001).

In the current study, using a model of *permanent* focal cerebral ischemia, albumin treatment provided only a moderate improvement of the neurological score and reduction of total infarct volume compared to its efficacy in transient middle cerebral artery occlusion. Previous studies in focal ischemia have demonstrated that the degree of injury depends upon the duration of ischemia as well as the decrement in blood flow (Jones et al., 1981). Middle cerebral artery occlusion for 1–6 h produces progressively larger infarcts (Weinstein et al., 1986), and vascular occlusion for 4–8 h produces nearly the same size infarction as permanent occlusion (Jones et al., 1981; Weinstein et al., 1986).

Treatment strategies in experimental stroke can be regarded as attempts to salvage the ischemic penumbra, the region surrounding the most intensely ischemic core of a focal ischemic lesion. The ischemic penumbra, an area characterized by levels of blood flow slightly greater than in the ischemic core itself, is a zone exhibiting preserved or even accentuated metabolic rate, apparently driven by recurrent ischemic depolarizations (Back et al., 1995; Belayev et al., 1997b; Ginsberg, 1997). In the 2-h middle cerebral artery suture-occlusion model, the size of the penumbra is substantial, constituting nearly one-half of the total lesion (Belayev et al., 1997b). In contrast, the penumbral region in *permanent* middle cerebral artery occlusion appears to be substantially smaller (Back et al., 1994).

Brain swelling was not affected by albumin treatment in the current study. This finding contrasts with the dramatic effect of albumin therapy in reducing brain swelling by 80% or more in the setting of *transient* focal ischemia (Belayev et al., 1997a, 1998, 2001). Various studies have reported that, in the setting of permanent focal ischemia, the blood–brain barrier remains intact for several hours after vascular occlusion (Shigeno et al., 1985; Ishimaru et al., 1993) while water content is significantly increased (Schuier and Hossmann, 1980). Glial swelling and edema can become sufficiently severe to impair capillary flow in the ischemic core (Menzies et al., 1993). The normally tight blood–brain barrier prevents cells of the central nervous system from coming into contact with albumin and other protein components of the blood.

Several reports strongly support a physiological role for human serum albumin as a scavenger of oxygen free radicals (Halliwell and Gutteridge, 1990; Wasil et al., 1987). The potential importance of this mechanism in ischemic injury is emphasized by the fact that albumin is present in relatively high concentrations in both plasma and interstitial fluid; hence, it is strategically situated to scavenge oxygen radicals and also to interrupt the damaging oxidative process of lipid peroxidation (Emerson, 1989). Albumin can also bind copper ions, thereby inhibiting copper ion-dependent lipid peroxidation and hydroxyl radical formation (Halliwell and Gutteridge, 1990). In addition, albumin is a specific inhibitor of endothelial-cell apoptosis (Zoellner et al., 1996).

The results of the present experiments indicate that albumin at a dose 1.25 mg/kg improves cortical perfusion by 45% after permanent focal cerebral ischemia. We have recently shown that albumin treatment also leads to increased cortical perfusion after transient middle cerebral artery occlusion in rats (Belayev et al., 2000). In the normal brain, in which autoregulation is intact, oxygen delivery remains constant over a range of hematocrit values because cerebral blood flow adjusts so as to compensate for the changes in oxygen-carrying capacity (Brown et al., 1985). However, in the ischemic brain, in which autoregulation is lost and resistance vessels are already maximally dilated, hemorheological factors may play a major role in determining cerebral blood flow and oxygen transport to ischemic tissue (Cole et al., 1994). The use of albumin solutions in the treatment of focal cerebral ischemia was initially prompted by the belief that the resulting hemodilution and lowering of blood viscosity would prove hemodynamically beneficial to ischemic tissue (Wood et al., 1983; Cole et al., 1994)—an assumption related in part to the fact that albumin also inhibits platelet aggregation (Jorgensen and Stoffersen, 1980) and influences erythrocyte aggregation, increasing low-shear viscosity but decreasing erythrocyte sedimentation under no-flow conditions (Reinhart and Nagy, 1995).

Human serum albumin has been studied as a hemodiluent in several previous experimental ischemia reports (Cole et al., 1996; Aronowski et al., 1996). 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 (Cole et al., 1996; Korosue et al., 1990), whereas other authors were unable to detect a positive effect (Little et al., 1981; Sundt et al., 1967). Many explanation have been proposed for this inconsistency, including differences among the various hemodilution protocols with respect to the magnitude of hematocrit reduction, treatment delay, and concentration of the agent.

The benefits of hemodilution in patients with cerebral infarction continue to be debated. While some recent clinical studies have shown a benefit with hemodilution (Goslinga et al., 1992; Strand, 1992), two large multi-center trials undertaken to study the effect of hemodilution in acute stroke failed to show benefit (Italian Acute Stroke Study Group, 1988; Scandinavian Stroke Study Group, 1987). Interpretation of these studies are difficult, however, because of the large variety of hemodiluents used (human albumin, low molecular weight dextran, hydroxyethyl starch, di-aspirin cross-linked hemoglobin, and hetastarch) obtained from various sources and administered at differing times and in diverse concentrations and resulting in differing degrees of hematocrit reduction.

Other mechanisms may have contributed to neuroprotection with albumin. As the major protein of blood plasma, albumin is in fact a unique and complex molecule having a variety of physiochemical properties. It is a principal transporter of plasma fatty acids: most circulating long-chain fatty acids exist as albumin-complexes (Curry et al., 1998). Albumin also accounts for the majority of drug binding in the plasma (Koch-Weser and Sellers, 1976). Albumin 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 (Halliwell and Gutteridge, 1990). Indeed, albumin is responsible for 80% of the plasma's colloid oncotic pressure (Albright et al., 1984). In the present study, albumin therapy reduced the hematocrit acutely by 22–28%. This is similar to our previous results, in which albumin therapy produced an acute reduction in hematocrit from 40–42% to 23–28% that recovered to normal levels by 1 day (Albright et al., 1984).

The present study has demonstrated the ability of Laser-Doppler perfusion imaging to measure perfusion changes in rapid sequence. The Laser-Doppler imager scans a low-power laser beam over cranial window. Moving blood in the microvasculature causes a Doppler shift that is processed to build up a color-coded image of relative perfusion. The measurement is non-contact and requires only a small craniotomy with the dura remaining intact. Thus, Laser-Doppler perfusion imaging can register dynamic changes and is capable of depicting regional differences (Lauritzen and Fabricius, 1995). By contrast, conventional Laser-Doppler flowmetry is a real-time measure but is restricted to a single point on the cortical surface.

In summary, the present study has shown that human albumin therapy is moderately neuroprotective in permanent focal cerebral ischemia and produces no observable adverse effects in young-adult experimental animals. These results offer additional rationale for clinical trials to investigate the efficacy of albumin in the treatment of acute ischemic stroke.

Acknowledgements

These studies were supported by Program Project Grant NS 05820 of the National Institutes of Health (MDG), American Heart Association Initial Investigator Award (LB), AHA Undergraduate Student Research Program (AB) and an equipment loan from Moor Instruments. The authors thank Guillermo Fernandez for his expert technical assistance.

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