# Small Volume Resuscitation in a Rat Model of Heatstroke

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**Abstract:** *Background:* Herein, we compared the effectiveness of different small volume resuscitation in a rat model of heatstroke. *Methods:* Anesthetized rats, immediately after the onset of heatstroke, were randomizedly divided into 5 groups and given the following: (*a*) nothing; (*b*) 0.9% NaCl (1–10 mL/kg of body weight, i.v.); (*c*) hydroxyethyl starch (HAES) (6%, 1–10 mL/kg of body weight, i.v.); (*d*) 7.2% NaCl (1–10 mL/kg of body weight, i.v.); and (*e*) hyper-HAES (6% HAES plus 7.2% NaCl, 1–10 mL/kg of body weight, i.v.). *Results:* When the untreated or 0.9% NaCl (1–5 mL/kg of body weight)-treated rats underwent heat stress, their survival time values were found to be 20 to 22 minutes. Resuscitation with 10 mL/kg of body weight of 0.9% NaCl, 6% HAES, 7.2% NaCl, or hyper-HAES, their survival time values, respectively, are 93 ± 6, 101 ± 12, 154 ± 18, or 286 ± 21. Apparently, the order of effectiveness in resuscitation of heatstroke is hyper-HAES > 7.2% NaCl > 0.9% NaCl or 6% HAES. The heatstroke-induced hypotension, cerebral ischemia and hypoxia, hypercoagulable state, activated inflammation, and hepatic and renal dysfunction can be significantly reduced by hyper-HAES. *Conclusions:* Our results suggest that hyper-HAES seems superior to 7.2% NaCl or HAES alone in resuscitation of heatstroke. The benefit of hyper-HAES during heatstroke is related to restoration of normal multiorgan function.

**Key Indexing Terms:** Hyper-HAES; Heatstroke; Multiorgan dysfunction; Coagulation; Inflammation; Hypotension; Cerebral ischemia. **[Am J Med Sci 2009;337(2):79–87.]**

Heatstroke is characterized by hyperpyrexia and multiple organs (in particular, the central nervous system) dysfunction or failure.[1,2] Cerebral dysfunctions that occurred during heatstroke, include delirium, convulsion, and coma. These neurological syndromes have been attributed to brain edema, ischemia, and/or injury during heatstroke.[2,3] Other organ dysfunctions such as renal and hepatic dysfunction or failure, hypercoagulable state, and systemic inflammation ensue from severe heatstroke.[2,4]

Small volume resuscitation (SVR) means the rapid administration of a small volume of a hypertonic solution to immediately restore the macro and microcirculation. For example, when patients with refractory hypovolaemic shock were given a small volume of 7.5% NaCl, hemodynamics and renal function were improved.[5] In animal studies, infusion of 4 mL/kg of a 7.5% NaCl-solution normalized both; the mean arterial pressure (MAP) and the acid-base balance in hypovolaemic dogs.[6] The rapid (2–5 minutes) infusion of a small volume (4 mL/kg b.w.) of a hypertonic solution causes an instant increase of the plasma osmolarity leading to an osmotic gradient between the extra and intravascular space. The mobilization of endogenous fluid leads to an immediate increase of the intravascular volume within seconds of bolus administration of the hypertonic solution.[7] In addition, the combination of hypertonic saline and a colloidal component is superior to hypertonic saline alone.[8] The additional administration of a colloid prolongs the circulatory stabilization and increases the survival rate.[8–10]

Indeed, as shown in the present study, hyper hydroxyethyl starch (HAES) (the combination of 7.2% NaCl and 6% HAES) is superior to 7.2% NACl or 6% HAES alone in amelioration of multiorgan dysfunction that occurred during heatstroke in a rat model.

## SUBJECTS AND METHODS

### Animals

Experiments were performed in male adult Sparague-Dawley rats (weighing 265–288 g) obtained from the Animal Resource Center of the National Science Council (Taipei, Taiwan, Republic of China). The animals were housed 4 to a cage at an ambient temperature of 22 ± 1°C, with a 12-hour light/dark cycle. Pelleted rat chow and tap water were available *ad libitum*. The experimental protocols were approved by the Animal Committee of the Chi Mei Medical Center. Animal care and experiments were conducted according to the National Science Council guidelines. They were allowed to become acclimated for 1 week. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinching throughout all experiments by an i.p. dose of urethane (1.4 g/kg of body weight). At the end of the experiments, control rats and any rats that had survived heatstroke were killed with an overdose of urethane.

Rats under anesthesia were randomized into 7 major groups: (*a*) normothermic controls (NC, n = 8); (*b*) untreated heatstroke rats [(HS), n = 8]; (*c*) HS rats treated with 0.9% NaCl solution (1–20 mL/kg, i.v.); (*d*) HS rats treated with 7.2% NaCl solution (1–10 mL/kg, i.v.); (*e*) (HAES; 1–10 mL/kg, i.v., 6%; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany); and (*f*) HyperHAES (1–10 mL/kg, i.v.; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). Before induction of HS, the core temperature (Tco) of urethane-anesthetized rats was maintained at about 36°C with a folded heating pad except during heat stress at a room temperature of 24°C. HS was induced by increasing the temperature of the folded heating pad to 43°C with circulating hot water. The instant at which MAP dropped to a value of 25 mm Hg from the peak level was about 70 minutes after the initiation of heat stress, this time

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point was arbitrarily taken as the onset of HS.[11] Then, the heating pad was removed and the animals were allowed to recover at room temperature (24°C).

### Surgery and Physiologic Parameter Monitoring

The right femoral artery and vein of rats were cannulated with polyethylene tubing under urethane anesthesia for blood pressure monitoring and drug administration, respectively. The animals were positioned in a stereotaxic apparatus (Kopf 1406; Grass Instrument, Quincy, MA) to insert probes for measurement of brain temperature, $PO_2$ and cerebral blood flow (CBF).[12]

A 100-$\mu$m-diameter thermocouple and two 230-$\mu$m fibers were attached to the organ probe. This combined probe measures oxygen, temperature, and microvascular blood flow. The measurement requires OxyLite and OxyFlo instruments; OxyLite 2000 (Oxford Optronix, Oxford, UK) is a two-channel device (measuring $PO_2$ and temperature at 2 sites simultaneously), whereas OxyFlo 2000 is a two-channel laser Doppler perfusion monitoring instrument. The OxyLite has been designed to operate in conjunction with the OxyFlo. The combination of these 2 instruments provides simultaneous tissue blood flow, oxygenation, and temperature data. Under anesthesia, the animal was placed in a stereotaxic apparatus, and the combined probe was implanted into the hypothalamus using the atlas and coordinates of Paxinos and Watson.[13] Tco was monitored continuously by a thermocouple, whereas MAP and heart rate were continuously monitored with a pressure transducer.

### Biochemical Determination

For biochemical determination, each animal was killed at each time point, and whole blood (7 mL) was obtained from the heart puncture and collected into sodium citrate tubes for plasma. The 3 different time points were the following: (1) 0 minutes before the initiation of heat stress, (2) 70 minutes after the start of heat exposure (or immediately after the onset of HS), and (3) 85 minutes after initiation of heat exposure (or 15 minutes after the onset of HS). The plasma levels of activated partial thromboplastin time, prothrombin time, and D-dimer were measured by automated coagulation instruments (SYSMEX CA-1500, Kobe, Japan). The platelet counts were measured by automated blood cell counting instruments (Beckman Coulter LH750, Miami, FL), whereas the plasma levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were determined by spectrophotometry (HITACHI 7600, Tokyo, Japan). For determination of protein C, to obtain the plasma, 1 part of sodium citrate solution (0.1 mol/L) was mixed carefully with 9 parts of venous blood, avoiding the formation of foam. Protein C, in the sample, was activated by specific venom activator. The resulting protein C activator was assayed in a kinetic test by measuring the increase in absorbance at 405 nm. The reagents for the determination of protein C activity were provided by Berichrom Protein C (Dade Behring Marburg GmbH, Marburg, Germany). Arterial blood hematocrit was measured via a blood gas analyzer (Nova Biochemical, Waltham, MA).

### Measurements of Cellular Ischemia and Damage Markers

For determination of extracellular glutamate, glycerol, and lactate-to-pyruvate ratio, the microdialysis probe was stereotaxically implanted into the hypothalamus, according to the atlas and coordinates of Paxinos and Watson.[13] After a midline incision, the skull was exposed and a burr hole was made in the skull for the insertion of a dialysis probe (4 mm in length, CMA/12, Carnegie Medicine, Stockholm, Sweden). The procedures for measurements of cellular ischemic and damage markers were described previously.[11]

### Determination of Serum Tumor Necrosis Factor-alpha

Blood samples were allowed to clot for 2 hours at room temperature or overnight at 2 to 8°C before centrifuging for 20 minutes at approximately 2000*g*. Serum was quickly removed from these plasma samples and assayed for tumor necrosis factor-alpha (TNF-$\alpha$) immediately. The Duo-Set Enzyme-linked immunosorbent assay (ELISA) Development System rat TNF-$\alpha$ kit (R&D Systems, Minneapolis, MN) was used for measuring the levels of active rat TNF-$\alpha$ present in serum. This assay employs the quantitative colorimetric sandwich ELISA technique.

### Histologic Verification

At the end of each experiment, the brain would be removed, fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Serial (10 $\mu$m) sections of the brain through the striatum were stained with hematoxylin and eosin for microscopic evaluation.

The extent of striatal neuronal damage was scored on a scale of 0 to 3, modified from the grading system of Pulsinelli et al,[14] in which 0 is normal, 1 means that ~30% of the neurons are damaged, 2 means that ~60% of neurons are damaged, and 3 means that 100% of that neurons are damaged. Each hemisphere was evaluated independently without the examiner knowing the experimental conditions. When examined for neuronal damage in gray matter, only areas other than those invaded by probes were assessed.

### Statistical Analysis

Data are presented as the mean ± standard deviation. For the data presented in Table 1 and Figures 1–4, Kruskal-Wallis *H* test was used for factorial experiments, whereas Dunn's test was used for *post hoc* multiple comparisons among means. The Wilcoxon tests were used for evaluation of neuronal damage scores. The Wilcoxon test converts the scores or values of a variable to ranks, requires calculation of a sum of the ranks, and provides critical values for the sum necessary to test the null hypothesis at a given significant levels. These data were presented as "median", followed by first (Q1) and third (Q3) quartile. A *P* value less than 0.05 was calculated as statistical significance.

## RESULTS

### Small Volume Resuscitation Improves Survival During Heatstroke

Table 1 summarizes the effects of heat exposure (43°C for 70 minutes) on survival time in different groups of rats. It can be seen from the table that the survival time was found to be 17 to 23 minutes for untreated HS rats. Treatment with 10 mL per kg of body weight of 0.9% NaCl, the survival time values were increased to new values of 36 ± 5 minute. In addition, immediately after the onset of heatstroke, intravenous injection of 10 mL per kg of body weight of 7.2% NaCl solution, 6% HAES, or Hyper-HAES, respectively increased the survival time to a new value of 154 ± 18, 101 ± 12, or 286 ± 21 minutes.

### Hyper-HAES Attenuates Cerebrovascular Dysfunction During Heatstroke

Figures 1–2 summarize the Tco, MAP, intracranial pressure (ICP), cerebral perfusion pressure (CPP), brain $PO_2$, brain temperature, CBF, and hypothalamic levels of glutamate, glyc-


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