Therapeutic Whole-body Hypothermia Protects Remote Lung, Liver, and Kidney Injuries after Blast Limb Trauma in Rats

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ABSTRACT

Background: Severe blast limb trauma (BLT) induces distant multiple-organ injuries. In the current study, the authors determined whether whole-body hypothermia (WH) and its optimal duration (if any) afford protection to the local limb damage and distant lung, liver, and kidney injuries after BLT in rats.

Methods: Rats with BLT, created by using chartaceous electricity detonators, were randomly treated with WH for 30 min, 60 min, 3 h, and 6 h (n = 12/group). Rectal temperature and arterial blood pressure were monitored throughout. Blood and lung, liver, and kidney tissue samples were harvested for measuring tumor necrosis factor-α, interleukin-6 and interleukin-10, myeloperoxidase activity, hydrogen sulfide, and biomarkers of oxidative stress at 6 h after BLT. The pathologic lung injury and the water content of the lungs, liver, and kidneys and blast limb tissue were assessed.

Results: Unlike WH for 30 min, WH for 60 min reduced lung water content, lung myeloperoxidase activity, and kidney myeloperoxidase activity by 10, 39, and 28% (all $P < 0.05$), respectively. WH for 3 h attenuated distant vital organs and local traumatic limb damage and reduced myeloperoxidase activity, hydrogen peroxide and malondialdehyde concentration, and tumor necrosis factor-α and interleukin-6 levels by up to 49% (all $P < 0.01$). Likewise, WH for 6 h also provided protection to such injured organs but increased blood loss from traumatic limb.

Conclusions: Results of this study indicated that WH may provide protection for distant organs and local traumatic limb after blast trauma, which warrants further study. **(Anesthesiology 2016; 124:1360-71)**

WITH the development of armor, the number of survivors with blast limb trauma (BLT) increased in modern military conflicts and terrorist attacks.[1,2] When multiple blast trauma is very severe and complicated, it often induces remote multiorgan injuries including the lungs, liver, and kidneys *via* systemic inflammatory responses and oxidative stress and suppression of cystathionine γ-lyase (CSE)/hydrogen sulfide pathway as reported previously.[3,4] The remote multiple-organ injuries not only perplex the condition and treatment, but also prolong recovery. These injuries could lead to multiple-organ dysfunction (MODS) or even failure if no proper interventions are promptly in place.

Whole-body hypothermia (WH) is a therapeutic intervention involving controlled reduction of core temperature to protect organs at risk of injury.[5] To date, WH has principally been used as a protective therapy for various brain injuries[6]; moreover, there is emerging evidence that it may also be useful to protect other organs when at risk of injury. For example, WH can improve the prognosis of severe sepsis and severe hemorrhagic shock.[7] It has been also reported that WH attenuates the heart,[8] lung, kidney,[9,10] and liver injuries.[11,12] It has been recognized that the therapy window and the duration of WH are very

What We Already Know about This Topic

- Blast limb trauma induces organ injury due to multiple mechanisms that produce a systemic inflammatory response

What This Article Tells Us That Is New

- Whole-body hypothermia for 3 h immediately after injury in an experimental animal model provides multiorgan protection for traumatic injury after blast trauma

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important for achieving promising protective outcome. In general, early application of therapeutic hypothermia can be more effective than late application.[13,14] Mild hypothermia is preferable because it can provide considerable protective effects and has minimal adverse effects.[15,16] It has been reported that prolonged hypothermia did not improve the protective effects, but increased adverse effects.[17,18] Mild hypothermia for 24 h is recommended for brain protection after cardiac arrest.[19] It has also been demonstrated that mild hypothermia for 1 h improved the final outcome in uncontrolled hemorrhagic shock rats[20] and 2.5 h of hypothermia afforded hepatic protection in multiple-trauma swine model.[21,22] Interestingly, regional cooling for 30 min of traumatic limb was enough to afford maximum protection for the remote lung injury.[4] Clearly, the optimal duration of hypothermic therapy varies with different diseases or pathologic conditions.

To extend our previous findings,[4] we hypothesized that WH could also attenuate organs injuries after BLT, but may be even more effective than regional hypothermia. Therefore, the aim of the current study is to determine whether WH affords protection to the local limb damage and distant lung, liver, and kidney injuries and the optimal duration of WH for such injuries after BLT in rats.

Materials and Methods

After approval of the protocol by the Third Military Medical University Animal Care Committee (Chongqing, China), the experiments were performed according to the Chinese Institutes of Health Guidelines on the Use of Laboratory Animals.

Animal Model

Anesthesia and methods of creation of BLT in rats have been previously described in detail.[4,23] Briefly, anesthesia was induced with intraperitoneal injection of pentobarbital (50 mg/kg of weight), and adequate anesthesia was achieved *via* injection of pentobarbital intraperitoneally according to the lash flash throughout the experiment. Two catheters (outer diameter, 0.965 mm; inside diameter, 0.58 mm) were inserted: one into the right femoral artery to monitor blood pressure and collect the blood sample and one into the right jugular vein for fluid infusion. Subsequently, rats were randomized into the Sham, which received identical treatment (anesthesia) and manipulations (surgery) but without blast injury, and the blast groups.

Blast limb trauma was induced by using chartaceous electricity detonators (845 Factory, China) containing 80 mg diazodinitrophenol. The injuries were manifested as hemorrhage, open comminuted fracture, Tscherne-Gotzen 3- to 4-degree soft tissue injury, and first-degree burn injury. The blasted limbs were packed with sterile sponges after injury. Normal saline at 4 ml kg^{-1} h^{-1} was infused, and spontaneous breathing was maintained throughout the experiment.

Experimental Protocol

After injury, the BLT rats were randomized using a random number generated by a computer to the normothermia group (BLT) and the groups of hypothermia (32° ± 0.5°C) for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h; n = 12/group; Supplemental Digital Content 1, fig. 1, http://links.lww.com/ALN/B266). WH was initiated for the hypothermic group immediately after blast by spraying alcohol onto abdominal skin while an electric fan circulated cool air until rectal temperature reached 33.5°C and rectal temperature was maintained at 32° ± 0.5°C for 30 min, 60 min, 3 h, or 6 h. Then the rats in the groups BLT-WH30, BLT-WH60, and BLT-WH3h were dried with electric hair drier and then placed onto the thermo mattress (40°C) for rewarming to more than 35°C, and this rectal temperature was maintained until the termination of experiment, and the rate of rewarming was kept at 1.5° to 2°C/h (Supplemental Digital Content 2, table 1, http://links.lww.com/ALN/B267). Adequate anesthesia was maintained to avoid shivering during the period of cooling and rewarming.

Measurements

Arterial blood pressure and rectal temperature were continuously monitored with PowerLab (AD Instruments, USA) throughout the experiments. Blood loss induced by blast was calculated by the following formula: blood loss = wet gauze weight – dry gauze weight. After the animals were euthanized by intraperitoneal injection of overdose of pentobarbital at 6 h postinjury, blood and tissue samples of the damaged limb, the right lower lobe of lung, the right kidney, and the left hepatic lobe were harvested for following measurements.

Histology. The right lower robe of lung and damaged limb muscle tissue were fixed in 10% formalin for 24 h and then embedded in paraffin. The blocks were sectioned at 5-μm thickness and then stained with hematoxylin–eosin. The pathologic changes of the lungs were scored in a blinded manner through analyzing the following variables: lung edema, hemorrhage, infiltration of the inflammatory cells, thickness of alveolar wall, and pulmonary architecture, as described previously.[4,23] Each variable was graded on a scale of 0 to 4 (0, absent; 1, mild; 2, moderate; 3, severe; and 4, very severe injury). The total histopathology score was expressed as the sum of the scores for statistical analysis.

Biological Measurements. Tumor necrosis factor-α (TNFα), interleukin-6, and interleukin-10 levels were measured by enzyme-linked immunosorbent assay according to instructions from the manufacturer (R&D systems, USA). Hydrogen sulfide level was determined using a modified methylene blue assay described previously.[4] Other parameters, including serum creatinine, aspartate aminotransferase, and alanine aminotransferase, were measured by using commercial kits (Jiancheng Biotechnology CO, China). All the biologic measurements were determined in a blinded manner.

Statistical Analysis

The group size (n = 12) was determined at set of 1-β = 0.9 and α = 0.05 by one-way ANOVA (PASS 10.0 software NCSS, LLC, USA) based on the data of lung histopathology scores from the pilot experiments. No data were missing for final analysis, and data were expressed as mean ± SD unless indicated otherwise. Histologic injury scoring data were expressed as a Box-and-whisker plot and analyzed by Kruskal-Wallis nonparametric test followed by Dunn test for comparison. The rest of the data were analyzed by two-tailed ANOVA followed by Tukey comparison test (GraphPadPrism 5, USA). A $P < 0.05$ was considered to be statistically significant.

Results

Physiological Variables

The blood loss from the traumatic limb in the BLT, BLT-WH30, BLT-WH60, and BLT-WH3h groups did not differ significantly (11.4%, 16.3%, 16.1%, and 17.1% of the body weight, respectively), but WH for 6 h significantly increased the blood loss when compared with that of group BLT (11.4% *vs.* 17.8%, $P = 0.037$).

BLT resulted in reduced mean arterial blood pressure (MBP), and this MBP level was maintained throughout experiments. Hypothermia for any duration did not affect the MBP further (Supplemental Digital Content 2, table 1, http://links.lww.com/ALN/B267).

WH Treatment Attenuated Muscle and Remote Lung Injury after Blast Limb Trauma

In contrast to the normal muscle in the naive rats (fig. 1A), muscle myofilaments were ruptured in the all BLT rats, and the rats also had hemorrhage and muscle swelling (fig. 1, B to F). In the rats treated with WH for 3 (fig. 1E) and 6 h (fig. 1F), muscle swelling was attenuated compared to the BLT rats. These observations were corroborated with the traumatic muscle water content (fig. 1G).

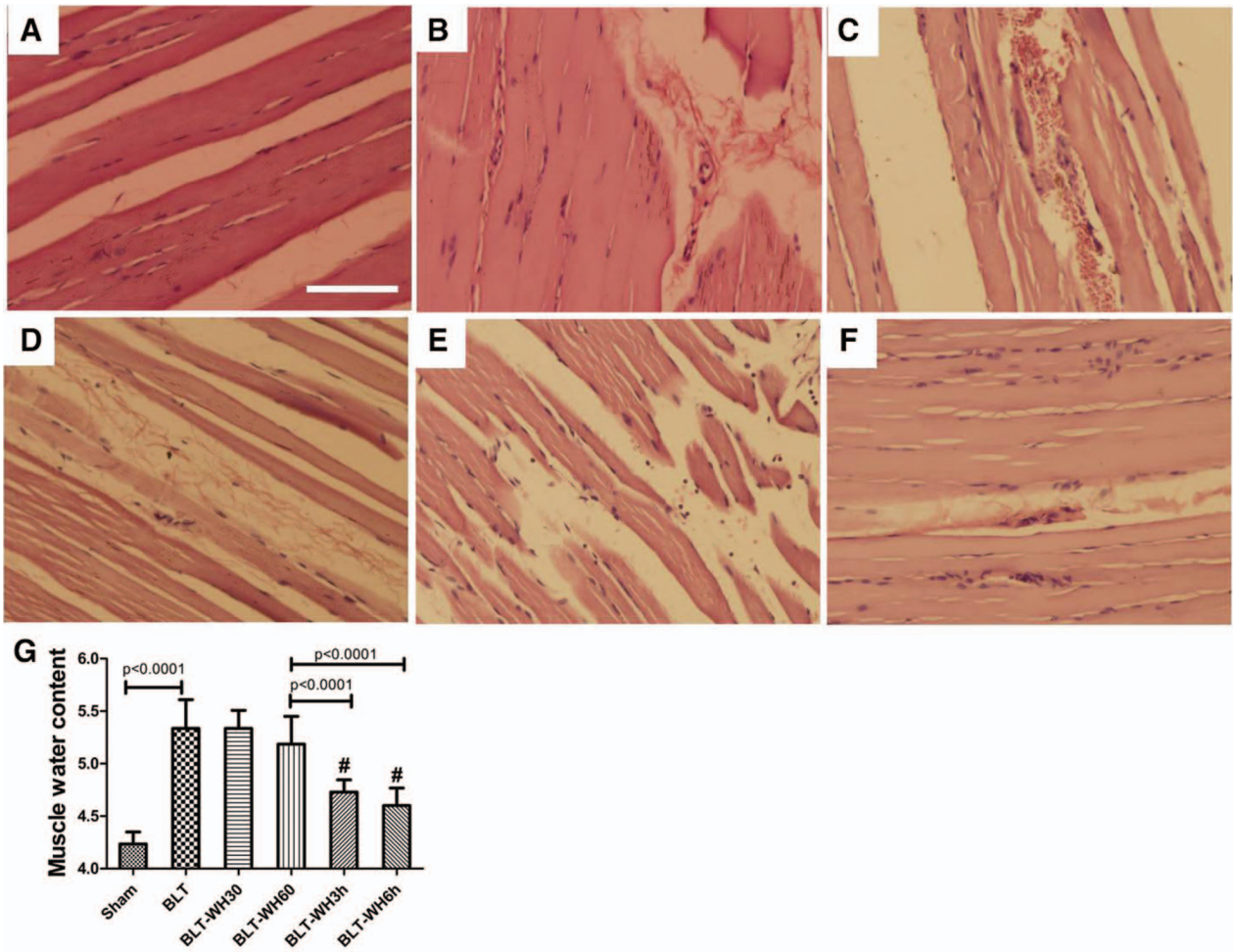

Fig. 1. The effects of whole-body hypothermia treatment on the muscle histopathology changes and traumatic tissue edema induced by blast limb trauma. Representative microphotographs were taken from Sham (*A*), blast limb trauma (BLT; *B*), and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30; *C*), 60 min (BLT-WH60; *D*), 3 h (BLT-WH3h; *E*), and 6 h (BLT-WH6h; *F*). Traumatic tissue water content was represented by wet/dry weight (*G*). Data are presented as mean ± SD (n = 12). #$P < 0.05$ *versus* BLT.

In contrast to the normal lung in the Sham rats (fig. 2A), lung histologic changes including congestions, hemorrhage, alveolar wall thickening, and cell infiltration were found in BLT rats (fig. 2B). WH treatment for 30 min (fig. 2C), 60 min (fig. 2D), 3 h (fig. 2E), and 6 h (fig. 2F) alleviated these lung morphologic changes induced by BLT to varying degrees, but only WH treatment for 3 and 6 h significantly decreased the pathology scoring (fig. 2G). Lung water content was decreased in the rats treated with WH for 30 min (P = 0.001), 60 min (P = 0.002), 3 h (P < 0.0001), and 6 h (P < 0.0001) compared with that in the BLT rats (fig. 2H).

The Effect of WH Treatment on Liver and Kidney Function

Liver water content was decreased in the BLT-WH3h or BLT-WH6h rats compared with that of the BLT rats (fig. 3A). WH for 3 and 6 h attenuated increased serum aspartate aminotransferase (fig. 3B) and alanine aminotransferase (fig. 3C) induced by BLT. WH for 3 and 6 h alleviated the increased kidney water content induced by BLT (fig. 3D), but serum urea level was found to be not changed in the all groups (fig. 3E).

The Effect of WH Treatment on Cytokines

As shown in figure 4A, plasma TNFα was increased 20-fold than that of the Sham rats (73.6 ± 15.4 ρg/ml, P < 0.0001). WH for 3 and 6 h reduced plasma TNFα to 58% (P = 0.002) and 66% (P = 0.008) of that in the BLT rats, respectively. Plasma interleukin-6 was increased 8.7-fold than that of the Sham rats (268.7 ± 31.7 ρg/ml, P < 0.0001), which was reduced to 63% and 59% by WH for 3 (P < 0.0001) and 6 h (P < 0.0001), respectively (fig. 4B). Plasma interleukin-10 was increased 2.6-fold than that of the Sham rats (139.8 ± 19.3 ρg/ml, P < 0.0001), but WH for 6 h decreased plasma interleukin-10 significantly (P = 0.018; fig. 4C).

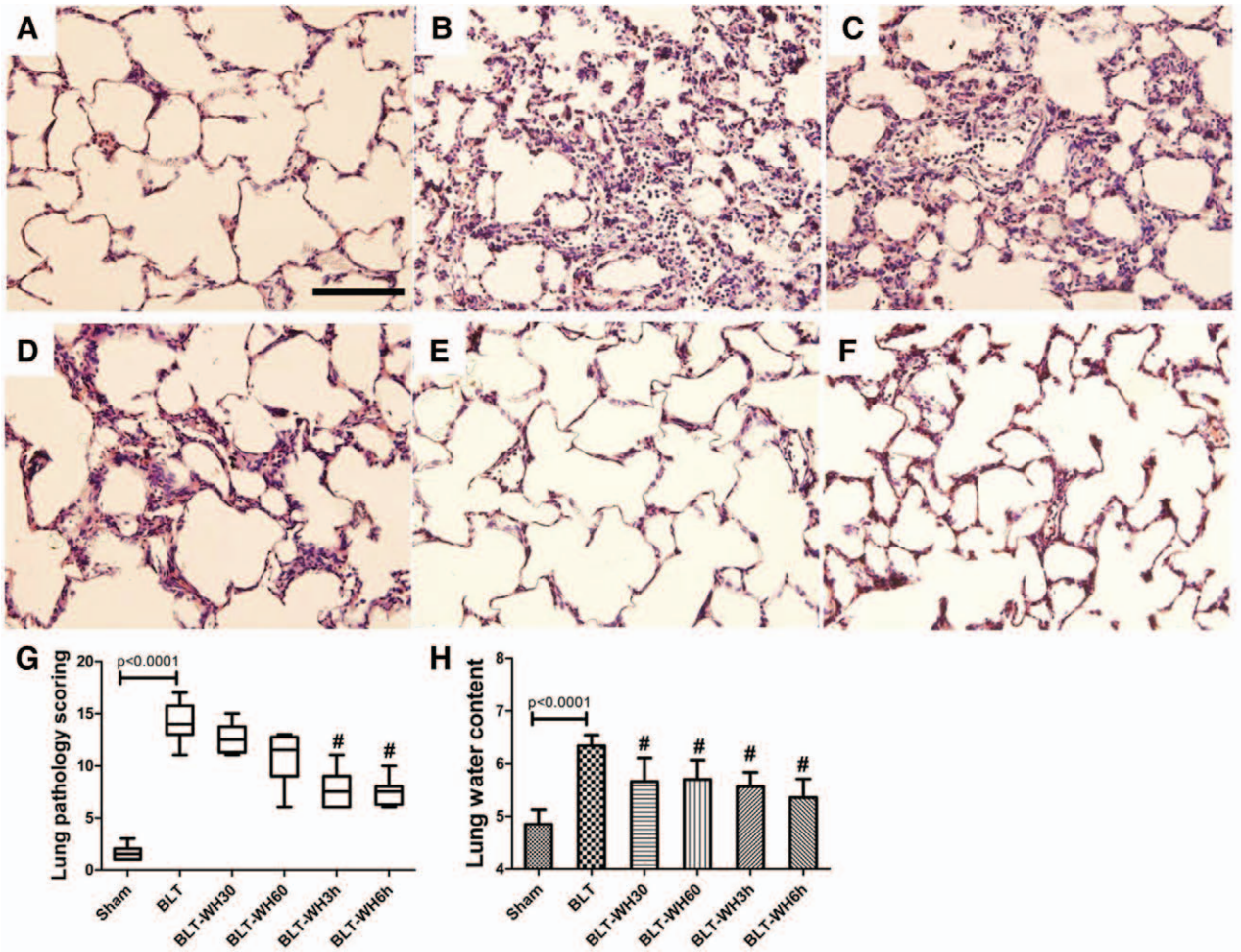

Fig. 2. The effects of whole-body hypothermia treatment on the lung histopathology changes and pulmonary edema induced by blast limb trauma. Representative microphotographs were taken from Sham (*A*), blast limb trauma (BLT; *B*), and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30; *C*), 60 min (BLT-WH60; *D*), 3 h (BLT-WH3h; *E*), and 6 h (BLT-WH6h; *F*). Histopathologic scoring data of lung injury are presented in a box-and-whisker plot (the *boxes* are constructed with 25% and 75% CIs and median and maximum or minimum individual values; *G*). Lung water content was represented by wet/dry weight (*H*). Data are presented as mean ± SD (n = 12). #P < 0.05 *versus* BLT.

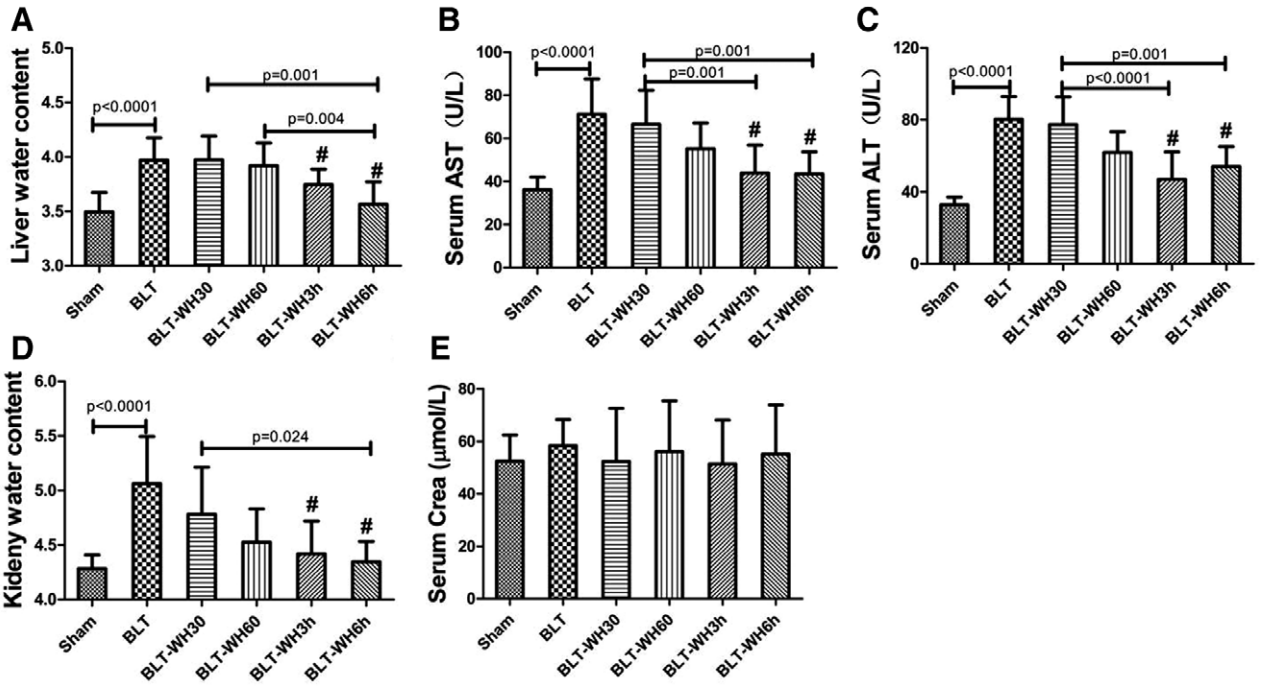

Fig. 3. The effects of whole-body hypothermia treatment on liver and kidney water content and biochemistry marker of liver and kidney function in blast limb trauma rats: liver water content (*A*); serum aspartate aminotransferase (AST [U/l]; *B*); serum alanine aminotransferase (ALT [U/l]; *C*); kidney water content (*D*); and serum creatinine (Crea [μmol/l]; *E*). Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #*P* < 0.05 *versus* BLT.

The Effect of WH on Plasma Hydrogen Sulfide

BLT resulted in a decreased plasma hydrogen sulfide level (36.8 ± 9.5 μM *vs.* 68.4 ± 11.7 μM of controls, $P < 0.001$). WH for 3 h (59.2 ± 4.2) almost revered this decrease ($P = 0.450$), and plasma hydrogen sulfide level was lower in the BLT-WH30 ($P < 0.001$), BLT-WH60 ($P = 0.003$), and BLT-WH6h ($P < 0.001$) rats compared with that in the Sham rats (fig. 4D).

The Effect of WH on Myeloperoxidase Activity

Myeloperoxidase activity was determined as a surrogate of neutrophil activity. Myeloperoxidase activity in plasma (fig. 5A), muscle (fig. 5B), lung (fig. 5C), liver (fig. 5D), and kidney (fig. 5E) was increased in the BLT rats compared with that in the Sham rats. WH for 3 and 6 h reduced plasma (fig. 5A) and muscle myeloperoxidase activity (fig. 5B) induced by BLT. WH for any durations reduced lung myeloperoxidase when compared with that of BLT rats, but WH for 6 h further reduced lung myeloperoxidase compared with WH for 3 h ($P = 0.047$; fig. 5C). Unlike hypothermia for 30 min, hypothermia for 3 ($P = 0.014$) and 6 h ($P < 0.0001$) reduced liver myeloperoxidase (fig. 5D). WH for 3 ($P = 0.002$) and 6 h ($P = 0.001$) alleviated the increased kidney myeloperoxidase activity induced by BLT, respectively (fig. 5E).

WH Attenuated Oxidative Stress in the BLT Rats

As shown in figure 6, BLT resulted in a decreased total antioxidation capacity (T-AOC) in the muscle (fig. 6A), lung (fig. 6B), liver (fig. 6C), and kidney (fig. 6D). Both WH for 3 and 6 h elevated a decrease of T-AOC in the muscle (fig. 6A), lung (fig. 6B), and kidney (fig. 6D), and WH for 6 h also increased T-AOC in the liver (fig. 6C).

BLT caused a decrease in superoxide dismutase (SOD) activity in plasma (fig. 7A), muscle (fig. 7B), lung (fig. 7C), liver (fig. 7D), and kidney (fig. 7E). All durations of WH had no effects on the SOD activity in plasma (fig. 7A) and kidney (fig. 7E), but the prolonged hypothermia for 3 and 6 h elevated SOD activity in the muscle (fig. 7B), lung (fig. 7C), and liver (fig. 7D).

BLT resulted in a decrease in the glutathione level in the plasma and all the organs measured ($P < 0.05$), but WH treatment did not alter such changes (fig. 8).

BLT resulted in an increase in the malondialdehyde level in the plasma (fig. 9A), muscle (fig. 9B), lung (fig. 9C), liver (fig. 9D), and kidney (fig. 9E). WH for 30 ($P = 0.033$) or 60 ($P = 0.04$) min reduced lung malondialdehyde level in BLT rats (fig. 9, B and C), but WH for 3 or 6 h decreased malondialdehyde level in the plasma and all organs measured (fig. 9).

BLT increased hydrogen peroxide in the plasma and all organs studied (fig. 10). WH for 60 min reduced lung ($P = 0.016$) and liver ($P = 0.011$) hydrogen peroxide level in BLT rats (fig. 10, C and D), and WH for 3 and 6 h decreased hydrogen peroxide in the plasma and concerned organs (fig. 10).

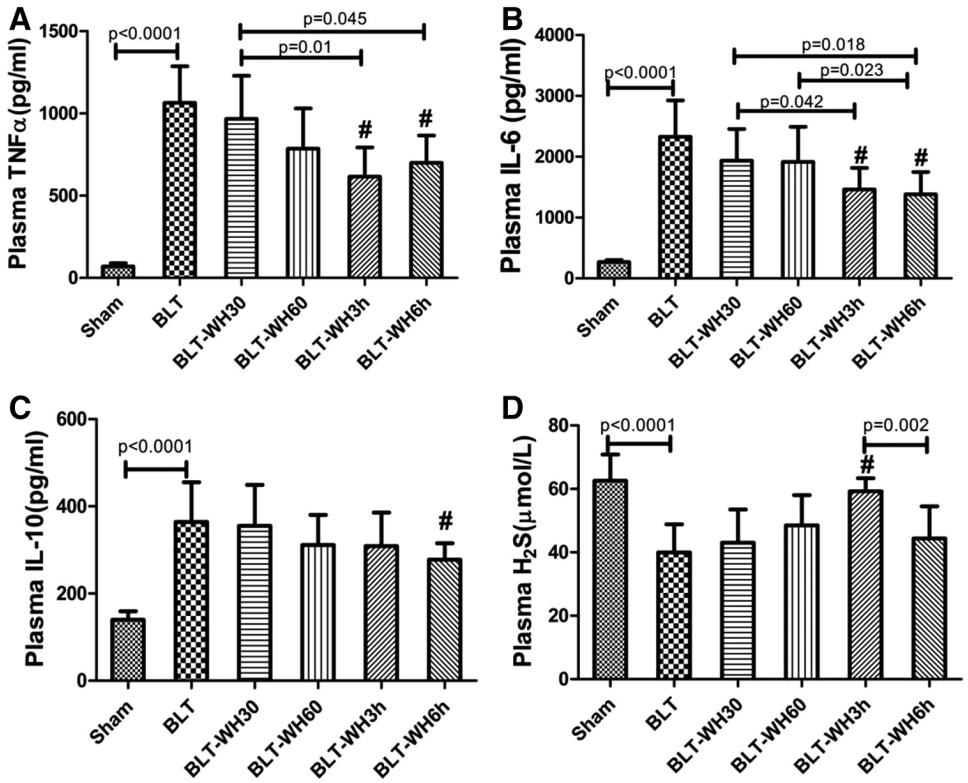

Fig. 4. The effects of whole-body hypothermia treatment on plasma cytokines and hydrogen sulfide (H_2S) level in blast limb trauma rats. Tumor necrosis factor (TNF)-α (*A*), interleukin (IL)-6 (*B*), IL-10 (*C*), and H_2S (*D*) in plasma. Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #$P < 0.05$ *versus* BLT.

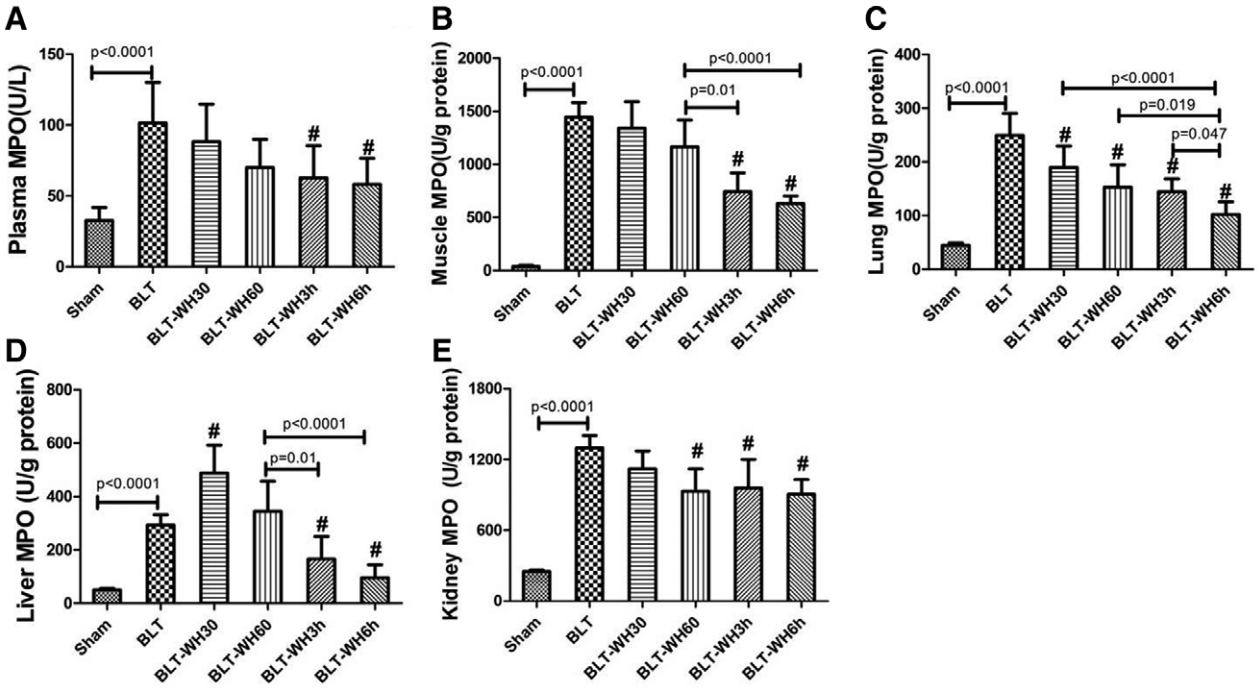

Fig. 5. The effects of whole-body hypothermia treatment on myeloperoxidase (MPO) activity in blast limb trauma rats. MPO activity is shown in plasma (*A*), muscle (*B*), lung (*C*), liver (*D*), and kidney (*E*). Data are shown for blast limb trauma (BLT) and blast limb trauma + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #$P < 0.05$ *versus* BLT.

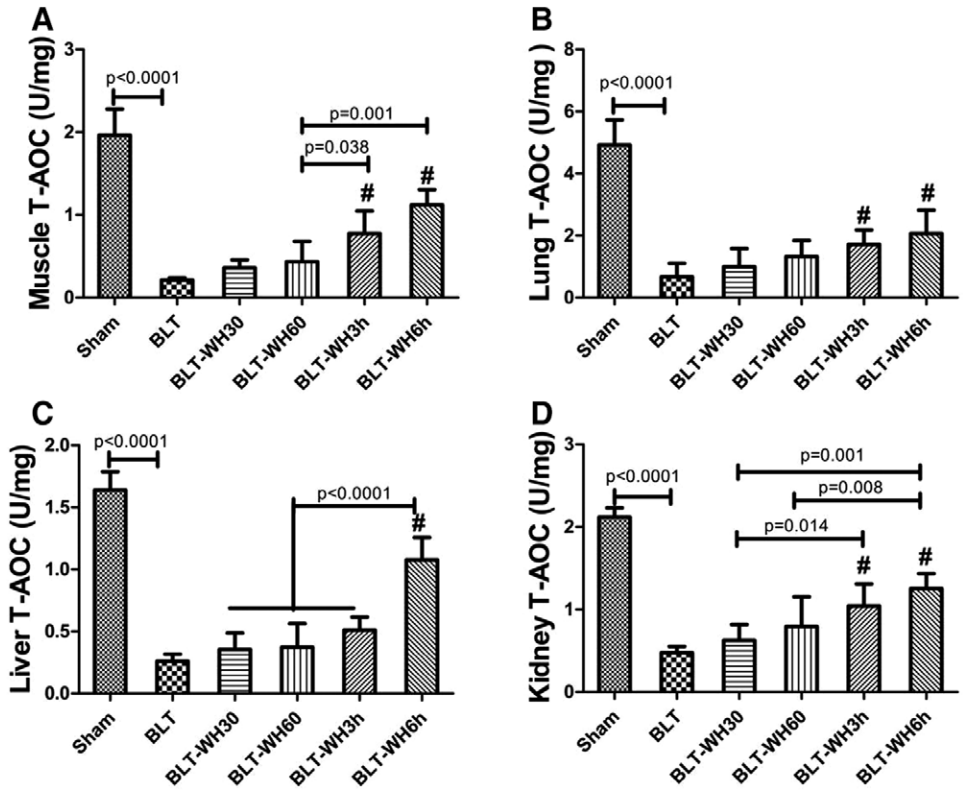

Fig. 6. The effects of whole-body hypothermia treatment on total antioxidation capacity (T-AOC) activity in blast limb trauma rats. T-AOC activity is shown in muscle (*A*), lung (*B*), liver (*C*), and kidney (*D*). Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and for 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #$P < 0.05$ *versus* BLT.

Discussion

BLT results in not only local injury but also in distant-organ injuries, including the lung injury, as reported previously.[4,23] The current data demonstrate that WH treatment for 3 and 6 h protected multiple-organ injuries such as the traumatic limb, lungs, liver, and kidneys after BLT. The underlying mechanism may be associated with its suppression of neutrophil infiltration, restoration of the balance of proinflammatory and antiinflammatory responses, inhibition of lipid peroxidation and production of reactive oxygen species, and elevation of hydrogen sulfide production.

In the current study, the protective effects of different durations of mild hypothermia were assessed in the BLT rats; WH for 3 h was shown to be the effective and optimal duration that afforded protection to all organs tested without remarkable adverse effects when compared with WH for 30 min, 60 min, or 6 h. Although WH for 30 or 60 min alleviated the distant lung injury and water content, but it seems not long enough to attenuate local traumatic limb, liver and kidney injury. WH for 3 h corrected the changes of the most of biochemical variables, while WH for 6 h could further correct T-AOC in the lungs and liver but increased blood loss. Therefore, WH for 3 h after spontaneous rewarming is sufficient to provide both local tissue and distant-organ protection in the BLT rats and limits the adverse effects of hypothermia. Our data reported here and those reported previously strongly suggest that different conditions need different durations of mild hypothermia for optimal outcome to be achieved.[3,4,20,21]

Hydrogen sulfide is a powerful biologic signal participating in many pathologic conditions[24] and was shown to be involved in the process of distant-organ injury after BLT.[4,23] Endogenous hydrogen sulfide is produced from L-cysteine by enzymes such as cystathionine β-synthase, CSE, 3-mercaptopyruvate sulfurtransferase, and cysteine aminotransferase.[25] Disturbance of hydrogen sulfide metabolism has been found in several disease conditions, such as ischemia–reperfusion injury,[26] acute lung injury,[27] hypertension,[28] atherosclerosis,[29] cirrhosis,[30] and kidney fibrosis.[31] Unlike other durations of hypothermia, WH for 3 h reversed the decreased plasma hydrogen sulfide level. The causes for this discrepancy remain unknown, but restoration of CSE activity could be one of many mechanisms. CSE is mainly expressed in the liver, kidney, and lungs,[32] and its activity was found to be decreased in these organs in the BLT rats, indicating that the decreased CSE activity is likely the main cause of decreased plasma hydrogen sulfide level after BLT.[23] It is reasonable to assume that WH for 6 h was too long and resulted in decreased metabolism rate because hydrogen sulfide is the metabolic product of L-cysteine, and all of

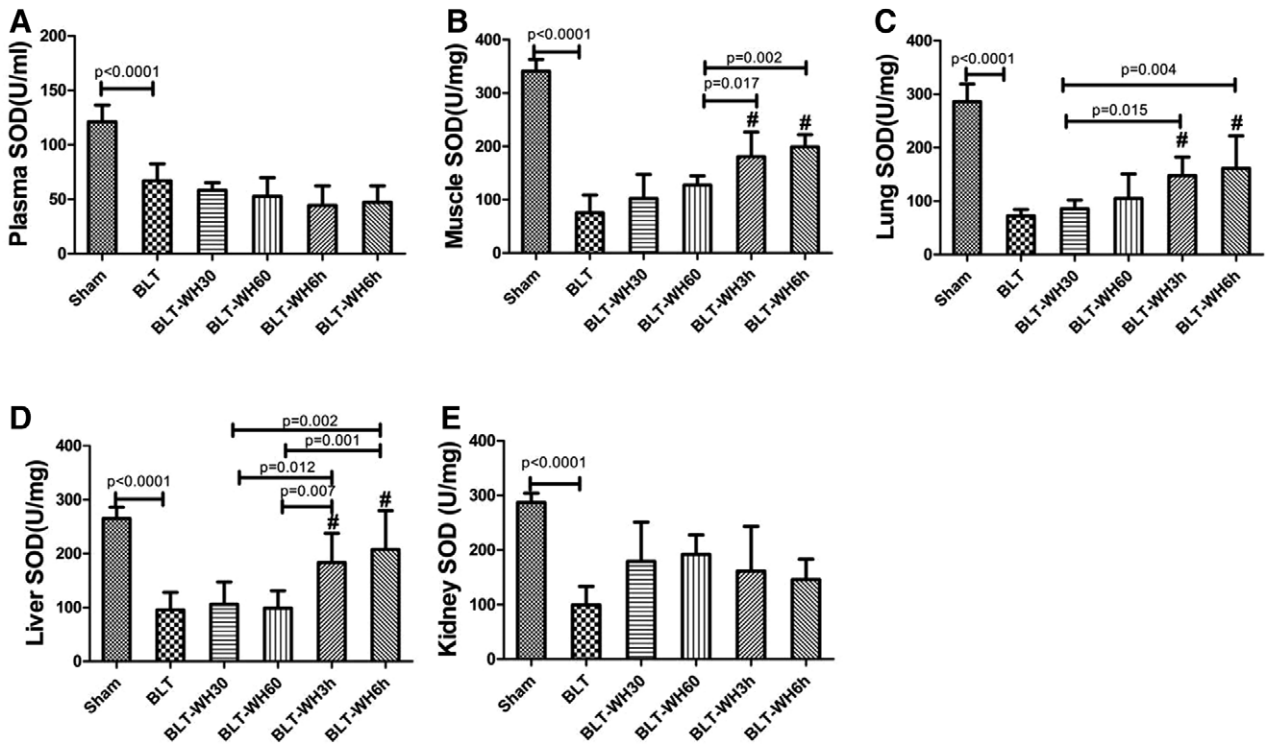

Fig. 7. The effects of whole-body hypothermia treatment on superoxide dismutase (SOD) activity in blast limb trauma rats. SOD activity is shown in plasma (*A*), muscle (*B*), lung (*C*), liver (*D*), and kidney (*E*). Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #*P* < 0.05 *versus* BLT.

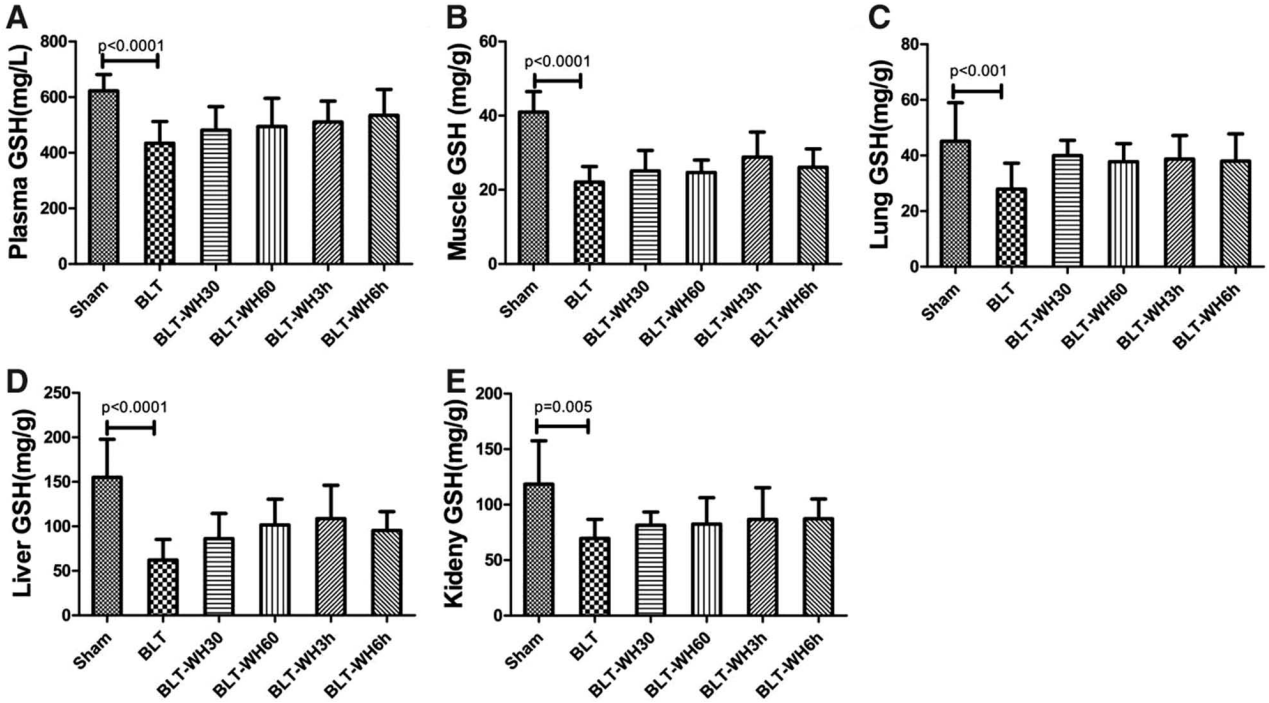

Fig. 8. The effects of whole-body hypothermia treatment on glutathione (GSH) level in blast limb trauma rats. GSH activity is shown in plasma (*A*), muscle (*B*), lung (*C*), liver (*D*), and kidney (*E*). Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12).

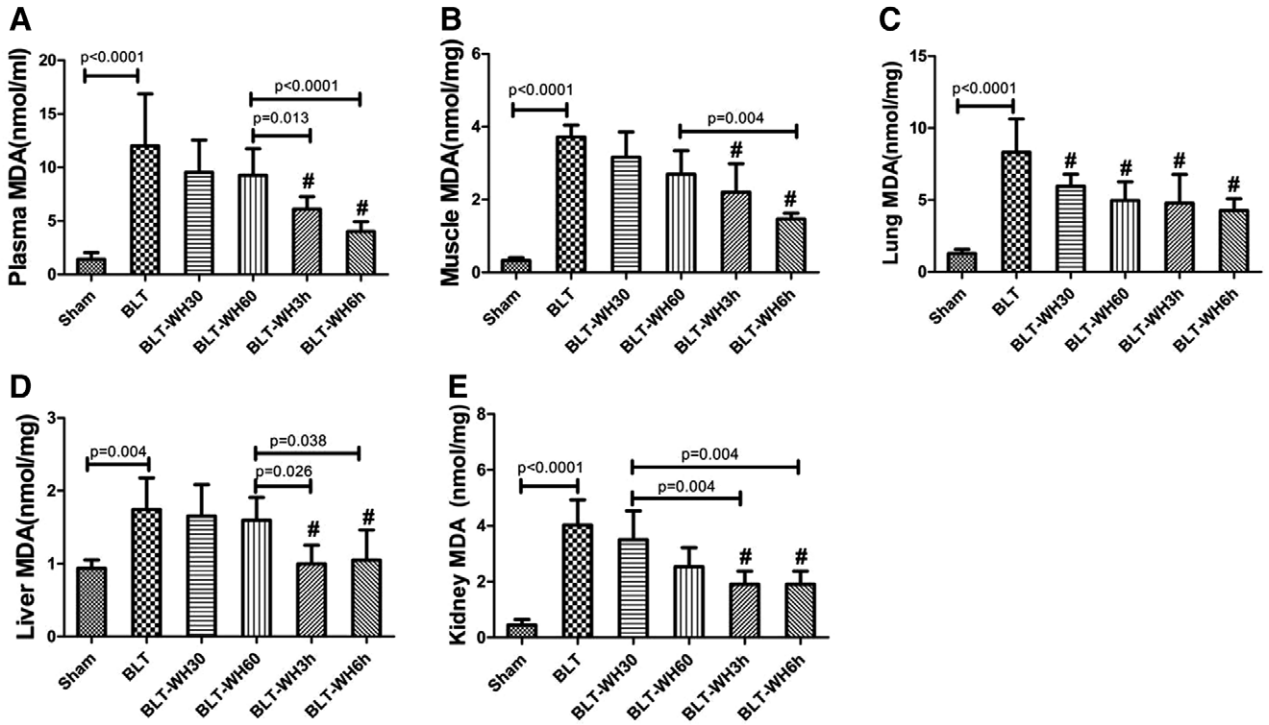

Fig. 9. The effects of whole-body hypothermia treatment on malondialdehyde (MDA) level in blast limb trauma rats. MDA activity is shown in plasma (*A*), muscle (*B*), lung (*C*), liver (*D*), and kidney (*E*). Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #$P < 0.05$ *versus* BLT.

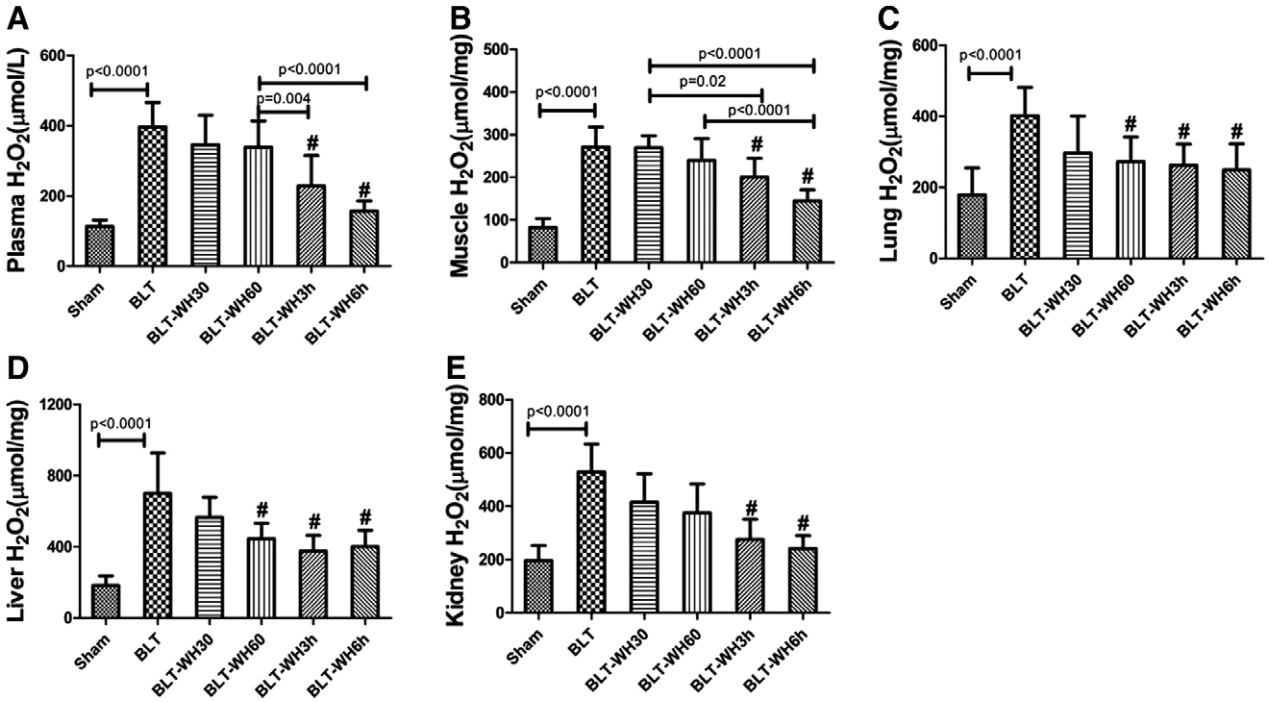

Fig. 10. The effects of whole-body hypothermia treatment on hydrogen peroxide (H_2O_2) level in blast limb trauma rats. H_2O_2 activity is shown in plasma (*A*), muscle (*B*), lung (*C*), liver (*D*), and kidney (*E*). Data are shown for blast limb trauma (BLT) and BLT + whole-body hypothermia treatment for 30 min (BLT-WH30), 60 min (BLT-WH60), 3 h (BLT-WH3h), and 6 h (BLT-WH6h). Data are presented as mean ± SD (n = 12). #$P < 0.05$ *versus* BLT.

substance metabolism is closely associated with reaction temperature and thus reduced the hydrogen sulfide production in the BLT rats, which warrants further study. Therapeutic hypothermia for 3 h *per se* may restore hydrogen sulfide production *via* increasing the release of dopamine and serotonin and other biogenic amines in the BLT rats.[33,34] Hydrogen sulfide has been found to afford multiple-organ protection, and it is true that exogenous hydrogen sulfide donor has been shown to alleviate ischemia–reperfusion injury of the lungs,[35] liver,[36] and kidney.[37] In addition, exogenous hydrogen sulfide donor, sodium hydrosulfide, was found to attenuate distant lung injury after BLT in our previous study,[38] which is very likely to be due to inhibition of nuclear factor-κB activity, suppression of oxidative stress, preservation of function and structure of mitochondria, and up-regulation of prosurvival signal pathway.[39] Thus, restoring hydrogen sulfide may be considered to be one of mechanisms underlying multiple-organ protection afforded by WH for 3 h in the BLT rats. However, WH for 6 h also provided protection for the distant organs although it did not restore plasma hydrogen sulfide level when compared with 3 h, which may suggest that multiple mechanisms are responsible for organ protection afforded by WH.

Inflammatory response is considered to play a key role in distant-organ injury. An early increase of proinflammatory cytokines after tissue trauma and hemorrhage has been well described in animal and clinical studies. TNFα and interleukin-6 were found to be increased earlier than interleukin-10 after BLT in rats.[23] It is well established that excessive TNFα plays a critical role in systemic inflammation and a physiopathologic role in the development of MODS. Interleukin-6 is an important cytokine in the progress of inflammatory response *via* delaying apoptosis of neutrophil[32] and mediating the hepatic acute phase response,[30] and it adopts a central regulatory role in primary cellular and humoral immune activation.[32,33] Antiinflammatory cytokines that are up-regulated after hyperinflammatory response triggered by insults have been considered to keep the balance between proinflammatory and antiinflammatory reactions. Interleukin-10 is the pleiotropic antiinflammatory cytokine, and its main biologic function is to limit inflammatory response *via* regulating the differentiation and proliferation of several immune cells such as T, B, natural killer, and antigen-presenting cells.[40] Both proinflammatory and antiinflammatory cytokines have been reported to be involved in the protective effects of hypothermia.[41] Indeed, hypothermia has been reported to block the inflammatory "cascade" reaction and thus attenuate the organ damage.[42] Plasma TNFα, interleukin-6, and interleukin-10 were found to be reduced by WH treatment in the current study; however, proinflammatory cytokines were prone to be suppressed by WH treatment when compared with antiinflammatory cytokines, which is confirmed by the elevated TNFα/interleukin-10 ratios by WH treatment for 3 h (BLT: 2.92 *vs.* BLT-WH3h: 1.99), which could be at least partly contributed to the organ protection by WH as well.

Oxidative stress is also an important component of systemic inflammatory response and MODS. Oxidative stress damages the parenchyma cells and microvessel endothelium and results in increased capillary permeability and edema. It is has been suggested that hypothermia suppresses mitochondrial oxidative stress and also preserves energy supply.[43] WH treatment for 3 or 6 h reduced production of hydrogen peroxide and malondialdehyde and preserved T-AOC in concerned organs, and WH for 3 or 6 h were shown to elevate SOD activity in the muscle, lungs, and liver; however, it had no pronounced effects on the glutathione level in all concerned organs. All these data suggested that WH treatment for 3 or 6 h may be more effective in the suppression of oxidation production than in the promotion of endogenous antioxidative protein expression, which is different from regional hypothermia. This viewpoint was supported by data showing that WH for 3 or 6 h does not increase Nrf2 (a key transcription factor for antixenobiotic genes) activity in the lungs (data not shown), but rather that regional hypothermia for 30 min could upregulate Nrf2 activity.[4] Activated macrophage is the main source of reactive oxygen species,[44] and reduced macrophage infiltration contributed to the suppression of oxidative stress by WH in the BLT rats, which confirms that WH for 3 or 6 h reduced myeloperoxidase activity in concerned organs and blood circulation.

WH has been reported to improve the hemodynamics states in different disease conditions, such as cardiogenic shock syndrome,[45] hemorrhagic shock,[46] and sepsis.[47] WH could affect cardiac output, systemic vascular resistance, left ventricular contractility, and heart rate.[45] However, MAP was not profoundly affected by WH treatment in this study, which suggested that stabilization of hemodynamics did not contribute to the WH-associated organ protection in the BLT rats. In the current study, although significant benefit could be achieved, WH treatment for 6 h was found to increase blood loss because prolonged WH treatment may impair coagulation function.[48,49] In addition, prolonged WH treatment also causes other well-known adverse effects including acid–base imbalance and immune suppression.[50,51] Yet, it is clinically important to keep traumatic patients warm for better tissue or cellular oxygenation. Our data reported here may suggest that cooling patients at earlier stage after trauma may have therapeutic value, but, undoubtedly, data obtained from rodents in the current study are far from clinical situation. Therefore, better designed preclinical studies with large animals as study subjects and clinical trials comparing therapeutic effectiveness of "warm," global (current data) or regional hypothermia[4] in treating traumatic patients are urgently needed.

Our study had several limitations. First, this blast model did not include penetrating wounds and severe contamination, and debridement was not performed in this severe blast limb model, which are different from clinical situation. Second, the time course of this study was only 6 h, and the

long-term outcomes of the treatment are not known. Third, vital-organ injuries observed in this study may not be necessarily due to subsequent BLT only but may also be due to explosion wave produced by blast or the combination of both.[23] Fourth, our data reflect blast trauma specifically, but not surgical "trauma," which has also been shown to cause remote lung injury recently.[52,53] Lastly, preclinical rodent model is far from clinical settings, and also the "favorable" effects of hypothermia on the biomarkers measured in this study are only "observable" changes; the causal relationship is unknown and therefore the translation of our findings to clinical condition requires more studies in big animal models and preclinical studies.

In conclusion, the current data show that WH for 3 h provided multiple-organ protection in the BLT rats *via* suppressing oxidative stress, restoring balance between proinflammatory and antiinflammatory reactions and elevating endogenous hydrogen sulfide production without adverse effects. Our current study could facilitate more studies in this area of research; until then, any therapeutic values of systemic hypothermia will remain unknown.

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Competing Interests

The authors declare no competing interests.

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