ORIGINAL ARTICLE

Combination Treatment with Gua Sha and Blood-letting Causes Attenuation of Systemic Inflammation, Activated Coagulation, Tissue Ischemia and Injury during Heatstroke in Rats*

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ABSTRACT **Objective**: Gua Sha and Blood-letting at the acupoints were Chinese traditional therapies for heatstroke. The purpose of present study was to assess the therapeutic effect of Gua Sha on the DU Meridian and Bladder Meridian combined with Blood-letting acupoints at Shixuan (EX-UE 11) and Weizhong (BL 40) on heatstroke. **Methods**: Anesthetized rats, immediately after the onset of heatstroke, were divided into four major groups: Gua Sha group, Blood-letting group, Gua Sha combined with Blood-letting group and model group. They were exposed to ambient temperature of 43 ℃ to induce heatstroke. Another group of rats were exposed to room temperature (26 ℃) and used as normal control group. Their survival times were measured. In addition, their physiological and biochemical parameters were continuously monitored. **Results**: When rats underwent heatstroke, their survival time values were found to be 21–25 min. Treatment of Gua Sha combined with Blood-letting greatly improved the survival time (230±22 min) during heatstroke. All heatstoke animals displayed and activated coagulation evidenced by increased prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, and decreased platelet count, protein C. Furthermore, the animals displayed systemic inflammation evidenced by increased the serum levels of cytokines interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α) and malondialdehyde (MDA). Biochemical markers evidenced by cellular ischemia and injury/dysfunction included increased plasma levels of blood urea nitrogen (BUN), creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) were all elevated during heatstroke. Core temperatures (Tco) were also increased during heatstroke. In contrast, the values of mean arterial pressure were significantly lower during heatstroke. These heatstroke reactions were all significantly suppressed by treatment of Gua Sha and Blood-letting, especially the combination therapy. **Conclusion**: Gua Sha combined with Blood-letting after heatstroke may improve survival by ameliorating systemic inflammation, hypercoagulable state, and tissue ischemia and injury in multiple organs.

KEYWORDS Gua Sha, Blood-letting, heatstroke, ischemia, inflammation, coagulation, Chinese medicine

The consequence of a thermal trauma such as a heat wave is disastrous. It is becoming a serious clinical problem when humans and mammals are exposed to high ambient temperatures. Heatstroke is a life-threatening disease, which is a typical kind of thermal trauma results from thermoregulatory failure coupled with an exaggerated acute phase response. However, few pharmacologic treatment has been proven to be effective.(1) In severe cases with multiple organ dysfunction, the mortality remains high.(2)

Heatstroke is characterized by hyperthermia, multiple organ failure, and central nervous system disorders.(3) The syndrome is comprised of a wide range of thermoregulatory, coagulation, immune, and tissue injury. Both disseminated intravascular coagulation and inflammatory activation are believed to be responsible for multiple organ dysfunction and death.(4) Diffuse bleeding, hemorrhagic necrosis, and widespread microthrombi occurred in most tissues of the body in patients with heatstroke.(5,6) Circulating levels of inflammatory

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cytokines are greatly elevated and related to severity and outcome during heatstroke.[7,8]

Gua Sha, therapeutic surface friction that intentionally raises transitory petechiae and ecchymosis, is a traditional East Asian healing technique also known as scraping, coining, and spooning. Gua Sha therapy is based on the Chinese principles of the 12 meridians and collaterals. It extravasates blood and metabolic waste that congest in surface tissues and muscles, promoting normal circulation and metabolic processes.[9,10] It is generally regarded as effective for acute or chronic pain and for mild to severe conditions such as colds, flu, fever, heatstroke, and myalgia.[11]

Blood-letting at the acupoints by a three-edged needle is also a traditional Chinese medicinal therapy, which has the function of dispelling blood stasis and assisting resuscitation, promoting the flow of qi and blood in meridians.[12,13] The combination of Gua Sha on the back (DU Meridian and Bladder Meridian) together with Blood-letting at Shixuan (EX-UE11) and Weizhong (BL40) has been proven to be effective to heatstroke in clinic and ancient literature. However, there were few reports in present literature and the mechanisms underlying the effects remain unclear.

Diffuse bleeding, hemorrhagic necrosis, and widespread microthrombi occurred in most tissues of the body in patients with heatstroke. Evidence has accumulated to indicate that rodents share with humans almost the same heatstroke syndromes such as hyperpyrexia, hypotension, activated inflammation, and multi-organ dysfunction.[14,15] Both disseminated intravascular coagulation and inflammatory activation are believed to be responsible for multiple organ dysfunction and death. Circulating levels of inflammatory cytokines e.g., interleukin-1 β (IL-1 β) and tumor necrosis factor α (TNF-α) are greatly elevated and related to severity and outcome during heatstroke.[16]

Based on these concepts, we proposed whether applications of the treatments of Gua Sha or Blood-letting or combined Gua Sha with Blood-letting immediate had efficacy to elongate the survival time and improve the heatstroke-induced circulatory shock. In addition, current experiments were performed to assess their therapeutic effects on the inflammatory and hemostatic responses, cellular ischemia and injury during heatstroke.

METHODS

Experimental Animals

Male Sprague-Dawley rats (285 ± 18 g) were obtained from the Center of Laboratory Animal Care of Wenzhou Medicine College, China. Rats were housed individually at a temperature of 22 ± 1 ℃ with a 12 h light/dark cycle and were fed food and water *ad libitum*. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinch throughout the course of all experiments (about 8 h) following a single dose of urethane (1.4 g/kg body weight, intraperitoneal). At the end of the experiments, control rats and any other rats that had survived heatstroke were killed with an overdose of urethane. All experiments were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical College.

Surgery and Physiological Parameter Monitoring

The right femoral artery and vein of rats were cannulated with polyethylene tubing (PE 50) under sodium pentobarbital anesthesia for blood pressure monitoring and drug administration. The core temperature (Tco) was monitored continuously by a thermocouple, and mean arterial pressure (MAP) and heart rate (HR) were continuously monitored with a pressure transducer.

Experimental Groups

A completely randomized design was used. Rats were randomly divided into five groups and all the rats were unhaired in the back before the experiment. One group of rats were normal control rats which were exposed to an ambient temperature of 26 ℃ for at least 90 min to reach thermal equilibrium (normal control group, *n*=16). In the following heatstroke groups of rats, all animals were exposed to 43 ℃ for 70 min at the onset of heatstroke and then allowed to recover at room temperature (26 ℃). One group of rats following heatstroke without treatment (model group, *n*=16). Another group then received the combination treatment of Gua Sha and Blood-letting (GB group, *n*=16). The third group of rats following heatstroke then received the treatment of Gua Sha (Gua Sha group, *n*=16). The fourth group of heatstroke rats received the treatment of Blood-letting (Blood-letting group, *n*=16).

Heatstroke was induced by exposing the animals to an ambient temperature (Ta) of 43 ℃

with a relative humidity of 60% in a temperature-controlled chamber).[17] The moment in which MAP and HR began to sharply decrease from their peak levels was arbitrarily defined as the onset of heatstroke.[18,19] The interval between the initiation of heatstroke onset and animal death were taken as values of survival time. Their physiological parameters were continuously recorded for up to 480 min (at the end of experiment). Their Tcos were maintained at about 36 ℃ using the electric thermal mat before the start of experiments. The rats of these groups were continually monitored from physiological parameters such as Tco, MAP and HR and survival time during heatstroke.

Manipulation of Gua Sha and Blood-letting

Gua Sha therapy was administered after initiation of heat stress, which was applied on the back at the centerline where DU Meridian and Bladder Meridian were located. The tool for Gua Sha was a slice of water-buffalo horn. A smooth, rounded edge was press-stroked into the flesh enough to contact the fascial layer. The scraping line was about 3 inches long. Scraping was repeated in one direction until the petechiae appeared, typically 50 strokes before producing ecchymosis (Figure 1).

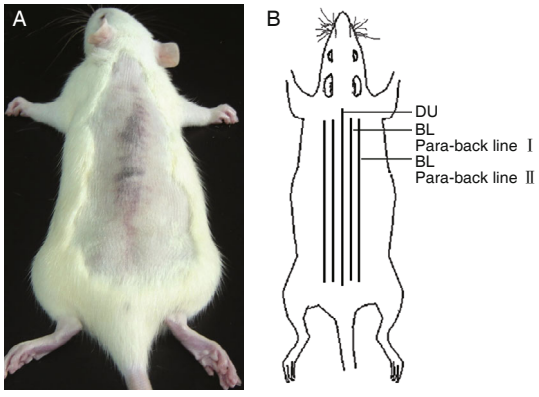

Figure 1. Drawing Showing Where Gua Sha Was Applied

Notes: A: Gua Sha therapy produces ecchymosis; B: schematic diagrams of the DU Meridian and Bladder Meridian in rat

Blood-letting at the acupoints was applied after Gua Sha therapy. Shixuan is including ten points on forelimbs. Pricking Shixuan and Weizhong bilateral with a three-edged needle induced bleeding, then, squeezed the skin around the pricked spot, two drops of blood (approximate 0.1 mL) from Shixuan and two drops of blood from Weizhong. The total volume of Blood-letting from Shixuan and Weizhong is approximate 1 mL.

Biochemical Analysis

Blood samples at 0, 70, and 85 min after initiation of heat stress were drawn by arterial femoral cannulation. The plasma levels of activated partial thromboplastin time (aPTT), prothrombin time (PT), 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 LH 750, Miami, USA) whereas the plasma levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) were determined by spectrophotometry (HITACHI 7600, Tokyo, Japan). The blood urea nitrogen (BUN) and serum creatinine were measured by the Randox® assay kit (*Randox Laboratories* Ltd., United kingdom). For determination of protein C, to obtain the plasma, one part of sodium citrate solution (0.11 mol/L) was mixed carefully with nine parts of venous blood, avoiding the formation of foam. Protein C in the sample was activated by a specific snake 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).

Measurement of Serum Malondialdehyde Levels

The biochemical kits (Beyotime Institute of Biotechnology, China) were used to measure lipid peroxidation end product malondialdehyde (MDA). Blood samples at 85 min after initiation of heat stress were drawn by arterial femoral cannulation. The 0.25 mL of serum was added to 25 μL of 0.2% butylated hydroxytoluene (BHT) and 12.5 μL of 10 N NaOH (to adjust to pH13) and incubated at 60 ℃ for 30 min in a shaking water bath. To this was added 1.5 mL of 0.44 mol/L (or 7.2%) trichloroacetic acid (TCA) containing 1% KI. Then the mixture was placed in ice for 10 min and centrifuged at 1,000 × *g* for 10 min. After that, 1 mL of the supernatant was added to 0.5 mL of 0.6% thiobarbituric acid (TBA), and the mixture was heated at 95 ℃ for 30 min. After cooling the mixture was extracted with 1.5 mL of n-butanol, and 20 μL of the butanol layer was injected to a C-18 (4.6 mm × 150 mm) column fitted with a guard and eluted at 1 mL/min by using 65% (v/v) 50 mm KH_2PO_4-KOH and 35% (v/v) methanol with spectrophotometric (532 nm) detector.

Measurement of Serum IL-1β and TNF-α Levels

Blood samples at 85 min after initiation of heat stress was withdrawn from the veinal femoral cannulation of each rat for measurement of serum IL-1β or TNF-α. Blood samples were allowed to clot for 2 h at room temperature or overnight at 2–8 ℃ before centrifuging for 20 min at approximately 2000 × *g*. Serum was quickly removed from these plasma samples and assayed for IL-1β or TNF-α immediately. The DuoSet enzyme-linked immunosorbent assay (ELISA) development system rat IL-1β or TNF-α kit (R&D systems, Minneapolis, MN, USA) was used for measuring the levels of active rat IL-1β or TNF-α present in serum. This assay employs the quantitative colorimetric sandwich ELISA technique.

Statistical Analysis

Data was presented as the mean ± standard deviation. Repeated-measures analysis of variance was used for factorial experiments, whereas Duncan's multiple-range test (multi-time point experiments) was used for post hoc multiple comparisons among means. Student's *t*-test was used when only two groups were compared. A *P*-value <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS Inc., Chicago.IL, USA).

RESULTS

Gua Sha and Blood-letting Improves Survival Time in Heatstroke

All heatstroke rats had exposure (43 ℃) withdrawn about at 70 min and were then allowed to recover at room temperature (24 ℃). Normal control rats were killed approximately 480 min after the initiation of experimentation with an overdose of sodium pentobarbital. It can be seen that the survival time was found to be 23 ± 2 min (*n*=8) for heatstroke model rats. At the time point of onset of heatstroke, treatment with Blood-letting increased the survival time to 56 ± 17 min, while treatment with Gua Sha significantly increased the survival time to 170 ± 34 min. While combination therapy (Gua Sha + Blood-letting) at the onset of heatstroke increased the survival time during heatstroke to 230 ± 22 min.

Gua Sha and Blood-letting Attenuates Heatstroke-induced Physiologic Dysfunction

Figure 2 summarizes the values of time-course changes in Ta, Tco, MAP and HR for normal control, model, Gua Sha, Blood-letting or the GB groups. During heatstroke, the Tco was all significantly higher at 80 to 100 min after the start of heat exposure than they were in normal control group. In contrast, the values for MAP and HR were significantly lower than those of normal control group. The combination of Gua Sha and Blood-letting at the onset of heatstroke significantly attenuated the heat stress.

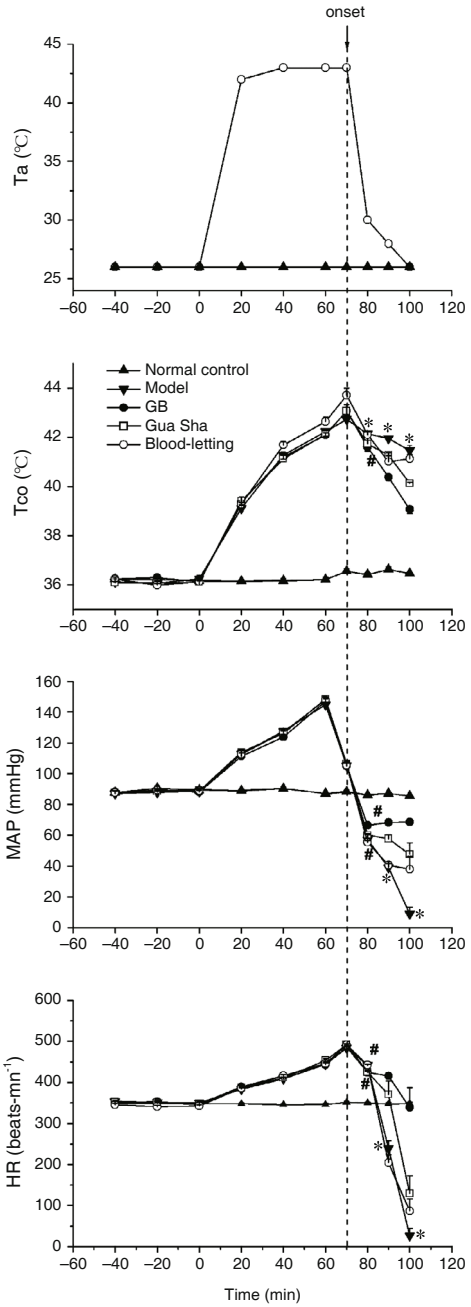

Figure 2. Effects of a High Ambient Temperature (Ta=42 ℃) on Tco, MAP and HR in Rats in Different Groups

Notes: *P<0.05, compared with normal control group; △P<0.05, compared with model group; ▲P<0.05, compared with GB group; the arrow indicates the time point of heatstroke onset

Gua Sha and Blood-letting Attenuates Heatstroke-induced Hypercoagulable State

It can be seen that aPTT, PT, and D-dimer values during heatstroke were all significantly higher at 70–85 mins after the initiation of heat stress than they were for the normal control group. In contrast, the values for plasma levels of both protein C and platelet count were all significantly lower than those of the normal control group. Resuscitation with (Gua Sha or Blood-letting), or the combination therapy, at the time point of onset of heatstroke, significantly attenuated the increased plasma levels of both aPTT and PT as well as the decreased plasma levels of both protein C and platelet count during heatstroke. In addition, it was found that the combination therapy, at the time point of heatstroke onset significantly reduced the heatstroke induced increased levels of D-dimer in plasma (Table 1).

Gua Sha and Blood-letting Attenuates Heatstroke-induced Cellular Injury and Organ Dysfunction

It can be seen from the Table 2 that the BUN values and plasma levels of creatinine, SGOT, SGPT, and ALP during heatstroke were all significantly higher at 70 to 85 min after the start of heat exposure than they were for normal control. Resuscitation with Gua Sha or Blood-letting, or the combination therapy, 70 min after initiation of heat exposure significantly attenuated the heat stress-induced increased plasma levels of BUN, creatinine, SGOT, SGPT and ALP. The BUN values and plasma levels of creatinine, SGOT, SGPT and ALP measured for rats treated with Gua Sha or Blood-letting or the combination therapy were indistinguishable from those of normothermic rats without treatment (Table 2).

Gua Sha and Blood-letting Reduces the Levels of MDA, IL-1β and TNF-α in Peripheral Blood Stream During Heatstroke

It can be seen that the serum IL-1β, TNF-α, and MDA levels following heatstroke were all significantly higher at 20 min after the onset of heatstroke than those in the normal controls. The immediate treatment with Gua Sha or Blood-letting alone and Gua Sha + Blood-letting at the onset of heatstroke attenuated the heatstroke-induced increased serum lipid peroxidation, as well as attenuating it increased the serum levels of IL-1β and TNF-α. However, these serum levels were more significantly diminished by treatment with the combined agent immediately at the onset of heatstroke (as shown in Figures 3 and 4).

DISCUSSION

It was claimed that Gua Sha therapy could extend blood vessels ends, improving partial blood circulation and metabolism.[20] Many researchers have noted that Gua Sha therapy stimulates the surface skin,

Table 1. Plasma Levels of aPTT, PT, Protein C, D-dimer, and Platelet Counts in Different Groups of Rats Measured at Different Times after the Initiation of Heat Exposure in Heatstroke Rats (n=8, $\bar{x} \pm s$)

Group	Time (min)	aPTT (s)	PT (s)	Protein C (μg/L)	D-dimer (μg/L)	Platelet counts (×1000 / μL)
Normal control	0	25.13±0.23	9.01±0.12	3.06±0.03	46.75±0.25	1117.50±4.95
	70	24.75±0.25	9.18±0.05	3.10±0.03	46.50±0.42	1112.13±4.29
	85	25.25±0.25	9.14±0.05	3.09±0.02	47.50±0.38	1110.63±3.02
Model	0	25.38±0.18	9.06±0.06	3.10±0.03	46.75±0.25	1114.13±4.29
	70	94.75±0.88*	13.41±0.08*	2.08±0.04*	81.88±1.47*	713.25±2.58*
	85	105.62±0.86*	14.79±0.09*	0.54±0.04*	109.62±1.60*	709.63±2.52*
GB	0	24.88±0.30	9.01±0.05	3.13±0.02	46.88±0.23	1111.25±0.82
	70	92.88±1.13	13.39±0.08	2.00±0.03	82.00±0.60	708.25±1.57
	85	42.63±0.60△	9.59±0.05△	1.76±0.05△	69.50±2.24△	993.75±12.65△
Gua Sha	0	24.75±0.25	9.08±0.04	3.11±0.03	47.13±0.30	1111.63±1.21
	70	93.25±0.73	13.25±0.08	2.05±0.03	82.25±0.56	710.50±1.50
	85	86.38±2.16△▲	11.30±0.27△▲	1.36±0.10△	98.88±1.65△▲	923.25±8.70△
Blood-letting	0	24.63±0.26	9.06±0.05	3.06±0.04	47.25±0.25	1111.50±0.98
	70	93.50±0.33	13.26±0.07	2.06±0.03	81.63±0.63	710.75±2.03
	85	96.00±1.66△▲	12.15±0.19▲	0.69±0.05△▲	92.75±1.89▲	855.88±16.68△▲

Notes: *P<0.05, compared with normal control group; △P<0.05, compared with model group; ▲P<0.05, compared with GB group

Table 2. Plasma Levels of Creatinine, BUN, SGOT, SGPT, and ALP in Different Groups at Different Times (n=8, $\bar{x} \pm s$)

Group	Time (min)	Creatinine (mg/dL)	BUN (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal control	0	0.43 ± 0.003	13.75 ± 0.37	165.75 ± 2.82	82.25 ± 1.19	459.12 ± 2.21
	70	0.43 ± 0.004	13.38 ± 0.53	170.12 ± 2.14	83.13 ± 0.79	461.88 ± 1.99
	85	0.42 ± 0.008	14.13 ± 0.44	168.88 ± 1.30	82.38 ± 0.73	464.25 ± 1.22
Model	0	0.43 ± 0.004	14.13 ± 0.52	160.25 ± 2.36	82.63 ± 0.94	461.00 ± 1.76
	70	0.98 ± 0.005*	27.50 ± 0.38*	464.25 ± 3.63*	229.50 ± 1.80*	645.25 ± 2.89*
	85	1.25 ± 0.014*	34.50 ± 0.42*	514.88 ± 3.78*	243.88 ± 1.98*	763.50 ± 5.02*
GB	0	0.43 ± 0.006	14.50 ± 0.42	166.38 ± 2.11	81.38 ± 0.65	462.25 ± 1.39
	70	0.98 ± 0.009*	27.75 ± 0.445*	460.62 ± 3.69*	227.50 ± 2.15*	646.50 ± 4.14*
	85	0.55 ± 0.012△	23.75 ± 0.59△	196.38 ± 2.51△	103.88 ± 1.85△	527.38 ± 4.14△
Gua Sha	0	0.42 ± 0.004	13.38 ± 0.53	164.00 ± 2.22	82.88 ± 1.19	461.62 ± 1.91
	70	0.98 ± 0.012*	28.50 ± 0.33*	462.88 ± 4.09*	230.75 ± 2.27*	645.12 ± 4.79*
	85	0.78 ± 0.021△▲	28.75 ± 0.53△	385.88 ± 4.18△▲	138.75 ± 3.32△	575.88 ± 4.45△
Blood-letting	0	0.42 ± 0.006	14.63 ± 0.38	165.75 ± 1.83	81.25 ± 1.28	458.88 ± 1.57
	70	0.98 ± 0.006*	27.63 ± 0.60*	461.00 ± 4.13*	228.62 ± 1.68*	638.00 ± 5.01*
	85	0.88 ± 0.018△▲	31.38 ± 0.32▲	403.88 ± 4.82△▲	201.25 ± 2.06△▲	596.50 ± 6.17△▲

Notes: *P<0.05, compared with normal control group; △P<0.05, compared with model group; ▲P<0.05, compared with the GB group

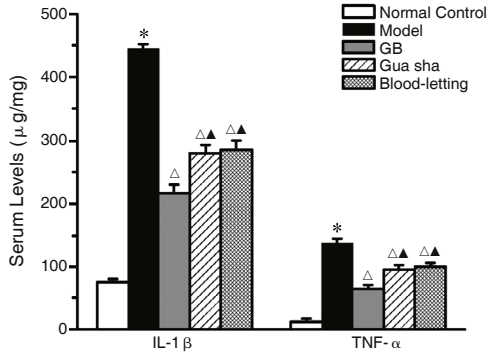

Figure 3. Serum Levels of IL-1 β and TNF-α

Notes: *P<0.05, compared with normal control group; △P<0.05, compared with model group; ▲P<0.05, compared with GB group; the blood samples were acquired after 85 min the initiation heat exposure in heatstroke rats or the equivalent time in normal control rats

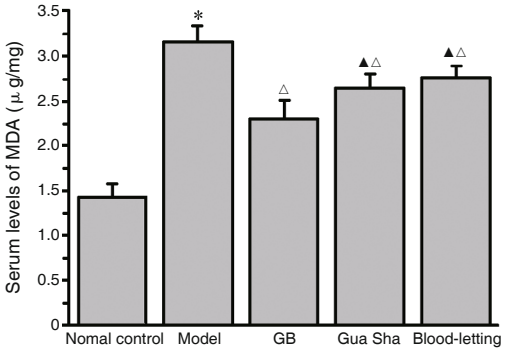

Figure 4. Serum Levels of MDA (n=8, $\bar{x} \pm s$)

Notes: *P<0.05, compared with normal control group; △P<0.05, compared with model group; ▲P<0.05, compared with GB group; the blood samples were acquired after 85 min the initiation heat exposure in heatstroke rats or the equivalent time in normal control rats

which facilitated the evacuation of heat and poisons. Their study showed that Gua Sha therapy caused a fourfold increase in microcirculation perfusion units in the treated area during the first 5–7.5 min following treatment and a significant increased in surface microcirculation during the entire 25 min of the study period following treatment.[9]

To expel out the vitiated blood, Blood-letting as a method of treatment is indicated in gouty arthritis, herpes zoster, acne, hordeolum, cervical spondylosis, abscess, inflammatory condition, cellulitis, painful ulcers, chronic ulcers resulting from snake bite, etc.[21] Blood-letting in our experiment means priching by a three-edged needle, not by phlebotomy or by leeches.

In a rat experimental model for heatstroke, as demonstrated in the present and previous results, hypercoagulable state (e.g., an increase in PT, aPTT, and D-dimer and a decrease in platelet count and protein C) and tissue ischemia and damage (e.g., an increase in blood urea nitrogen; plasma levels of creatinine, SGOT, SGPT, and ALP) occurred during heatstroke.[22,23] Apparently, the coagulopathy, inflammatory, and cardiovascular responses of a rodent model to heat stress matched closely the physiologic response to a certain extent in a primate model of heat stress. The heatstroke reactions resembled severe septic responses in many aspects: clots, not free circulating fibrin or fibrinogen.

Inflammatory activation has also been shown to be related to the severity of acute heart failure, septic shock, and circulatory shock.[24,25]

The serum concentrations of inflammatory cytokines (such as IL-1β and TNF-α) were elevated in humans and animals with heatstroke. Indeed, as it was shown in the present results, an increase of serum IL-1β and TNF-α levels was observed in heatstroke rats. The increase in the levels of these inflammatory cytokines was associated with arterial hypotension, cerebral ischemia and neuronal damage.

Currently, the treatment of heatstroke was cooling.[26,27] Despite adequate body hypothermia, heatstroke was often fatal. In addition, tissue injury continues to develop after cooling to normal body temperature in 25% of heatstroke patients.

Our results indicated that following heatstroke, arterial hypotension, increased serum levels of IL-1β, TNF-α and MDA, increased serum levels of PT, aPTT, and D-dimer and decreased in platelet count and protein C and increased in blood urea nitrogen, plasma levels of creatinine, SGOT, SGPT, and ALP. It was also obtained that the values of MAP were significantly lower during heatstroke. This raises of the values of MAP the possibility that might be a beneficial treatment for heatstroke. As a result of present study, we see that acute treatment with Gua Sha or Blood-letting alone at the onset of heatstroke can alleviate the heatstroke-induced arterial hypotension, and hydroxyl radical production overload, systemic inflammation, hypercoagulable state and tissue ischemia and damage. However, combination therapy has more effective therapy than treatment with Gua Sha or Blood-letting alone to maintain appropriate levels of MAP by attenuating the heatstroke-induced abnormal physiological and pathological changes, and results in prolongation in survival.

Therefore, Gua Sha and Blood-letting treatment showed partial effects on those parameters after heatstroke induction. According to our present results, it is reasonable to assume that acute combination treatment with Gua Sha and Blood-letting has a better effectiveness on reducing the heatstroke-induced damage, and augmenting survival time. Of course, this needs further investigation.

The results presented here indicate that the combination treatment with Gua Sha on the back (DU Meridian and Bladder Meridian) and Blood-letting acupoints at Shixuan and Weizhong therapy at the onset of heatstroke improved survival by ameliorating systemic inflammatory, hypercoagulable state, and tissue ischemia and injury in multiple organs.

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