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Decrease of Heatstroke-Induced Multiorgan Dysfunction by Whole Body Cooling in Streptozotocin-Induced Diabetic Rats

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Abstract

The present study was conducted to assess the effects of whole body cooling on multiorgan dysfunction that occurred during heatstroke in streptozotocin (STZ)-induced diabetic rats. The rats were randomly divided into four groups: [1] the normal control, [2] diabetic control, [3] diabetic heatstroke, and [4] diabetic heatstroke-whole body cooling (WBC). They were exposed to ambient temperature of 43°C for exactly 58 min to induce heatstroke. When the diabetic heatstroke rats underwent heat stress, their survival time values were found to be 11-13 min. Immediately after the onset of heatstroke, resuscitation with body cooling greatly improved survival (221-257 min). Compared with the diabetic (STZ-treated) controls, the diabetic-heatstroke rats displayed higher levels of body temperature, intracranial pressure, serum nitric oxide metabolite, tumor necrosis factor-α and dihydroxybenzoic acid, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. In contrast, the values of mean arterial pressure, cerebral perfusion pressure, and brain levels of local blood flow, and partial pressure of oxygen were all significantly lower during heatstroke. The cerebrovascular, renal, and hepatic dysfunction, the increased levels of nitric oxide metabolites, tumor necrosis factor-α, and dihydroxybenzoic acid in the serum during heatstroke were significantly reduced by WBC. Although the serum interleukin-10 maintained at a negligible levels before heat stress, they were significantly elevated by WBC in diabetic-heatstroke rats. The data demonstrate that heatstroke-induced multiorgan dysfunction in streptozotocin-induced diabetic rats can be decreased by WBC.

Key Words: diabetes, heatstroke, body cooling, cytokines, cerebrovascular dysfunction

Introduction

Heatstroke is characterized by hyperpyrexia and multiorgan dysfunction. Animal heatstroke models fulfill the empirical triad used for the diagnosis of classic human heatstroke (5). Multiorgan dysfunctions ensue from severe heatstroke, including hepatic and renal dysfunction, pulmonary and brain edema, hypotension, cerebral ischemia and injury, and activated inflammation. Currently, the treatment of heatstroke is whole body cooling (WBC) (4, 11, 19, 24). In rats, WBC immediately after the onset of

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heatstroke reduced the heatstroke-induced circulatory shock, cerebral ischemia, neuronal damage, and surge of tissue ischemia and damage markers in the hippocampus, and resulted in prolongation of survival time (8).

According to the report of al-Harthi and colleagues (1), among the patients with heatstroke at Mecca Pilgrimage, most of them had diabetes with hyperglycemia. This is consistent with the findings observed in streptozotocin (STZ)-induced diabetic rats (16). In comparison with normal rats, STZ-induced diabetic rats had shorter survival duration, which could be reversed by insulin replacement (16). However, the effects of heatstroke on multiorgan dysfunctions mentioned above have not been determined in STZ-diabetic rats. In addition, it is still unknown whether the survival of STZ-diabetic rats during heatstroke can be improved by WBC.

To deal with the question, the present investigation was performed to assess the effects of heatstroke on the serum levels of nitric oxide metabolites (NO_x^-), dihydroxy benzoic acid (DHBA), tumor necrosis factor-alpha (TNF-α), and interleukin-10 (IL-10), and hepatic, renal, and cerebrovascular functions in diabetic rats with or without WBC therapy. TNF-α is a pro-inflammatory cytokine whereas IL-10 is a potent anti-inflammatory cytokine (9).

Materials and Methods

Animals and Treatment

The experimental procedures were performed in accordance with the animal care guidelines of the National Science Council of the Republic of China (Taipei, Taiwan). Male Sprague-Dawley rats weighing 248 ± 12 g (9 weeks in age) were used in this experiment. Rats were housed under controlled temperature (22 ± 1°C) and lighting conditions (07:00 to 19:00), with food and water made available ad libitum throughout the experiments. The rats were randomly divided into four groups: [1] the normal control, [2] diabetic control, [3] diabetic heatstroke, and [4] diabetic-heatstroke-whole body cooling (WBC), with 8 rats in each group. Diabetes was produced by injecting streptozotocin (STZ) (Sigma, St. Louis, MO, USA) at 30 mg/kg in the tail veins. The rats were maintained for 4-5 weeks before heat stress was performed. A blood sample was collected and body weights, plasma glucose, and plasma insulin were determined at this time.

Induction of Heatstroke

For the induction of heatstroke, rats were anesthetized with pentobarbital sodium (40 mg/kg i.p.; Sigma) and placed in a stereotaxic frame. Before induction of heatstroke, the core temperature (Tco) was maintained at about 36°C with a folded heating pad with no heat stress at a room temperature of 24°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 mmHg from the peak level and the Tco reached over 42°C was taken as the onset of heatstroke (13). Immediately after the onset of heatstroke, the heating pad was removed, and the rats were allowed to recover at room temperature (24°C).

Induction of WBC

Immediately after the onset of heatstroke (or 58 min after the start of heat stress), WBC was accomplished by decreasing the blanket temperature from 43°C to 16°C for 20 min. Then, the heating pad was removed, and the rat was allowed to recover at room temperature (24°C).

Physiologic Parameters Monitoring

The rats received a prophylactic injection of the antibiotic, gentamicin sulfate (18 mg/kg, i.m.), and were anesthetized 1 h later with sodium pentobarbital. The standard aseptic procedures were conducted to avoid contamination. The right femoral artery of rats were cannulated with polyethylene tubing (PE-50) under anesthesia and standard aseptic procedures for blood pressure monitoring. For measurement of intracranial pressure (ICP), the rats were positioned in a stereotaxic apparatus to insert probes for Statham P23AC transducer *via* 20-gauge stainless steel needled probe (diameter, 0.90 mm; length 38 mm), which was introduced into the right cerebral ventricle according to the stereotaxic coordinates of Paxinos and Watson (17): 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. MAP and heart rate (HR) were monitored continuously with a pressure transducer.

A 100-μm diameter thermocouple and two 230-μm fibers were attached to the oxygen probe. This combined probe measured oxygen, temperature, and microvascular blood flow. The measurement required OxyLite™ and OxyFlo™ instruments. OxyLite 2000 (Oxford Optronix Ltd., Oxford, UK) is a 2-channel device (measuring PO_2 and temperature at two sites simultaneously), whereas OxyFlo 2000 is a 2-channel Laser Doppler perfusion monitoring instrument. The OxyLite has been designed to operate in conjunction with OxyFlo. The combination of these 2 instruments provided simultaneous tissue blood flow, oxygenation,

Table 1. Body weight, plasma glucose, and plasma insulin concentrations in normal and streptozotocin-induced diabetic rats

	Body weight (gm)	Plasma glucose (mg/dl)	Plasma insulin (μU/ml)
Normals	298 ± 9 (16)	107 ± 7 (16)	49 ± 6 (16)
Diabetics	261 ± 11* (24)	445 ± 19* (24)	10 ± 5* (24)

*$P < 0.01$ compared to normals.

and temperature data. Under anesthesia, the rat was placed in a stereotaxic apparatus, and the combined probe was implanted into the striatum using the atlas and coordinates of Paxinas and Watson (17).

Measurement of both Tumor Necrosis Factor-α (TNF-α) and Interleukin-10 (IL-10)

For determination of TNF-α and IL-10, blood samples were taken 0, 58, or 68 min after the start of heat stress. The blood samples were allowed to clot for 2 h at room temperature and then were centrifuged (2,000 × g, 20 min, 4°C). The concentrations of TNF-α and IL-10 in serum were determined using double-antibody sandwich ELISA (R&D, Systems, Minneapolis, MN, USA) according to the manufacture's instructions. Optical densities were read on a plate reader set at 450 nm for TNF-α and IL-10. The concentrations of TNF-α or IL-10 in the samples was calculated from the standard curve multiplied by the dilution factor and was expressed as pg/ml.

Measurement of Nitric Oxide Metabolites (NO_x^-) and Dihydroxybenzoic Acid

For determination of NO_x^- or DHBA, a microdialysis probe (CMA/20 20/40 PC; CMA/Microdialysis AB, Stockholm, Sweden) was put into jugular vein/right atrium as described previously (22). The NO_x^- concentrations in the dialysates were measured with Eicom ENO-20 NO_x^- analysis system (Eicom, Kyoto, Japan) (21). In the Eicom ENO-20 NO_x^- analysis system, after the NO_2^- and NO_3^- in the sample have been separated by the column, the NO_2^- reacted in the acidic solution with the primary aromatic amine to produce an azo compound. Following this, the addition of aromatic amines to the azo compound results in a coupling that provides a diazo compound and the absorbance rate of the red color in this compound was then measured. The concentrations of hydroxyl radicals in the dialysates were measured by a modified procedure based on the hydroxylation of sodium salicylate by hydroxyl radicals, leading to the production of 2,3-dihydroxylbenzoic acid and 2,5-dihydroxylbenzoic acid (25).

Biochemical Determinations

Blood samples at 0, 58, or 68 min after initiation of heat stress were drawn by arterial femoral cannulation. Properly calibrated and controlled automated devices were used to determine complete blood counts (Cell Dyn 400; Abbott Diagnostics, Santa Clara, CA, USA), and liver profiles (Hitachi 912; Mannheim-Boehringer, Mannheim, Germany).

Statistics

Data are presented as the means ± SD. Unpaired *t*-test was used for comparisons of two groups. $P < 0.05$ was considered evidence of statistical significance.

Results

Animal Characteristics

Diabetic rats had smaller body weight, higher plasma glucose levels, and lower plasma insulin when compared to normal (Table 1).

Survival Improvement by WBC during Heatstroke

Table 2 summarizes both latency (interval between onset of heat exposure and onset of heatstroke) and survival time (interval between onset of heatstroke and animal death) values for normothermic controls or heatstroke rats with or without WBC. The latency and survival time values during heatstroke for diabetic rat without WBC were decreased from the control values of > 480 min to new values of 55-61 min and 11-13 min, respectively. Resuscitation with WBC increased the survival time values (221-257 min) during heatstroke.

Attenuation of Hypotension, Intracranial Hypertension, Cerebral Hypoperfusion, Ischemia and Hypoxia, and Hyperpyrexia by WBC during Heatstroke

Figure 1 depicts the effects of heat exposure (43°C for 58 min) on physiologic variables in diabetic-heatstroke rats treated with or without WBC. In diabetic-heatstroke rats without WBC treatment, the ICP and Tbr were both significantly higher at 58-68 min after the start of heat exposure than they were for

Table 2. Latency and survival time values for the normothermic control group, diabetic-normothermia group, diabetic group with heatstroke (hyperglycemia-heatstroke group), and hyperglycemia group with heatstroke and whole body cooling (hyperglycemia-heatstroke-whole body cooling) group

Treatment Group	Latency (min)	Survival Time (min)
1. Norglycomia-normothermia (NN)	> 480 (8)	> 480 (8)
2. Diabetic-normothermia controls (DNC)	> 480 (8)	> 480 (8)
3. Diabetic-heatstroke controls (DHC)	58 ± 3 (8)[a]	12 ± 1 (8)[a]
4. Diabetic-heatstroke-whole body cooling (DHWBC) group	58 ± 4 (8)[a]	239 ± 18 (8)[b]

All diabetic-heatstroke group had heat exposure (43°C) withdrawn exactly at 58 min and were then allowed to recover at room temperature (24°C). Data are means ± SD followed by the amount of animals (n) in parentheses. Whole body cooling was conducted at 58 min after start of heat stress. The interval between the start of heat exposure and the onset of heatstroke is defined as latency, while the interval between onset of heatstroke and animal death is defined as survival time.
[a]$P < 0.05$ in comparison with group DNC or group NN.
[b]$P < 0.05$ in comparison with DHC group.

normothermic controls. In contrast, the values for MAP, CPP, CBF, and brain PO_2 were significantly lower than those of the normothermic controls. Resuscitation with WBC 58 min after initiation of heat exposure (or immediately at the time point of onset of heatstroke) significantly attenuated the heat stress-induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion, cerebral ischemia and hypoxia, and hyperpyrexia.

Improvement of Renal and Hepatic Dysfunction during Heatstroke by WBC

Figure 2 depicts the effects of heat exposure (43°C for 58 min) on renal and hepatic function in diabetic heatstroke rats treated with or without WBC. In diabetic-heatstroke rats without WBC treatment, the values of creatinine, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were all significantly higher at 58-68 min after the start of heat exposure than they were for normothermic controls. In contrast, both renal and hepatic dysfunction during heatstroke were significantly reduced by WBC therapy immediately at the time point for heatstroke.

Attenuation of Inflammation Responses during Heatstroke by WBC

Figure 3 depicts the serum levels of NO_x^-, DHBA, IL-10, and TNF-α for normothermic controls, or diabetic-heatstroke rats treated with or without WBC. In untreated diabetic-heatstroke groups, the serum levels of NO_x^-, DHBA, and TNF-α were all significantly higher at 58-68 min after the start of heat exposure than they were for normothermic controls. Resuscitation with WBC 58 min after initiation of heat exposure significantly attenuated the heat stress-induced overproduction of NO_x^-, DHBA and TNF-α in the serum.

In normothermic diabetic controls and untreated diabetic-heatstroke rats, the serum levels of IL-10 were maintained at a negligible level. However, 10 min following WBC, the serum levels of IL-10 were greatly elevated in WBC-treated diabetic-heatstroke rats.

Discussion

It has been documented that normal rats display hypotension, hepatic and renal dysfunction (evidence by increased serum urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels in serum), hypercoagulable state, activated inflammation (evidenced by increased TNF-α levels in serum), and cerebral dysfunction (evidenced by intracranial hypertension, cerebral hypoperfusion and hypoxia, and cerebral ischemia and injury) during heatstroke (6). The present results further demonstrated that diabetic-heatstroke rats shared with normoglycemia-heatstroke rats the same pattern of heatstroke reactions. The major discrepancy between those 2 groups was that the latency for the onset of heatstroke was significantly shorter in the former than in the latter. Compared to normal controls, diabetic rats seemed to be more susceptible to heatstroke. As compared to normal controls, diabetic rats had shorter survival time. The reduced survival was related to hypotension, multiple organ dysfunction, overproduction of cytokines, ROS, and RNS, and cerebral ischemia and damage. In fact, diabetes is a major risk factor for the development of cerebrovascular disease. For example, patients with diabetes are about two times more likely to have a stroke than non-diabetic subjects, and they

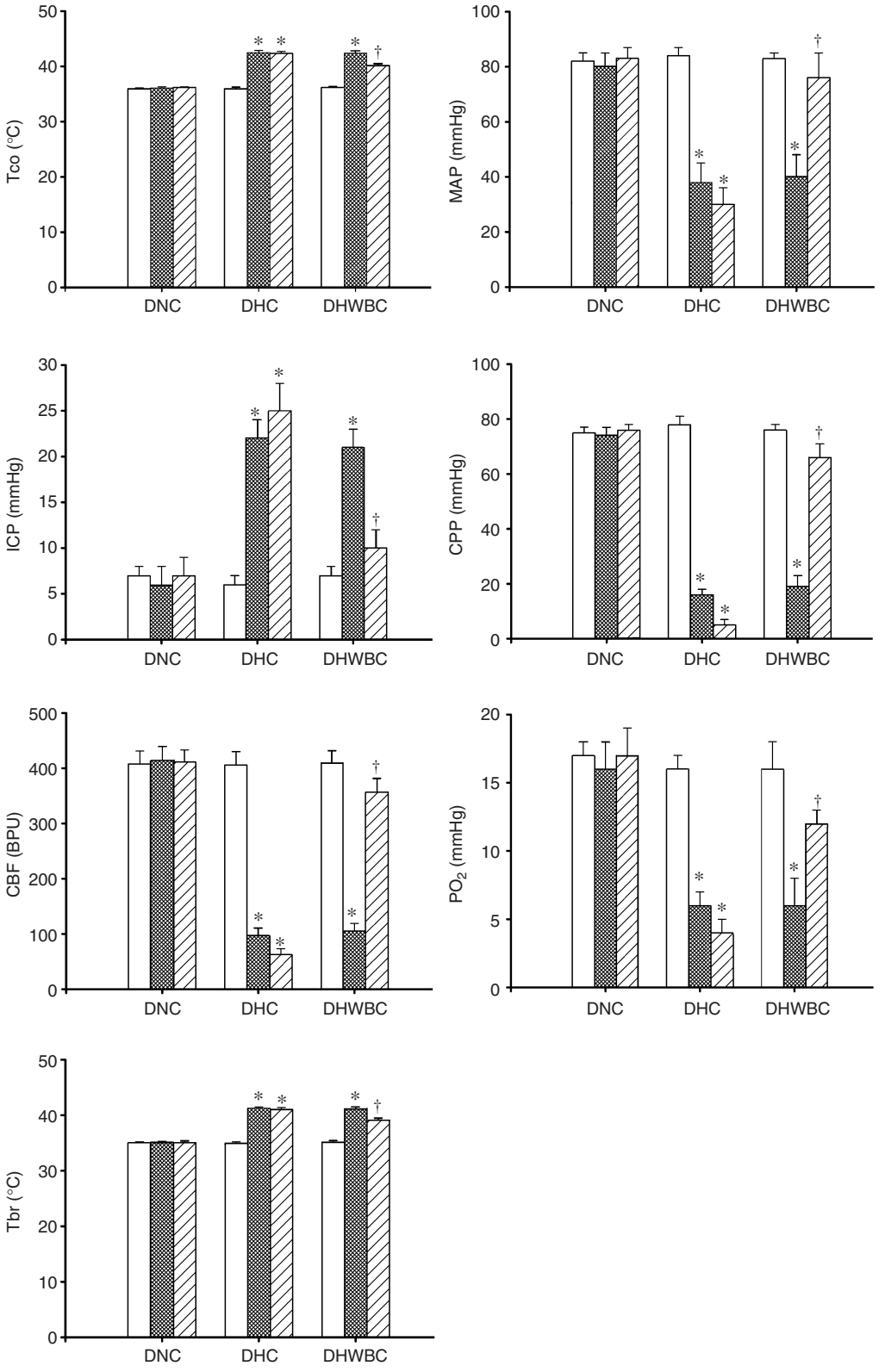

Fig. 1. Values of core temperature (Tco), mean arterial pressure (MAP), intracranial pressure (ICP), cerebral perfusion pressure (CPP), cerebral blood flow (CBF), brain PO_2, and brain temperature (Tbr) for diabetic – normothermia rats (DNC), diabetic-heatstroke rats (DHC), and diabetic-heatstroke-WBC (DHWBC) rats. $^{*}P < 0.05$ in comparison with DNC group; $^{\dagger}P < 0.05$ in comparison with DHC group. All heatstroke groups had heat exposure (43°C) withdrawn exactly at 58 min and were then allowed to recover at room temperature (24°C). Bars are means ± SD of 8 rats for each group. The values were obtained at 0 (□), 58 (▩), or 68 (▨) min after the initiation of heat exposure in heatstroke rats or the equivalent times in the normothermia controls.

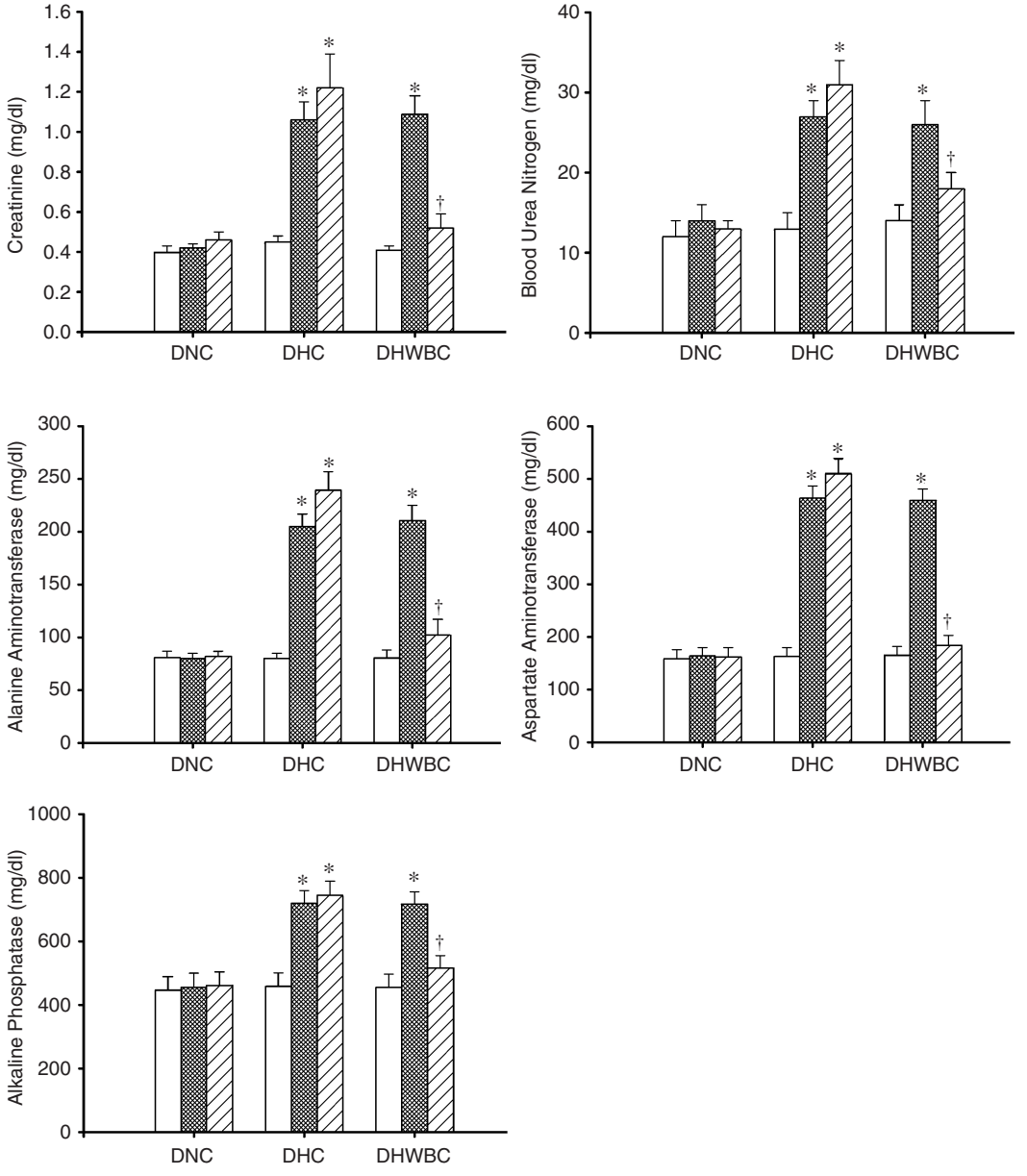

Fig. 2. Values of serum levels of creatinine, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase for diabetic-normothermia rats (DNC), diabetic-heatstroke rats (DHC), and diabetic-heatstroke-WBC rats (DHWBC). *$P < 0.05$ in comparison with DNC group; †$P < 0.05$ in comparison with DHC group. All heatstroke groups had heat exposure (43°C) withdrawn exactly at 58 min and were then allowed to recover at room temperature (24°C). Bars are means ± SD of 8 rats for each group. The values were obtained at 0 (□), 58 (▩), or 68 (▨) min after the initiation of heat exposure in heatstroke rats or the equivalent times in normothermia controls.

are more likely to suffer increased morbidity and mortality after stroke (3, 23). Diabetes is believed to be associated with a series of vascular disorders (23). It has been promoted that cerebrovascular dysfunction is an attractive target for therapy in heatstroke (7). Indeed, as shown in the present study, WBC therapy raised the survival rate of STZ-induced diabetic rats during heatstroke by reducing cerebrovascular disorders (*e.g.*, intracranial hypertension, cerebral hypoperfusion, ischemia and injury).

Many of the cerebrovascular responses observed during heatstroke can be mimicked by systemic administration of interleukin-1 (14). The authors' previous (12, 14, 15) and present results showed that overproduction of interleukin-1 and TNF-α in both the peripheral blood stream and the central nervous system occurs during heatstroke in the rat. Both circulatory shock and cerebral ischemia and damage during heatstroke could be attenuated by prior administration of corticosteroids or cytokine receptor antagonists before

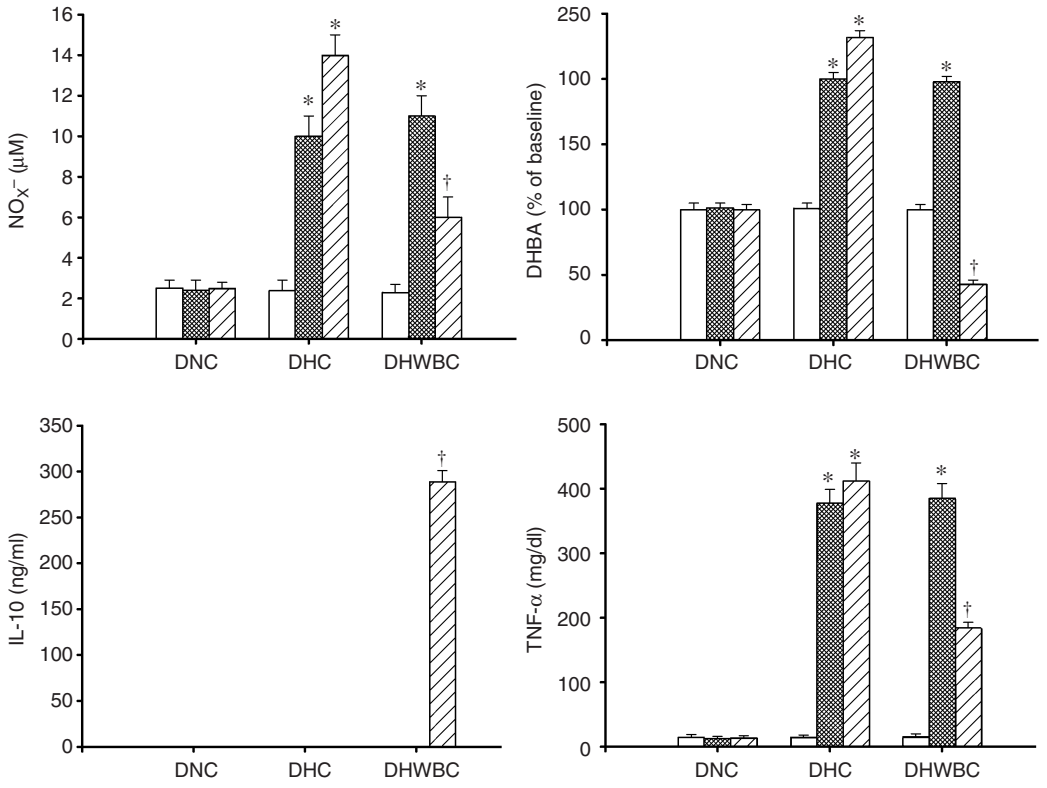

Fig. 3. Values of nitric oxide metabolites (NO_x^-), dihydroxybenzoic acid (DHBA) interleukin-10 (IL-10), and tumor necrosis factor-α (TNFα) for diabetic-normothermia controls (DNC), diabetic-heatstroke rats (DHC), and diabetic-heatstroke rats (DHWBC). *$P < 0.05$ in comparison with DNC group; †$P < 0.05$ in comparison with DHC group. All heatstroke groups had heat exposure (43°C) withdrawn exactly at 58 min and were then allowed to recover at room temperature (24°C). Bars are means ± SD of 8 rats for each group. The values were obtained at 0 (□), 58 (▩), or 68 (▨) min after the initiation of heat exposure in heatstroke rats or the equivalent times in normothermia controls.

the initiation of heat stress. The current findings further showed that WBC protected against heatstroke by decreasing TNF-α overproduction but increasing IL-10 production in the serum. In fact, TNF-α is best known as a proinflammatory cytokine necessary for activating immune response (2) and inducing septic shock (18). On the other hand, IL-10 has been shown to protect mice from lethal endotoxemia by reducing TNF-α release (9). In endotoxemic mice, neutralization of endogenous produced IL-10 resulted in an increased production of proinflammatory cytokines and enhanced mortality (20).

Figure 4 depicts a scheme showing events between the exposure of rats to a hot environment and death from heatstroke, with known or proposed interrelation ships derived from the published data. It has been proposed (10) that heat stress stimulates metabolism and progressively reduces blood flow to critical organs. Increased metabolic demand coupled with reduced splanchnic and cerebral blood flow generates cellular hypoxia, increases mitochondrial ROS production and stimulates cellular oxidase and Ca^{2+}-dependent NOS activities. As heat stress continues, hypoxic cells export oxidases into the extracellular space, where oxidative stress can produce multifocal cellular injury and inflammation. The large increase in both ROS and RNS leads to splanchnic dilation, systemic hypotension, circulatory shock (10; present results), and cerebral ischemia and injury (present results; 7). WBC can be used to limit hypotension, splanchnic ischemia, cerebral ischemia and neuronal damage during heatstroke by restoring the appropriate levels of blood flow to both the peripheral organs and the brain and to intervene the secondary toxic cascades by reducing overproduction of both RNS and ROS in both the peripheral organs and the brain.

If more indicators for multiorgan dysfunction were examined after WBC, it would benefit more the clinical benefit for treatment of diabetic patient during heatstroke. For example, cardiovascular index and severity of pulmonary edema will be good indicators for evaluation of the cardio respiratory status of a diabetic patient. Glomerulus filtration rate should be measured in addition to plasma creatinine and BUN for the evaluation of renal dysfunction. Moreover, whether the body temperature and level of blood

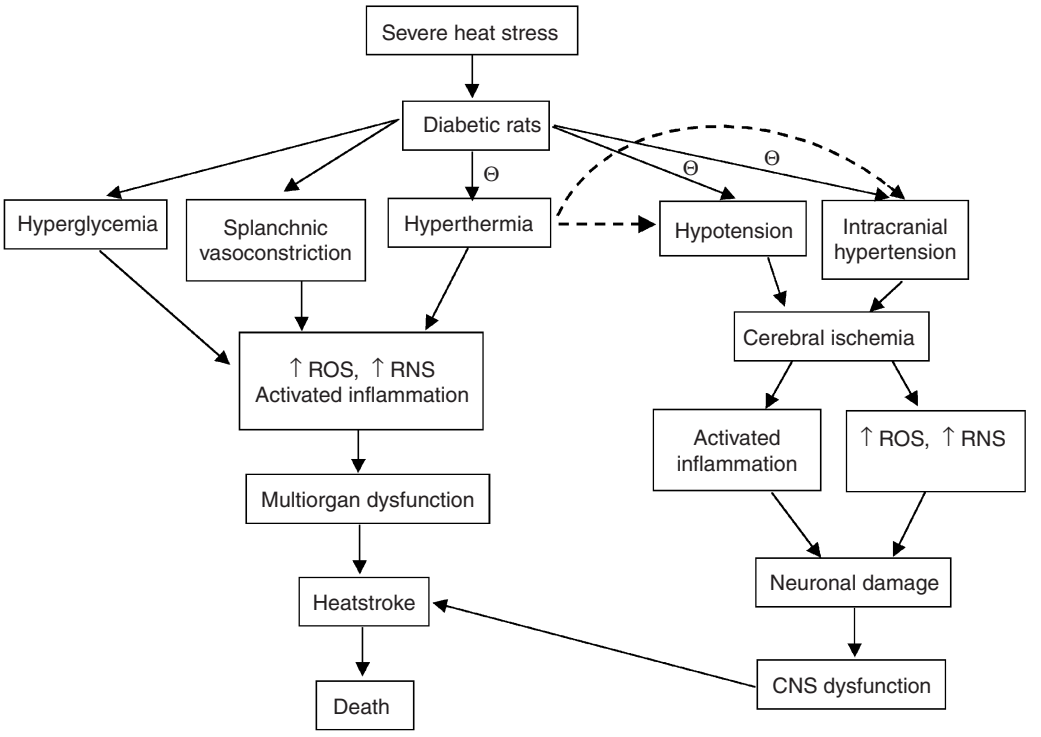

Fig. 4. Proposed scheme of the interaction sequence of events occurring from the beginning of exposure to a hot environment to heat stroke occurrence in diabetic rats. Uparrows indicate increased parameter. Dash inside circles denote the inhibitory action exerted by whole body cooling. CNS: central nervous system; ROS: reactive oxygen species; RNS: reactive nitrogen species.

sugar of diabetic rat maintain well after WBC needs to be clarified in futural studies.

In summary, as demonstrated in the present findings, STZ-induced diabetic rats had cerebrovascular, hepatic and renal dysfunction as well as large increase of RNS, ROS, and TNF-α in the peripheral blood stream during heatstroke. Whole body cooling, in addition to increasing the serum levels of IL-10, reduced the heatstroke-induced cerebrovascular, hepatic and renal dysfunction and overproduction of ROS, RNS, and TNF-α in the serum. The results suggest that WBC may resuscitate diabetic animals who had heatstroke by reducing multiorgan dysfunction.

Acknowledgments

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