Effects of hypertonic/hyperoncotic treatment after rat cortical vein occlusion*

A. Heimann, DVM; T. Takeshima, MD; B. Alessandri, PhD; R. Noppens, MD; O. Kempski, MD, PhD

Objective: **To examine the effects of hypertonic/hyperoncotic treatment on physiologic variables and regional cerebral blood flow and to test its neuroprotective efficiency in a model of permanent venous ischemia.**

Design: **Randomized prospective study.**

Setting: **University research institute.**

Subjects: **Adult male Wistar rats, weighing 359 ± 54 g (n = 38).**

Interventions: **Rats were subjected to photochemical occlusion of two adjacent cortical veins. A randomized infusion with vehicle (0.9% NaCl), 10% hydroxyethyl starch 200000 (HES), or 7.5% saline plus 10% hydroxyethyl starch 200000 (HHES) was started 30 mins after two-vein occlusion. Effects on physiologic variables and regional cerebral blood flow (assessed by laser Doppler flowmetry) were studied up to 120 mins after two-vein occlusion. Two days after occlusion, the brains were removed for histologic evaluation.**

Measurements and Main Results: **After occlusion, regional cerebral blood flow decreased by 50%, significantly in all groups (from 47.3 ± 3 to 22.2 ± 2.2 laser Doppler units). In the vehicle and HES groups, regional cerebral blood flow further decreased to 12.9 ± 1.9 and 17.8 ± 2.3 laser Doppler units, respectively. HHES improved regional cerebral blood flow significantly to 27.3 ± 3.5 laser Doppler units, particularly by reducing no-flow/low-flow areas and reducing infarct size.**

Conclusion: **We found that HHES reduced infarct size as a consequence of an improved regional cerebral blood flow and reduced no-flow/low-flow areas in the tissue at risk in the two-vein occlusion model. (Crit Care Med 2003; 31:2495–2501)**

KEY WORDS: cerebral ischemia; hypertonic solution; hydroxyethyl starch; cerebral blood flow; microcirculation

Small volume resuscitation is defined as rapid infusion of a small volume of hypertonic/hyperoncotic fluid (2400 mOsm/kg) and was originally designed for prehospital treatment of hemorrhagic shock (1–8). The rapid infusion of a hypertonic/hyperoncotic solution at 2–6 mL/kg of body weight leads to an osmotic gradient, with water being drawn into the intravascular compartment and a rapid mobilization of parenchymal fluid (1, 3), hemodilution, endothelial shrinkage, and improved microvascular flow (7, 9). These effects in combination with an increase of cardiac output (2, 4, 5) and a decrease of the perivascular resistance contribute to the microcirculatory and metabolic improvement, mentioned as the most important mechanisms of small volume resuscitation after shock (6, 7, 10). Beneficial effects on the microcirculation have been reported in kidney, heart, liver (4), and small intestine (1, 11).

Polytraumatized patients with head injury had an increased survival after hypertonic/hyperoncotic treatment (HHT) (12). Experimental investigations confirmed that HHT reduces elevated intracranial pressure after head injury (6, 10, 13–16). This suggests that the injured brain that is susceptible to ischemia should profit from that regimen. It is surprising that there are only a few investigations of HHT in focal cerebral ischemia, although the hypoperfusion involved is similar in many aspects to that occurring in shock. Endothelial blebs, tissue compression by swollen glial cells, viscosity changes due to erythrocyte sludging, and venous congestion are seen in cerebral ischemia/reperfusion injury (17). The literature provides limited and conflicting information about effects of HHT in ischemia. On the one hand, HHT significantly reduced no-reflow areas in a model of global ischemia (i.e., after cardiopulmonary resuscitation) (18). On the other hand, HHT followed by chronic hypernatremia exacerbated damage after transient focal ischemia from middle cerebral artery occlusion (19).

We have now used a two-vein occlusion (2VO) model in which two adjacent cortical bridging veins are permanently occluded (20–24). This leads to a slowly developing infarct and to a distinct distribution of low-flow and no-flow areas with characteristics of a large penumbra-like territory (24). Because of the slow changes within the microcirculation, this model is particularly suited to evaluate effects of HHT on regional cerebral blood flow (rCBF) using laser Doppler scanning.

The purpose of the current study was to test whether a single bolus of hypertonic/hyperoncotic fluid improves the microcirculation within the large penumbra-like territory seen after 2VO. We hypothesized that an effect on cerebral perfusion should result in a better neuropathological outcome. Two sets of experiments were performed: one to assess effects of HHT on regional blood flow and histologic outcome and another to evaluate acute effects on plasma sodium, osmolality, colloid oncotic pressure, and hematocrit with high time resolution.

*See also p. 2559.

From the Institute for Neurosurgical Pathophysiology, Johannes Gutenberg-University Mainz, Germany.

DOI: 10.1097/01.CCM.0000084893.44650.CB

MATERIALS AND METHODS

Surgical Preparation

All experiments were performed under the "Animal Welfare Guidelines" and were approved by the local ethics committee. Male Wistar rats, weighing 359 ± 54 g (Charles River), were subjected to 2VO, as described in detail previously (20–24). Briefly, rats were anesthetized with chloral hydrate (36 mg/100 g of body weight, intraperitoneally; premedication with 0.5 mg of atropine, subcutaneously). Body temperature was maintained constant at 37.5°C by a feedback-controlled homeothermic blanket (Harvard, South Natick, MA). Tail artery and femoral vein were cannulated with polyethylene tubing for continuous monitoring of mean arterial blood pressure (MABP), withdrawal of arterial blood samples (Po_2, Pco_2, pH, electrolytes), and venous infusion of fluids and drugs. The volume of each blood gas analysis was 210 μL (ABL615/EML105 for electrolytes, Radiometer, Copenhagen). The animals were placed into a stereotactic frame, and a craniectomy was made overlying the right parietal cortex (4 × 5 mm window; 1 mm lateral and caudal of the bregma).

Two-Vein Occlusion

Rose Bengal (50 mg/kg of body weight) was slowly infused intravenously. Two adjacent cortical veins were occluded by selective illumination for 10 mins via a light guide (200 μm fiber, 540 nm, 5000–6000 lx). Fluorescence angiography was used to control for complete permanent occlusion at the end of each experiment. Animals with incomplete occlusion were excluded from the study.

CBF Measurement

Local cerebral blood flow (lCBF) was assessed by laser Doppler flowmetry (Vasamedics BPM 403a, St. Paul, MN; 0.8-mm needle probe). We measured lCBF using a computer-assisted micromanipulator in 36 different locations 500 μm from each other within a rectangular cortical window centered around the two occluded veins. The probe always remained <100 μm from the surface of the dura. We expressed lCBF in laser Doppler units (LDU). CBF data from individual sites represent local measurements. As previously shown (25–27), the median of all lCBF acquired during a scanning procedure represents a reproducible measure of rCBF for individual animals. Many authors express local laser Doppler data as percentage of an initial baseline value. Considering the physiologic variability of lCBF values in rat, cerebral cortex flow values expressed in percentages can only be compared if locations with comparable baseline flow are selected. When using the scanning technique, on the other hand, it is merely necessary to include enough locations into the scan. Then, the median value of such a scan can be used as a reliable baseline value that will allow for comparisons not only with successive measurements from the same locations but also—more important—with data from other animals (26, 27). In addition, laser Doppler scanning allows the production of flow histograms with observation frequencies. Values <10 LDU are defined as low-flow/no-flow areas.

Histology

Two days after the occlusion, the brains were perfused-fixed with 4% paraformaldehyde (24 hrs postfixation) and then embedded in paraffin. Coronal frontoparietal sections (3 μm) were stained with hematoxylin and eosin, and the infarct area was measured on blinded brain slices by quantitative histomorphometry (24). The maximal infarct area after 2VO perfectly correlates with the total infarct volume ($r^2 = .9918$), which in contrast to the major coronary artery occlusion model has a lens-like symmetric shape and is restricted to the cortex. Therefore, several sections were cut at the center of the macroscopically visible infarct, and the slice with the maximal infarct area was used.

Experimental Studies and Groups

Study 1. After a 30-min baseline period, two adjacent cortical veins were occluded photochemically. Treatment started 30 mins after occlusion, and drugs were infused intravenously (4 mL/kg of body weight) during 4 mins. Animals were randomly assigned to three groups: a) vehicle (0.9% saline, 308 mOsm/kg, 0 mm Hg colloid oncotic pressure; n = 10); b) 10% hydroxyethyl starch 200000/0.5 (HES), 308 mOsm/kg, 68 mm Hg colloid oncotic pressure; n = 8); or c) 7.5% NaCl in 10% hydroxyethyl starch 200000/0.5 (HHES), 2567 mOsm/kg, 68 mm Hg colloid oncotic pressure; n = 9). The effects on MABP and CBF were observed every 15 mins during the baseline and the 120-min postocclusion period. Blood gases, pH, electrolyte concentration, and hematocrit were analyzed at baseline and at the end of the experiment. The resected bone flap was repositioned and the skin wounds were closed. Animals were returned to individual cages with free access to food and water and were perfusion-fixed for histology after 2 days in deep anesthesia.

Study 2. This experiment focused on acute plasma sodium concentrations after treatment with HHES. Hematocrit, blood gases, hemodynamics, and CBF also were evaluated. The experimental procedure was as in study 1 with two major modifications: First, no vein occlusion was induced, and, second, the frequency of blood samples was increased for higher temporal resolution. Data were collected three times during a 20-min baseline period, at the first and third minute of infusion, and 1, 5, 10, 20, 30, 40, 60, 75, and 90 mins after infusion. Animals were randomly assigned to groups receiving isotonic vehicle (n = 3) or HHES (n = 6). Because of the considerable volume that was needed to determine osmolality and colloid oncotic pressure, separate experiments were necessary. Thus, approximately 1 mL of blood was withdrawn at baseline and 0.5, 30, and 90 mins after infusion with vehicle (n = 3), HES (n = 4), or HHES (n = 4) to measure osmolality (Osmomat 030, Gonotec, Germany) and oncotic pressure (Onkometer BMT923, Delta Pharma, Germany) in plasma and for analyses of blood gases and electrolytes (whole blood).

Data Analysis

Data were expressed as mean ± SE and analyzed by repeated-measures analyses of variance followed by the Student-Newman-Keuls test for multiple comparisons between the three experimental groups. Nonparametric data were analyzed by analyses of variance on ranks for repeated measures according to Friedman and for differences between groups according to Kruskal-Wallis. Differences were considered statistically significant at an error probability <.05 (Sigmastat software, SPSS, Chicago, IL).

RESULTS

Study 1: Effects of HES/HHES After 2VO

Physiologic Variables. MABP was comparable in all groups and varied within physiologic ranges (Fig. 1). MABP was moderately higher in the HHES group at baseline and at the end of the acute phase of the experiments. Neither

body nor tympanic temperature changed after 2VO.

At baseline, arterial pH, Po_2, Pco_2, and hematocrit were similar in all experimental groups. Until the end of the experiments, pH and hematocrit became reduced in all three groups compared with baseline without differences between the individual groups (Table 1).

Baseline arterial sodium concentration was comparable in all groups (Table 1). In the HHES group, with 141.9 ± 2.2 mmol/L ($p < .001$), plasma sodium was still significantly elevated at 120 mins after 2VO (i.e., 90 mins after treatment) compared with baseline as well as with the vehicle and the HES group (Table 1).

At baseline, hematocrit ranged from 43.2 ± 4.0% to 44.6 ± 1.7% without any differences between the groups. At the end of observation, hematocrit had significantly decreased in all three groups compared with baseline ($p < .05$ for vehicle and HES; $p < .001$ for HHES).

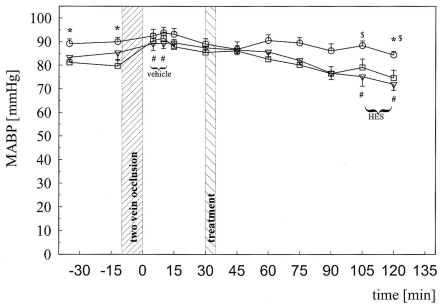

Figure 1. Effects of vehicle *squares*, n = 10), 7.5% saline plus 10% hydroxyethyl starch 200000 (HHES, *circles*, n = 9) and 10% hydroxyethyl starch 200000 (*HES, triangles*, n = 8) on mean arterial blood pressure (*MABP*) after two-vein occlusion ($p < .05$, #vs. baseline; $HHES vs. HES; *HHES vs. vehicle).

Regional Cerebral Blood Flow. Baseline CBF was comparable in all groups with no difference either in median flow (47 ± 3 LDU) or in the distribution pattern of local cerebral blood flow in the area between the adjacent cortical veins assigned for later photothrombosis (data not shown). Immediately after 2VO, rCBF decreased by approximately 50% (Fig. 2). During the next 30 mins, rCBF medians decreased further and were identical (22 ± 2 LDU) in all groups before the beginning of therapy. After treatment, rCBF continued to decrease in the vehicle and HES group, whereas HHES infusion improved the cerebral perfusion significantly and with a continuing, significant effect during the next 90 mins (Fig. 2). Although HHES could not restore rCBF completely, rCBF remained elevated until the end of observation compared with vehicle and HES ($p < .05$).

After 2VO, the observation frequency pattern revealed a dramatic increase of observations in low-flow classes, especially in classes with 0–5 and 6–10 LDU in all experimental groups (Fig. 3). Increasing numbers of no-flow/low-flow areas reflect an increasing perfusion deficit. During the postocclusion observation period, no-flow/low-flow areas increased after vehicle or HES treatment. In contrast, the number of low-flow areas was <10 LDU after HHES infusion and thereafter did not increase further (Fig. 3). At 120 mins after HHES, only 10.5 ± 3.6% of all measurements were within the low-flow range, that is, significantly fewer observations than after HES (28.8 ± 5.5%) or saline (37.6 ± 6.5%).

Histology. 2VO induced a reproducible infarct in the expected area. The infarct was clearly demarcated (Fig. 4), and the narrow border zone included swollen and some pyknotic cells. The infarction size was 4.8 ± 0.6 mm^2 in the vehicle group, 3.2 ± 0.5 mm^2 in the HES group, and 1.5 ± 0.1 mm^2 after HHES. Relative to the ipsilateral hemisphere, the infarction area was 12.4 ± 4.8% in the vehicle and 9.0 ± 4.3% in the HES group and only 4.1 ± 1.1% after HHES. Only HHES had a significant neuroprotective effect (Fig. 5).

Study 2: Acute Effects of HHES Without 2VO

MABP was constant over the entire observation time in both groups. Neither treatment had a significant effect on heart rate, Pco_2, Po_2, or arterial potassium concentration.

The sodium concentration increased immediately after HHES infusion from 136.3 ± 1.9 at baseline up to 146 ± 1.8 mmol/L during infusion. This transient increase never exceeded a maximum of 153 mmol/L in individual animals and reverted to moderately elevated levels. Compared with the vehicle group, significantly higher concentrations persisted during the 90-min posttreatment period (Fig. 6*A*).

Table 1. Physiologic variables at baseline and 90 mins after treatment

Group	pH	Pco_2	Po_2	Na^+	K^+	Htc
Baseline						
Study 1						
Vehicle	7.36 ± 0.007	46.28 ± 0.6	85.09 ± 1.5	135.80 ± 0.4	4.38 ± 0.1	43.18 ± 1.3
HES	7.37 ± 0.005	46.06 ± 1.5	87.74 ± 2.4	135.75 ± 1.0	4.39 ± 0.2	44.59 ± 0.5
HHES	7.37 ± 0.004	42.72 ± 1.2	84.23 ± 2.1	135.78 ± 0.7	4.59 ± 0.1	44.59 ± 0.6
Study 2						
Control	7.35 ± 0.002	42.62 ± 0.8	75.27 ± 2.2	137.56 ± 0.3	3.94 ± 0.2	39.04 ± 1.8
HHES	7.36 ± 0.007	42.35 ± 1.2	83.27 ± 1.4	136.33 ± 0.8	3.95 ± 0.1	39.86 ± 1.3
90 mins after treatment						
Study 1						
Vehicle	7.31 ± 0.006[a]	45.55 ± 0.7	81.98 ± 1.1	135.60 ± 0.6[b]	3.79 ± 0.1	41.48 ± 0.9[a]
HES	7.31 ± 0.009[a]	47.53 ± 0.4	82.53 ± 2.6	135.88 ± 0.8[b]	4.00 ± 0.1	43.25 ± 0.7[a]
HHES	7.31 ± 0.007[a]	45.19 ± 1.4	82.73 ± 1.4	141.89 ± 0.7[a]	3.78 ± 0.2	42.03 ± 0.8[a]
Study 2						
Control	7.33 ± 0.006	42.30 ± 1.3	81.17 ± 4.3	136.33 ± 0.3[b]	4.03 ± 0.2	35.07 ± 0.6
HHES	7.33 ± 0.014	39.07 ± 1.9	89.45 ± 6.2	140.83 ± 0.4[a]	3.38 ± 0.2[a]	32.83 ± 1.3[a]

Pco_2, carbondioxid pressure, mm Hg; Po_2, oxygen pressure, mm Hg; Na^+, sodium, mmol/L; K^+, potassium, mmol/L; Htc, hematocrit, %; Study 1, with 2-vein occlusion; vehicle, 0.9% NaCl; HES, 10% hydroxyethyl starch 200000/0.5; HHES, 7.5% NaCl plus 10% hydroxyethyl starch 200000/0.5; Study 2, infusion only (no ischemia).

[a] $p < .05$ vs. baseline; [b] $p < .05$ vs. HHES. Values are mean ± SE.

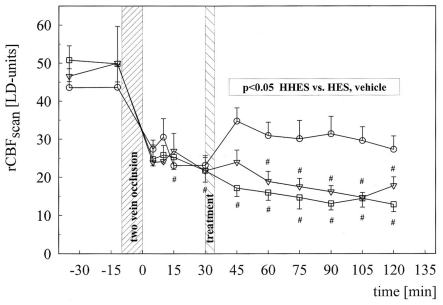

Figure 2. Effects of vehicle (*squares*, n = 10), 7.5% saline plus 10% hydroxyethyl starch 200000 (*HHES, circles*, n = 9), and 10% hydroxyethyl starch 200000 (*HES, triangles*, n = 8) on regional cerebral blood flow (*rCBF*; assessed by laser Doppler scanning) after two-vein occlusion. At each scan, local cerebral blood flow was measured over 36 cortical locations. After occlusion, rCBF decreased significantly by 5% in the area at risk ($^{\#}p < .05$ vs. baseline) in all groups and deteriorated with time after vehicle or HES infusion. The critically reduced cortical perfusion was significantly improved by HHES and remained elevated compared with vehicle and HES.

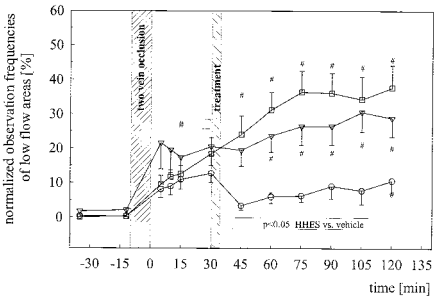

Figure 3. Effects of vehicle (*squares*, n = 10), 7.5% saline plus 10% hydroxyethyl starch 200000 (*HHES, circles*, n = 9), and 10% hydroxyethyl starch 200000 (HES, *triangles*, n = 8) on low-flow areas after two-vein occlusion. The low-flow local CBF values (<10 laser Doppler units, LDU) from each laser Doppler scanning procedure (36 local cerebral blood flow measurements/scan) were selected and normalized (36 observations/scan equal 100%). The increased occurrence of low-flow areas demonstrates that the microcirculatory perfusion is critically reduced ($^{\#}p < .05$ vs. baseline), whereas the reduced occurrence after HHES indicates improved microcirculation.

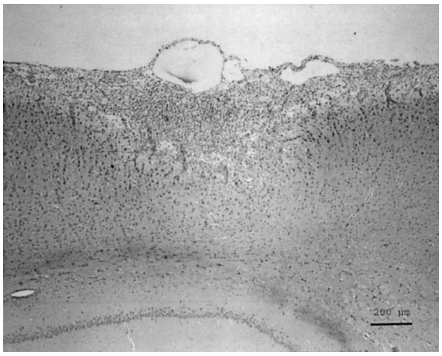

Figure 4. Micrograph of a cortical infarction 2 days after two-vein occlusion.

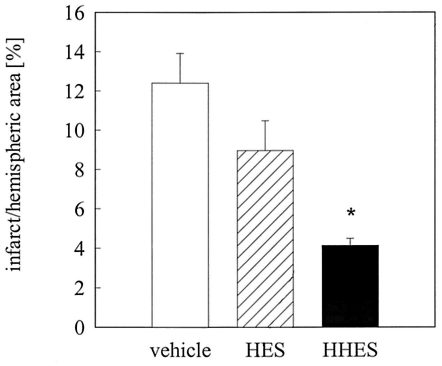

Figure 5. Infarct size as determined at 2 days after two-vein occlusion (2VO; percent ipsilateral hemisphere). 7.5% saline plus 10% hydroxyethyl starch 200000 (*HHES*) significantly reduced infarct size after 2VO ($^{*}p < .05$ vs. vehicle). *HES*, 10% hydroxyethyl starch 200000.

The hematocrit at baseline was identical in both groups. After isotonic saline, hematocrit was reduced compared with baseline (39.04 ± 3.2% baseline; 35.07 ± 1.1% at 90 mins after infusion, nonsignificant). HHES caused a significantly larger decrease of hematocrit throughout the entire experiment ($p < .05$ vs. baseline). Compared with vehicle, hematocrit decreased significantly only between the third minute of infusion and the first minute after the end of infusion (Fig. 6*B*).

pH decreased significantly during the first minutes of HHES infusion. Thereafter, there was no difference in the further time course or compared with the control group.

Local cerebral blood flow measured continuously by stationary laser Doppler was constant in both groups at baseline (33.71 ± 1.6 LDU in the control and 28.86 ± 2.8 LDU in the HHES group). The infusions had no long-term influence on lCBF, which increased moderately immediately after infusion without reaching statistical significance and after 90 mins again was not different from baseline (control, 33.07 ± 1.3 LDU; HHES, 31.10 ± 2.4 LDU).

The physiologic data of animals used to measure osmolality/colloid oncotic pressure were comparable in level and in time course with study 2. Furthermore, there was no baseline difference between the groups in osmolality and colloid oncotic pressure (Table 2). The osmolality significantly increased immediately after HHES infusion to 313.25 ± 2.2 mOsm/L and remained elevated up to 90 mins after infusion. Likewise, the colloid oncotic pressure was significantly increased 0.5 mins after HES infusion compared with baseline, vehicle, or HHES from 14.90 ± 0.7 mm Hg to 17.40 ± 0.1 mm Hg and at 90 mins, respectively (Table 2). Together with osmolality, the plasma sodium concentration increased significantly, whereas hematocrit decreased. There were no changes in sodium concentration after HES infusion, but hematocrit remained significantly reduced for 90 mins. At that end point, hematocrit (40.0 ± 0.9%) was significantly higher compared with vehicle or HHES (35.0 ± 1.3% and 34.0 ± 0.2%, respectively).

DISCUSSION

The data demonstrate two major findings: First, the 4-min infusion of hypertonic hyperoncotic saline (7.5% HHES) acutely improved rCBF if applied 30 mins after 2VO, and this short treatment was followed by a long-lasting rCBF recovery seen during the total observation period. Second, in contrast to vehicle or HES alone, HHT reduced infarct size in the 2VO model. The reduction of regional blood flow that goes along with brain damage indicates that the degree of flow reduction determines outcome from venous circulation disturbances in the brain.

Mechanisms of Action

One basic mechanism of HHT is the generation of an osmogradient by the fast infusion of a hypertonic bolus, which rapidly mobilizes endogenous fluid (1). Mazzoni and Borgström (9) demonstrated that the fluid is mobilized from blood cells, endothelial cells, and the surrounding parenchyma. Thereby, an immediate fluid shift into the microvasculature and hemodilution are initiated (6). The decreased capillary hydraulic resistance contributes to the improved microcirculation and is, in fact, most pronounced in capillaries that had previously exhibited swollen endothelial cells (9, 28). In the present study, an improvement of the microcirculation due to these mechanisms could be demonstrated as a better rCBF and a reduction of no-flow/low-flow areas after 2VO. Albumin therapy also had been shown to improve lCBF, lower the hematocrit (37%), raise plasma colloid oncotic pressure (56%), decrease the total infarct volume (66%), or improve the neurologic score throughout the 3-day

survival period. The neuroprotection, however, is not attributed to hemodilution alone, and other mechanisms of albumin in ischemia (rheologic mechanism, inhibition of platelet aggregation, reduced water content, scavenger of oxygen free radicals) have been discussed (29, 30). In addition, numerous experimental and clinical trials of hemodilution with dextran or other agents have been inconclusive. Our study cannot substantially evaluate the isolated effects of the hyperosmotic or hyperoncotic component, since the effect of hyperosmotic treatment alone was not investigated. However, HHES treatment in normovolemic subjects induced a rather moderate hemodilution (<10%, Fig. 6*B*) or a discrete increase of colloid oncotic pressure (<17%, Table 2). In addition, since HES (alone) certainly increased the colloid oncotic pressure compared with vehicle or hypertonic HES (Table 2) but did not reduce the infarct volume as hypertonic HES did (study 1), our data suggest that the osmotic component plays the major role in the neuroprotection induced.

Adverse effects, such as the suspicion that the arterial oxygen capacity might be reduced by hemodilution, are pathophysiologically negligible for two reasons. On the one hand, treatment did not lower hematocrit dramatically. Even a more important, initial decrease of hematocrit by 10% after HHES did not impair the oxygen supply of brain tissue under initially normovolemic conditions (31). On the other hand, a decreased hematocrit in normal and ischemic regions of the brain will be compensated by an increased microflow during isovolemic hemodilution (32). Neither should hypernatremia or hyperosmolarity become a problem (7). Toda et al. (33) suggested that the increased osmotic pressure stimulates Na^+-H^+ exchange, resulting in Na^+ accumulation that activates the Na^+ pump and causes hyperpolarization, arterial relaxation, and, thereby, an even better perfusion. Indeed, HHT did not affect acute and outcome variables negatively, supporting the notion that short-lasting hyperosmolarity, hypernatremia, or a potentially reduced oxygen tension produce no adverse effects following 2VO.

This study did not consider changes related to immune functions. Thus, we cannot exclude that modulatory effects of hypertonic saline on early inflammation (34, 35) or on late immunosuppression also may play a role in the improvement of rCBF and infarct reduction (34, 36).

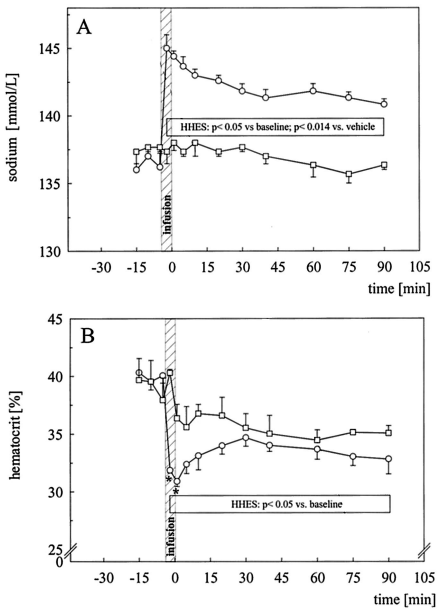

Figure 6. Acute changes of arterial plasma sodium concentration and hematocrit in study 2 (7.5% saline plus 10% hydroxyethyl starch 200000, *HHES, circles*, n = 6; vehicle, *squares*, n = 3). There was a rapidly occurring peak due to the infusion with HHES. Sodium concentration remained increased during the observation period (*A*). In addition, HHES caused a fluid shift from the extracellular space into plasma, leading to a sharp initial decrease in hematocrit (*B*; $p < .05$).

HHT in Shock

There is a general consensus concerning the circulatory effectiveness and metabolic improvement of hypertonic/hyperoncotic solutions after shock (see review in Ref. 6). In shock, cardiac output and MABP are the first variables that profit from HHT/small-volume resuscitation, independently of whether hyperosmotic solution alone or in combination with hyperoncotic components is applied (4, 7). These variables seem to play a minor role for therapy of focal ischemic diseases that do not affect systemic blood pressure, as evident from the absence of MABP changes in our setup. This is in good agreement with the observation that effects of HHT on cardiac output and MABP are far less pronounced in normovolemic subjects than in hypovolemia (1, 31). This is not surprising: In shock, where cardiac output is critically reduced, any supply of fluid helps to normalize hemodynamics. In normovolemia, on the other hand, the additional fluid load will cause hypervolemia and systemic blood pressure, and cardiac output can be maintained normal by vasodilation and, hence, a reduction of peripheral resistance. Therefore, neither arterial hypertension nor myocardial insufficiency develops (1, 6).

HHT in Cerebral Ischemia

Although beneficial effects have been reported, there are controversial results in global ischemia (8, 18, 37), traumatic brain injury, and intracerebral hemorrhage (10, 14, 32, 38), and no treatment regimen for patients has been established yet. This might be due to differences in study design, goals, and pathologies. There is only little information concerning HHT in cerebral ischemia, although beneficial effects on the microcirculation and on intracranial pressure preventing secondary ischemia after brain injury suggest a general improvement and a better functional outcome. In a model of global cerebral ischemia, cerebral perfusion and somatosensory evoked potentials were improved by HHT (37, 39).

Table 2. Osmolality and colloid oncotic pressure (COP) in study 2

	Vehicle	HES	HHES
Baseline			
Osmolality, mOsmol/kg	296.3 ± 3.3	298.8 ± 4.4	298.3 ± 1.9
COP, mm Hg	15.3 ± 1.1	14.9 ± 0.7	16.4 ± 1.0
0.5 min			
Osmolality, mOsmol/kg	296.3 ± 1.8	301.0 ± 4.8	313.3 ± 2.2[a,b]
COP, mm Hg	15.4 ± 0.2	17.4 ± 0.1[a,c]	15.4 ± 0.6
30 mins			
Osmolality, mOsmol/kg	295.7 ± 2.8	299.3 ± 5.3	305.8 ± 3.1[a]
COP, mm Hg	15.3 ± 0.2	16.5 ± 0.1	15.6 ± 0.4
90 mins			
Osmolality, mOsmol/kg	300.7 ± 0.9	300.3 ± 5.5	307.5 ± 3.3[a]
COP, mm Hg	13.9 ± 0.4	15.4 ± 0.3[c]	14.1 ± 0.3

[a] $p < .05$ vs. baseline; [b] $p < .05$ vs. vehicle, HES; [c] $p < .05$ vs. vehicle, HHES.
Values are mean ± SEM.

Fischer and Hossmann (18) confirmed that microvascular reperfusion after 15 mins of ventricular fibrillation and reanimation was significantly improved by HHES as assessed by microvascular dye filling. HHES reduced cerebral no-reflow 30 mins after resuscitation, and the tissue showed a nearly homogeneous capillary filling after treatment with 2 mL/kg/10 mins 7.5% NaCl plus 6% HES. There were no differences in MABP compared with untreated cats. These findings are in excellent agreement with the current hemodynamic, microcirculatory and histologic results in focal ischemia after 2VO.

So far there is only one publication concerning HHT in focal ischemia. That study was performed to test the hypothesis that inducing hypernatremia during reperfusion decreases infarct size after temporary (2 hrs) middle cerebral artery occlusion in rats (19). The results revealed an increase of infarct volume. It has to be stressed, however, that to induce hypernatremia during reperfusion, the treatment protocol had to be adjusted: After an initial bolus of 10 mL of HHES/kg, which is double that usually administered in a short interval (2–6 mL/kg in approximately 5 min; see review in Ref. 6), a continuous infusion of 3% NaCl was applied over 22 hrs (0.5 mL/hr). The authors themselves could not exclude that the aggravation of infarct volume was related to the steep initial osmogradient or the long-lasting continuous infusion following the initial bolus, which generated a considerable sodium load. As expected, animals after 22 hrs had a serum osmolality of 338 mOsm/L and a serum sodium of 154 mEq/L (19).

Therefore, the purpose of that study and the model were quite different from those chosen for the current study. The infusion of hypertonic fluid after temporary major coronary artery occlusion model implies reperfusion of a developing infarct core which, without treatment, is sealed off by swollen glial end-feet. It cannot be excluded that mediators of secondary tissue damage are released or activated and negatively affect the penumbra. A recent study, in fact, has unveiled remarkably different ultrastructural changes of neuronal mitochondria after transient and permanent cerebral ischemia (40). It was seen that damage occurs more acutely and to a greater extent during reperfusion in comparison to ischemic conditions alone. HHT improves reperfusion and, therefore, might have aggravated damage in the temporary major coronary artery occlusion study (19). As a conclusion of their study, the authors indeed speculated that hypertonic saline "may have therapeutic efficacy if used in a setting of permanent focal ischemia without reflow, if initiated at a later time than immediately after occlusion, or if given as a single large bolus during early reperfusion rather than as a continuous infusion." All these conditions are met in the current approach. The 2VO model is a model of permanent focal cortical ischemia. Treatment was given as a single bolus and initiated 30 mins after vein occlusion.

The occlusion of two adjacent bridging veins goes along with a rather widespread 50% reduction of cortical blood flow within the area at risk (24). This flow reduction is more homogeneous than after arterial occlusion since outflow is occluded. Initially, some locations show even hyperperfusion indicative of reverse flow through collateral venous outflow pathways. After 30–90 mins, flow usually equilibrates and in many aspects resembles the penumbra zone found around arterial infarct cores (24). Hence, in that setting, the hypertonic bolus will primarily affect tissue at risk in a low perfusion zone. The osmotic gradient will possibly open up collateral pathways which, once open, can contribute to maintain viability in the area at risk. The laser Doppler data indeed demonstrate not only an immediate effect of HHES on rCBF but also a reduction of low-flow/no-flow areas (Fig. 3) and significantly better perfusion during the observation time long after termination of the infusion (Fig. 2). The data, therefore, are in favor of a therapeutic efficacy of HHT in focal cerebral ischemia and suggest that even after arterial ischemia, area at risk may benefit from that regimen.

CONCLUSION

Our results suggest that the most important mechanism of HHT in focal venous ischemia is linked to the improvement of the microcirculation, which goes along with neuroprotection. These results perfectly agree with findings in global ischemia. Since the infusion regimen (dosage, time course) is important for achieving neuroprotective effects, additional experiments using focal permanent arterial ischemia models are recommended.

We found that 7.5% saline plus 10% hydroxyethyl starch 200000 reduced infarct size as a consequence of an improved regional cerebral blood flow and reduced no-flow/low-flow areas in the tissue at risk in the two-vein occlusion model.

ACKNOWLEDGMENTS

The first two authors contributed equally to this article. We thank B. Kempski, L. Kopacz, M. Malzahn, and A. Schollmayer for their excellent technical assistance. We thank Dr. C. Fischer (Institute for Clinical Chemistry and Laboratory Medicine, Johannes Gutenberg-University) for measuring colloid oncotic pressure. We are also grateful to Dr. Thun (Fresenius, Bad Homburg, Germany) for the gift of HES/HHES solutions.

REFERENCES

1. Velasco IT, Pontieri V, Rocha e Silva M, et al: Hyperosmotic NaCl and severe hemorrhagic shock. *Am J Physiol* 1980; 239:H664–H673
2. de Felippe J Jr, Timoner J, Velasco IT, et al: Treatment of refractory hypovolaemic shock by 7.5% sodium chloride injections. *Lancet* 1980; 2:1002–1004
3. Nakayama S, Sibley L, Gunther RA, et al: Small-volume resuscitation with hypertonic saline (2,400 mOsm/liter) during hemorrhagic shock. *Circ Shock* 1984; 13:149–159
4. Maningas PA: Resuscitation with 7.5% NaCl in 6% dextran-70 duratin hemorrhagic shock in swine: Effects on organ blood flow. *Crit Care Med* 1987; 15:1121–1126
5. Bitterman H, Triolo J, Lefer AM: Use of hypertonic saline in the treatment of hemorrhagic shock. *Circ Shock* 1987; 21:271–283
6. Kreimeier U, Messmer K: Small-volume resuscitation. *Baillieres Clin Anaesthesiol* 1988; 2:545–577
7. Messmer K, Kreimeier U: Microcirculatory therapy in shock. *Resuscitation* 1989; S51–S61
8. Kreimeier U, Brückner UB, Niemczyk S, et al: Hyperosmotic saline dextran for resusci-

tation from traumatic- hemorrhagic hypotension: Effect on regional blood flow. *Circulatory Shock* 1990; 32:83–99
9. Mazzoni MC, Borgström P: Dynamic fluid redistribution in hyperosmotic resuscitation of hypovolemic hemorrhage. *Am J Physiol* 1988; 255:H629–H637
10. Qureshi AI, Suarez JI: Use of hypertonic saline solutions in treatment of cerebral edema and intracranial hypertension. *Crit Care Med* 2000; 28:3301–3313
11. Jonas J, Heimann A, Strecker U, et al: Hypertonic/hyperoncotic resuscitation after intestinal superior mesenteric artery occlusion: Early effects on circulation and intestinal reperfusion. *Shock* 2000; 14:24–29
12. Vassar MJ, Fischer RP, O'Brien PE, et al: A multicenter trial for resuscitation of injured patients with 7.5% sodium chloride. The effect of added dextran 70. The Multicenter Group for the Study of Hypertonic Saline in Trauma Patients. *Arch Surg* 1993; 128: 1003–1013
13. Berger S, Schürer L, Härtl R, et al: Reduction of post-traumatic intracranial hypertension by hypertonic/hyperoncotic saline/dextran and hypertonic mannitol. *Neurosurgery (Baltimore)* 1995; 37:98–108
14. Doyle JA, Davis DP, Hoyt DB: The use of hypertonic saline in the treatment of traumatic brain injury. *J Trauma* 2001; 50: 367–383
15. Gunnar W, Jonasson O, Merlotti G, et al: Head injury and hemorrhagic shock: Studies of the blood brain barrier and intracranial pressure after resuscitation with normal saline solution, 3% saline solution, and dextran-40. *Surgery* 1988; 103:398–407
16. Hartl R, Schurer L, Goetz C, et al: The effect of hypertonic fluid resuscitation on brain edema in rabbits subjected to brain injury and hemorrhagic shock. *Shock* 1995; 3:274–279
17. Hossmann KA: Reperfusion of the brain after global ischemia: Hemodynamic disturbances. *Shock* 1997; 8: 95–103
18. Fischer M, Hossmann KA: Volume expansion during cardiopulmonary resuscitation reduces cerebral no-reflow. *Resuscitation* 1996; 32:227–240
19. Bhardwaj A, Harukuni I, Murphy SJ, et al: Hypertonic saline worsens infarct volume after transient focal ischemia in rats. *Stroke* 2000; 31:1694–1701
20. Heimann A, Takeshima T, Horstick G, et al: C1-esterase inhibitor reduces infarct volume after cortical vein occlusion. *Brain Res* 1999; 838:210–213
21. Nakase H, Kakizaki T, Miyamoto K, et al: Use of local cerebral blood flow monitoring to predict the brain damage after disturbance to the circulation: Cortical vein occlusion model by photochemical dye. *Neurosurgery (Baltimore*) 1995; 37:1–7
22. Nakase H, Heimann A, Kempski O: Local cerebral blood flow in a rat cortical vein occlusion model. *J Cereb Blood Flow Metab* 1996; 16:720–728
23. Nakase H, Kempski OS, Heimann A, et al: Microcirculation after cerebral venous occlusions as assessed by laser Doppler scanning. *J Neurosurg* 1997; 87:307–314
24. Otsuka H, Ueda K, Heimann A, et al: Effects of cortical spreading depression on cortical blood flow, impedance, DC potential, and infarct size in a rat venous infarct model. *Exp Neurol* 2000; 162:201–214
25. Heimann A, Kroppenstedt S, Ulrich P, et al: Cerebral blood flow autoregulation during hypobaric hypotension assessed by laser Doppler scanning. *J Cereb Blood Flow Metab* 1994; 14:1100–1105
26. Kempski O, Heimann A, Strecker U: On the number of measurements necessary to assess regional cerebral blood flow by local laser Doppler recordings: A simulation study with data from 45 rabbits. *Int J Microcirc Clin Exp* 1995; 15:37–42
27. Soehle M, Heimann A, Kempski O: On the number of measurement sites required to assess regional cerebral blood flow by laser-Doppler scanning during cerebral ischemia and reperfusion. *J Neurosci Methods* 2001; 110:91–94
28. Mazzoni MC, Borgstrom P, Warnke KC, et al: Mechanisms and implications of capillary endothelial swelling and luminal narrowing in low-flow ischemias. *Int J Microcirc Clin Exp* 1995; 15:265–270
29. Belayev L, Zhao W, Pattany PM, et al: Diffusion-weighted magnetic resonance imaging confirms marked neuroprotective efficacy of albumin therapy in focal cerebral ischemia. *Stroke* 1998; 29:2587–2599
30. Huh PW, Belayev L, Zhao W, et al: The effect of high-dose albumin therapy on local cerebral perfusion after transient focal cerebral ischemia in rats. *Brain Res* 1998; 804: 105–113
31. Schurer L, Dautermann C, Hartl R, et al: Treatment of hemorrhagic hypotension with hypertonic/hyperoncotic solutions: Effects on regional cerebral blood flow and brain surface oxygen tension. *Eur Surg Res* 1992; 24:1–12
32. Chan R, Leniger-Follert E: Effect of isovolemic hemodilution on oxygen supply and electrocorticogram in cat brain during focal ischemia and in normal tissue. *Int J Microcirc Clin Exp* 1983; 2:297–313
33. Toda N, Ayajiki K, Enokibori M, et al: Monkey cerebral arterial relaxation caused by hypercapnic acidosis and hypertonic bicarbonate. *Am J Physiol* 1993; 265:H929–H933
34. Rizoli SB, Rotstein OD, Sibbald WJ: The immunological effects of hypertonic saline. *In:* Year Book 2002 of Intensive Care and Emergency Medicine. Berlin, Springer Verlag, 2002, pp 446–453
35. Hartl R, Medary MB, Ruge M, et al: Hypertonic/hyperoncotic saline attenuates microcirculatory disturbances after traumatic brain injury. *J Trauma* 1997; 42:S41–S47
36. Junger WG, Hoyt DB, Davis RE, et al: Hypertonicity regulates the function of human neutrophils by modulating chemoattractant receptor signaling and activating mitogen-activated protein kinase p38. *J Clin Invest* 1998; 101:2768–2779
37. Strecker U, Dick W, Obert C, et al: Hypertonic-hyperoncotic HES enhances recirculation after global cerebral ischemia. *In:* Microcirculatory Stasis in the Brain. Tomita M (Ed). New York, Elsevier Science, 1993, pp 63–68
38. Prough DS, Whitley JM, Taylor CL, et al: Regional cerebral blood flow following resuscitation from hemorrhagic shock with hypertonic saline. Influence of a subdural mass. *Anesthesiology* 1991; 75:319–327
39. Kempski O, Obert C, Mainka T, et al: "Small volume resuscitation" as treatment of cerebral blood flow disturbances and increased ICP in trauma and ischemia. *Acta Neurochir Suppl (Wien*) 1996; 66:114–117
40. Solenski NJ, diPierro CG, Trimmer PA, et al: Ultrastructural changes of neuronal mitochondria after transient and permanent cerebral ischemia. *Stroke* 2002; 33:816–824