An early bolus of hypertonic saline hydroxyethyl starch improves long-term outcome after global cerebral ischemia

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Objective: **The beneficial effect of hypertonic saline solutions in the emergency treatment of shock and traumatic brain injury is well described. The present study determines effects of a single bolus of hypertonic saline on long-term survival, neurologic function, and neuronal survival 10 days after global cerebral ischemia. In addition, we evaluated the therapeutic window for hypertonic saline treatment (early vs. delayed application).**

Design: **Laboratory experiment.**

Setting: **University laboratory.**

Subjects: **Male Wistar rats weighing 240–330 g.**

Interventions: **Rats were submitted to temporal global cerebral ischemia using temporary bilateral carotid occlusion combined with hypobaric hypotension. Animals received 7.5% saline/6% hydroxyethyl starch (HHS) or vehicle (NaCl 0.9%) at either 1.5 mins (early treatment) or 31.5 mins (delayed treatment) of reperfusion. Regional cerebral blood flow (rCBF) and physiologic variables were measured during insult and early reperfusion. Animal survival and neurologic function were evaluated throughout the 10-day observation period. Quantification of brain injury was performed on day 10.**

Measurements and Main Results: **Early treatment with HHS resulted in a robust restoration of rCBF after ischemia, reduced postischemic mortality by 77% (9% vs. 39% in vehicle-treated controls), ameliorated neurologic performance (Neuro-Deficit-Score 10 days after insult, 96 ± 0.7 vs. 85 ± 1.4, mean ± SE), and almost blunted neuronal cell death (hippocampal CA1, 2150 ± 191 vs. 884 ± 141 neurons/mm^2; cortex, 1746 ± 91 vs. 1060 ± 112). In contrast, delayed treatment resulted in no sustained effects.**

Conclusions: **Timing of HHS treatment is critical after experimental global cerebral ischemia to reduce mortality, improve neurologic function, and neuronal survival. Our results suggest that early application of HHS may be a potential neuroprotective strategy after global cerebral ischemia. (Crit Care Med 2006; 34:2194–2200)**

KEY WORDS: rats; cerebral ischemia; cerebral resuscitation; hypertonic saline; hydroxyethyl starch; histopathology; neurological deficit; survival

Incomplete reperfusion after transient cerebral ischemia aggravates brain damage and is known to significantly impair long-term functional outcome following various brain insults (1–3). Postischemic events, like increased vascular permeability and perivascular edema, increased blood viscosity, thrombus formation, and leukocyte-to-endothelium interaction, may critically impair blood flow in the microcirculatory bed (4–7).

Hypertonic solutions (HS) have been used for some time for fluid resuscitation from shock, specifically in prehospital emergency treatment (8, 9). They have been found to improve the perfusion of kidney, liver, small intestine, and heart following various injuries (1, 10–13). More recently, they have also been shown to reduce brain edema and the associated elevated intracranial pressure and to increase survival after traumatic brain injury in humans (14–16).

Despite the positive results in traumatic brain injury, few studies have been done on effects of HS after global cerebral ischemia, and little is known about effects on long-term survival and neurologic function (1, 17–19). A single-bolus HS treatment regime has not been thoroughly investigated in global cerebral ischemia models. We therefore examined whether a single bolus of HS improves neurologic performance and neuronal survival 10 days after transient global cerebral ischemia in rats. In addition, we evaluated the therapeutic window for HS treatment (immediate vs. delayed application). We used a novel commercially available HS (7.5% hypertonic saline + 6% hydroxyethyl starch, HHS), which is widely used for advanced trauma life support.

MATERIALS AND METHODS

Subjects

With institutional approval, male Wistar rats (240–330 g, Charles River) were treated in accordance with international and institutional guidelines. All animals were housed in a temperature-controlled environment (22°C) under a 12:12 hr dark/light cycle and had free access to food and water throughout the experiment.

Paradigm

The insult consisted of 15 mins of global cerebral ischemia and subsequent reperfusion, followed by 10-day observation period. The intervention consisted of 7.5% saline, 6% HHS, or vehicle (NaCl 0.9%) at two time points: at 1.5 mins of reperfusion (early treatment) or at 31.5

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mins of reperfusion (delayed treatment). End points were the regional cerebral blood flow and physiologic variables during insult and the first 120 mins of reperfusion; survival, neurologic performance, and body weight during 10 days of postischemic reperfusion; and neuronal survival 10 days after the brain insult.

Global Brain Ischemia and Reperfusion

The experimental procedures on rats were performed as previously described (20, 21). In brief, animals were anesthetized (induction, halothane, 3 Vol%; maintenance, intraperitoneal chloral-hydrate 120 mg/kg/hr), orally intubated, and ventilated (30%/ 70% inspiratory oxygen/air mixture at all times; end-tidal carbon dioxide pressure controlled to 35–40 mm Hg). The right jugular vein and both common carotid arteries were exposed. The right jugular vein was catheterized using polyethylene tubing (PE-50) for intravenous drug administration. The left common carotid artery was catheterized (PE-50) for blood sampling and continuous monitoring of arterial blood pressure. The right common carotid artery was loosely looped with a nylon thread (5-0).

The head of the rat was fixed in a stereotaxic frame. The skull bone above the right hemisphere was thinned over an area of approximately 24 mm^2 (1.3–5.3 mm lateral to the right and 1.5–7.5 mm occipital from the bregma). A laser-Doppler flow probe (BPM 403A; TSI, St. Paul, MN) moved by a computer-driven robotic micromanipulator was used to determine regional cerebral blood flow (rCBF) at 30 locations within this window (scanning technique) (21–23). A small closed cranial window (approximately 4 mm^2) was established over the left hemisphere to measure local cerebral blood flow (lCBF) using a stationary laser-Doppler flow probe (BPM 2, Vasamedics, St. Paul, MN). lCBF measurements were used only to confirm the onset and persistence of ischemia during the insult (data not shown) (23). CBF measurements are expressed in laser-Doppler units.

Temperature was monitored within the left auricular tube and the rectum, as previously described (20). Pericranial temperature was controlled to 37°C using a thermostatically controlled heating blanket and a near-infrared heating lamp.

To induce cerebral ischemia, after a 30-min postsurgical stabilization period, the right common carotid artery was occluded (tightening of the previously placed nylon thread by attaching a defined weight; 2.2 g) and the mean arterial blood pressure (MAP) was reduced to 35 mm Hg by vacuum-induced venous pooling using an airtight chamber around the lower part of the animal's body (hypobaric hypotension technique) (21, 24). Measurements of lCBF and rCBF confirmed forebrain ischemia. After 15 mins, the nylon thread was removed and hypobaric hypotension was terminated to allow reperfusion of the brain.

Physiologic variables (cerebral blood flow, MAP, temperatures) were monitored for 120 mins of reperfusion. Arterial blood gases, pH, base excess, hematocrit, hemoglobin, blood glucose, and lactate levels were measured 10 mins before brain ischemia (baseline) and at 5, 35, 60, and 90 mins of reperfusion. Subsequently, catheters were removed, incisions were closed, and animals were weaned from the ventilator. After extubation, rats were returned to their housing cages that were continuously warmed to avoid heat loss (warming lamp) for the next 24 hrs.

Experimental Groups

Seventy-three animals were randomized to receive 4 mL/kg of either a 7.5 % saline plus 6% hydroxyethyl starch solution (mean molecular weight 200,000; HyperHAES, Fresenius, Germany; HHS) or normal saline (0.9% NaCl; vehicle). The respective solution was infused via the jugular venous catheter over a period of 3 mins (bolus treatment) using a programmable motor-driven syringe pump. Sham-operated animals underwent the same experimental procedures, omitting hypobaric hypotension and brain ischemia. Rats were assigned to eight different experimental groups: Forty-nine animals were exposed to transient global cerebral ischemia and received early or delayed HHS or vehicle treatment, respectively (early vehicle [at 1.5 mins reperfusion], n = 11; early HHS [E-HHS], n = 11; delayed vehicle [at 31.5 mins reperfusion], n = 12; delayed HHS [D-HHS], n = 15). Twenty-four animals served as respective sham controls (sham early vehicle, n = 4; sham early HHS, n = 8; sham delayed vehicle, n = 4; sham delayed HHS, n = 8).

Behavioral- and Neurologic-Deficit Evaluation

All neurologic and behavioral testing was performed in a quiet room with dimmed light by one investigator, who was unaware of the experimental group assignment. The evaluation procedure was performed on the last 2 days before brain ischemia (baseline) and on days 1, 2, 3, 4, 6, 8, and 10 of reperfusion. Consciousness, motor function, overall activity, ability to interact with the environment, orientation, and presence of seizures were scored according to a neurologic assessment score adapted for this experiment (0–100 scale; 100 = normal performance, no deficit; 0 = equivalent to brain death, most severe deficit) (25). Animals that died prematurely during the observation period were excluded from further data analysis.

Neurohistopathologic Evaluation

At 10 days of reperfusion, animals were deeply anesthetized and transcardially perfused with freshly prepared 4% buffered paraformaldehyde. The brains were carefully removed and paraffin-embedded. Coronal sections (3 μm) were prepared and stained with hematoxylin/eosin. Neuronal cell density (number of viable neurons/mm^2) in sensomotoric parietal neocortex and different regions of hippocampus (CA1, CA2, CA3, CA4; interaureal 5.2 mm, bregma −3.8 mm) was determined by an investigator blinded to the treatment, using a video microscope and a computer system (26). Briefly, images of the region of interest were obtained using a color CCD camera (SSC-C370P, Sony) connected to a light microscope (×10 lens, Zeiss). Images were projected on the monitor of an Amiga 2000 computer (Commodore) via a Genlock interface. Standardized frames (calibrated using a Leitz microscope ruler) were superimposed over the video image with the use of custom software developed in this laboratory. Frames were adjusted over region of interest in a defined manner. Viable neurons were marked on the screen by mouse clicks, minimizing the chance of double counting objects. The software calculated objects/mm^2 after the whole frame was counted. Neuronal viability was assumed in the absence of eosinophilic cytoplasm, cytoplasmic vacuolation, and nuclear pyknosis.

Statistical Analyses

Data are expressed as mean ± SE. Comparison of treatment groups for physiologic variables, neurologic deficits, and neuronal densities was performed using one-way analyses of variance and *post hoc* Bonferroni test (SigmaStat 2.03, SPSS, Chicago, IL). Mortality was analyzed using Fisher's exact test. Differences were considered statistically significant at $p < .05$.

RESULTS

Ischemic animals receiving early or delayed vehicle treatment did not differ in physiologic variables, neurologic performance, or neuronal survival. Neither did sham animals receiving early or delayed vehicle treatment show differences in these parameters. For further analysis, animals were therefore pooled into two groups only: ischemia vehicle and sham vehicle.

Physiologic Variables

Blood Analysis. Blood gases, pH, base excess, blood glucose, and lactate were affected by the ischemic insult but not by treatment (HHS, vehicle, immediate, delayed, respectively; Table 1). Administration

Table 1. Physiologic variables

	Sham			Ischemia		
Reperfusion, Mins	Vehicle	E-HHS	D-HHS	Vehicle	E-HHS	D-HHS
pH						
Baseline	7.38 ± 0.02	7.41 ± 0.02	7.38 ± 0.02	7.35 ± 0.01	7.40 ± 0.01	7.37 ± 0.01
4.5	7.35 ± 0.01	7.36 ± 0.01	7.36 ± 0.02	7.25 ± 0.01	7.24 ± 0.01	7.28 ± 0.02
34.5	7.35 ± 0.01	7.36 ± 0.01	7.31 ± 0.02	7.3 ± 0.01	7.3 ± 0.01	7.3 ± 0.01
60	7.33 ± 0.01	7.33 ± 0.01	7.34 ± 0.02	7.32 ± 0.01	7.32 ± 0.01	7.33 ± 0.01
90	7.33 ± 0.01	7.33 ± 0.01	7.32 ± 0.01	7.31 ± 0.01	7.32 ± 0.01	7.31 ± 0.01
$Paco_2$, mm Hg						
Baseline	37.6 ± 2.3	37.8 ± 1.7	38.5 ± 1.5	37.9 ± 0.9	36.3 ± 1.2	38.7 ± 1.1
4.5	41.2 ± 0.9	39.9 ± 0.9	38.7 ± 1.5	40.4 ± 1	41 ± 1	40.6 ± 1.6
34.5	38.6 ± 0.8	39.4 ± 0.8	40.9 ± 1.4	39.3 ± 0.9	39 ± 0.8	42 ± 1.5
60	39.4 ± 1.2	39.6 ± 1.3	39 ± 1.2	39.5 ± 1	38.9 ± 1.7	37.1 ± 0.9
90	38.7 ± 0.9	39.4 ± 0.6	39.6 ± 1.3	38.7 ± 0.8	38.4 ± 0.8	37.7 ± 1.4
Pao_2, mm Hg						
Baseline	153.1 ± 5.1	153.3 ± 8.6	152.8 ± 8.9	139.8 ± 4.4	138.9 ± 4.5	144.8 ± 4.6
4.5	148.2 ± 6.4	140.6 ± 7.5	146.6 ± 8.8	160.2 ± 5.1	166.2 ± 5	168.7 ± 4
34.5	145.1 ± 6	136.7 ± 5.3	136.6 ± 8.7	134 ± 4.9	140.2 ± 5.2	151 ± 4
60	141 ± 5.8	126.2 ± 6	130.6 ± 8.8	131.5 ± 4.7	138.5 ± 6.7	143.5 ± 4
90	136.5 ± 3.8	124.1 ± 6.4	129 ± 7	132 ± 5.3	141 ± 6.2	143.6 ± 5.2
Base excess, mEq/L						
Baseline	−2 ± 0.6	−0.2 ± 0.7	−1.9 ± 0.8	−4.0 ± 0.5	−1.8 ± 0.4	−2.8 ± 0.6
4.5	−2.7 ± 0.3	−2.6 ± 0.4	−2.9 ± 1	−4.0 ± 0.5	−1.8 ± 0.4	−2.8 ± 0.6
34.5	−4.4 ± 0.6	−2.2 ± 1	−5.3 ± 1	−6 ± 0.4	−5 ± 0.4	−7.1 ± 0.5
60	−4.7 ± 0.4	−4 ± 0.9	−4.6 ± 0.9	−5.7 ± 0.5	−5.2 ± 0.4	−5.5 ± 0.6
90	−5 ± 0.4	−4.6 ± 0.8	−5.2 ± 0.9	−6.7 ± 0.5	−5.2 ± 1	−7.0 ± 0.8
Glucose, mg/dL						
Baseline	163.7 ± 22.5	181.1 ± 15.6	165.3 ± 14.6	157.4 ± 5.3	164.5 ± 9.1	144.7 ± 8.5
4.5	147.7 ± 16.9	160.4 ± 13.5	170.3 ± 12.9	145.4 ± 5.3	139.2 ± 9.7	138.7 ± 17.8
34.5	160.4 ± 22.5	166 ± 16.4	164.6 ± 14.6	165.8 ± 5.2	164.8 ± 6.1	144.5 ± 9.2
60	161.3 ± 21.6	161.6 ± 18.7	172.6 ± 14.1	173.095 ± 6	171.615 ± 6.6	153.125 ± 8.1
90	163.6 ± 21.9	173.7 ± 15.8	166.3 ± 10.2	172.5 ± 7.1	172.9 ± 7.4	156.6 ± 7.8
Lactate, mmol/dL						
Baseline	1.2 ± 0.3	1 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
4.5	1.3 ± 0.3	1 ± 0.1	1.4 ± 0.1	3.5 ± 0.2	3.3 ± 0.3	3.5 ± 0.4
34.5	1.1 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1 ± 0.1	1.2 ± 0.1
60	1.1 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1 ± 0.1	1.6 ± 0.5
90	1 ± 0.1	1 ± 0.1	0.9 ± 0.1	1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Sodium, mmol/L						
Baseline	137.7 ± 0.7	137.4 ± 0.5	137.9 ± 0.7	139 ± 0.5	138.2 ± 0.3	138.1 ± 0.5
4.5	137.143 ± 0.6	150.143 ± 1[a]	138.571 ± 0.9	138.5 ± 0.3	149.6 ± 0.7[a]	137.9 ± 0.5
34.5	138.3 ± 1.6	143.9 ± 1.5[a]	148.4 ± 2.2[a]	138.5 ± 0.4	143.8 ± 0.2[a]	149.9 ± 0.6[a]
60	137.7 ± 0.9[a]	145 ± 0.6[a]	144.7 ± 0.5[a]	138.3 ± 0.3	143.8 ± 0.5[a]	144.5 ± 0.3[a]
90	137.9 ± 0.7	143.4 ± 1.5[a]	144.3 ± 0.6[a]	138.5 ± 0.4	144.2 ± 0.3[a]	144.6 ± 0.5[a]
Potassium, mmol/L						
Baseline	4.2 ± 0.1	4.3 ± 0.1	4.4 ± 0.2	3.8 ± 0.1	3.9 ± 0.1	4.2 ± 0.1
4.5	4.1 ± 0.2	3.6 ± 0.1[b]	4.5 ± 0.3	4.1 ± 0.1	3.5 ± 0.2[b]	4.3 ± 0.1
34.5	4.2 ± 0.1	3.7 ± 0.1[b]	3.5 ± 0.1[a]	4 ± 0.1	3.9 ± 0.1	3.6 ± 0.1[b]
60	4.3 ± 0.2	3.5 ± 0.1[b]	3.9 ± 0.2	3.9 ± 0.1	3.8 ± 0.1	3.7 ± 0.1
90	4.3 ± 0.1	3.8 ± 0.1	3.9 ± 0.2	3.8 ± 0.1	3.7 ± 0.1	3.6 ± 0.1
Hematocrit, %						
Baseline	44.6 ± 1.5	46.5 ± 1.2	45.7 ± 0.8	41.5 ± 0.9	43.9 ± 1.5	44.4 ± 1.1
4.5	43.6 ± 1.6	39.7 ± 1.2	42.8 ± 0.9	43.7 ± 0.6	36.8 ± 1[a]	45.5 ± 1
34.5	41.2 ± 2.2	42.4 ± 1.8	36.3 ± 1.1[a]	42.2 ± 0.9	41.1 ± 1.4	36.6 ± 1[a]
60	40.4 ± 1.7	43.2 ± 1.5	37.7 ± 0.7	42.2 ± 0.8	41.8 ± 1.3	39 ± 1
90	40 ± 1.9	43.3 ± 1	40.1 ± 2	41.2 ± 0.8	40.5 ± 1.4	39.7 ± 1

Vehicle, NaCl 0.9%; E-HHS, hypertonic hydroxyethyl starch 1.5 mins after ischemia; D-HHS, hypertonic hydroxyethyl starch 30 mins after ischemia. [a]$p < .01$ vs. vehicle; [b]$p < .05$ vs. vehicle. Values are mean± SE.

of HHS resulted in elevated sodium levels for ≥90 min after infusion, compared with vehicle ($p < .001$). In contrast, potassium serum levels and hematocrit transiently decreased following HHS infusion ($p < .001$; Table 1).

Regional Cerebral Blood Flow. During the early phase of reperfusion, rCBF was above baseline levels in all postischemic animals (postischemic hyperperfusion, Fig. 1*A*). However, rats receiving early HHS (at 1.5 mins of reperfusion, E-HHS) showed a more rapid restoration of rCBF compared with vehicle-treated animals ($p < .05$). After 30 mins of reperfusion, rCBF was lower than baseline in vehicle-treated rats (postischemic hypoperfusion), whereas E-HHS animals maintained rCBF at or above baseline levels throughout the experiment ($p < .05$, Fig. 1*A*). In contrast, delayed HHS treatment (at 31.5 mins of reperfusion, D-HHS) did not raise rCBF values compared with vehicle treatment. HHS had no effect on rCBF in sham-operated animals (data not shown).

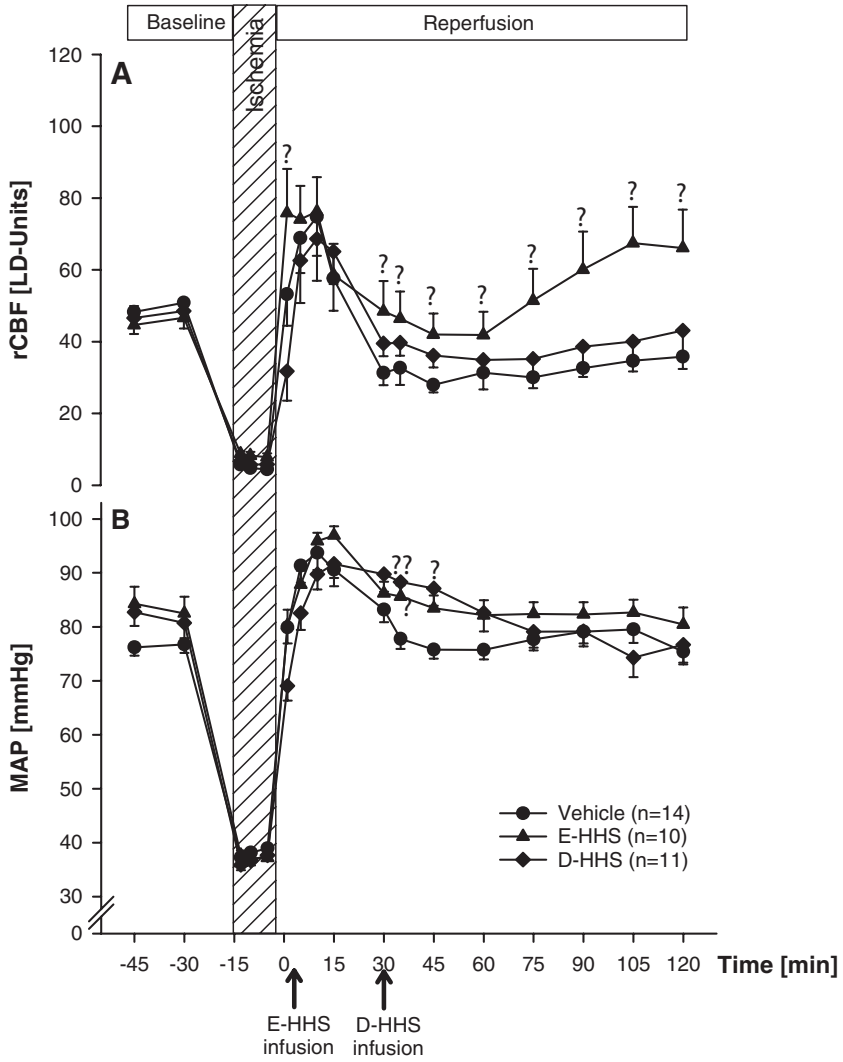

Figure 1. *A*, regional cerebral blood flow (*rCBF*) expressed as laser-Doppler units throughout the experimental procedure. *B*, mean arterial blood pressure (*MAP*) expressed in mm Hg. Mean ± SE; *$p <$ .05 vs. vehicle; **$p <$ .01 vs. vehicle. *E-HHS*, early hydroxyethyl starch; *D-HHS*, delayed hydroxyethyl starch.

Mean Arterial Blood Pressure. Blood pressure did not differ between groups at baseline or during ischemia (Fig. 1*B*). At reperfusion, MAP rose well above baseline levels in all groups. Postischemic MAP was back to baseline at 35 mins of reperfusion in vehicle-treated animals. In contrast, both HHS treatment groups maintained elevated MAP levels until 45 min after ischemia ($p < .05$ and $p < .01$, respectively). HHS treatment did not influence MAP in sham animals (data not shown).

Mortality

Fourteen of 73 randomized animals (19%) did not survive the entire 10-day observation period. Most animals (11, 15%) died within the first 72 hrs after the insult. E-HHS (9%; one of 11, $p < .001$), but not D-HHS (27%; four of 15, p not significant), reduced postischemic mortality compared with vehicle treatment (39%; nine of 23).

Neurologic Deficit and Weight Gain

Postischemic neurologic deficit was most pronounced on the first day after the insult and improved throughout the observation period, although baseline levels were not regained (Fig. 2*A*). Neurologic presentation was most profoundly impaired in vehicle-treated animals, whereas early treatment with HHS was associated with a significantly ameliorated neurologic performance throughout the entire 10-day observation period ($p < .01$). In contrast, delayed HHS treatment had only transient effects on neurologic performance (days 3–4, $p < .05$).

Changes in body weight showed a similar pattern (Fig. 2*B*). Vehicle-treated animals lost weight for 3 days after ischemia and started to regain weight only after 6 days, without reaching baseline levels. E-HHS animals, in contrast, regained weight much faster ($p < .05$ vs. vehicle) and surpassed baseline levels between day 4 and 6 of reperfusion, comparable to sham-operated animals. D-HHS, however, did not result in significant improvement.

Neurohistopathology

Neuronal density was profoundly decreased in hippocampal CA1 and neocortex of vehicle-treated animals 10 days after global cerebral ischemia ($p < .001$ vs. sham; Fig. 3). Similarly, the number of viable neurons was reduced in hippocampal CA2, CA3, and CA4 (Table 2). Early application of HHS was associated with a robust neuroprotection in hippocampal CA1 ($p < .001$), CA2 ($p < .05$), and parietal neocortex ($p < .001$), compared with vehicle. Effects were less pronounced in the other regions of the hippocampus. Delayed HHS treatment caused some improvement of neuronal survival, but differences failed to reach statistical significance (CA1, $p = .069$; neocortex, $p = .1$).

DISCUSSION

The presented data demonstrate that a bolus dose of 7.5% hypertonic saline + 6% hydroxyethyl starch (HHS, 4 mL/kg) dramatically improves long-term survival, neurologic function, and neuronal survival after global cerebral ischemia in rats. Timing of treatment was crucial in this experimental setting: Administration immediately after the ischemic insult resulted in robust neuroprotection, whereas the delayed treatment paradigm produced no significant changes in outcome. We saw an impressive beneficial effect of an early treatment on all end points, cell survival as well as actual neurologic function, that was sustained throughout the 10-day observation period. This strongly suggests that the observed effects may be of actual clinical relevance.

It is well known that cerebrovascular reperfusion after transient ischemia remains inhomogeneous for hours. Areas of impaired microvascular perfusion have been observed in various species and different models of brain ischemia (1, 27–29). This pattern was termed "no-reflow

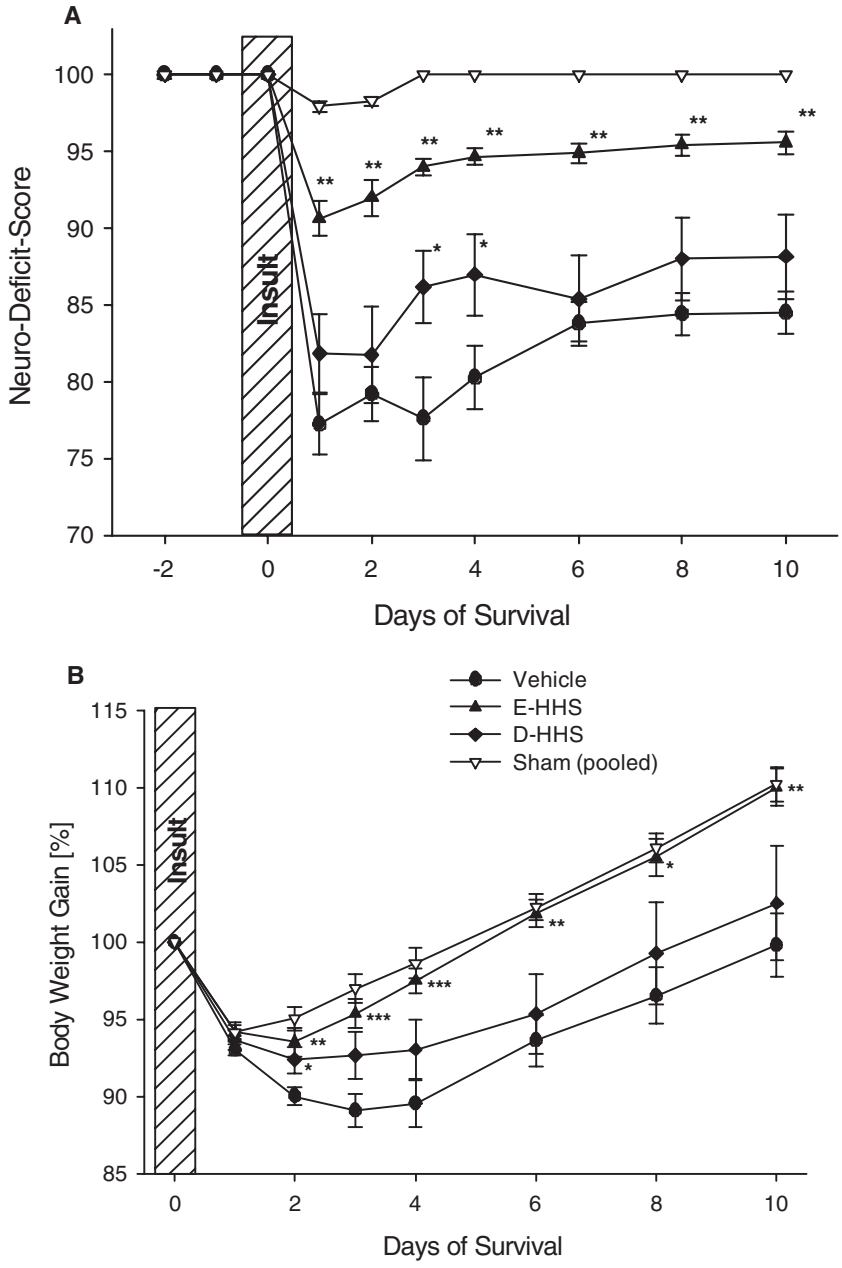

Figure 2. Neuro-Deficit-Score (*A*) and weight gain (*B*) throughout the 10-day postischemic observation period. Mean ± SE; *$p < .05$ vs. vehicle; **$p < .01$ vs. vehicle; ***$p < .001$ vs. vehicle. *E-HHS*, early hydroxyethyl starch; *D-HHS*, delayed hydroxyethyl starch.

phenomenon" (27). Factors like postischemic increased blood viscosity, thrombus/corpuscle formation in small blood vessels, reduced capillary diameter due to perivascular edema, and leukocyte-to-endothelium interaction have been identified to play a role in reperfusion dysfunction (4–7). Hypertonic saline has been shown to reduce the number of "no-reflow" areas after cardiopulmonary resuscitation in cats (1), and microcirculation was particularly promoted in areas showing edematous endothelial cells before administration (30). More recently, we observed a reduction in infarct size accompanied by an improved cerebral perfusion with HHS administration in a model of cortical vein occlusion (31). The early and profound restoration of cerebral microcirculation that we saw with early HHS treatment in our present study may therefore be responsible, at least in part, for the improved survival and outcome in this group.

Possible mechanisms by which HHS may improve the restoration of microperfusion after cerebral ischemia include a direct reduction of endothelial and perivascular edema, due to an increased osmogradient after administration of HHS, which forces free water to shift from endothelial cells and from the interstitium back into the intravascular space, as well as an interference with leukocyte adhesion to the endothelium, due to a decreased expression of relevant adhesion molecules (32). Beyond the direct negative effect that adherent leukocytes will have on blood flow in small-diameter vessels, they transmigrate into the parenchyma and have also been implicated in postischemic inflammation and the exacerbation of ischemic damage (33). Neutrophils have been widely studied in this respect. They can come to a complete standstill and transmigrate through the vessel wall after ischemia, causing additive injury due to direct cytotoxicity and further microvascular injury (32, 34). Activation and neutrophil transmigration correlated with the development of brain edema in a model of traumatic brain injury in rats (35). *In vitro* exposure to hypertonic solutions attenuated neutrophil activation, cytotoxicity, and oxygen burst in a time-dependent manner (36, 37). However, the lack of beneficial effects of delayed HHS treatment in our model of transient global ischemia argues against a relevant contribution of neutrophil transmigration to ischemic damage in this model, at least within the first 10 days after reperfusion.

The HHS used in our experiments contains 7.5% sodium chloride, resulting in a tonicity comparable to hypertonic saline solutions (31). Many of the effects that we saw may be attributed to this hypertonic component. It has been suggested that the addition of hydroxyethyl starch (HES) to HS may prolong the intravascular half-life of HS and therefore extend its beneficial effects (38, 39). When administered after successful resuscitation from cardiac arrest, both HS and HHS infusions ameliorated myocardial blood flow and improved short-term survival, but HES administration alone had no effect on these end points (40). This is well in accordance with our recent observation that HS, but not HES, reduced infarct size after cortical vein occlusion (31). An independent protective effect of HES after cerebral ischemia therefore seems unlikely.

HHS administration was well tolerated in our present study. We did not see cardiovascular side effects or severe or sustained derangements of electrolytes after a single dose of HHS in our population of young and healthy adult ani-

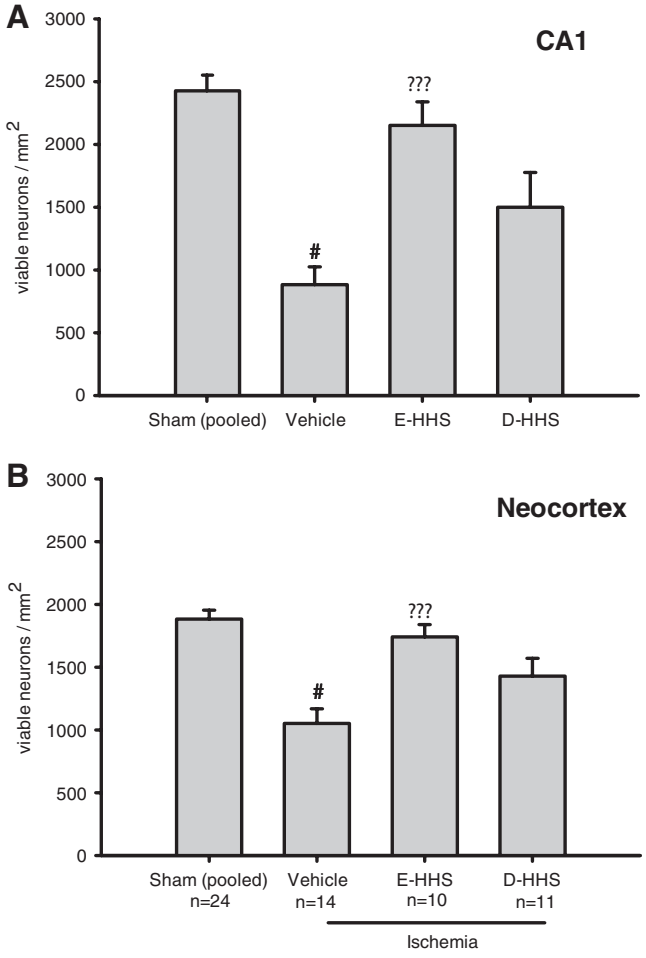

Figure 3. Neurohistopathologic outcome in hippocampal CA1 (*A*) and parietal neocortex (*B*) 10 days after global cerebral and reperfusion. Mean ± SE; #$p < .001$ vs. sham; ***$p < .001$ vs. vehicle. *E-HHS*, early hydroxyethyl starch; *D-HHS*, delayed hydroxyethyl starch.

Table 2. Neurohistopathology (viable neurons/mm^2) of hippocampal subregions CA2, CA3, and CA4

		Ischemia		
	Sham (Pooled)	Vehicle	E-HHS	D-HHS
CA 2	968 ± 44	696 ± 69[a]	981 ± 89[b]	740 ± 87
CA 3	1259 ± 47	1008 ± 56[a]	1172 ± 101	951 ± 60
CA 4	471 ± 17	446 ± 19	450 ± 30	397 ± 21

Vehicle, NaCl 0.9%; E-HHS, hypertonic hydroxyethyl starch 1.5 mins after ischemia; D-HHS, hypertonic hydroxyethyl starch 30 mins after ischemia.

[a]$p < .01$ vs. sham; [b]$p < .05$ vs. vehicle. Values are mean ± SE.

mals. A previous study reported severe chronic hypernatremia and exacerbated brain damage after a continuous infusion of hypertonic saline over 22 hrs after transient middle cerebral artery occlusion in rats (41). In accordance with this, long-lasting hypernatremia (increase in serum sodium of 33–39 mmol/L) was able to induce myelinolysis in healthy rats (42). Previous data suggest that a single bolus of hypertonic saline may result in acute myelinolysis in case of preexisting long-term hyponatremia (42, 43). This was not a concern in the healthy animals used in our study. Moreover, our single-dose treatment regimen resulted in mild increases of serum sodium only (maximum increase 12 mmol/L; maximum serum sodium 150 mmol/L). It is therefore highly unlikely that the proposed single-dose HHS treatment may result in additional damage in this experimental setting, and we have indeed no histologic evidence of myelinolysis. Further work is needed to assess a risk/benefit relation of HHS for critically ill subjects.

CONCLUSION

A single bolus (4 mL/hr, 3 mins) of a premixed solution containing hypertonic saline (7.5%) and hydroxyethyl starch (6%) improved overall survival, long-term neurologic outcome, and neuronal damage when administered early after global cerebral ischemia in adult rats. This suggests a potential neuroprotective strategy for the immediate postinjury treatment of cerebral ischemia in humans after, for example, successful cardiopulmonary resuscitation. Further research is necessary to determine the clinical feasibility and safety and to elucidate the protective mechanisms involved.

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