Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

GSK2193874 treatment at heatstroke onset reduced cell apoptosis in heatstroke mice

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Received April 17, 2018; **Accepted** May 10, 2018; **Published** May 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.7.7

Abstract: Heatstroke is still a potentially fatal threat during summer heat waves, despite improved prevention and treatment. It is reported that the transient receptor potential vanilloid 4 (TRPV4) inhibitor may protect septicemia mice. Many aspects of heatstroke have been defined, from the sepsis-mimic inflammatory response to hyperthermia. Hence, TRPV4 may be a therapeutic target for heatstroke. The results in murine models of heatstroke verified that GSK2193874, as a selected TRPV4 inhibitor, was injected at heatstroke onset, and then reduced the reduction of core temperature, the death rate, wet/dry ratio of the lung, levels of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, coagulation indicators, the degree of organ injury, and caspase-3/7 activity ($P<0.05$). But GSK2193874 treatment before heat stress did not improve the symptoms of heatstroke mice. Therefore, TRPV4 should be involved in heatstroke-induced injury. Timely GSK2193874 administration may be useful to reduce heatstroke-induced injury. TRPV4 may be a potential new therapeutic target in fatal heatstroke.

Key words: Heatstroke; TRPV4; GSK2193874; Core temperature; Coagulation indicator.

Introduction

The increase of greenhouse gas emissions has induced more frequent summer heat waves, and as a result, heatstroke still threatens many people, such as children, the elderly, and construction personnel (1). However, despite decades of research , the ability of modern medicine is limited by the lack of pharmacological therapy of heatstroke (2-3). Recently, heatstroke was defined as a systemic inflammatory response to excessive hyperthermia, which results in cellular apoptosis and multiple organ dysfunction (4-6). Whether in animals or patients with severe heatstroke, the levels of inflammatory cytokines, including tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are increased. Histochemical analysis shows endothelial injury, the elevation of vascular permeability, disseminated intravascular coagulation, and widespread haemorrhage (7). The purpose of treatment is mainly to reduce the inflammatory response and improve prognosis, but it still cannot meet the clinical needs (7-9).

Transient receptor potential vanilloid 4 (TRPV4) is a vanilloid receptor-related non-selective cation channel that is widely expressed in many tissues, such as the lung, kidney, heart, and brain (10-13). It was reported that TRPV4 plays an important role in many physiological and disease processes (14-16). Inhibition of TRPV4 was shown to have an anti-inflammatory effect and vascular barrier stabilization (14). Therefore, the modulation of TRPV4 as a therapeutic target has been applied to studies on the treatment of multiple diseases (14,17).

TRPV4 is sensitive to heat stimuli (28-42°C) (18), cell swelling (19), and chemical stimuli (such as arachidonic acid metabolites) (17,20-21). It is presented as a polymodal sensory protein and contributes to the downstream physiological responses (17,22). During the heatstroke process, patients develop an up-to-42°C core body temperature (7), cell swelling (3), and a sepsis-mimic inflammatory response (6). Furthermore, the inflammation process is often accompanied by the generation of arachidonic acid metabolites (23). In addition, TRPV4 expression is up-regulated under inflammatory states (24). Multiple stimulating factors subsequently induce the high levels of TRPV4 activation (19). The high levels of TRPV4 activation induced excessive heat loss which resulted in hypothermia (25).

In addition, it was reported that GSK2193874, a selective TRPV4 inhibitor, suppresses the secretion of inflammatory factors and attenuates inflammation symptoms in sepsis (26). Considering that many aspects of heatstroke are similar to the inflammatory response of sepsis (6), we speculated that GSK2193874 might have a therapeutic effect under the critical conditions of heatstroke. In this study, we measured the changes of core temperature, the death rate, pulmonary edema, systemic inflammatory biomarkers (serum levels of TNF-α and IL-6), coagulation indicators, markers of liver and kidney injury, and apoptosis state (caspase-3/7 activity in the lung) in murine heatstroke models with different treatments. GSK2193874 administration at heatstroke onset induced the trend of improving these markers.

Materials and Methods

Murine model of heatstroke

All protocols were approved by the Animal Ethics Committee of Hangzhou Normal University, and the experiments were performed in accordance with the Na-

tional Institutes of Health Guidelines. Male C57BL/6J mice were given food and water ad libitum and lived in a controlled environment with a 12-hour (h) dark/light cycle at room temperature (23 ± 1°C) with a relative humidity of 50±8%. Mice 9 to 10 weeks old were used in this study, and their weights were 23-25 grams.

The animals were randomized into four groups: a control group, a heatstroke group (HS), a heatstroke plus GSK2193874 pre-treatment group (HS-pre-GSK) and a heatstroke plus GSK2193874 group (HSGSK). Before heat stress, mice in the HS-pre-GSK group were intraperitoneally injected (i.p.) with 1 mg/kg GSK2193874 (26). HSGSK group was injected with the same dose at heatstroke onset. DMSO was used as the vehicle for GSK2193874.

Heatstroke was induced as previously described by Xu et al. (7). Briefly, the animals were placed in an artificial climate chamber at 38 ± 0.5°C and a relative humidity of 60 ± 5%. The core temperature (Tc) was continuously measured using a data acquisition system (VitalView; Starr Life Sciences). The criterion for heatstroke onset was 42°C Tc. Next, animals that experienced heatstroke were recovered at 24 ± 0.5°C. Mice in the control group were sham-heated with an artificial climate chamber maintained at 24 ± 0.5°C.

Eight animals in each group (n=8) were assessed for serum biomarkers. The animals were anaesthetized and sacrificed at 6 h after heatstroke onset (27). The blood and organ samples were then collected.

Core temperature monitoring and mortality observation

During recovery after heatstroke onset, the core temperature of mice reduced, which in turn induced hypothermia (6,28). The lowest Tc value as an indicator of heatstroke severity was observed during recovery (6). We devised the hypothermia index for statistical analysis, which was defined as (normal Tc – the lowest Tc) ÷ normal Tc. Recovering time represents the time from 42°C to normal Tc during recovery. The mouse death rate of each group (n=10) was assessed at 72 h after heatstroke onset (27).

Analysis of pulmonary edema

Heatstroke-induced pulmonary edema was assessed by the W/D ratio of the left lung. The left lungs of anaesthetized mice (n=8) were excised from the thoracic cavity, and the wet weights were recorded. The lung samples were then dried to a constant weight of 50°C for approximately 3 days, and the constant dry weights were recorded. The ratio of the wet weight: dry weight (W/D) was finally compared among the various treatments groups.

Inflammation marker and homeostasis analysis

Blood specimens at 6 h after heatstroke onset were collected from the hearts of anaesthetized mice. The blood was centrifuged to isolate the plasma. The plasma concentrations of tumour necrosis factor (TNF)-α and interleukin (IL)-6 were determined using the enzyme-linked immunosorbent assay kit (eBiosciences, Thermo, Waltham, MA) and Infinite® 200 Pro NanoQuant microplate reader (Tecan, Männedorf, CH) according to the manufacturer's protocols. T`he soluble endothelial protein C receptor (sEPCR) was also assessed by enzyme linked immuno sorbent assay (Novatein Biosciences, Cambridge, USA). The concentration of TNF-α, IL-6 or sEPCR in the plasma samples was calculated from the standard curve.

The prothrombin time (PT), activated partial thromboplastin time (APTT) and D-dimer were assessed using a full-automatic coagulation analyzer (Sysmex CA-1500, Kobe, Japan), according to the manufacturer's protocols. Reagents for PT, APTT and D-dimer were from Siemens Healthcare (Erlangen, Germany).

Liver and kidney injuries assay

Liver and kidney profiles, such as alanine aminotransferase (ALT), aspartic aminotransferase (AST), lactate dehydrogenase (LDH), plasma creatinine (Cr), and urea nitrogen (BUN) levels, were assessed by the HITACHI 7600 automated biochemistry analyzer (Tokyo Japan), according to the manufacturer's protocols. Reagents for ALT, AST, LDH, Cr, and BUN were taken from the Medical System (Ningbo, China).

Caspase-3/7 activity assay

Caspase-3/7 protease activity in the right lung tissue was measured using the Caspase-3/7 Activity Apoptosis Assay Kit (Sangon, Shanghai, China) according to the manufacturer's protocols. Briefly, after homogenization of lung tissue in cell lysis buffer, the homogenates were centrifuged for 1 min at 10,000 × g. The supernatant with equal amounts of protein was incubated with (Z-DEVD)2 R110 substrates for caspase-3/7 and reaction buffer for 90 min at room temperature, protected from light. Caspase-3/7 activity was assessed according to the fluorescence intensity at Ex/Em=490/525 nm and was normalized against the control (29).

Statistical analysis

All data was analyzed for statistical significance using SPSS 13.0 software (SPSS, Chicago, IL, USA). Biological triplicates were performed, and values were presented as means ± standard error of the mean (SEM). Survival data was analyzed using Kaplan-Meier log-rank test. Students ' paired t-tests and one-way ANOVA were applied for statistical analysis of other results. A p-value < 0.05 was considered to indicate significance.

Results

GSK2193874 treatment at heatstroke onset enhanced the core temperature and the survival ratio.

Heatstroke can develop into physiologic function disorder and lethality. Recovering time represents the time 42°C decreases to a normal Tc during recovery. The mice in HS-Pre-GSK group showed a longer recovering time when compared with HS or HSGSK group ($p = 0.012$, $p = 0.018$, respectively; Fig. 1A). It demonstrated that the GSK2193874 pre-treatment before heat stress was untimely and interfered with thermoregulation. Heat loss was then inhibited during hyperthermia. In contrast, during heat stress, the physiological function of heat dissipation had been extremely activated in HS and HSGSK groups, which caused a quick drop in body temperature during recovery.

Hypothermia is a necessary response during recov-

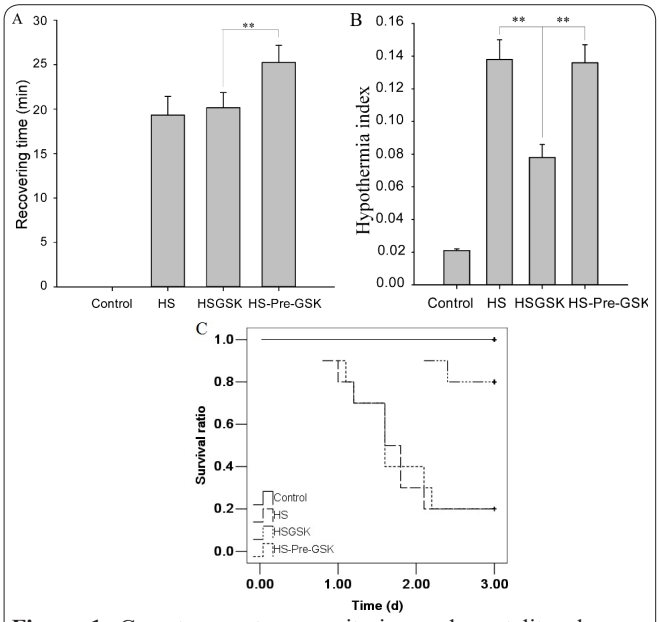

Figure 1. Core temperature monitoring and mortality observation during the recovery. Effects of GSK2193874 (GSK) on time from 42 °C to reach normal Tc, the lowest Tc and mortality were assessed during the recovery in heatstroke mice (n=10). (A)The recovering time in HS-Pre-GSK was longer than that in the HS or HSGSK groups (p = 0.012, p = 0.018; respectively). (B) Hypothermia depth was assessed by Hypothermia index [Hypothermia index = (the normal Tc- the lowest Tc) ÷ the normal Tc]. Hypothermia indexes in HSGSK were significantly lower than those in the HS or HS-Pre-GSK groups (p < 0.01, p < 0.01; respectively). (C) The survival ratios were assessed within 72 h after heatstroke onset. The survival ratio in HSGSK mice was higher than that in HS or HS-Pre-GSK mice (p = 0.036, p = 0.041; respectively). *: P<0.05, **: P<0.01.

ery, and the lowest core temperature can be used as an indicator of heatstroke severity (6). The reduction of core body temperature was represented by the hypothermia index, which was lower in the HSGSK group than in the HS or HS-Pre-GSK groups (p < 0.01, p < 0.01, respectively; Fig. 1B). However, there was not a significant difference between HS-Pre-GSK and HS group (p = 0.89; Fig. 1B). This demonstrated that the GSK2193874 treatment at heatstroke onset attenuated excessive heat loss during hypothermia.

Most heatstroke mice in HS and HS-Pre-GSK groups died within 72 h of recovery. The survival ratios were only 40% (4/10) in the HS and HS-Pre-GSK groups. By contrast, few heatstroke mice died in the HSGSK group, and the survival ratio was 80% (8/10). GSK2193874 significantly enhanced the survival ratio of HSGSK group when compared with HS or HS-Pre-GSK group (p = 0.036, p = 0.041, respectively; Fig. 1C).

GSK2193874 injection at heatstroke onset reduced pulmonary edema in heatstroke mice.

The W/D ratios of lung tissue were also significantly elevated in heatstroke mice compared with that in the control group (Fig. 2), suggesting that heatstroke induced significant disruption of the pulmonary capillary barrier and elevation of pulmonary capillary permeability. The W/D values in HSGSK group were significantly reduced compared with those in HS or HS-Pre-GSK group (p = 0.022, p = 0.031, respectively; Fig. 2). It suggested that the GSK2193874 injection at heatstroke

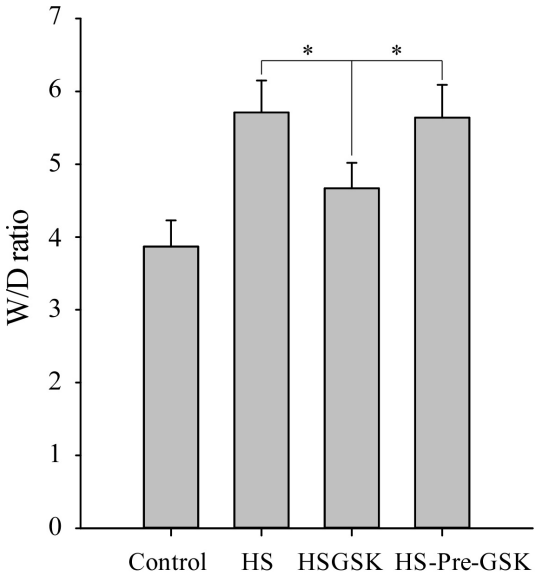

Figure 2. The lung injury was assessed using the W/D ratio. The lung injury was assessed using the W/D ratio (n=8). The W/D values were increased in heatstroke mice compared with that in the controls. The W/D ratio in HSGSK was lower than that in HS or HS-Pre-GSK mice (p = 0.022, p = 0.031; respectively). *: P<0.05.

onset attenuated pulmonary capillary injury and maintained barrier stabilization in heatstroke mice.

GSK2193874 reduces the plasma levels of TNF-α and IL-6 in HSGSK mice.

It is reported that many inflammatory factors are increased in the heatstroke process, such as TNF-α, IL-6, IL-1β, and IL-8 (6,7,30). Our study showed that the plasma concentrations of TNF-α and IL-6 in heatstroke mice were significantly increased compared with that in the controls (Fig. 3A, B). The levels of serum TNF-α and IL-6 in HS were markedly higher than those in HSGSK (p = 0.012, p = 0.01, respectively) or HS-Pre-GSK (p = 0.021, p = 0.016, respectively) (Fig. 3A, B). The levels of TNF-α and IL-6 were not significantly different between HSGSK and HS-Pre-GSK groups (p = 0.89, p = 0.85, respectively; Fig. 3A, B). Further , GSK2193874 shows low clearance (31). Therefore, in heatstroke mice, GSK2193874 administration suppressed the secretion of TNF-α and IL-6 at 6 h post-heatstroke.

TNF-α induces endothelial apoptosis via the death

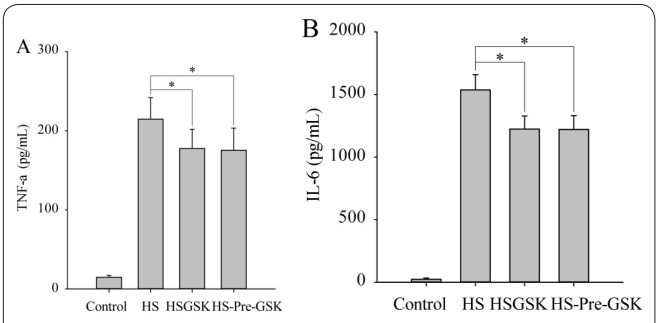

Figure 3. The levels of serum TNF-α and IL-6 were assessed at 6 h after heatstroke onset. The levels of serum TNF-α and IL-6 were assessed at 6 h after heatstroke onset (n=8). Heatstroke induced the secretion of TNF-α and IL-6. (A) The levels of serum TNF-α in HS were higher than those in HSGSK or HS-Pre-GSK (p=0.012, p = 0.021; respectively). (B) The levels of serum IL-6 in HS mice were also higher than those in HSGSK or HS-Pre-GSK (p=0.01, p = 0.016; respectively). *: p < 0.05.

receptor pathway and promotes vascular barrier dysfunction (31-32). IL-6 is also involved in inflammatory injury (33). Next, pulmonary edema was aggravated as above. The hyposecretion of TNF-α and IL-6 induced by GSK2193874 was an important reason to attenuate organic injury and enhance the survival ratio in HSGSK mice. However, the cause of low survival ratio in HS-Pre-GSK still needs to be further explored.

GSK2193874 treatment at heatstroke onset attenuated coagulation dysfunction.

Heatstroke induces endothelial injury, disseminated intravascular coagulation, and widespread haemorrhage (7). The endothelial protein C receptor (EPCR) is present primarily on the endothelium of vessels (34). Endothelial injury increases the plasma levels of sEPCR, which has been used as a marker of endothelial injury (35). The comparison among groups showed that the plasma levels of sEPCR in HSGSK were lower than those in HS or HS-Pre-GSK ($p = 0.011$, $p = 0.023$; respectively; Fig 4A). This demonstrated that endothelial injury in HSGSK was mild compared with HS and HS-Pre-GSK. In addition, sEPCR reduces the anticoagulant effect of EPCR, which interferes with the coagulation balance (35).

PT, APTT and D-dimer were markers reflecting coagulation function (36). Results showed that the values of PT in HSGSK were shorter than those in HS or HS-Pre-GSK ($p = 0.019$, $p = 0.027$; respectively; Fig 4B). APTT also had similar changes in HSGSK compared with HS or HS-Pre-GSK ($p = 0.016$, $p = 0.023$; respectively; Fig 4C). The plasma levels of D-dimer in HSGSK were lower than those in HS or HS-Pre-GSK ($p = 0.025$, $p = 0.033$; respectively; Fig 4D). This demonstrated that coagulation function in HSGSK was less damaged than those in HS or HS-Pre-GSK. Correspondingly, the survival ratio in HSGSK was higher than that in HS or HS-Pre-GSK.

GSK2193874 attenuated liver and kidney injuries in HSGSK mice.

The markers of liver and kidney injuries were examined at 6 h after heatstroke onset. Heatstroke induced liver and kidney injuries, characterized by markedly increased plasma levels of ALT, AST, LDH, Cr, and BUN compared with control (Table 1). The levels of ALT, AST, LDH, Cr, and BUN in HSGSK were significantly lower than those in HS or HS-Pre-GSK (Table 1). There were no significant differences between HS and HS-Pre-GSK.

GSK2193874 reduces caspase-3/7 activity in HSGSK mice.

Caspases in apoptotic cells are activated, which then execute apoptosis by cleaving various cellular proteins (37). To further confirm the effect of GSK2193874 on heatstroke-induced injuries, we measured the expression of apoptosis-related caspase-3/7. Heatstroke markedly increased the levels of cleaved caspase-3/7 in lung tissue compared with those in the controls at 6 h after heatstroke onset (Fig. 5). However, the levels of caspase-3/7 in HSGSK mice were significantly attenuated compared with those in the HS or HS-Pre-GSK group ($p = 0.023$, $p = 0.032$, respectively; Fig. 4). They suggested that GSK2193874 injection at heatstroke onset reduced cell apoptosis, but GSK2193874 administration before heat stress did not attenuate cell apoptosis.

The stimulating factors of TRPV4 were increased during the heatstroke process (3,6,7,23). The levels of

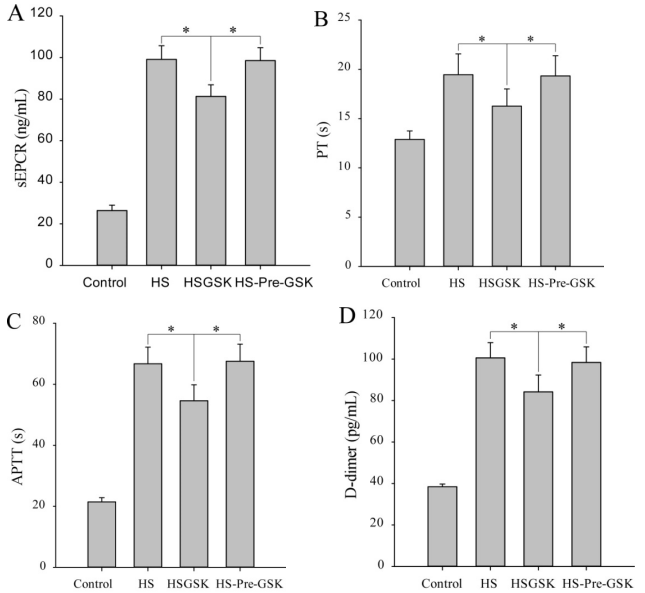

Figure 4. The comparison of the coagulation function among groups. (A)The plasma levels of sEPCR were assessed at 6 h after heatstroke onset (n=8). The levels of sEPCR in HSGSK were significantly lower than those in HS or HS-Pre-GSK ($p = 0.011$, $p = 0.023$; respectively). (B) The values of PT in HSGSK were shorter than those in HS or HS-Pre-GSK ($p = 0.019$, $p = 0.027$; respectively). (C) APTT also had similar changes in HSGSK compared with HS or HS-Pre-GSK ($p = 0.016$, $p = 0.023$; respectively). (D) The plasma levels of D-dimer in HSGSK were lower than those in HS or HS-Pre-GSK ($p = 0.025$, $p = 0.033$; respectively).*: $p < 0.05$.

Table 1. The markers of liver and kidney injury in heatstroke mice.

	Control (n=8)	HS (n=8)	HSGSK (n=8)	HS-Pre-GSK (n=8)
ALT (U/L)	25.7 ± 3.4	300.9 ± 29.1	80.6 ± 8.5**	294.7 ± 32.5
AST (U/L)	22.3 ± 3.6	420.2 ± 38.7	169.4 ± 23.1**	422.4 ± 41.8
LDH (U/L)	77.5 ± 7.1	433.4 ± 40.6	170.2 ± 24.7**	434.1 ± 45.2
Cr (μmol/L)	30.6± 5.8	184.3± 20.1	109.8 ± 13.6*	182.9 ± 19.8
BUN (mmol/L)	3.1 ± 0.5	19.6 ± 2.3	10.3 ± 1.2*	19.7 ± 2.3

Mice were randomized into four groups: Control, HS (heatstroke), HSGSK (intraperitoneally injected with 1 mg/kg GSK2193874 at heatstroke onset) and HS-Pre-GSK (intraperitoneally injected with 1 mg/kg GSK2193874 before heat stress). Values were presented as means ± standard error of the mean (SEM). Alanine aminotransferase (ALT), aspartic aminotransferase (AST), lactate dehydrogenase (LDH), plasma creatinine (Cr), nitrogen (BUN). * $P < 0.01$,** $P < 0.001$, compared with HS.

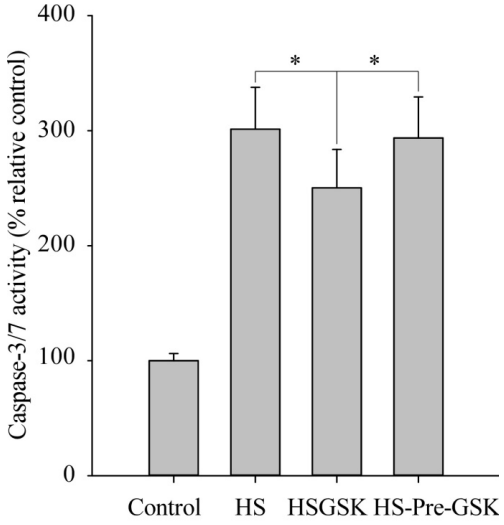

Figure 5. The comparison of the caspase-3 activation among groups. Heatstroke induced caspase-3/7 activity at 6 h after heatstroke onset. The levels of caspase-3/7 activity in HSGSK were lower than those in HS or HS-Pre-GSK ($p = 0.023$, $p = 0.032$; respectively). *: $p < 0.05$.

TRPV4 activation should be elevated by stimulating factors. According to the above results, GSK2193874 inhibited TRPV4 activation to protect HSGSK mice. Therefore, we speculated that TRPV4 might be excessively activated, which contributed to the increase in the levels of inflammation factors, vascular permeability, cell apoptosis, and death in HS mice. In addition, GSK2193874 treatment before heat stress inhibited heat loss during hyperthermia. Sustained hyperthermia might aggravate cell injuries in HS-Pre-GSK mice.

Discussion

When an individual is exposed to high temperatures, body heat dissipation is difficult, which causes an elevated core temperature (> 40 °C) and accompanies cardiovascular strain and multi-organ damage (1). Hence, it is very important that core temperature returns to basal body temperature as soon as possible (1). Additionally, TRPV4 activation is involved in heat loss, and TRPV4 antagonist can inhibit the response (25). GSK2193874 shows low clearance (31), hence, when injected before heat stress, the regulation of body temperature in HS-Pre-GSK was disturbed, which resulted that the recovering time (from 42 °C to normal core temperature) in the HS-Pre-GSK group was significantly longer than the HS group (Fig. 1A). The injury induced by hyperthermia in HS-Pre-GSK mice should be more serious than that in the HS group. Because the physiological function of heat dissipation in HSGSK group had been extremely activated during heat stress (1), their core temperature showed a quick drop during recovery, and recovering time in HSGSK was shorter than that in the HS-Pre-GSK group (Fig. 1A). Therefore, heatstroke severity in HSGSK mice should be milder than that in HS-Pre-GSK.

The heatstroke mice must experience hypothermic state during recovery, hypothermia depth being a biomarker of systemic disorder severity (38-39). The hypothermia reduces the survival rate in heatstroke murine model (1). It is reported that TRPV4 activation by chemical stimulation increased tail heat loss, and TRPV4 antagonist inhibited the response (25). In our study, GSK2193874 injection at heatstroke onset enhanced the lowest Tc in HSGSK group during recovery, which was represented by a smaller reduction of core body temperature (hypothermia index) compared to the HS group (Fig. 1B). Arguably, GSK2193874 inhibited excessive hypothermia and attenuated systemic disorder in HSGSK mice. Further, HS-Pre-GSK mice suffered more hyperthermia injuries because of a longer recovering time than HSGSK mice; the hypothermia index in HS-Pre-GSK group was larger than that in the HSGSK group (Fig. 1B). Hence, the heatstroke severity in HSGSK group should be significantly milder than in HS or HS-Pre-GSK groups.

The W/D ratio of the lung is often used as a marker of vascular barrier injury in mice (40-41), whilst TRPV4 activation is responsible for high vascular permeability and edema formation (42-43). In HSGSK mice, GSK2193874 reduced the W/D ratio (Fig. 2). This demonstrated that appropriate GSK2193874 administration at heatstroke onset attenuated heatstroke injury and then reduced the mortality (Fig. 1C). In addition , the serum levels of inflammatory factors, such as TNF-α and IL-6, are increased in heatstroke mice (7). TNF-α and IL-6 play an important role in organ injuries, including vascular barrier injuries (40,44). GSK2193874 administration inhibits the secretion of TNF-α and IL-6 in sepsis (26). Our results also showed that GSK2193874 reduced the increased levels of TNF-α and IL-6 in HSGSK mice (Fig. 3A, B) which are involved in cell apoptosis (29-31). Further apoptosis analysis showed that GSK2193874 reduced the caspase-3/7 activities in HSGSK mice (Fig. 5). This suggested that timely GSK2193874 treatment suppressed the secretion of inflammatory factors, reduced inflammatory injury and enhanced the survival ratio in HSGSK mice.

Soluble endothelial protein C receptor (sEPCR) is the extracellular domain of the endothelial protein C receptor (45). sEPCR levels are increased in patients with systemic inflammatory diseases (46) and has been used as a marker of endothelial damage (35). In our study, sEPCR levels in HSGSK mice were significantly lower than those in HS or HS-Pre-GSK group (Fig 4A) suggesting that vascular injury in HSGSK mice was milder compared with HS or HS-Pre-GSK.

In addition, the endothelial protein C receptor, which is almost exclusively expressed on vascular endothelial cell, can bind and activate protein C to limit the progression of the coagulation cascade (34,47-48). The sEPCR can also bind protein C and activated protein C but inhibits anticoagulant activity of activated protein C (49). Heatstroke induces coagulation dysfunction. The histochemical result shows disseminating intravascular coagulation, which then promotes organ failure and induces death (36). Prothrombin time (PT), activated partial thromboplastin time (APTT) and D-dimer are used as markers reflecting disseminating intravascular coagulation (36). In HSGSK group, GSK2193874 treatment at heatstroke onset significantly reduced the value of PT, APTT or D-dimer compared with HS or HS-Pre-GSK group (Fig 4B, C and D), and improved coagulation dysfunction. The results were consistent with the plasma level of sEPCR.

Research showed that exocytosis and endocytosis depend on the perfectly tuned orchestration of the intracellular calcium signalling and the actin dynamics (50). The intracellular calcium was precisely regulated in a spatiotemporal manner through various calcium ion channels and membrane pumps (51). TRPV4 is one such channel (26). In HSGSK group, GSK2193874 inhibited excessive TRPV4 activation, reduced Ca2+ influx, partly interfered with actin dynamics, suppressed the secretion of TNF-α and IL-6, attenuated the inflammatory response, enhanced the lowest core temperature, and protected mice against heatstroke-induced death.

In summary, our results suggest that TRPV4 may be a therapeutic target of fatal heatstroke. GSK2193874 administration at heatstroke onset may be useful, but prophylactic administration before heat stress may be not beneficial. Furthermore, GSK2193874 as an orally active TRPV4 inhibitor is storable and portable. It may be timely taken orally to attenuate heatstroke-induced injury at the scene of heatstroke.

Acknowledgments
We thank the members of the Pei laboratory for their discussion and support.

Interest conflicts
None.

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