Effects of Combination Treatment with Dexamethasone and Mannitol on Neuronal Damage and Survival in Experimental Heat Stroke

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There is evidence that increased plasma cytokines, elevated brain levels of monoamines and hydroxyl radical production may be implicated in pathogenesis during heat stroke in rats. Acute treatment with a combined therapeutic approach has been repeatedly advocated in cerebral ischemia experiments. The aim of this study was to investigate whether the combined agent (mannitol and dexamethasone) has beneficial efficacy to improve the survival time (ST) and heat stroke-induced damage in experimental heat stroke. Urethane-anesthetized rats underwent instrumentation for the measurement of colonic temperature, mean arterial pressure (MAP), striatal cerebral blood flow (CBF), heart rate, and neuronal damage score. The rats were exposed to an ambient temperature (43 °C) to induce heat stroke. Concentrations of the ischemic and damage markers, dopamine, serotonin, and hydroxyl radical production in corpus striatum, and the plasma levels of tumor necrosis factor-α (TNF-α) were observed during heat stroke. After the onset of heat stroke, the heat stroke rats displayed decreased MAP, decreased CBF, increased the plasma levels of TNF-α, increased cerebral striatal monoamines and hydroxyl radical production release, and severe cerebral ischemia and neuronal damage compared with those of normothermic control rats. However, immediate treatment with the combined agent confers significant protection against heat stroke-induced arterial hypotension, systemic inflammation, cerebral ischemia, cerebral monoamines and hydroxyl radical production overloads, and improves neuronal damage and the ST in heat stroke rats. Our data suggest that administration of this combined agent seems to have more effective to ameliorate the heat stroke-induced neuronal damage and prolong the ST.

Key words heat stroke; combined treatment; dexamethasone; mannitol; cerebral ischemia; neuronal damage

Unless immediately recognized and treated, heat stroke is often lethal, and victims who do survive may sustain permanent neurological damage. 1) The clinical diagnosis of heat stroke is demonstrated when hyperthermia is accompanied with circulatory shock (arterial hypotension), intracranial hypertension, and cerebral ischemia and injury. 2) 3) Meanwhile, the heat stroke-induced central nervous system dysfunction includes delirium, convulsion, or coma. 5) 6) Hence, prolonging survival time in heat stroke victims may offer more sufficient time for urgent treatment, thereby ameliorating the heat stroke-induced damage.

Several lines of evidence indicate that rodents share with humans almost the same heat stroke syndromes, such as arterial hypotension, activated inflammation, and multiorgan dysfunction (in particular, cerebral ischemia, injury, and dysfunction). 5 — 7) In the rodents heat stroke model, significant decrements in both mean arterial pressure (MAP) and cerebral blood flow (CBF), but increments of cerebral monoamines levels and free radical productions are obtained in urethane-anesthetized rats after heat stroke. 8 , 9) These pathophysiological changes are known to aggravate the conditions of cerebral ischemia and neuronal damage during heat stroke in rats. 10 , 11) Activated inflammation is evidenced by overproduction of pro-inflammatory cytokines (e.g., tumor necrosis factor-α (TNF-α)) in plasma of heat stroke rats. 12 , 13) High levels of TNF-α in the peripheral blood stream, as well as excessive accumulation of glutamate, hydroxyl radicals, dopamine (DA) and serotonin (5-HT) in the central brain, correlate with the severity of circulatory shock, cerebral ischemia and neuronal damage during heat stroke in rats. 6, 9, 11, 14, 15) Various clinical and experimental investigations have shown that single doses of dexamethasone (DXM; exogenous glucocorticoids) or mannitol are extensively used in the treatment of cerebral ischemia and/or cerebral injury. 16 — 18) In the studies of heat stroke, pretreatment with DXM attenuated serum IL-1β levels and improved survival in heat stroke. 19) Additionally, pretreatment with mannitol before the onset of heat stroke caused significant reduction of the heat stroke-induced increased free radical formation and intracranial hypertension. 20) Although, many therapeutic agent show potential promise in many animal models, the results of most single-agent clinical trials are sobering. Consequently, various authors advocate studies to estimate the efficacy of combined therapeutic approaches. 21 , 22) Hence, the mannitol that acts to decrease intracranial pressure and radical formation, and a potent inflammatory agent (such as DXM) might be combined to develop an improved fluid therapy for attenuation or amelioration of heat stroke-induced damage. Furthermore, there is less attention to evaluate immediate effects of both DXM and mannitol (the combined agent) on heat stroke-induced pathophysiological changes, let alone their neuroprotective underlying influences, especially in the aspects of striatal monoamines and hydroxyl radical production release. In the present study, we first observe whether application of the combined agent immediate treatment has efficacy to elongate the survival time, and improve the heat stroke-induced circulatory shock, cerebral ischemia, and neuronal damage in rats. Furthermore, we also attempt to ascertain whether the neuroprotective effects of the combined...
agent treatment are associated with inhibition of cerebral release of glutamate, DA, 5-HT, hydroxyl radicals, and the plasma TNF-α levels after heat stroke.

MATERIALS AND METHODS

Experimental Animals Male Sprague-Dawley rats weighing between 250 and 350 g were obtained from the National Science Council of Republic of China (Taipei, Taiwan). Between experiments the animals were housed individually at an ambient temperature of 24±1°C with a 12-h light–dark cycle, with the lights being switched on at 0600 h. Animal chow and water were allowed ad libitum. All protocols were approved by the Animal Ethics Committee of the Chia-Nan University of Pharmacy and Science, Tainan, Taiwan (approved no. CN-IACUC-94007) in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Guidelines of the Animal Welfare Act. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes by tail-pinchng throughout all experiments (approximately 8 h) by a single intraperitoneal dose of urethane (1.4 g·kg⁻¹ b.w., intraperitoneally (i.p.)). At the end of the experiments, control rats were killed with an overdose of urethane.

Animal Surgery and Physiological Parameter Monitoring Under urethane anesthesia, the right femoral artery of the rats was cannulated with polyethylene tubing (PE50) for physiological monitoring, the right femoral vein was also cannulated for blood sampling and drug administration. The animals were then positioned in a stereotaxic apparatus (Kopf model 1460) for measurement of CBF and microdialysis experiment. Physiological monitoring included colon temperature (TCo), MAP, heart rate (HR) and CBF values in the corpus striatum.

Induction of Heat Stroke and Experimental Design Rats were randomly assigned to one of five groups. One group of rats (n=8) with heat stroke received saline treatment (2 ml/kg body wt, 0.9% saline, intravenously (i.v.)) at the onset of heat stroke. Heat stroke was induced by exposing the animals to an ambient temperature of 43 °C (with a relative humidity of 60% in a temperature-controlled chamber). The moment in which MAP and local CBF began to sharply decrease from their peak levels was arbitrarily defined as the onset of heat stroke. The interval between the initiation of heat stroke onset and animal cardiac arrest were taken as values of survival time (ST). Another three group of rats (n=8) with heat stroke respectively received DXM (4 mg/kg, i.v.), mannitol (10%), 1 ml/kg i.v., and DXM (4 mg/kg) together with mannitol (10%, 1 ml/kg) i.v. at the onset of heat stroke. The dose of DXM or mannitol for this study is accordance with the previous studies of heat stroke.19,20) The other group of rats (n=8) were normothermia, control rats which were exposed to an ambient temperature of 24 °C for at least 90 min to reach thermal equilibrium. Their colon temperature were maintained at about 36 °C using the electric thermal mat before the start of experiments. The rats of these groups were continually monitored from physiological parameter (such as TCo, MAP, HR, and CBF) and ST during heat stroke. The ST of the normothermic control rats (kept at 24 °C) is over 8 h, the value of ST in this study is expressed as “>480 min” (as shown in Table 1).

Table 1. Effects of Heat Exposure (HE; Tc=43 °C) on Survival Time in Rats Treated with Normal Saline (NS), in Rats Treated with Dexamethasone (DXM), in Rats Treated with Mannitol, and in Rats Treated with the Combined Agent (DXM and Mannitol).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rats treated with NS and kept at 24 °C</td>
<td>&gt;480*†,‡,##</td>
</tr>
<tr>
<td>2. Rats treated with NS (2 ml/kg, i.v.) and kept at 43 °C</td>
<td>22±3*†,‡</td>
</tr>
<tr>
<td>3. Rats treated with DXM (4 mg/kg, i.v.) and kept at 43 °C</td>
<td>34±6*†,‡</td>
</tr>
<tr>
<td>4. Rats treated with mannitol (10%, 1 ml/kg, i.v.) and kept at 43 °C</td>
<td>24±4*†,‡</td>
</tr>
<tr>
<td>5. Rats treated with DXM (4 mg/kg, i.v.)+mannitol (10%, 1 ml/kg, i.v.) and kept at 43 °C</td>
<td>107±8*†,‡</td>
</tr>
</tbody>
</table>

NS or drugs were administered immediately after the onset of heat stroke. Values are the means±S.E.M. of 8 rats per group. Groups 2—5 exposed to 43 °C had heat exposure withdrawn at the onset of heat stroke. *p<0.05, compared with the corresponding control values (rats kept at 24 °C; treatment group 1, normothermic control rats) (one-way ANOVA, followed by Duncan’s test). †p<0.05, compared with the corresponding control values (treatment 2) (one-way ANOVA, followed by Duncan’s test). ‡p<0.05, compared with the corresponding control values (treatment 3) (one-way ANOVA, followed by Duncan’s test). #p<0.05, compared with the corresponding control values (treatment 4) (one-way ANOVA, followed by Duncan’s test).

Measurements of Cellular Ischemia and Injury Markers After cunnalation of vessels, the animal’s head was mounted on 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 corpus striatum according to the atlas and coordinates of Paxinos and the coordinates of Paxinos and Watson (1982).23) As the methods described previously24,25–27) an equilibrium period of 2 h without sampling was allowed after probe implantation. The dialysis probe was perfused with Ringer’s solution (147 mM Na⁺, 2.2 mM Ca²⁺, 4 mM K⁺, pH 7.0) at 2 μl/min using a CMA/100 microinfusion pump. Dialysates were collected every 10 or 20 min in a CMA140 fraction collector. Aliquots of dialysates (5 μl) were injected onto a CMA600 Microdialysis Analyzer (Carnegie Medicine) for measurement of lactate, glycerol, pyruvate and glutamate. Four analytes can be analyzed per sample and the result is displayed graphically within minutes. The thermal experiments were started after showing stabilization in four consecutive samples.

The lactate/pyruvate ratio is a well-known marker of cell ischemia, that is, an inadequate supply of oxygen and glucose.24,25) Glycerol is a marker of how severely cells are affected by the ongoing pathology.26,27) Glutamate is released from neurons during ischemia and initiates a pathological influx of calcium leading to cell damage. It is an indirect marker of cell damage in the brain.28,29)
calibrated by dialysis of a known amount of the standard mixture, and recovery of all analyses was then determined. Brain concentrations of DA and 5-HT were calculated by determining each peak height ratio relative to the internal standard and were also corrected by each probe performance. The internal standard 3-methoxy-tyramine and standard mixtures were prepared fresh daily. The mobile phase was prepared by adding 60 ml of acetonitrile, 0.42 g of sodium dodecyl sulfate (SDS) (2.2 mM), 200 g of sodium citrate (30 mM), 10 mg of ethylenediaminetetraacetic acid (EDTA) (0.027 mM), and 1 ml of diethyamine in double-distilled water.

**Production of Hydroxyl Radical Production Monitoring** The concentrations of hydroxyl radicals were measured by a modified procedure based on the hydroxylation of sodium salicylate by hydroxyl radicals, leading to production of 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-DHBA. These two compounds were then measured in dialysates by HPLC with electrochemical detection. A Ringer’s solution (0.860 g of NaCl, 0.030 g of KCl and 0.033 g of CaCl2 per 100 ml) containing 2 mmol/l sodium salicylate was perfused through the microdialysis probe at a constant flow rate (1.2 µl/min). A reverse-phase C18 column [phase II; particle size, 3 µm; 100×3.2 mm (length×internal) diameter]; Bio-Analytic Systems, West Lafayette, IN, U.S.A.] was used, and the mobile phase consisted of a mixture of 75 mmol/l monochloroacetic acid, 0.7 mmol/l disodium EDTA, 1.5 mmol/l sodium 1-octanesulphonate and 45 ml/l acetonitrile (pH 3.0). The retention times of 2,3-DHBA and 2,5-DHBA were 9.07

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**Fig. 1.** Effects of Heat Stress (Ambient Temperature; \( T_a = 43 ^\circ C \) for 70 min) on Colonic Temperature (\( T_c \)), Mean Arterial Pressure (MAP), Heart Rate (HR), and Cerebral Blood Flow (CBF), and Extracellular Levels of Glutamate, Glycerol, and Lactate/Pyruvate Ratio of Cerebral Corpus Striatum in Normothermic Control Rats (—), Normal Saline (NS)-Treated Rats (—), Dexamethasone (DXM)-Treated Rats (—), Mannitol-Treated Rats (—) or the Combined Agent-Treated Rats (—). 

The arrow indicates time of heat stroke onset and drug injection. * \( p < 0.05 \), compared with those of normothermic controls (ANOVA followed by Duncan’s test). † \( p < 0.05 \), compared with NS-treated group (43°C) (ANOVA followed by Duncan’s test).
and 5.44 min respectively.

**Measurement of Plasma TNF-α** The blood samples were acquired 85 min after the initiation of heat exposure in heat stroke rats or the equivalent time in normothermic controls. Five milliliters of blood was withdrawn from the femoral vein of each rat for measurement of plasma TNF-α. Blood samples were allowed to clot for 2 h at room temperature or overnight at 2—8 °C before centrifuging for 20 min at approximately 2000×g. Serum was quickly removed from these plasma samples and assayed for TNF-α immediately. The DuoSet Enzyme-linked Immunosorbent Assay (ELISA) Development System rat TNF-α kit (R&D Systems, Minneapolis, MN, U.S.A.) was used for measuring the levels of active rat TNF-α present in serum. This assay employs the quantitative colorimetric sandwich ELISA technique.

**Neuronal Damage Score** At the end of each experiment, the brain was removed, fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Serial (10 μm) sections 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—3, modified from the grading system of Pulsinelli et al. (1982), in which 0 is normal, 1 means that ca. 30% of the neurons are damaged, 2 means that ca. 60% of those neurons are damaged, and 3 means that 100% of those 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 means±S.E.M. Repeated-measures ANOVA is conducted to test the treatment by time interactions and the effect of treatment over time on each score. The Duncan multiple-range test is used for post hoc multiple comparisons among means. A p value less than 0.05 is calculated as statistical significance.

**RESULTS**

**ST of Heat Stroke** ST of DXM-treated and mannitol-treated rats after heat stroke are respectively 34±6 min and 24±4 min, neither is statistically different from that of the normal saline (NS)-treated rats. In contrast, ST (values are 107±8 min) in heat stroke rats with combined agent is greater increase than that in heat stroke rats with either DXM or mannitol treatment alone (as shown in Table 1).

**Cerebral Ischemia and Injury Markers** Figure 1 shows the effects of a high $T_a$ (43 °C) on $T_{CO}$, MAP, HR, CBF, glutamate, glycerol and lactate/pyruvate ratio in 8 rats with the combined treatment at the onset of heat stroke. Another 8 rats treated with 0.9% saline expose to the same $T_a$ served as heat stroke controls. The other 8 rats treated with 0.9% saline expose to the room temperature (24 °C) served as normothermic controls. After heat stroke, the values of MAP and CBF are significantly decreased in the NS-treated group, in the DXM-treated group, or in the mannitol-treated group; however, it displays better maintenance in these two parameters in the combined treatment group. At the same time, the high concentration of glutamate and high values of lactate/pyruvate ratio, which are viewed as the markers of cerebral ischemia, are observed in rats’ brain of the NS-treated group after heat stroke, and the high levels of glycerol, which is viewed as the marker of cellular injury in rats’ brain, are also obtained in the same group. These heatstroke-induced conditions of cerebral ischemia and cellular injury can be alleviated by immediate treatment with the combined agent, but not by treatment with DXM or mannitol alone.

**The Striatal Levels of DA, 5-HT, and Hydroxyl Radical Production** Figure 2 reveals that the levels of DA, 5-HT and total production of dihydroxybenzoic acid (DHBA, indirectly stood for production of hydroxyl radicals) are increased in saline-treated group after heat stroke, and these results agree with that of other previous studies. Nevertheless, in our present results, these high levels in cerebral corpus striatum of rats are apparently diminished by treatment with the combined agent, not by treatment with DXM or
mannitol alone, at the onset of heat stroke.

The Plasma Levels of TNF-α Figure 3 summarizes the values of plasma TNF-α in rats (n=8) kept at 24 °C for 85 min, in normal saline (NS)-treated rats (n=8) kept at 43 °C for 70 min plus 15 min room temperature exposure, and in the rats with combined treatment (n=8) kept at 43 °C for 70 min plus 15 min room temperature exposure. The values of plasma TNF-α are significantly increased in NS, DXM, or mannitol-treated rats kept at 43 °C as compared with those rats kept at room temperature (24 °C). Additionally, the values of plasma TNF-α in the rats with combined treatment kept at 43 °C are significantly lower than those of NS-treated rats kept at 43 °C.

Neuronal Damage Score In separate studies, the heat stroke-induced severe neuronal damage is estimated by staining with hematoxylin and eosin for microscope evaluation. Pictures are showed in Fig. 4, and data are shown in the Table 2. The cerebral striatal section of heat stroke rats treated with NS displays severe ischemia neuronal damage, as well as the section of heat stroke rats treated with DXM or mannitol alone dose (as shown in Fig. 4B). The values of neuronal damage score in heat stroke rats treated with NS were 2.73±0.28 after heat stroke. Additionally, the values of neuronal damage score in heat stroke rats treated with DXM or mannitol alone were respectively 2.66±0.31 or 2.88±0.29 after heat stroke. In contrast, in rats treated with the combined agent, the values are decreased to 1.55±0.18, as shown in Table 2. As a result of this treatment, compared with saline-treated rats, the rats with combined treatment has a lower value of neuronal damage score and accompanies by a higher value of striatal CBF 20 min after the onset of heat stroke (Fig. 1). However, the heatstroke-induced neuronal injury and cerebral ischemia were not affected in the heat stroke controls group after heat stroke.

DISCUSSION

Previous studies have shown that systemic pretreatment with mannitol or DXM alone before heat stress could in-
crease the ST in rats by attenuating plasma levels of free radicals or interleukins\textsuperscript{19,20}, however, there are less studies showing the immediate treatment with the combined agent at onset of heat stroke. In fact, it will be more meaningful if the combined treatment shows neuroprotection after heat stroke attacks. After all, it is not practical to give pretreatment in clinical practice. Moreover, the hemodynamic, histological and biological changes by DXM, mannitol, or the combined treatment immediately after the onset of heat stroke were not observed in detail.

Pretreatment with DXM or high dose of DXM is shown to improve survival during heat stroke by reducing overproduction of cytokines.\textsuperscript{19} Although our previous results\textsuperscript{19} have shown an insignificant therapeutic effect of DXM (4 mg/kg) administered immediately after the onset of heat stroke alone, the combined agent dose provides a better therapeutic effect for rats with heat stroke in the present study. Additionally, mannitol has been used extensively on brain injury, and various clinical and experimental studies have demonstrated that single doses of mannitol reduce increased intracranial pressure.\textsuperscript{32} Although mannitol cannot cross the blood brain barrier, it has a potent anti-edema action by drawing water from intracellular spaces into the intravascular space.\textsuperscript{33} As a result, the brain has been thought to be rescued by lowering intracellular pressure, and thus by lowering intracranial pressure. Moreover, one of the nonosmotic mannitol effect is the protection against free radical-mediated ischemic cell damage.\textsuperscript{34} Indeed, in agreement with previous study of heat stroke, pretreatment of rats with mannitol attenuated heat stroke-induced free radical production and decreased intracranial pressure, which result in restoration of normal cerebral perfusion.\textsuperscript{20} Based on these concepts, these raise that the combination of an anti-inflammatory agent and an osmotherapeutic agent/a radicals scavenger agent may provide a better survival for victims with heat stroke.

In this study, administration of the combined agent appears more effective to prolong the ST in rats with heat stroke, by comparison to acute treatment with DXM or mannitol alone (shown in Table 1). Similarly, in agreement with the present results, treatment of the combined agent (DXM and mannitol) can also offer beneficial amelioration from ischemic condition and therapeutic influence in the focal and global brain ischemia experiments.\textsuperscript{22,35,36} There is evidence that cerebral ischemia (due to arterial hypotension and intracranial hypertension) may be one of the major causes to induce further damage after heat stroke onset.\textsuperscript{14,19,37} After heat stroke induction, the CBF is instantly drops from highest peak, and it is concomitant with significant increments of cerebral ischemia and injury indexes, as shown in Fig. 1. The lactate/pyruvate ratio is a well known marker of cellular ischemia, whereas glycerol is a marker of how severely cells are affected by ongoing pathology.\textsuperscript{24,25,27} Excessive accumulation of glutamate has been shown in ischemic brain tissue.\textsuperscript{28,29} Indeed, both present and previous results\textsuperscript{3,37} have demonstrated that extracellular levels of glutamate, glycerol and lactate/pyruvate in ischemic brain are greater in heat stroke rats compared with those of normothermic controls. Meanwhile, evidences of histopathological morphology and neuronal damage score also reveal severe neuronal damage (shown in Fig. 3, Table 2) in heat stroke rats. However, as shown in the present results, all these heat stroke-induced cerebral ischemia and injury can be alleviated by acute treatment with the combined agent.

It has been reported\textsuperscript{9,15,38} that the increased DA, 5-HT and glutamate in the brain during the rat heat stroke were mediated in the development of neuronal damage. Cerebral DA, 5-HT ot/and glutamate overload resulting from arterial hypotension and intracranial hypertension might be responsible for occurrence of central nervous system syndromes associated with heat stroke.\textsuperscript{15,38} Systemic administration of dopaminergic or serotoninergic nerve depletors or receptor antagonists, or glutamate receptor antagonists cloud protect against ischemic neuronal injury in experimental heat stroke.\textsuperscript{11,20} Evidence had accumulated to suggest that heat stroke-induced cerebral ischemia and neuronal damage might be associated with an increased production of free radicals, specifically hydroxyl radicals.\textsuperscript{11} Pretreatment with hydroxyl radicals scavengers, such as \textit{α}-tocopherol, prevented production of hydroxyl radicals, reduced lipid peroxidation and ischemic neuronal damage in several brain areas (corpus striatum, hypothalamus and cortex) of rats exposed to heat stroke and prolonged subsequent survival.\textsuperscript{20} In brief, as demonstrated by Chang \textit{et al.}, after the onset of heat stroke, cessation or reduction of blood flow to the brain induced neuronal damage. This neurotoxic cascade involved overproduction of glutamate, DA, and 5-HT as well as oxidative stress in the brain.\textsuperscript{5} Likewise, in the present study, heat stroke also produces similar increases in cerebral striatal DA, 5-HT, glutamate and hydroxyl radical productions in heat stroke rats. Indeed, according to our present findings, the heat stroke-induced high levels of DA, 5-HT, glutamate, and hydroxyl radicals in rats’ corpus striatum can be prevented by acute treatment with the combined agent. This probably implies that the immediate

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### Table 2. Neuronal Damage Score of Corpus Striatum from Normothermic Control Rats and Rats in Different Heat Stroke Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neuronal damage score (0—3) corpus striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normothermic control rats</td>
<td>0.18±0.15</td>
</tr>
<tr>
<td>2. Heat stroke rats treated with NS (2 ml/kg b.w., i.v.)</td>
<td>2.73±0.28*</td>
</tr>
<tr>
<td>3. Heat stroke rats treated with both DXM (4 mg/kg b.w., i.v.)</td>
<td>2.66±0.31*</td>
</tr>
<tr>
<td>4. Heat stroke rats treated with mannitol (10%, 1 ml/kg b.w., i.v.)</td>
<td>2.88±0.29*</td>
</tr>
<tr>
<td>5. Heat stroke rats treated with both DXM (4 mg/kg b.w., i.v.) and mannitol (10%, 1 ml/kg b.w., i.v.)</td>
<td>1.55±0.18*</td>
</tr>
</tbody>
</table>

Normal saline (NS), dexamethasone (DXM), or mannitol were administered immediately after the onset of heat stroke. Values are the means±S.E.M. of 8 rats per group. Neuronal damage score were evaluated by a Kruskal–Wallis non-parametric test followed the Mann–Whitney U-test when appropriate. *p<0.05, compared with normothermic control rats. September 2010 1527
administration of the combined agent during heat stroke may be mediated with the decrements of cerebral monoamines and oxidative stress to prolong the ST and improve the cerebral neuronal damage in rats. Meanwhile, rats immediately treated with DXM or mannitol alone and rats treated with NS at the onset of heat stroke had similar results in these physiological and biochemical parameters. These treatments displayed insignificant therapy effects on the heat stroke-induced arterial hypotension, cerebral monoamines and hydroxyl radical production overload, systemic inflammation, and severe cerebral ischemia and injury, as shown in Figs. 1—3 and Table 2.

Furthermore, Nakamura et al. demonstrated that TNF-α might play a pathophysiological role in the progression of acute heart failure, and septic shock could be mimicked by systemic administration of TNF-α. As for heat stroke in our previous studies, the plasma levels of TNF-α were greatly elevated during heat stroke in rats. The increased levels of the inflammatory cytokines in the peripheral blood stream were showed to be well related to the severity of heat stroke. Hence, the plasma levels of TNF-α in heat stroke rats are also observed in this study. As it is shown in the present results, we further showed that the combined agent treatment significantly ameliorated overproduction of TNF-α in the plasma during heat stroke. The immediate administration of this combined agent might exert its protective effects by attenuating the increased plasma level of TNF-α during heat stroke.

In the present study, the heat stroke-induced increases in arterial hypotension, cerebral ischemia and neuronal damage are associated with elevated levels of DA, 5-HT, glutamate and hydroxyl radicals in rat brain, and increased circulating TNF-α in plasma. The immediate systemic treatment with the combined agent (both DXM and mannitol), in addition to attenuating the elevating levels of TNF-α in plasma, diminishes monoamines, glutamate, and hydroxyl radicals formation, and ischemia injury in brain, and improves ST in rats with heat stroke. These results demonstrated that although acute treatment with the combined agent dose not entirely prevent the heat stroke syndrome, an attenuation of the syndrome and prolongation of survival time are observed.

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