Resuscitation from experimental heatstroke by transplantation of human umbilical cord blood cells*

Sheng-Hsien Chen, MD; Fong-Ming Chang, MD, PhD; Yung-Chieh Tsai, MD; Kuo-Feng Huang, MD; Mao-Tsun Lin, PhD, DDS

Objective: **Human umbilical cord blood cells (HUCBCs) are effective in the treatment of conventional stroke in experimental models. In the study described herein, we administered HUCBCs into the femoral vein or directly into the cerebral ventricular system and assessed their effects on circulatory shock, cerebral ischemia, and damage during heatstroke.**

Design: **Controlled, prospective study.**

Setting: **Hospital medical research laboratory.**

Subjects: **Sprague-Dawley rats (287 ± 16 g body weight, males).**

Interventions: **Anesthetized rats, immediately after the onset of heatstroke, were divided into four major groups and given the following: a) normal saline or AIM-V medium intravenously (0.3 mL) or intracerebroventricularly (10 μL); b) peripheral blood mononuclear cells (5×10^6 in 0.3 mL AIM-V medium, intravenously, or 5×10^5 in 10 μL AIM-V medium, intracerebroventricularly); or c) HUCBCs (5×10^6 in 0.3 mL AIM-V medium, intravenously, or 5×10^5 in 10 μL AIM-V medium, intracerebroventricularly). Another group of rats, under urethane anesthesia, were exposed to room temperature (26°C) and used as normothermic controls. Urethane-anesthetized animals were exposed to an ambient temperature of 43°C to induce heatstroke. Their physiologic and biochemical parameters were continuously monitored.**

Measurements and Main Results: **When the vehicle-treated rats underwent heat exposure, their survival time values were found to be 21–23 mins. Resuscitation with intravenous or intracerebroventricular doses of HUCBCs, but not peripheral blood mononuclear cells, immediately at the onset of heatstroke significantly improved survival during heatstroke (61–148 mins). As compared with values for normothermic controls, the vehicle-treated heatstroke rats had lower mean arterial pressure, cerebral blood flow, and brain Po_2 values but higher intracranial pressure and cerebral ischemia values and more injury markers. The circulatory shock, intracranial hypertension, cerebral hypoperfusion and hypoxia, increment of cerebral ischemia, and damage markers during heatstroke were all significantly attenuated by intravenous or intracerebroventricular delivery of HUCBCs but not peripheral blood mononuclear cells.**

Conclusions: **We successfully demonstrate that HUCBC therapy may resuscitate heatstroke victims by reducing circulatory shock and cerebral ischemic injury; central delivery of HUCBCs seems superior to systemic delivery of HUCBCs in resuscitating patients with heatstroke. (Crit Care Med 2005; 33:1377–1383)**

KEY WORDS: heatstroke; rat; human umbilical cord blood cells; brain ischemia; nitric oxide

Heatstroke is characterized by hyperthermia, central nervous system dysfunctions, and multiple organ failure (1, 2). In rodents, hyperthermia, circulatory shock, and cerebral ischemia and injury occur during heatstroke (3–5). Unless promptly recognized and treated, these heatstroke reactions would result in a high rate of mortality (1, 2).

Human umbilical cord blood cells (HUCBCs) are rich in hematopoietic stem cells (6). Two percent of the HUCBCs are stem cells capable of reconstituting blood lineages. These HUCBCs have been used to reconstitute bone marrow and blood cell lineages in children with malignant and nonmalignant diseases (7). These cells could be induced to express neural proteins (8–10). Some of these cells, when transplanted into the neonatal subventricular zone, differentiated into neuronal and glial phenotypes within this neurogenic region (11). When the HUCBCs were administered via the tail vein, surviving HUCBCs were identified in the cortex and striatum of the injured hemisphere (12, 13). The behavioral dysfunctions produced by stroke (12–14), traumatic brain injury (15), and spinal cord injury (16) were significantly improved by intravenous delivery of HUCBCs. Among these cells, only 2% expressed neuronal markers, and 6% expressed glial fibrillary acidic protein. These results indicated that there is a signal that can attract the HUCBCs toward the site of ischemia and damage and stimulate development of neural markers. However, it is not known whether administration of HUCBCs attenuates circulatory shock and cerebral ischemia during heatstroke.

In this study, to test the hypothesis, we administered HUCBCs intravenously into the femoral vein or directly into the cerebral ventricular system and assessed which route of cell administration produced the greatest protection in heatstroke rats with both circulatory shock

*See also p. 1458.

From the Institute of Clinical Medicine, School of Medicine, National Cheng Kung University (SHC, FMC); and Departments of Obstetrics and Gynecology (SHC, YCT, KFH) and Medical Research (MTL), Chi-Mei Medical Center, Tainan, Taiwan.

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Address requests for reprints to: Mao-Tsun Lin, DDS, PhD, Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan 710. E-mail: 891201@mail.chimei.org.tw

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and cerebral ischemia. In addition, we assessed the effects of HUCBC administration on the concentrations of glutamate (a cellular ischemia marker), glycerol (a cellular injury marker), the lactate/pyruvate ratio (a cellular ischemia marker), and nitric oxide (NO, a cellular ischemia marker) in the extracellular fluid of rat brains associated with heatstroke (3, 17). At the same time, we also tested the therapeutic effects of human peripheral blood mononuclear cells (PBMCs) on heatstroke and compared them with HUCBCs to ascertain which were more effective in treating heatstroke.

MATERIALS AND METHODS

Experimental Animals. Adult Sprague-Dawley rats (weight, 287 ± 16 g) were obtained from the Animal Resource Center of the National Science Council of the Republic of China (Taipei, Taiwan). The animals were housed four in a group at an ambient temperature of 22 ± 1°C, with a 12-hr light/dark cycle. Pellet rat chow and tap water were available *ad libitum*. All protocols were approved by the Animal Ethics Committee of the Chi-Mei Medical Center (Tainan, Taiwan) in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health as well as the guidelines of the Animal Welfare Act. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail-pinching throughout all experiments (approximately 8 hrs) by a single intraperitoneal dose of urethane (1.4 g/kg body weight). At the end of the experiments, control rats and any rats that had survived heatstroke were killed with an overdose of urethane.

Induction of Heatstroke. Before induction of heatstroke, the colon temperature of urethane-anesthetized rats was maintained at about 36°C with a folded heating pad, except during heat stress at a room temperature of 26°C. Heatstroke was induced by increasing the temperature of the folded heating pad to 43°C with circulating hot water. The moment at which the mean arterial pressure (MAP) dropped to 25 mm Hg from the peak level was taken as the onset of heatstroke (4, 5). Immediately after the onset of heatstroke, the heating pad was removed and the animals were allowed to recover at room temperature (26°C). Our pilot study showed that the latency for the onset of heatstroke (interval between the start of heat exposure and the onset of heatstroke) was found to be 68 ± 3 for the vehicle-treated heatstroke group (n = 10). Then, both physiologic parameters and survival times (intervals between the initiation of heat exposure and animal death) were observed to 450 mins (or the end of experiments). For comparison with the vehicle-treated heatstroke group, all cell-treated heatstroke group animals were exposed to heat for exactly 68 mins and then allowed to recover at room temperature (26°C).

Experimental Groups. The animals, under urethane anesthesia, were divided into the following groups. In the normothermic group (n = 8), the colon temperature was maintained at about 36°C with a folded heating pad at a room temperature of 26°C throughout the entire experiments. In the vehicle-treated heatstroke groups, the animals were treated with a dose of normal saline or AIM-V medium (GIBCO, BRL, Grand Island, NY) intravenously (iv; 0.3 mL per rat) or intracerebroventricularly (icv; 10 μL per rat) 68 mins after initiation of heat exposure (or immediately at the onset of heatstroke). In the PBMC-treated heatstroke groups, the animals received an intravenous dose (5×10^6 in 0.3 mL) or an icv dose (5×10^5 in 10 μL) of PBMCs 68 mins after initiation of heat exposure. In the HUCBC-treated heatstroke group, the animals received an intravenous dose (5×10^6 in 0.3 mL) or an icv dose (5×10^5 in 10 μL) of HUCBCs 68 mins after initiation of heat exposure.

Surgery and Physiologic Parameter Monitoring. The right femoral artery and vein of rats were cannulated with polyethylene tubing (PE 50), under urethane anesthesia, for blood pressure monitoring and drug administration. The animals were positioned in a stereotaxic apparatus (Kopf 1406, Grass Instrument, Quincy, MA) to insert probes for measurement of intracranial pressure (ICP). The ICP was monitored with a Statham P23AC transducer via a 20-gauge stainless-steel needle probe (diameter, 0.90 mm; 38 mm), which was introduced into the right lateral cerebral ventricle according to the stereotaxic coordinates of Paxinos and Watson (18): A, interaural, 7.7 mm; L, 2.0 mm from the midline; and H, 3.5 mm from the top of the skull. All recordings were made on a four-channel Gould polygraph. Colonic temperature was monitored continuously by a thermocouple, while both MAP and heart rate were continuously monitored with a pressure transducer. Different groups of animals were used for the different sets of experiments: measurement of latency for onset of heatstroke (n = 8); measurement of survival time (n = 104); measurement of ambient temperature, MAP, ICP, cerebral perfusion pressure (MAP − ICP), cerebral blood flow, brain Po_2, and brain temperature (n = 24); measurement of ambient temperature, colon temperature, heart rate, and cerebral levels of glutamate, glycerol, lactate/pyruvate, and NO (n = 24); and measurement of neuronal damage (n = 80).

Preparation of PBMCs and HUCBCs. Human PBMCs and HUCBCs were obtained from freshly collected buffy coat fraction from healthy donors at the Tainan Blood Bank Center (Tainan City, Taiwan, ROC) and Chi-Mei Medical Center (Tainan Hsien, Taiwan, ROC), respectively. The fraction was isolated by centrifugation over a Ficoll-Paque (Famacia, Uppsala, Sweden) density gradient at 400 × *g* for 30 mins at room temperature in a Sorvall RT6000B (Du Pont, DE). The cells collected at the interface were washed thrice with serum-free RPMI-1640 (GIBCO, BRL, Grand Island, NY) and subsequently resuspended in AIM-V medium containing 100 U/mL of penicillin and 100 μg/mL of streptomycin. The PBMCs and HUCBCs, at concentrations of 5×10^5 and 5×10^6 cells in 0.3 mL and 10 μL, respectively, were prepared and stored in a 37°C incubator. For intravenous administration, a 26-gauge needle was inserted into the lumen of the femoral vein, and cells (0.3 mL) were delivered over a 5-min period. For intracerebroventricular administration, 10 μL of the cell suspension was injected into the third cerebral ventricle over a 10-sec period, according to the atlas and coordinates of Paxinos and Watson (18).

Measurements of Cerebral Blood Flow, Brain Oxygen, and Brain Temperature. A 100-μm-diameter thermocouple and two 230-μm fibers were attached to the oxygen probe. This combined probe measures oxygen, temperature, and microvascular blood flow. The measurement requires OxyLite and OxyFlo instruments (Oxford Optronix, Oxford, UK). OxyLite 2000 is a two-channel device (measuring Po_2 and temperature at two sites simultaneously), whereas OxyFlo 2000 is a two-channel laser Doppler perfusion monitoring instrument. The OxyLite has been designed to operate in conjunction with OxyFlo. The combination of these two instruments provides simultaneous tissue blood flow, oxygenation, and temperature data. Under urethane anesthesia, each animal was placed in a stereotaxic apparatus, and the combined probe was implanted into the striatum with use of the atlas and coordinates of Paxinos and Watson (18). The detailed procedures for measurement of brain temperature, Po_2, and temperature were described previously (3, 19).

Measurement of Extracellular Glutamate, Glycerol, and Lactate/Pyruvate Ratio in the Striatum. Each animal was anesthetized with urethane administered intraperitoneally. The animal's head was mounted in a stereotaxic apparatus (Davis Kopf Instruments) with the nose bar positioned 3.3 mm below the horizontal line. Following 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 microdialysis probe was stereotaxically implanted into the striatum according to the atlas and coordinates of Paxinos and Watson (18). The detailed procedures for measurements of cellular ischemic and damage markers were described previously (3).

Extracellular NO Monitoring. A microdialysis probe (CMA20; Carnegie Medicine) with a 4-mm-long dialysis membrane was vertically implanted in the striatum. A Ringer's solution (0.860 g NaCl, 0.30 g KCl, and 0.033 g $CaCl_2$ per 100 mL) was perfused through the microdialysis probe at a constant flow (2.0 mL/min). After 6 hrs of stabilization, the dialysates from

the striatum were collected at 20-min intervals. The NO concentrations in the dialysates were measured with the ENO-20 NO analysis system (Eicom, Kyoto, Japan) (20).

Neuronal Damage Score. At the end of the experiments, animals were killed by an overdose of urethane and the brains were fixed *in situ* and left in the skull in 10% neutral buffered formalin for at least 24 hrs before removal from the skull. The brain was removed and embedded in paraffin blocks. Serial (10-μm) sections through the striatum, hippocampus, hypothalamus, and frontal cortex were stained with hematoxylin and eosin for microscopic evaluation. The extent of cerebral neuronal damage in different brain structures was scored on a scale of 0 to 3, modified from the grading system of Pulsinelli et al. (21), in which 0 is normal, 1 indicates approximately 30% of the neurons are damaged, 2 indicates that approximately 60% of the neurons are damaged, and 3 indicates that 100% of the neurons are damaged. Each hemisphere was evaluated independently by the examiner, who knew nothing of the experimental conditions.

Statistical Analysis. Data are presented as mean ± SEM. Repeated-measures analysis of variance was conducted to test the treatment-by-time interactions and the effect of treatment over time on each score. The Duncan's multiple-range test was used for *post hoc* multiple comparison among means. Wilcoxon's tests were used for evaluation of neuronal damage scores. Wilcoxon's tests convert the scores or values of a variable to ranks, require calculation of a sum of the ranks, and provide critical values for the sum necessary to test the null hypothesis at a given significant level. These data were presented as "median," followed by first (Q_1) and third (Q_3) quartile. $p < .05$ was considered evidence of statistical significance.

RESULTS

HUCBCs Improve Survival During Heatstroke. Table 1 summarizes the survival time values for normothermic controls, vehicle-treated heatstroke rats, PBMC-treated heatstroke rats, and HUCBC-treated rats. It can be seen from the table that survival time values during heatstroke for rats resuscitated with normal saline (0.3 mL iv or 10 μL icv) or AIM-V medium (0.3 mL iv or 10 μL icv) were found to be about 21–23 mins. Resuscitation with PBMCs (5×10^6/0.3 mL iv or 5×10^5/10 μL icv) did not affect survival time values exerted by respective normal saline or AIM-V medium controls. However, resuscitation with HUCBCs (5×10^6/0.3 mL iv or 5×10^5/10 μL icv) was associated with significantly greater survival time values than for the respective PBMC controls. In addition, as compared with those for resuscitation with intravenous administration of 5×10^6 HUCBCs, values for intracerebroventricular administration of 5×10^5 HUCBCs were higher in terms of survival time during heatstroke. It was also found that rats treated with HUCBCs (5×10^6/0.3 mL iv) had higher survival time values than were produced by a lower dose of HUCBCs (5×10^5/0.3 mL iv).

HUCBCs Attenuate Heatstroke-Induced Physiologic Dysfunction and Cerebral Ischemia and Damage. Figures 1 and 2 depict the effects of heat exposure (43°C for 68 mins) on several physiologic and biochemical parameters in rats treated with 5×10^5 PBMCs (10 μL icv), rats treated with 5×10^5 HUCBCs (10 μL icv), and normothermic controls. In PBMC-treated heatstroke groups, the ICP, brain temperature, and cellular levels of glutamate, glycerol, lactate/pyruvate, and NO were all significantly higher at 80–100 mins after the start of heat exposure than they were for normothermic controls. In contrast, the values for MAP, cerebral perfusion pressure, cerebral blood flow, and brain P_{O_2} were all significantly lower than those of normothermic controls. Resuscitation with 5×10^5 HUCBCs icv 68 mins after initiation of heat exposure (or immediately at the onset of heatstroke) significantly attenuated the heat stress–induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion, and cerebral hypoxia and increased levels of cellular ischemia and damage markers in striatum. Resuscitation with an intravenous dose of 5×10^6 HUCBCs, but not 5×10^5 HUCBCs, yielded a pattern of beneficial effects similar to that exerted by an intracerebroventricular dose of 5×10^5 HUCBCs (data not shown).

HUCBCs Attenuates Heatstroke-Induced Neuronal Damage. Table 2 summarizes the neuronal damage scores of different brain structures in normothermic rats, vehicle-treated heatstroke rats, PBMC-treated heatstroke rats, and HUCBC-treated heatstroke rats. It was found that the scores for neuronal damage in heatstroke rats resuscitated with vehicle solutions, 5×10^6 PBMCs (0.3 mL iv), 5×10^5 PBMCs (10 μL icv), or 5×10^5 HUCBCs (0.3 mL iv) 68 mins after initiation of heat exposure (median [Q_1, Q_3], 2 [2, 2]) were all significantly greater ($p < .05$) than those for the normothermic controls (median [Q_1, Q_3], 0 [0, 0.75]). However, the neuronal damage scores for heatstroke rats resuscitated with 5×10^6 HUCBCs (0.3 mL iv) or 5×10^5 HUCBCs (10 μL icv) (median [Q_1, Q_3], 0 [0, 1]) were all significantly lower ($p < .05$) than those for the respective heatstroke controls. A typical example for neuronal damage is depicted in Figure 3. The figure shows that intracerebroventricular delivery of HUCBCs reduced the heatstroke-induced neuronal damage in different brain structures, including striatum.

DISCUSSION

This is the first study to examine the efficacy of transplanting HUCBCs for resuscitation from heatstroke. It was found

Table 1. Survival time values for normothermic controls, vehicle-treated heatstroke rats, peripheral blood mononuclear cells (PBMC)-treated heatstroke rats, and human umbilical cord blood cells (HUCBC)-treated heatstroke rats

Treatment Group	Survival Time (Mins)
Normothermic controls (no treatment)	450 ± 2 (8)
Normal saline (0.3 mL iv)	21 ± 2 (8)[a]
Normal saline (10 μL icv)	22 ± 3 (8)[a]
AIM-V (0.3 mL iv)	23 ± 2 (8)[a]
AIM-V (10 μL icv)	22 ± 2 (8)[a]
PBMCs (5×10^6 in 0.3 mL iv)	23 ± 2 (8)[a]
PBMCs (5×10^5 in 10 μL icv)	22 ± 4 (8)[a]
HUCBCs (5×10^5 in 0.3 mL iv)	24 ± 2 (8)[a]
HUCBCs (5×10^6 in 0.3 mL iv)	61 ± 7 (10)[a,b]
HUCBCs (5×10^5 in 10 μL icv)	148 ± 23 (10)[a,b,c]

iv, intravenously; icv, intracerebroventricularly.

[a] $p < .05$ in comparison with group 1; [b] $p < .05$ in comparison with group 2, 4, or 6, as well as group 3, 5, or 7; [c] $p < .05$ in comparison with group 9 (analysis of variance followed by Duncan's test). All vehicle-treated or cell-treated heatstroke rats had heat exposure (43°C) withdrawn exactly at 68 mins and then were allowed to recover at room temperature (26°C). Data are mean ± SEM, followed by number of animals (n) in parentheses. Vehicles or cells were administered 68 mins after initiation of heat exposure (or at the onset of heatstroke). Group 1 was killed about 450 mins after the initiation of heat exposure (or at the end of the experiments) with an overdose of urethane.

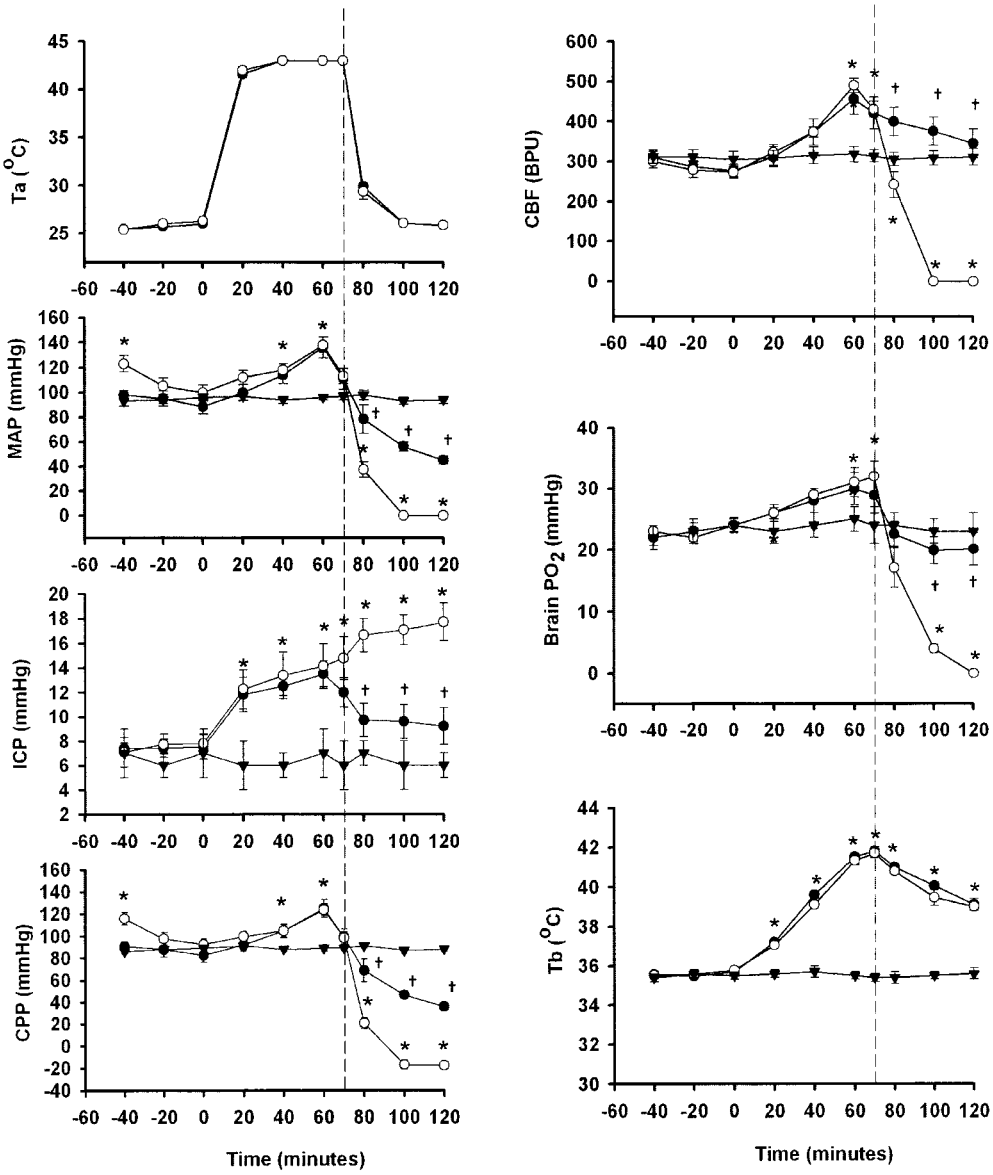

Figure 1. Effects of heat stress (ambient temperature [*Ta*] of 43°C for 68 mins) on mean arterial pressure (*MAP*), intracranial pressure (*ICP*), cerebral perfusion pressure (*CPP*), cerebral blood flow (*CBF*), brain Po_2, and brain temperature (*Tb*). *Open circles* are values at Ta of 43°C in eight rats treated with peripheral blood mononuclear cells (PBMCs; 5×10^5/10 μL intracerebroventricularly [icv]) immediately after the onset of heatstroke. Another eight rats exposed to a Ta of 26°C served as a control (*filled triangles*). *Filled circles* are values at Ta of 43°C in eight rats treated with human umbilical cord blood cells (HUCBCs; 5×10^5/10 μL icv) immediately after the onset of heatstroke. *Points* represent mean ± SEM (*$p < .05$ in comparison with normothermic control values [at Ta of 26°C]; +$p < .05$ in comparison with PBMC-treated group [at Ta of 43°C]) (analysis of variance, followed by Duncan's test). The onset of heatstroke is indicated by the *dashed line*. Tb during heatstroke is insignificantly different between the HUCBC and PBMC groups.

that the survival during heatstroke was much improved following systemic or central administration of HUCBCs, but not PBMCs, immediately at the onset of heatstroke. Central delivery seems superior to systemic delivery of HUCBCs in resuscitating patients with heatstroke, in terms of survival time (148 ± 23 vs. 61 ± 7 mins; n = 8–10 for each group), suggesting a central route of action. The present results further show that transplantation of HUCBCs, but not PBMCs, attenuate heatstroke by reducing circulatory shock, intracranial hypertension, cerebral hypoperfusion and hypoxia, and cerebral ischemia and injury. This suggests that the administration of HUCBCs can become pleuropotentially better than that of PBMCs. The present results, in part, are consistent with previous findings concerning the efficacy of transplanting HUCBCs as a treatment for conventional stroke. It has been shown that intravenous delivery of either whole bone marrow (22, 23) or umbilical cord blood (12, 15) can produce behavioral recovery after stroke or traumatic brain injury. In addition, Willing et al. (24) have demonstrated that intravenous delivery of HUCBCs may be more effective than striatal delivery of HUCBCs in producing long-term functional benefits to rats after stroke. However, the present results show that intracerebroventricular delivery of HUCBCs may be more effective than intravenous delivery of HUCBCs in improving survival during heatstroke. The discrepancy between these two groups of results is not apparent now. It should be

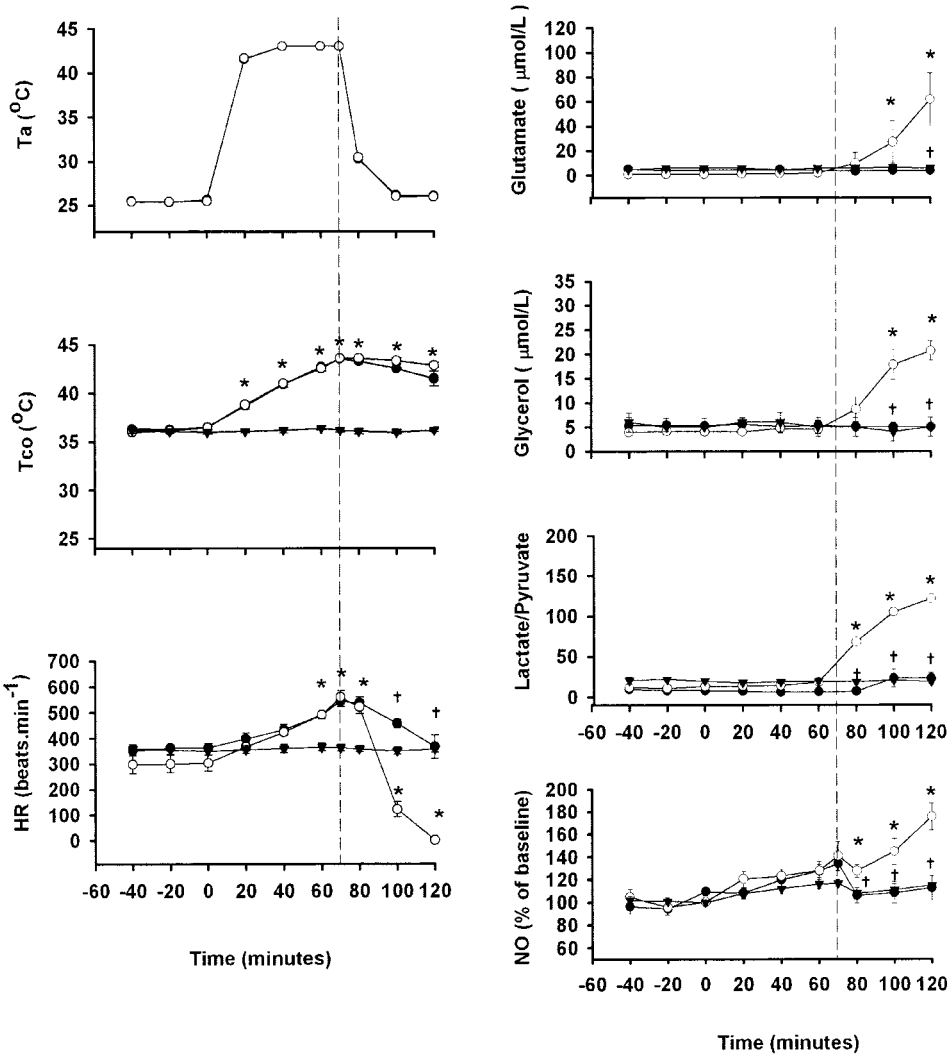

Figure 2. Effects of heat stress (ambient temperature [*Ta*] of 43°C for 68 mins) on levels of glutamate, glycerol, lactate/pyruvate, and nitric oxide metabolites (*NO*) in the extracellular fluids of the striatum. *Open circles* show values at Ta of 43°C in eight rats treated with peripheral blood mononuclear cells (PBMCs; 5×10^5/10 μL intracerebroventricularly [icv]). Another eight rats exposed to a Ta of 26°C served as a control (*filled triangles*). *Filled circles* show values at Ta of 43°C in eight rats treated with human umbilical cord blood cells (HUCBCs; 5×10^5/10 μL icv). The PBMC or HUCBC solution was administered immediately after the onset of heatstroke, as indicated by the *dashed line* (*$p < .05$ in comparison with normothermic controls [at Ta of 26°C]; +$p < .05$ in comparison with PBMC-treated group [at Ta of 43°C]) (analysis of variance, followed by Duncan's test). *HR*, heart rate.

stressed that systemic or central delivery of AIM-V medium or normal saline had an insignificant effect on heatstroke.

In fact, previous results have revealed that microvascular disturbances, including cerebral ischemia, disruption of the blood-brain barrier permeability, and formation of cerebral vasogenic edema, occur during heatstroke in rodents (25, 26). Therefore, the present results suggest that after the onset of heatstroke, disruption of the blood-brain barrier may facilitate selective entry of HUCBCs into the ischemic sites within the brain. Apparently, intracerebral administration had some advantages in reaching the target sites in the brain more easily than with the intravenous route of injection. This can explain why intracerebroventricular delivery is superior to intravenous delivery in resuscitation from heatstroke. The contention is supported by previous results. For example, *in vitro* studies have demonstrated that there is significant HUCBC migrative activity in the presence of ischemic cerebral tissue harvested at 24 hrs after cerebral ischemia, in comparison with normal nonischemic brain tissue (12).

When rodents are exposed to external heat stress, both depressed depolarization and decreased cardiac stroke volume produce arterial hypotension (26). After the onset of heatstroke, both arterial hypotension and intracranial hypertension eventually lead to cerebral ischemia and hypoxia. As demonstrated in the present study, the prolongation of survival among rats with HUCBC transplants was found to be related to maintenance of appropriate levels of both MAP and cerebral perfusion pressure, as well as reduction in both intracranial hypertension and cerebral neuronal damage exhibited during the onset of heatstroke. The maintenance of cerebral blood flow and Po_2 in animals treated with HUCBCs might be brought about by higher cerebral perfusion pressure resulting from lower ICP (due to reduction in cerebral edema and cerebrovascular congestion) and higher MAP during the development of heatstroke, as demonstrated by the present and previous results (22).

It has been shown that an endotoxin given intravenously can elicit an increase

Table 2. The neuronal damage scores for different brain structures in normothermic rats, vehicle-treated heatstroke rats, peripheral blood mononuclear cells (PBMC)-treated heatstroke rats, and human umbilical cord blood cells (HUCBC)-treated heatstroke rats

Treatment Group	Neuronal Damage Score (0–3)			
	Striatum	Hippocampus	Hypothalamus	Frontal cortex
Normothermic controls (no treatment)	0 (0, 0.75)	0 (0, 0.75)	0 (0, 0)	0 (0, 0)
Normal saline (0.3 mL iv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
Normal saline (10 μL icv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
AIM-V (0.3 mL iv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
AIM-V (10 μL icv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
PBMCs (5×10^6 in 0.3 mL iv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
PBMCs (5×10^5 in 10 μL icv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
HUCBCs (5×10^5 in 0.3 mL iv)	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]	2 (2, 2)[a]
HUCBCs (5×10^6 in 0.3 mL iv)	0 (0, 1)[b]	0 (0, 0.75)[b]	0 (0, 0.75)[b]	0 (0, 1)[b]
HUCBCs (5×10^5 in 10 μL icv)	0 (0, 1)[b]	0 (0, 0.75)[b]	0 (0, 0.75)[b]	0 (0, 1)[b]

iv, intravenously; icv, intracerebroventricularly.

[a]$p < .05$ in comparison with group 1; [b]$p < .05$ in comparison with group 3 or group 4. The data were evaluated by a Wilcoxon's signed-rank test, followed by Duncan's test. Data, for eight rats per group, are presented as median values, with Q1 and Q3 in parentheses. For determination of neuronal damage score, animals were killed 80 mins after the initiation of heat exposure or 12 mins after the onset of heatstroke.

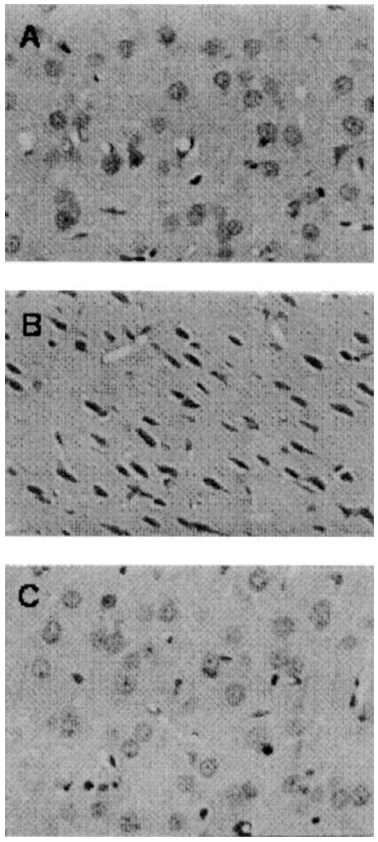

Figure 3. Histologic examination of neuronal damage. The photomicrographs of the striatum in a normothermic control rat (*A*), in a heatstroke rat treated with peripheral blood mononuclear cells (PBMCs; 5×10^5/10 μL icv) (*B*), and in a heatstroke rat treated with human umbilical cord blood cells (HUCBCs; 5×10^5/10 μL icv) (*C*) at the onset of heatstroke. Twelve minutes after the onset of heatstroke, the striatum of the rat treated with PBMCs showed cell shrinkage and pyknosis of the nucleus (*B*). However, with HUCBC treatment, neuronal damage was reduced (*C*).

of inducible nitric oxidase–dependent NO production in the nucleus tractus solitarii and induce arterial hypotension (23). Exposure of animals to a hot environment induces heatstroke that is characterized by arterial hypotension, cerebral ischemia, overproduction of cytokines (24, 27, 28), and reduced baroreceptor reflex response (29). The hemodynamic changes associated with heatstroke were mimicked by direct interleukin-1β administration (30, 31). Our recent results showed that the heatstroke-induced arterial hypotension, cerebral ischemia, and inducible nitric oxidase overexpression and NO overproduction in rat brain can be suppressed by pretreatment with aminoguanide (an inducible nitric oxidase inhibitor) (17). In the present study we also demonstrated that HUCBC transplantation attenuated arterial hypotension, cerebral ischemia, and NO production in rat brain during heatstroke. These findings together suggest that HUCBC transplantation may maintain appropriate levels of systemic and cerebral circulation during heatstroke by reducing cytokine production as well as inhibiting inducible nitric oxidase–dependent NO production in the brain.

It has been shown that HUCBCs could be induced to express neural proteins (8), class III β-tubulin, glial fibrillary acidic protein, Galc (a marker of oligodendrocytes) (9–10), and neurofilament microtubule–associated protein 2 (32). When these cells were transplanted into the neonatal subventricular zone, some of them differentiated into neuronal and glial phenotypes within the neurogenic region (11). In addition, there was an increase in glial-derived neurotrophic factor levels following HUCBC transplantation after stroke (33). It is likely that HUCBCs, rather than PBMCs, may release any mediators (i.e., cytokines, NO, and endothelial or other neurotrophic factors), reduce hypotension, decrease intracranial hypertension, and confer neuronal protection during heatstroke.

As shown by our previous findings (17, 19, 34, 35), ischemic neuron damage in different brain structures occurred 10–15 mins after the onset of heatstroke. The heatstroke-induced neuron damage in different brain structures was reduced by intravenous infusion of albumin (19) or HUCBCs (present study) as well as brain cooling (34, 35), initiated immediately after the onset of heatstroke. Apparently, this histopathologic profile was noted in rats killed 12–15 mins after the onset of heatstroke. Furthermore, if therapy was given after the onset of heatstroke, the cells or albumin was injected or cooling was initiated, and then an effect on neuronal survival was produced in <12 mins. Probably, severe hyperthermia during heatstroke is responsible for such a very rapid effect. In addition, as mentioned in the former paragraph, HUCBCs may release any unknown mediators to attenuate the neuronal damage. According to a more recent report (14), at 4 wks after intravenous infusions, HUCBCs were localized by polymerase chain reaction analysis only in the injured brain hemisphere and spleen. In our study, although we provided no data concerning the HUCBCs located at the end of the experiment or 12–15 mins after systemic or central administration, we did observe that HUCBC transplantation at the onset of heatstroke ameliorated the circulatory shock, cerebral ischemia and damage, and NO overproduction during heatstroke. More recently, our unpublished data showed that overproduction of tumor necrosis factor-α in the serum exhibited during heatstroke was greatly suppressed by HUCBC transplantation at the onset of heatstroke.

Owing to their greater availability, feeble immunogenicity, and lower risk of mediating viral transmission, HUCBCs have come out as an alternative to bone marrow (14). Moreover, the HUCBCs can

We successfully demonstrate that human umbilical cord blood cells (HUCBC) therapy may resuscitate heatstroke victims by reducing circulatory shock and cerebral ischemic injury; central delivery of HUCBCs seems superior to systemic delivery of HUCBCs in resuscitating patients with heatstroke.

mediate therapeutic effects in several animal models of neurologic diseases, including stroke (12, 13, 15, 16). On the other hand, the recovery of nucleated cells from rat umbilical cord blood is relatively difficult and inconvenient (36). Therefore, HUCBCs were used for this study rather than rat umbilical cord blood cells.

CONCLUSION

Our studies involving rats indicate that HUCBCs could potentially be an excellent source of cells for the treatment of heatstroke, because they are widely available and have been used clinically. Additional studies in experimental models are needed.

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