Effects of a single-dose hypertonic saline hydroxyethyl starch on cerebral blood flow, long-term outcome, neurogenesis, and neuronal survival after cardiac arrest and cardiopulmonary resuscitation in rats*

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Objective: **The beneficial effects of hypertonic saline on neuronal survival and on cerebral blood flow have been shown in several animal models of global and focal brain ischemia. Because of the potential benefits of hypertonic solutions, it is hypothesized that hydroxyethyl starch enhances cerebral blood flow and improves long-term outcome after cardiac arrest and cardiopulmonary resuscitation in an animal model.**

Design: **Laboratory animal study.**

Setting: **University animal research laboratory.**

Subjects: **Fifty-nine male Sprague-Dawley rats.**

Interventions: **Rats were randomized to receive either 7.2% saline/6% hypertonic saline hydroxyethyl starch (4 mL/kg) or vehicle (NaCl 0.9 %) after 9 mins of asphyxic cardiac arrest and cardiopulmonary resuscitation. Local cerebral blood flow and physiologic parameters were evaluated during arrest and early restoration of spontaneous circulation. Survival and neurologic assessment were evaluated over a 7-day observation period. Animals received 5-bromo-2-deoxyuridine for 6 days. Neuronal injury and neurogeneration (5-bromo-2-deoxyuridine positive neurons) were quantified on day 7 after cardiac arrest and cardiopulmonary resuscitation.**

Measurements and Main Results: **Hypertonic saline hydroxyethyl starch treatment resulted in an accentuated local cerebral blood flow during early reperfusion, compared to the vehicle group. Animal survival and neurologic outcome were not altered between groups. Neurohistopathological injury was present in hippocampal CA1 and neocortex with no effects of hypertonic saline hydroxyethyl starch on neuronal survival. Increased neurogeneration was found in the dentate gyrus after cardiac arrest/cardiopulmonary resuscitation, which was not influenced by hypertonic saline hydroxyethyl starch administration.**

Conclusions: **Despite promising results in other models of brain injury, hypertonic saline hydroxyethyl starch failed to improve the outcome when administered after asphyxic cardiac arrest/cardiopulmonary resuscitation in rats. One major difference between the cardiac arrest/cardiopulmonary resuscitation model and other models of brain ischemia is that the effects of asphyxic cardiac arrest involve the whole organism (post-cardiac arrest syndrome) and not exclusively the brain leading to a more severe injury. This might explain why hypertonic saline hydroxyethyl starch has failed to improve outcome in the present model. (Crit Care Med 2012; 40: 2149–2156)**

Key Words: asphyxia; cardiac arrest; cardiopulmonary resuscitation; cerebral blood flow; hypertonic saline; neurogenesis; neuronal survival

Sudden cardiac arrest (CA) is still associated with poor survival and neurological outcome (1). In Europe alone approximately 700,000 individuals do not survive when sudden CA occurs (2). Over the years, several techniques and treatments have been introduced to improve neurological outcome after cardiopulmonary resuscitation (CPR). Only very few techniques and interventions focus on improvement of brain function. Thus far, only the induction of mild hypothermia for 12–24 hrs after CA and CPR is generally accepted to improve neuronal survival and functional recovery (3). CA results in a complete standstill of brain perfusion, a state which is well-known as complete global cerebral ischemia known to cause neuronal death. Once CPR is initiated, microcirculatory blood flow in the brain is impaired (4). After restoration of spontaneous circulation (ROSC), microcirculation remains impaired. After global cerebral ischemia a "no-reflow" syndrome has been described (5). Additionally, post-CA events like increased vascular permeability, perivascular edema, increased blood viscosity, thrombus formation, and leukocyte-to-endothelium interaction may critically impair blood flow in the microcirculatory bed (6, 7).

Hypertonic saline (HS) has been successfully used for fluid resuscitation from hemorrhagic shock for decades (8, 9). Hypertonic solutions have been shown to improve microcirculation in several organs (e.g., heart, kidney, liver) in various injury models (10, 11). In a model of permanent stroke, hypertonic/hyperoncotic saline improved cerebral blood flow (CBF) and reduced infarct size (12). In a rat model of acute subdural hematoma, hypertonic/oncotic saline improved functional outcome and neuronal survival (13). These solutions have also been successfully used in the treatment of traumatic brain injury to reduce brain edema and increased intracranial pressure (14).

Only a few studies have been done on effects of HS treatment after global brain ischemia. In a model of temporal global cerebral ischemia using bilateral carotid occlusion and hypobaric hypotension, an

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early treatment with HS after the ischemic episode resulted in a robust restoration of CBF, reduced mortality, and improved neurological recovery (15). Despite the promising results in human traumatic brain injury and in models of incomplete brain ischemia, little is known of the effects of HS on long-term survival, neuronal survival, and neurological outcome after full body ischemia with complete "no-flow" caused by CA.

Therefore, the effect of a single bolus of hypertonic saline hydroxyethyl starch (HHS) on survival, neurologic performance, and neuronal survival 7 days after asphyxic CA and CPR in rats was investigated. A commercially available hypertonic isooncotic saline (7.2% HS + 6% HHS) was examined, which is widely used for fluid resuscitation of severe trauma.

MATERIALS AND METHODS

Subjects. After approval of the governmental animal care committee (Koblenz, Germany), 59 male Sprague-Dawley rats (275–325 g, Charles River, Sulzfeld, Germany) were treated in accordance with international and institutional guidelines. Animals were housed in a temperature-controlled environment (22°C) under a 12:12 hour dark/light cycle and had free access to food and water.

Animal Preparation. All animals were fasted overnight with free access to water before surgery. Experimental procedures on rats were performed as previously described (16). In brief, animals were anesthetized (induction, sevoflurane 4%; maintenance, sevoflurane 2.5 %), orally intubated, and ventilated (30%/70% inspiratory oxygen/air at all times); end-tidal carbon dioxide tension was controlled at values of 40 mm Hg using capnography. The right femoral artery and vein were catheterized using polyethylene tubing (polyethylene tubing 0.58 inner diameter) for blood sampling, continuous monitoring of mean arterial blood pressure (MAP), and intravenous drug administration, respectively. Tympanic temperature was monitored within the right auricular tube using an ear-bar thermocouple probe linked to an infra-red heating lamp and maintained at 37°C (17). A rectal probe was inserted, and body temperature was controlled at 37°C with a biofeedback-heating mat. The head of the rat was fixed in a stereotaxic frame. A small closed cranial window was created over the left and right hemisphere to measure local CBF (lCBF) using stationary laser-Doppler flow probes (Perimed Probe 403; PeriFlux 4001 Master, Perimed AB, Stockholm, Schweden).

CA and CPR. Deeply anesthetized rats were paralyzed with intravenous (IV) 0.5 mg/kg pancuronium bromide. After 15 mins, asphyxia was induced by stopping the controlled ventilation. CA was defined as a MAP <20 mm Hg. Nine minutes after induction of apnea, CPR was initiated with external chest compressions (200 min^{-1}) and controlled ventilation was started (16). A single dose of adrenaline was injected IV (10 µg/kg) via the right femoral vein catheter. ROSC was defined as spontaneous MAP over 40 mm Hg. ROSC had to be achieved within 3 mins, otherwise the experiment was out of protocol, and the animals were excluded. Controlled ventilation and sevoflurane anesthesia were continued for 60 mins after CA and CPR (Fig. 1). After the observation period, intravascular catheters were removed, the vessels ligated, and the incisions were closed. Anesthesia was discontinued, and the animals were weaned from the ventilator. After tube removal, animals were placed for 24 hrs in an incubator tempered at 36°C with oxygen-enriched air (17).

Experimental Groups. Animals were randomized to receive 4 mL/kg of either a 7.2% saline plus 6% hydroxyethyl starch solution (mean molecular weight 200,000; HyperHAES, Fresenius, Germany) or normal saline (0.9% NaCl; vehicle; Fig. 2) 1.5 mins after ROSC. The respective solution was infused via the femoral 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 CA/CPR and adrenaline application. Rats were assigned to five different groups: 41 animals underwent CA/CPR and received HHS bolus (HHS, n = 14) or vehicle (Vehicle, n = 14), respectively. Twelve served as sham controls, receiving either HHS bolus (sHHS, n=6) or Vehicle (sVehicle, n=6). Six rats were used as naïve animals for neurohistopathological controls.

Measurements. MAP, electrocardiogram, heart rate, lCBF, and tympanic and rectal temperature were monitored and recorded permanently using HSE Haemodyn W, Hugo Sachs Elektronik (Havard Apparatus GmbH, March-Hugstetten, Germany). lCBF measurements were recorded in blood perfusion units and expressed in percent of baseline. Arterial blood samples were taken 15 mins before CA (baseline) and at 1, 30, and 60 mins after CA and CPR (Fig. 1). Arterial blood gases, electrolytes, pH, base excess, hematocrit, hemoglobin, blood glucose concentration, and lactate levels were measured using an ABL 800 Basic blood gas analyzer (Radiometer GmbH, Willich, Germany).

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 assignment. The evaluation was performed 1 day before CA/CPR (baseline) and each day for 7 days after ROSC. Food/water intake, consciousness, breathing pattern, vibrissae movement, motoric function, and interaction with the environment were scored according to a neurologic assessment score adapted for this experiment (0 = normal performance, no deficit; 20 = most severe deficit) (18, 19). Animals that died before completion of the 7-day experimental protocol were excluded from further data analysis.

5-Bromo-2-Deoxyuridine Labeling and Tissue Preparation. For labeling of proliferating cells, 100 mg/kg 5-bromo-2-deoxyuridine (BrdU; Sigma-Aldrich ChemieGmbH, Munich, Germany) was injected intraperitoneally every day during the first 6 days of survival. As a thymidine analog, BrdU is incorporated into DNA on mitotic division.

Seven days after CA and CPR, animals were deeply anesthetized with sevoflurane 5% and transcardially perfused with fresh prepared 0.2 M phosphate buffered 4% formaldehyde solution. The brains were carefully removed and divided in left and right hemispheres. For analysis of neurogenesis, left side hemispheres were postfixed in paraformaldehyde phosphate

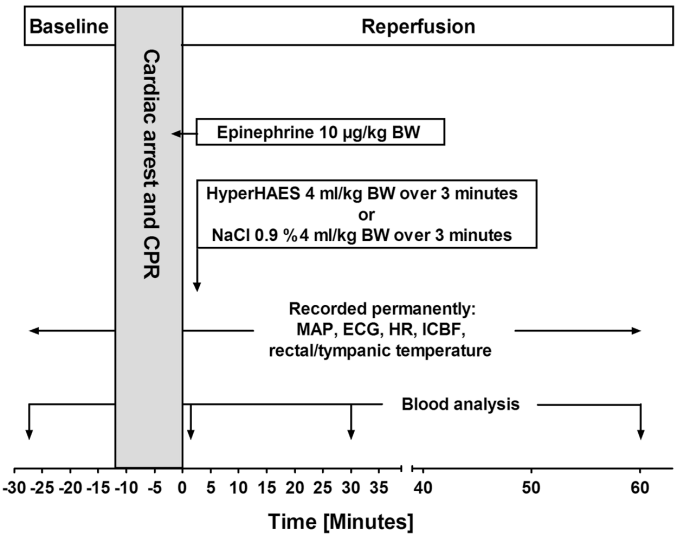

Figure 1. Experimental procedure and measurements during baseline, cardiac arrest/cardiopulmonary resuscitation (*CPR*), and reperfusion. Blood analysis including arterial blood gases, electrolytes, pH, base excess, hematocrit, hemoglobin, blood glucose concentration, and lactate levels. *MAP*, mean arterial blood pressure; *ECG*, electrocardiogram; *HR*, heart rate; *lCBF*, local cerebral blood flow.

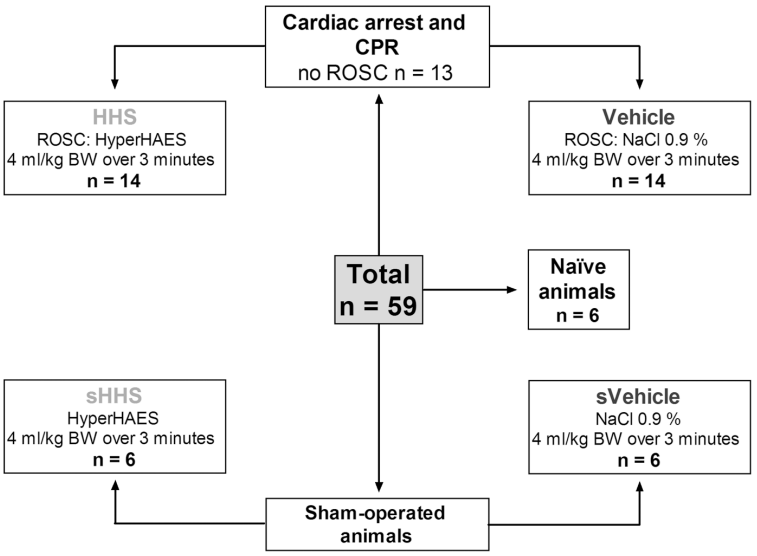

Figure 2. Flow diagram of the experimental groups. *HHS*, hypertonic saline hydroxyethyl starch; *s*, sham; *ROSC*, restoration of spontaneous circulation; *CPR*, cardiopulmonary resuscitation.

buffer for 24 hrs, and placed in 30% sucrose. Forty-micrometer sagittal brain sections were prepared and stored in a cryoprotection solution (glycerol, ethylene glycerol, and 0.1 M phosphate buffer) at –20°C (20). Evaluation of neurogenesis immunohistochemistry and immunofluorescence staining were performed as previously described (21). In brief, sections for immunohistochemistry staining were incubated with the primary mouse anti-BrdU antibody (monoclonal mouse immunoglobulin G [IgG], 1:500; Roche Diagnostics Corp., Indianapolis, IN) followed by an incubation with the biotinylated secondary donkey antimouse antibody (Biotin-SP–conjugated donkey antimouse IgG, 1:500; Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Sections for immunofluorescence staining were incubated with the primary rat anti-BrdU antibody (monoclonal rat IgG, 1:500; Oxford Biotechnology, Oxford, United Kingdom) and the primary goat anti-doublecortin antibody (polyclonal goat IgG, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), followed by incubation with the secondary antibodies (fluorescein isothiocyanate–conjugated donkey antirat IgG, 1:500, Rhodamine Red-X–conjugated donkey antigoat IgG, 1:500).

For neurohistopathologic evaluation, right-sided hemispheres were paraffin-embedded. Coronal sections (4 µm) were prepared and stained with hematoxylin/eosin.

Evaluation of Neurogenesis and Neurohistopathology. Blinded stereologic analysis of immunohistochemically stained BrdU-positive cells was performed manually using light microscopy assuming equal distribution of BrdU-positive cells throughout the dentate gyrus. BrdU positive cells were counted in a one-in-ten series of sections (400 µm apart) spanning the entire dentate gyrus, and results were multiplied by ten. To determine the percentage of newly generated neurons, BrdU-positive cells were analyzed for colabeling of BrdU and doublecortin or neuron-specific nuclear protein with an immunofluorescence microscope combined with an Apotome and Axiovision software (Axiovert 200; Zeiss GmbH, Göttingen, Germany). The resulting percentages were multiplied with the stereologically estimated amount of BrdU-positive cells assessed by immunohistochemical staining to estimate the amount of newborn neurons in the dentate gyrus.

Neuronal cell density in sensomotoric parietal neocortex (number of viable neurons/mm^2) and different regions of hippocampus (number of viable neurons/mm) known to be sensitive to ischemic injury (CA1, CA2, CA3, CA4; intraureal 5.2 mm, bregma –3.8 mm) was determined by an investigator unaware of group affiliation. A video microscope and a computer system were used for evaluation (22). Briefly, images of the region of interest were obtained using a video camera (SSC-C370P, Sony) connected to a light microscope (×10 lens, Zeiss). Images were projected on the monitor of a personal computer via a gunlock 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 the region of interest in a predefined manner. Viable neurons were marked on the screen by mouse clicks, minimizing the chance of double counting objects. Neuronal viability was assumed in the absence of eosinophilic cytoplasm, cytoplasmic vacuolation, and nuclear pyknosis.

Statistical Analyses. Data are expressed as mean ± SD. Neurohistopathological and neurogenesis data are presented as median (minimun–maximum and interquartile range), additionally the mean is reported. Exploratory comparisons between time-based measurements like MAP, lCBF, blood samples, and tympanic temperatures within the groups were conducted with two-way repeated measurement analysis of variance and Bonferroni posttests. Comparisons of weight and duration of CA were analyzed using a two-tailed unpaired *t* test. Neurogenesis and neuronal injury between groups were performed using a Kruskal–Wallis test and Dunn's multiple comparison posttest. Survival rate was compared using a log-rank test. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, La Jolla, CA). Differences are termed statistically significant if $p < .05$.

RESULTS

Nine animals of the HHS and 4 rats of the Vehicle group did not achieve ROSC within 3 mins after CA and were replaced accordingly (Fig. 2). Seven animals of the HHS group did not survive the first 24 hrs after ROSC. Five animals of the Vehicle group died within 24 hrs after ROSC; one died after 48 hrs and another 72 hrs after CA/CPR (p = not statistically significant). All animals of the sham-treated groups survived the 7-day observation period.

Physiologic Variables. Blood gases, pH, and blood glucose were affected by CA and CPR but not by treatment regimen (Table 1). Administration of HHS resulted in elevated sodium levels throughout the observation period, compared to vehicle (Table 1; $p < .001$). Hemoglobin, hematocrit, potassium, and base excess did not differ between groups (data not shown). Rectal temperature declined to a nadir of 36°C 10 mins after the arrest and returned to baseline levels 30 mins after ROSC. All groups were comparable. Accordingly, tympanic temperature did not differ between CA groups (Table 1). CA resulted in a decreased tympanic temperature, compared to baseline values. Temperature returned to baseline levels 5 mins after the arrest. Tympanic temperature was stable throughout the whole experiment in sham groups.

MAP did not differ between CA groups during baseline and asphyxia (Fig. 3*A*). Ten minutes after ROSC, MAP was elevated in CA groups and returned to baseline values within 30 mins. MAP was higher in the vehicle group during treatment, compared to HHS ($p < .01$). At 20 mins after ROSC, HHS treatment resulted in an elevated MAP, compared to vehicle group ($p < .01$). This difference was abolished throughout the remaining observation period. HHS treatment did not influence MAP in sham animals.

During early phase of ROSC, cortical lCBF was above baseline levels in both CA/CPR groups (postischemic hyperperfusion; Fig. 3*B*). Animals treated with HHS

Table 1. Physiologic variables

Group	Time (min)	pH	$Paco_2$ (mm Hg)	Pao_2 (mm Hg)	Glucose (mg/dL)	Na^+ (mmol/L)	Cl^- (mmol/L)	Tympanic Temperature (°C)
HHS	Baseline	7.4 ± 0.03	38.5 ± 3.7	97.0 ± 10.4	115.8 ± 18.1	137.9 ± 2.1	103.9 ± 3.6	37.1 ± 0.2
	1	7.1 ± 0.04	54.6 ± 6.8	159.7 ± 48.9	93.4 ± 47.9	139.6 ± 4.3	107.7 ± 6.2	35.8 ± 0.2
	30	7.3 ± 0.07	47.2 ± 8.9	135.9 ± 38.4	108.6 ± 21.3	143.7 ± 3.2[a]	110.5 ± 4.6[b]	37.0 ± 0.1
	60	7.4 ± 0.04	40.7 ± 4.8	104.7 ± 13.4	122.4 ± 23.0	142.9 ± 2.2[a]	109.7 ± 2.9	37.1 ± 0.1
Vehicle	Baseline	7.4 ± 0.03	38.9 ± 3.0	99.1 ± 17.3	112.4 ± 17.6	138.9 ± 2.1	103.6 ± 4.5	37.0 ± 0.0
	1	7.1 ± 0.03	54.1 ± 6.9	157.8 ± 44.3	86.1 ± 50.7	138.6 ± 2.5	106.4 ± 4.8	35.7 ± 0.3
	30	7.3 ± 0.05	44.4 ± 4.8	129.4 ± 19.0	105.0 ± 26.2	139.3 ± 2.0	105.9 ± 4.5	37.0 ± 0.1
	60	7.4 ± 0.03	39.3 ± 3.2	106.2 ± 11.9	115.6 ± 21.3	138.0 ± 1.9	105.6 ± 4.3	37.0 ± 0.1
sHHS	Baseline	7.4 ± 0.05	36.9 ± 3.5	101.8 ± 15.7	123.3 ± 29.4	137.5 ± 2.5	103.0 ± 2.5	37.1 ± 0.2
	1	7.4 ± 0.04	37.0 ± 5.6	363.7 ± 74.5	109.7 ± 31.9	139.3 ± 3.0	105.8 ± 7.3	36.6 ± 0.3
	30	7.4 ± 0.02	39.4 ± 4.7	211.1 ± 39.5	119.8 ± 14.5	143.5 ± 2.0[b]	108.8 ± 3.4	36.9 ± 0.2
	60	7.4 ± 0.02	39.7 ± 4.1	126.8 ± 16.7	121.0 ± 20.2	143.2 ± 2.6[b]	108.7 ± 3.9	36.9 ± 0.3
sVehicle	Baseline	7.4 ± 0.05	39.3 ± 4.8	88.7 ± 17.4	114.2 ± 15.0	137.8 ± 1.2	101.3 ± 2.9	37.1 ± 0.2
	1	7.4 ± 0.05	38.2 ± 4.1	284.2 ± 135	111.7 ± 17.4	137.8 ± 1.8	102.0 ± 4.5	36.7 ± 0.1
	30	7.4 ± 0.05	40.0 ± 5.7	191.1 ± 64.5	116.5 ± 34.0	139.8 ± 2.3	104.0 ± 4.7	36.9 ± 0.2
	60	7.4 ± 0.04	39.6 ± 5.2	124.7 ± 26.4	116.5 ± 26.7	139.3 ± 2.0	103.3 ± 3.5	36.9 ± 0.2

HHS, hypertonic saline hydroxyethyl starch; s, sham.
Vehicle: NaCl 0.9%. Vehicle vs. HHS Bonferroni Posttests.
[a]$p < .01$; sVehicle vs. sHHS Bonferroni Posttests, [b]$p < .05$; Mean ± SD.

at 1.5 mins after ROSC showed a more rapid restoration of lCBF in the neocortex, compared to vehicle-treated rats ($p < .001$). Postischemic hyperperfusion was also more accentuated in the HHS group at 5, 10, and 20 mins after CPR. Thirty minutes after CA and CPR, lCBF was lower than baseline values in both groups (postischemic hypoperfusion). lCBF was influenced by treatment regimen during postischemic hypoperfusion with higher values in the HHS group ($p = .0074$). Sham-treated animals showed no differences in cortical lCBF throughout the whole observation period, independent of treatment.

Weight Gain and Neurological Assessment Score. No differences in body weight were observed between sham and CA groups (Fig. 4*A*). However, CA and CPR resulted in a developing weight loss during the 7-day observation period, compared to baseline levels (HHS day –1 vs. HHS day 7 ($p < .05$), Vehicle day –1 vs. Vehicle day 7 [$p < .05$]). HHS did not alter weight after the arrest. Weight was not affected in sham animals.

Postarrest neurological deficit was most pronounced on the first day after CA and CPR and improved throughout the observation period, although baseline levels were not reached (Fig. 4*B*). No differences between arrest HHS and vehicle groups were observed ($p = .2701$). Sham-treated animals showed a minor neurological deficit after anesthesia and surgery for up to 2 days after the intervention.

Neurohistopathology. Neuronal density was decreased in the neocortex 7 days

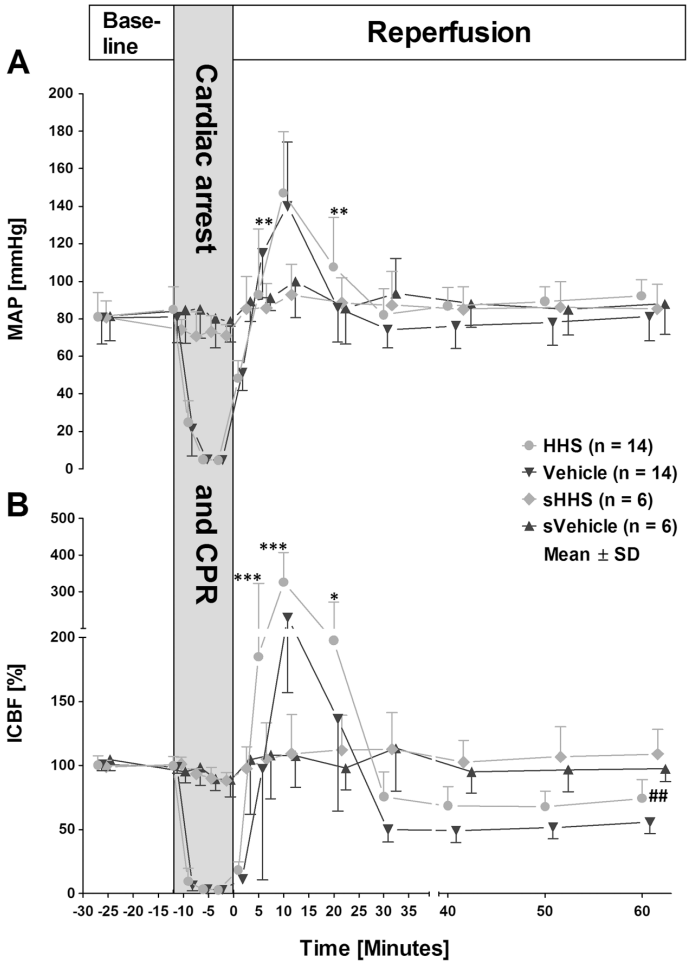

Figure 3. *A*, Mean arterial blood pressure (*MAP*) expressed in mm Hg throughout the experimental procedure. *B*, Local cerebral blood flow (*lCBF*) in the neocortex expressed as relative perfusion (%) from baseline values. Vehicle: NaCl 0.9%; vehicle vs. hypertonic saline hydroxyethyl starch (*HHS*) $^{\#\#}p < .01$, Bonferroni Posttests $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$; Mean ± SD. *s*, sham; *CPR*, cardiopulmonary resuscitation.

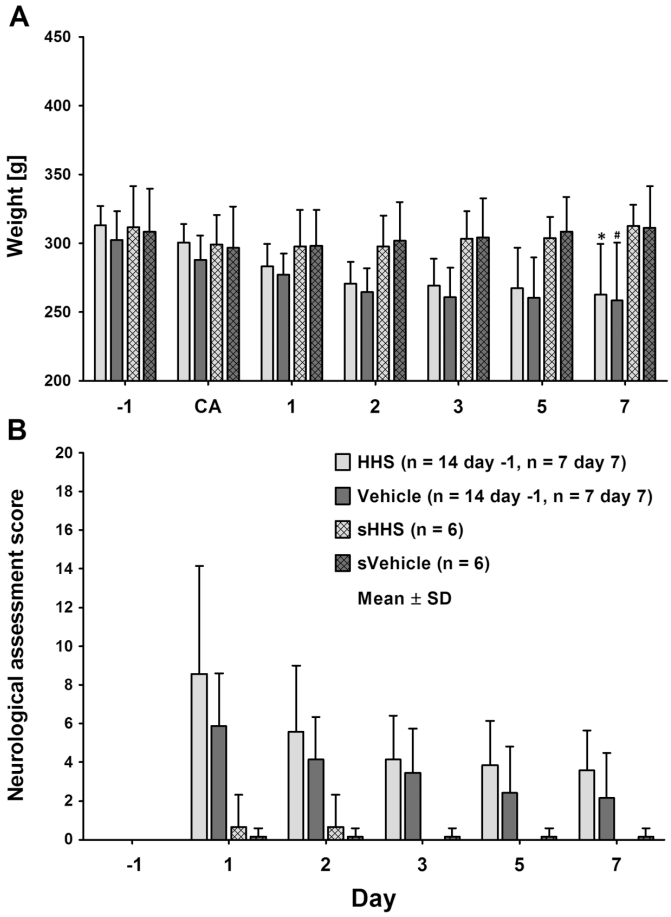

Figure 4. *A*, Weight gain throughout the 7-day postarrest observation period, cardiac arrest (*CA*), hypertonic saline hydroxyethyl starch (*HHS*) day –1 vs. HHS day 7 $^{*}p < .05$, vehicle day –1 vs. vehicle day 7. *B*, Neurological assessment score throughout the whole experiment. Vehicle: NaCl 0.9%. $^{\#}p < .05$; Mean ± SD. *s*, sham.

after CA and CPR in the HHS group ($p < .05$) and vehicle group ($p < .05$) compared to naïve animals (Fig. 5*A*). Neuronal density in the hippocampal CA1 showed a similar pattern (Fig. 5*B*). The number of viable neurons was reduced in HHS ($p < .01$) and vehicle-treated animals ($p < .05$), compared to naïve rats. HHS application did not alter neuronal survival in hippocampal CA1, compared to vehicle. Similarly, the number of viable neurons did not differ in the neocortex between HHS and vehicle-treated animals. In hippocampal CA1, CA resulted in neuronal injury compared to sham animals (HHS vs. sVehicle ($p < .05$). No statistical significance in neuronal injury was observed in the hippocampal CA2, CA3, and CA4 region between all groups (Table 2).

Neurogenesis. The number of newly generated neurons in the dentate gyrus of the left brain is shown in Figure 6. Seven days after CA/CPR, both CA groups presented an increased number of BrdU-positive neurons in the dentate gyrus, compared to naïve animals (HHS vs. Naïve [$p < .05$], Vehicle vs. Naïve [$p < .01$]) and in part to sham animals. Neurogenesis was not different between vehicle and HHS animals. HHS administration did not affect newly generated neurons in sham-operated animals, compared with naïve rats.

DISCUSSION

The present study examines for the first time possible beneficial effects of a single dose of 7.2% HS + 6% hydroxyethyl starch (HES) on neurological outcome and neuronal survival after asphyxial CA and CPR. HHS (4 mL/kg) improves lCBF in the neocortex during early reperfusion after experimental CA and CPR. Long-term survival was not influenced by treatment pattern. Neurologic function improved in all rats after CA and CPR over the 7-day observation period. However, HHS treatment had no effects on recovery. Neurohistopathological injury was present after CA and CPR in brain areas being known as vulnerable to ischemia. HS and 6% HHS application during early reperfusion after CA did not alter neuronal survival in this clinical relevant model of brain ischemia. This experiment is one of the few studies that could not show beneficial effects of HHS on long-term outcome after brain injury.

It is well-known that cerebrovascular reperfusion after CA and CPR follows a specific pattern. Early brain reperfusion after CA and CPR is associated with initial cerebral hyperemia (23). Accumulation of several metabolites such as hydrogen ions, adenosine, or lactate as potential vasodilators has been associated with early hyperemia (24). Hyperemia is followed by a reduction of lCBF, which has been described as "delayed hypoperfusion." Several mechanisms have been proposed for this phenomenon, including vasospasm caused by postischemic-impaired endothelial metabolism. The lCBF data of the present study are in agreement with these previous studies. After ROSC, initial hyperemia is followed by delayed hypoperfusion, which is present for at least 60 mins after CA and CPR.

HHS was chosen in the present study because this solution is commonly used in the clinical and prehospital setting. The HHS applied is commercially available and contains 7.2% sodium chloride, resulting in a tonicity comparable to HS solutions (12). HS has been shown to reduce the extension of no-reflow areas after CA and CPR in cats (25), and microcirculation was particularly improved in areas showing edematous endothelial cells after brain ischemia (26). HHS potentially improves restoration of microperfusion by direct reduction of endothelial perivascular edema by forcing free water to shift from endothelial cells and from interstitium back into intravascular space due to an increased osmogradient (27). Possible beneficial effects of HS may be attributed to this hypertonic component. HS but not HES alone reduced infarct size in a model of cortical vein occlusion. This is in agreement with studies examining HS and HES on myocardial blood flow after CA and CPR. HS and HHS infusion both ameliorated myocardial blood flow and survival. However, HES alone had no effect in this model (28). An independent effect of HES after CA and CPR, therefore, seems unlikely.

In a model of temporal global brain ischemia induced by bilateral carotid occlusion and hypobaric hypotension,

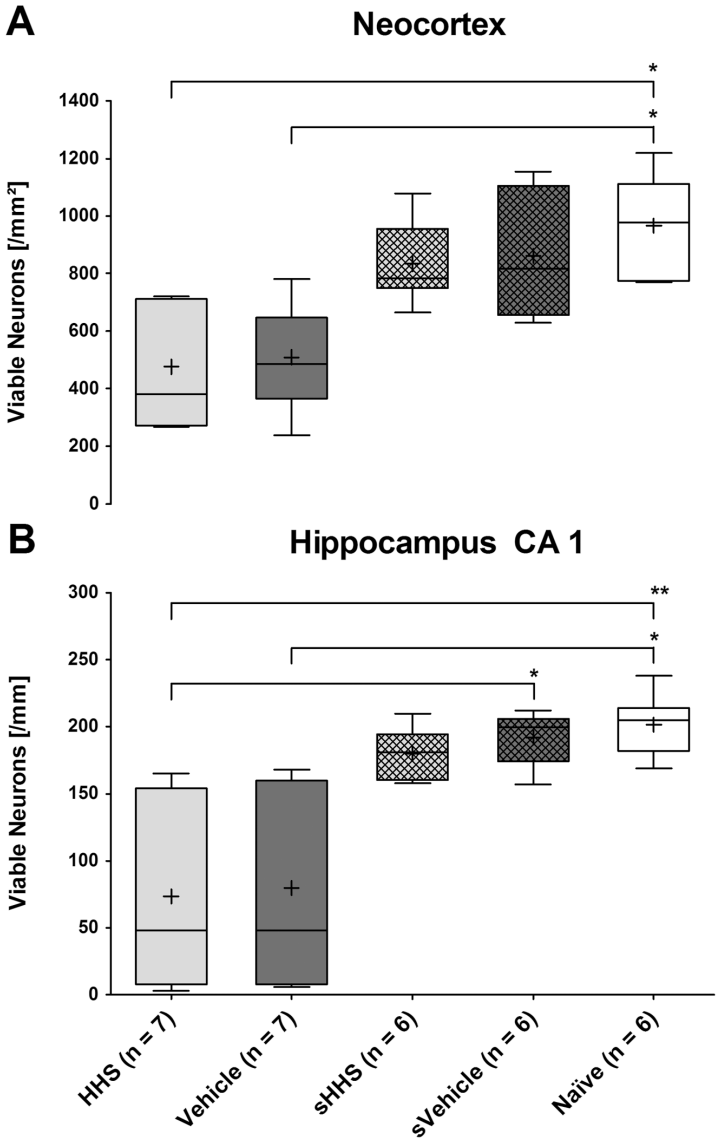

Figure 5. Neurohistopathologic outcome in neocortex (*A*) and hippocampal CA1 (*B*) 7 days after cardiac arrest and cardiopulmonary resuscitation. Vehicle: NaCl 0.9%. Dunn's multiple comparison posttest $*p < .05$, $**p < .01$, Median, interquartile range, (min–max, + mean). *HHS*, hypertonic saline hydroxyethyl starch; *s*, sham.

Table 2. Neurohistopathologic outcome in hippocampal subregions CA2, CA3, and CA4

Group	CA2 (Viable Neurons [/mm])	CA3 (Viable Neurons [/mm])	CA4 (Viable Neurons [/mm])
HHS (n = 7)	118 (12, 195)	99 (77, 121)	156 (91, 212)
Vehicle (n = 7)	54 (3, 146)	95 (12, 152)	155 (54, 190)
sHHS (n = 6)	155 (100, 197)	110 (87, 140)	164 (124, 204)
sVehicle (n = 6)	157 (124, 181)	115 (82, 147)	197 (131, 240)
Naïve (n = 6)	156 (104, 201)	132 (108, 164)	176 (160, 256)

HHS, hypertonic saline hydroxyethyl starch; s, sham.
Vehicle: NaCl 0.9%. Median (min–max).

a single bolus of HHS reduced mortality, improved neurologic function and neuronal survival 10 days after transient forebrain ischemia (15). Similar to this previous work, HHS was applied in the same dose (4 mL/kg) immediately after CA and CPR in the present study. In the previous study, the effect of HHS on lCBF in early reperfusion was more pronounced. The immediate application after transient brain ischemia resulted in an improved lCBF throughout the whole reperfusion period; hypoperfusion was absent after treatment. HS during CPR increased CBF during resuscitation in a pig model of cardic arrest and CPR (29). Similar to the present results, CBF did not differ to vehicle treatment after ROSC was achieved. Application of HS in combination with methylene blue during CPR did not alter CBF during reperfusion in a study of CA and CPR in pigs (30). The same group showed in an earlier publication that HS infusion during CPR had no effects on CBF during early recovery. Results from these different studies cannot be directly compared because different dosages of HS were used. Current literature is in favor of a lower dose (2 mL/kg) during CPR to improve CBF after CA and CPR. The use of very high doses HS (10 mL/kg) during CPR failed to show effects on CBF. In a recent study in human CA victims, the application of 2 mL/kg HS over a 10-min time period failed to improve survival to hospital admission or rate of hospital discharge (31). However, the authors show a trend towards an improved neurological outcome at hospital discharge in the HS-treated group.

The model of neuronal injury used for evaluation might also affect the neuroprotective effects of HHS. Most studies showing an improved CBF and better histologic outcome after HS treatment have in common that they are performed in models of brain ischemia with sustained circulation. Global brain ischemia induced by two-vessel occlusion and hypobaric hypotension reduces CBF and causes neuronal ischemia in vulnerable brain regions (14). However, minimal blood flow might be still present, resulting in a different type of injury than CA with no flow during cardiac standstill. In a model of experimental stroke using permanent middle cerebral artery occlusion, HS decreased brain injury and reduced brain edema (32). Similarly, HHS application in a stroke model using two-vein occlusion resulted in an improved regional CBF and a reduced infarct size (12). In contrast, CA does not only affect the brain. Ischemic damage also occurs to other vital organs, leading to so-called "postresuscitation disease" (33). Systemic inflammation occurs and coagulatory cascades are activated immediately following ischemia/reperfusion, which can contribute to cerebral noreflow (24). Therefore, animal models of CA and CPR are probably the most severe

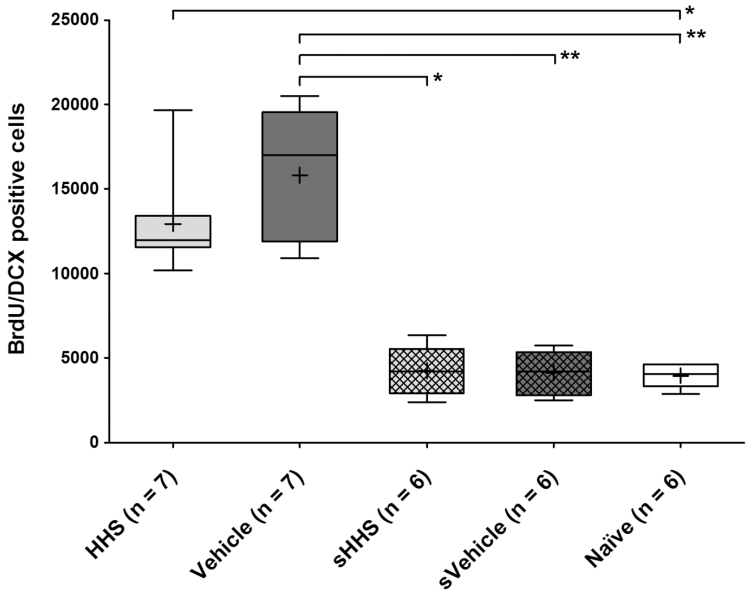

Figure 6. Newly generated neurons in the dentate gyrus 7 days after cardiac arrest and cardiopulmonary resuscitation. Vehicle: NaCl 0.9%. Dunn's multiple comparison posttest $*p < .05$, $**p < .01$, Median, interquartile range, (min–max, + mean). *BrdU*, 5-bromo-2-deoxyuridine; *DCX*, doublecortin; *HHS*, hypertonic saline hydroxyethyl starch; *s*, sham.

and most clinically relevant models of brain ischemia. Possibly because of the more complex model of whole body ischemia, HHS failed to improve long-term outcome in the present study.

In several models of brain injury, HHS effectively reduced neuronal injury. For example, HHS application after two-vein occlusion resulted in a reduced infarction seize. In this two-vein occlusion model, the reproducible infarction seize is small (12%) and administration of HHS reduced the infarct area to 4% (12). In a model of transient forebrain ischemia, neuronal survival in the hippocampal CA1 region was reduced to approximately 40% and neuronal survival in the neocortex was approximately 63%, compared to sham-operated animals (15). HHS treatment immediately at the start of reperfusion resulted in an improved neuronal survival of approximately 90% 10 days after the insult. In the present study after CA/CPR, only 25% of the neurons survived in the hippocampal CA 1 region, and 40% in the neocortex. These data suggest that the model of CA/CPR used in the present study resulted in a more severe brain injury than other models. Possibly the damage caused by CA/CPR was too severe for the potentially weak neuroprotective effect of HHS.

The neurological outcome after brain injury is influenced not only by the intensity of the neuronal damage but also by the ability of the neurons to regenerate. In the last decade, it became obvious that neuronal injury induces a regionally specific generation of new neurons even in adult brains, which then positively improve neurological outcome. This has been observed in animal models of cerebral vessel occlusion and after brain trauma. In the present study, the amount of newly generated neurons was tripled by CA/CPR, which is in line with a previous study (34).The infusion of HHS did not influence the amount of newly generated neurons, and therefore, it is unlikely that HHS has any direct beneficial effect on cerebral neuroregeneration after injury. A single dose of HHS did not have cardiovascular side effects or severe derangements of electrolytes in the present study, whereas in a previous study, infusion of HS over 22 hrs resulted in chronic hypernatremia and exacerbated brain injury after transient middle artery occlusion (35). A well-known complication of a single bolus of HS is acute myelinolysis in case of preexisting long-term hyponatremia (36).This was not a concern in the healthy adult animals used in the present study. A single bolus of HHS resulted in only a mild increase of serum sodium (maximum increase 10 mmol/L; maximum serum sodium observed 149 mmol/L). It seems very unlikely that a single dose of HHS resulted in an additional injury in this experimental setting.

CONCLUSIONS

In conclusion, a single bolus (4 mL/kg, 3 mins) of HS (7.2%) and HES (6%) increased early cerebral hyperperfusion and attenuated delayed hypoperfusion when administered after asphyxia CA and CPR in adult rats. Effects on overall survival, long-term neurologic outcome, neurogenesis, and neuronal damage were not observed, compared to vehicle treatment. Despite promising results in other models of brain ischemia, HHS failed to provide neuroprotective effects in a model of CA and CPR. This might be due to the more complex and severe injury pattern after asphyxia CA and CPR in this model.

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