Thermal Steam Aerosolization Protects Against Lipopolysaccharide-Induced Sepsis in Rats

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Background: *Sepsis is a severe systemic inflammatory state in response to infection. The induction of heat shock protein 70 (HSP70) by heat stress has been reported to protect against lethal effects of sepsis. In clinical situation, inhalation of thermal water has been used empirically in the treatment of chronic diseases of respiratory tract. Thus, thermal steam aerosolization may have beneficial effects on sepsis via HPS70 induction.*

Objective: *The present study tested the hypothesis that thermal steam aerosolization could protect against lipopolysaccharide-induced sepsis in rats.*

Material and Method: *Male Spraque-Dawley rats were subjected to steam aerosolization at 40°C for 1 hour before intraperitoneal injection of 10 mg/kg lipopolysaccharide (LPS). Blood pressure, heart rate and core temperature were recorded in two hours interval. At the end of the study period, vascular response to vasoconstrictor of isolated aortic rings ex vivo was assessed. Serum proinflammatory cytokines, IL-1beta and IL-6, were analyzed using ELISA technique. Plasma nitric oxide was determined using nitrate/nitrite fluorometric assay Kit. HSP70 expression, heat shock factor-1 (HSF-1) mRNA level and nuclear factor-kappa B (NF-kappa B) activity in the lungs of rats were investigated using western blot analysis, real-time quantitative PCR and transcription factor kits for NF-kappa B p65, respectively.*

Results: *Thermal steam aerosolization treatment prevented the fall in systolic, diastolic, mean arterial blood pressures induced by LPS and restored the vascular response to adrenaline. LPS significantly increased plasma nitrate/nitrite concentration, serum IL-1 beta and IL-6 levels, and NF-kappa B activity in rat lung lysate which were reduced by thermal steam aerosolization. Thermal steam aerosolization induced both HSP70 and HSF-1 mRNA expression.*

Conclusion: *The present study suggests that thermal steam aerosolization can delay the stage of shock in LPS-induced septic rats. It shows a beneficial therapeutic effect and may be applied to the clinical approach for septic shock patient.*

Keywords: *Heat shock protein 70, Hypotension, Lipopolysaccharide, Proinflammatory cytokines, Sepsis, Shock, Steam aerosolization*

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Sepsis is a systemic inflammation state in response to infection which could progress to hypovolemia, multiple organ failure and septic shock[1]. On cellular and subcellular levels, the pathophysiology of sepsis involves alterations or dysfunctions in immune function/signaling, the endothelium, the intestinal epithelium, and the coagulation cascade which result in systemic inflammation, hemodynamic abnormality, coagulopathy, and multiple organ dysfunction/injury. Sepsis and septic shock remain a major therapeutic challenge with a high mortality rate[1,2]. The treatment for sepsis is nonspecific, limited primarily to support of organ function and administration of intravenous fluids, antibiotics, vasopressor and oxygen[3]. The therapeutic guidelines and the novel therapeutic approach have been continuously developed. However, the overall incidence of sepsis and mortality remains high. The necessity for effective therapeutic approaches to this illness should be explored.

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The induction of heat shock proteins (HSPs), a highly conserved family of stress response proteins, has been shown to be beneficial to sepsis on the cellular and molecular levels[4-6]. These proteins are induced by a wide variety of stresses, such as elevated temperature, hypoxia, oxidative stress, physical injury or chemical stimuli. They play a crucial role to maintain cell homeostasis against these stressors. There is evidence that pretreatment with heat induces expression of the 70-kDa family of HSP (HSP70) and reduces both mortality and organ dysfunction in several animal models of experimental sepsis[4-6]. Thermal preconditioning-induced HSP70 decreases lung damage and increases animal survival in experimental sepsis-induced acute lung injury, either by cecal perforation, intravenous lipopolysaccharide (LPS) injection or by intrathecal administration of TNF-alpha[7]. Moreover, an expression of HSP70 among heat stress could suppress the activity of the transcription nuclear factor-kappa B (NF-kappa B), an activator of genes encoding inflammatory cytokines, which is a critical factor in pathophysiology of sepsis[8].

Inhalation thermal therapy has been used as a part of the treatment of chronic inflammations of upper and lower respiratory tract, such as pharyngitis, rhinosinusitis, obstructive chronic bronchitis and pulmonary diseases[9]. Previous clinical studies have demonstrated that inhaled humidified warm air or elevated intranasal temperature could alleviate the symptoms of common cold, perennial rhinitis and allergic rhinitis[10] but its mechanisms is not yet well clarified. The preliminary study showed that steam aerosolization with the temperature of 40°C for 1 hour could induce HSP70 expression in the rat lungs. So it is interesting to investigate whether thermal steam aerosolization could protect against sepsis through HSP70 induction. Consequently, the aim of the study has been designed to determine the effects of steam aerosolization on hemodynamic parameters, vascular responses to vasoconstrictor, pro-inflammatory cytokines and NF-kappa B activity in LPS-induced sepsis in rats.

Material and Method

Animals and study design

Forty male Sprague-Dawley rats provided by National Laboratory Animal Center of Salaya Campus, Mahidol University Thailand, weighing 350-450 g, were used. The study protocol was approved by the experimental animals ethics committee of Faculty of Medicine, Srinakharinwirot University, Thailand (license number. 4/2557). All rats were housed in a climate-controlled room (temperature of 25$\pm$2°C, relative humidity of 55$\pm$10%) with a 12-hour dark/light cycle and allowed to access free standard chow diet for 2 weeks before treatment. The rats were let to be used to the instruments before starting each experiment.

LPS-induced sepsis model was designed according to Fujiwara T et al[11] by a peritoneal injection of bacterial LPS (*Escherichia coli*, serotype 011:B4, Sigma Chemical, USA) at the amount of 10 mg/kg body weight. After the administration, the rats were considered to be in shock state when mean arterial blood pressure decreased to 25% to 30% of baseline level[12]. Rats were randomly divided into 5 groups of eight animals per group. Three groups of rats inhaled aerosolized steam at 40°C for 1 hour and were immediately sacrificed (Steam aerosolized group: S, n = 8) or were allowed to cool passively for 6 hours before sacrifice (Steam-recovery group: SR, n = 8) or were intraperitoneally injected with 10 mg/kg body weight of LPS and allowed to cool passively for 6 hours before sacrifice (Steam-LPS group: SL, n = 8). Another 2 groups of rats were placed into a box at room temperature without steam aerosolization for 1 hour and returned to their cages with free access food and water for 6 hours before sacrifice (Control group: C, n = 8) or were intraperitoneally injected with 10 mg/kg body weight of LPS before return to their cages with free access food and water for 6 hours and sacrifice (LPS group: L, n = 8).

Temperature, heart rate, systolic, diastolic and mean arterial blood pressures of rats in all groups were recorded before and after treatments at 1, 2, 4, 6 hours by a thermometer and an automatically non-invasive blood pressure measuring system LE 5002 with Seda program (Barcelona, Spain).

At the end of each experiment, blood samples were collected by cardiac puncture. Plasma and serum were separated by centrifugation at 3,500 rpm for 15 minutes and stored at -80°C until use for analysis of pro-inflammatory cytokines and nitric oxide assay. The lungs were removed, washed in 0.9% normal saline and snapped frozen in liquid nitrogen, and stored at -80°C until use for Western blot analysis. The aorta was isolated and immediately placed in fresh ice-cold Krebs buffer for the measurement of vascular reactivity.

Steam aerosolization

The condition of steam aerosolization was performed in according to Chaidee et al[13]. Aerosolized steam with optimal temperature at 40°C was produced

by steam generator machine (Thailand). Temperature and humidity were controlled by thermostat and hygrometer. The steam was produced by using distilled water and filled into a steam box until the inside temperature reached 40±2°C, then only the head and neck of a rat was placed in the steam box whereas it body left outside, for 1 hour. The insertion only part of head and neck was designed to mimic as closely as possible to inhaled condition which is used in pediatric patients. Temperature and humidity were recorded every 5 minutes.

Measurement of vascular reactivity

The aortas were dissected free of adhering fat and connective tissue. The specimens were cut into 3 mm segments in length. Each aortic ring was placed into 20 mL organ bath containing Krebs bicarbonate buffer solution at pH 7.4 of the following composition (in mM): $NaHCO_3$, 25; $MgSO_4$, 0.6; NaCl, 120; KCl, 4.75; KH_2PO_4, 1.2; $CaCl_2$, 1.28; glucose, 11; and EDTA, 0.026. The solution was maintained at 37°C with the temperature controlled circulating and continuously aerated with a gas mixture containing 95% O_2: 5% CO_2. The aortic rings were connected to force transducers (March, Germany) for the measurement of isometric tension. They were allowed to equilibrate at least 60 minutes under an optimal resting tension of 1 g which was maintained throughout the experiment and then subjected to contraction by accumulation dose of adrenaline (1 nM-100 μM). The isometric tension was measured with recorded on a chart recorder version 3.6 by Program in Computers, Macintosh (Power PC 7100/60, USA).

Determination of inflammatory mediators

The quantitative determinations of IL-1beta and IL-6 concentrations in rat serum were performed using ELISA kits (eBiosciences, USA) whereas the concentration of nitrate/nitrite in rat plasma was measured using nitrate/nitrite fluorometric assay kit (Cayman, USA), by following the manufacturers' protocol.

Determination of NF-kappa B activity

Rat lung homogenate which was prepared in NE-PER™ Nuclear and Cytoplasmic extraction reagents (Thermo Scientific, USA) was used to detect NF-kappa B activity using transcription factor kits for NF-kappa B p65 (a subunit of NF-kappa B transcription complex) (Thermo Scientific, USA) by following the manufacturer's instruction.

Determination of heat shock protein (HSP) 70 by immunoblotting

Rat lung homogenate was prepared in lysis buffer containing 10 mM Tris, 5 mM EDTA, 2% Triton X-100, 0.2 mM Na_3VO_4, 1 mM phenylmethyl sulphonyl fluoride, and 10 mg/ml leupeptin and aprotinin. The lung homogenate was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membrane (Pall Corporation, Mexico)[14]. The nonspecific binding was blocked with 5% nonfat dry milk (Bio-RAD, USA) in PBS containing 0.1% Tween-20 (PBST) and 0.1% of NaN_3 at 37 C overnight. The membrane was incubated with mouse anti-rat monoclonal antibody of HSP70 (Sigma, USA) diluted (1: 5,000) and mouse anti-actin monoclonal antibody diluted (1: 30,000) (Millipore, USA) at 4 C overnight. After washing in PBST, the membrane was stained with a mixture of rabbit anti-mouse peroxidase conjugate (Sigma, USA) diluted (1: 8,000) and streptavidin peroxidase complex diluted (1: 10,000) at room temperature for 1 hr. PBST containing 1% BSA was used as diluents for primary and secondary antibodies. Pierce western blot reagent (Thermo Scientific, USA) was used to develop stained blot and the protein bands on the membrane were visualized by an exposure with CL-X Posue™ autoradiograpy film. The film was developed with film developer and fixer (Kodak, USA). The intensity of HSP70 and actin bands was quantified using Gene Tools gel image analysis software (Syngene, UK).

Determination of HSF1 mRNA expression

Total RNA were extracted from lung tissue using the Trizol reagent (Invitrogen, USA) according to manufacturer's protocol. Complementary DNA (cDNA) was synthesized with 2 mg of total RNA using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, USA) by following manufacturer's instruction. The oligonucleotide primers specific for *HSF-1* (forward primer, 5'GATGAAGGGGA AACAGGAGT3' and reverse primer, 5'CCAGTGAGAT CAGGAATTGG3') were synthesized by Life Technologies, USA. cDNA reaction product was used to perform real-time PCR with the QuantiTect SYBR green PCR kit (Qiagen, USA) and an Applied Biosystems 7300 real-time PCR system. The amplification was carried out, starting with 1 cycle at 95°C for 15 min, followed by 40 rounds of 15 s at 95°C, 1 min at 58°C and 0.34 min at 72°C. An extension cycle of 1 min at 95°C, 30s at 55°C and 30s at 95°C was added to complete the dissociation curve. All data were

normalized to 18S house-keeping gene (18S QuantiTect Primer assay, Qiagen) to obtain ΔC_T. The data were shown as relative expression ($2^{-\Delta C_T}$)(15).

Statistical analysis

All data are expressed as mean $\pm$ standard error of mean (SEM). One-way ANOVA followed by LSD post hoc test was used to compare within a group at multiple time points and differences between groups. Statistical significance was accepted at $p<0.05$.

Results

Hemodynamic parameters

At baseline, the systolic, diastolic, mean arterial blood pressures and heart rate of the rats in five groups did not show significant difference among groups as shown in Fig. 1A-C, Fig. 2A, B. The systolic, diastolic, mean arterial blood pressures and heart rate remained stable throughout the experimental period in the control (C), steam (S) and steam-recovery (SR) groups. LPS caused a decrease in systolic, diastolic and mean arterial blood pressures at each time point on the same level (Fig. 1A-C) and an increase in heart rate (Fig. 2A) over the 6 hours course after LPS injection ($p<0.05$). Treatment with steam aerosolization prior to LPS administration (steam-LPS group) prevented the fall in systolic, diastolic, mean arterial blood pressures and the increase in heart rate at any time points ($p<0.05$).

Core temperatures were 37.43$\pm$0.13°C at baseline with no significant differences between the groups (Fig. 2B). They were significantly elevated by the steam aerosolization at 40°C for 1 hour (39.00$\pm$0.23°C, 39.50$\pm$0.18°C and 39.47 $\pm$ 0.11°C for steam aerosolized, steam-recovery and steam-LPS groups, respectively) and declined to the baseline level after 1 hour of the recovery at room temperature. Only the rats in LPS group showed a decreased core temperature at the time of 2, 4 and 6 hours (35.65$\pm$0.08°C, 36.20$\pm$0.22°C and 35.93$\pm$0.19°C, respectively) when compared to the baseline or control group ($p<0.05$).

Vascular reactivity

Compared with the control group, constriction of isolated aortic rings ex vivo in response to adrenaline was significantly attenuated in LPS-treated rat ($p<0.05$; maximum contraction (Emax) 0.34$\pm$0.02 and 0.99.00$\pm$0.07 g; ED50 -6.71$\pm$0.07 and -7.50$\pm$0.11, for LPS and control groups respectively; Fig. 3, Table 1). Treatment with the steam aerosolization partly, and significantly, restored the vascular response to adrenaline ($p<0.05$; Emax 0.34$\pm$0.02 and 0.55$\pm$0.02 g; ED50 -6.71$\pm$0.07 and

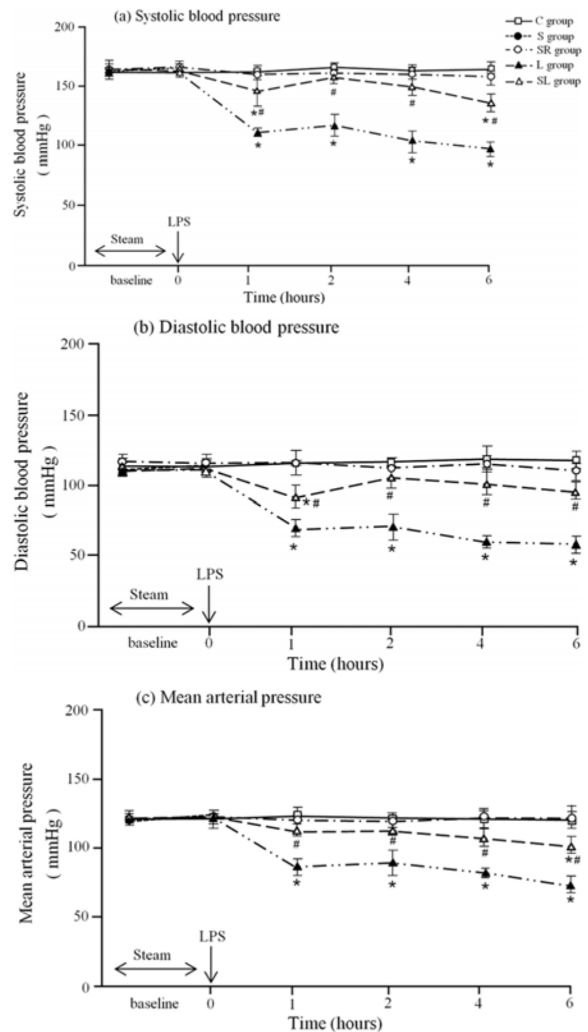

Fig. 1 Hemodynamic parameters of rats on various treatments. Systolic blood pressure (a), diastolic blood pressure (b) and mean arterial blood pressure (c). C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are mean $\pm$ SEM; * $p<0.05$ compared with control group, # $p<0.05$ compared with LPS group.

-6.91$\pm$0.04 for LPS and steam-LPS groups, respectively).

Plasma nitrate/nitrite and serum cytokines concentrations

LPS significantly increased plasma nitrate/nitrite concentration by more than 90% of that in control group (99.00$\pm$1.80 and 1.31$\pm$0.22 mM for LPS and control groups respectively, $p<0.05$, Fig. 4). Steam aerosolization treatment significantly reduced in total nitric oxide compared with the LPS group (99.00$\pm$1.80

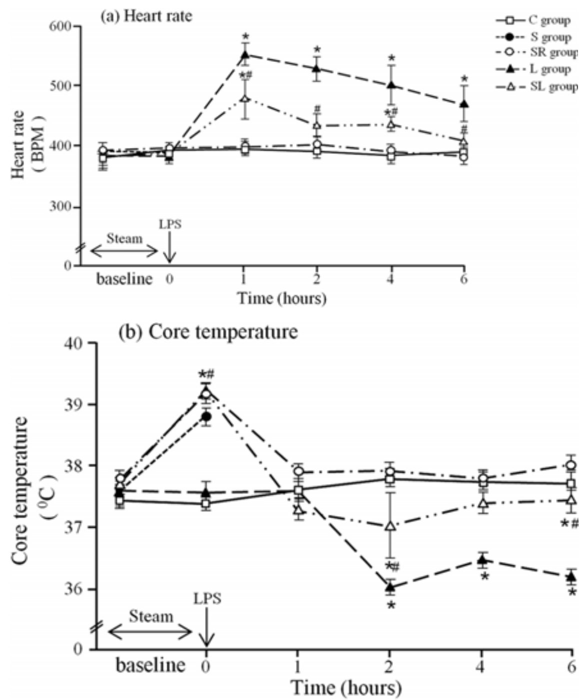

Fig. 2 Heart rate (a) and core temperature (b) of rats on various treatments. C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are means ± SEM; * $p<0.05$ compared with control group, # $p<0.05$ compared with LPS group.

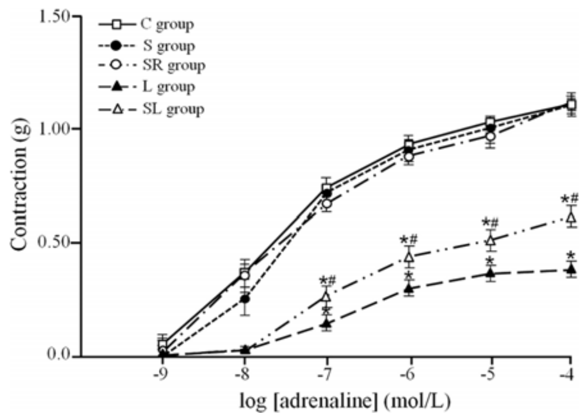

Fig. 3 Concentration response curves to adrenaline of isolated aortic rings from each group of rats. C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are means ± SEM; * $p<0.05$ compared to control group; # $p<0.05$ compared to LPS group.

and 78.79±7.47 mM for LPS and steam-LPS groups respectively, $p<0.05$).

Table 1. Maximal contractions (E_{max}) and median effective dose (ED_{50}) of adrenaline in the different groups of rats

Group	E_{max} (g)	ED50 (log mol/L)
Control	0.99±0.07	-7.50±0.11
Steam-aerosolized	0.98±0.05	-7.49±0.13
Steam-recovery	0.99±0.04	-7.51±0.08
LPS	0.34±0.02*	-6.71±0.07*
Steam-LPS	0.55±0.02*#	-6.91±0.04*

All values are means ± SEM
* $p<0.05$ compared to control group; # $p<0.05$ compared to LPS group

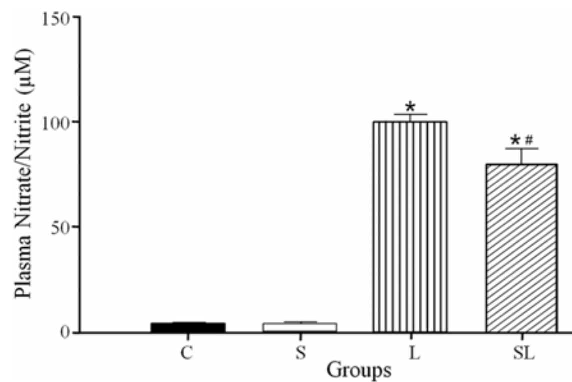

Fig. 4 Plasma nitrate/nitrite oxide level in rats. C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are mean ± SEM; * $p<0.05$ compared to C group; # $p<0.05$ compared to L group.

Serum IL-1beta and IL-6 levels in normal rats were too low to be detected by the immunoassay kits used in the experiment (the sensitivity of the assay 39 pg/mL and 31.33 pg/mL for IL-1beta and IL-6, respectively). A significant increase in both serum IL-1beta and IL-6 was found in LPS-treated rats (IL-1beta 1,385.25±106.78 and <39 pg/mL; IL-6 1,652.00±0.00 and <31.33 pg/mL; for LPS and control groups respectively, $p<0.05$, Table 2), and reduced by steam aerosolization (IL-1beta 623.82±89.07 and 1,385.25±106.78; IL-6 509.06±285.16 and 1,652.00±0.00 pg/mL; for steam-LPS and LPS groups respectively, $p<0.05$)

NF-kappa B activity

NF-kappa B p65, a subunit of NF-kappa B

transcription complex was monitored in rat lung lysate using an ELISA base kit. A significant increase in NF-kappa B p65 activity was observed in LPS-induced septic rats compared with the control (53.65±10.65 and 16.99±2.46 RLU/mg protein for LPS and control groups respectively, p<0.05, Fig. 5), which was markedly brought down to the same level of control group by steam aerosolization (21.94±1.61 RLU/mg protein).

HSP70 expression

The levels of HSP70 expression were carried out by western blot analysis. As shown in Fig. 6, a protein band at the molecular weight of 70 kilodaltons was observed in the lung of all groups of rats. Protein intensity was measured using Gene Tool and expressed as an intensity ratio of HSP70 to actin. Either steam aerosolization or LPS induced HSP70 expression in rat's lungs. HSP70 levels in steam, steam-recovery, LPS and steam-LPS groups were in the same levels and significantly different from the control group (0.574±0.022, 0.550±0.017, 0.538±0.014, 0.586±0.015 and 0.496±0.017 for steam, steam-recovery, LPS, steam-LPS and control groups respectively, p<0.05).

HSF-1 mRNA expression

Heat shock factor-1 (HSF-1) plays an important role in regulating HSP70 expression under conditions of heat or other stresses. HSF-1mRNA expression was performed by the quantitative reverse transcription polymerase chain reaction (qRT-PCR). The results revealed that HSF-1 mRNA expression was significantly increased (p<0.05) in response to steam aerosolization. LPS administration induced slightly increase of HSF-1 mRNA level but no significant difference when compared to the control group. However, the HSF-1 mRNA expression in the rats given aerosolized steam before LPS administration was significantly higher than those of LPS-induced septic rats (p<0.05, Fig. 7).

Table 2. IL-1beta and IL-6 level in the serum of rats by ELISA

Groups	IL-1beta	IL-6
Control	Undetectable	Undetectable
Steam-aerosolized	Undetectable	Undetectable
LPS	1,385.28±106.78*	1,562.00±0.00*
Steam-LPS	623.82±89.07*#	509.06±285.16*#

All values are means ± SEM
* p<0.05 compared to control group; # p<0.05 compared to LPS group

Discussion

The present study have shown that the pretreatment with steam aerosolization at 40°C for 1 hour could restore blood pressure and improve vascular response to vasoconstrictor in LPS-induced septic rats. This beneficial effect is paralleled by (1) an induction of HSF-1 mRNA and HSP70 expression and (2) a decrease of serum pro-inflammatory cytokines, plasma nitric oxide levels and NF-kappa B activity.

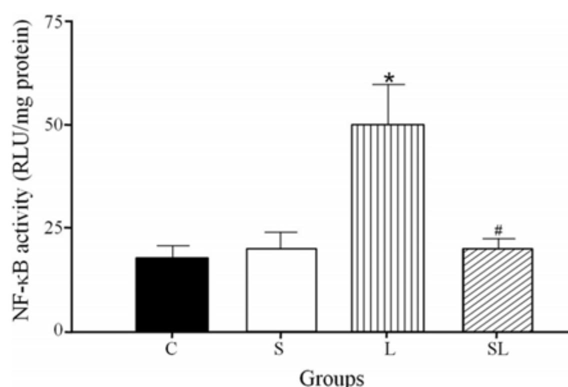

Fig. 5 NF-κB p65 activity in the lung lysate of tested rats. C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are mean ± SEM; * p<0.05 compared to C group; # p<0.05 compared to L group.

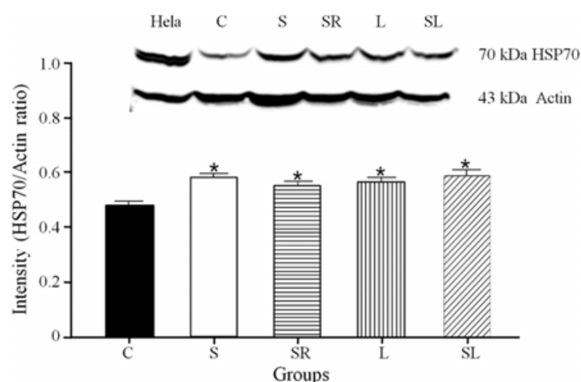

Fig. 6 HSP70 protein expression in the lungs of rats by Western Blot. The HSP70 bands were visualized on Clear Blue x-ray Film. The intensity ratio of HSP70 to actin protein. C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are mean ± SEM; * p<0.05 compared to C group.

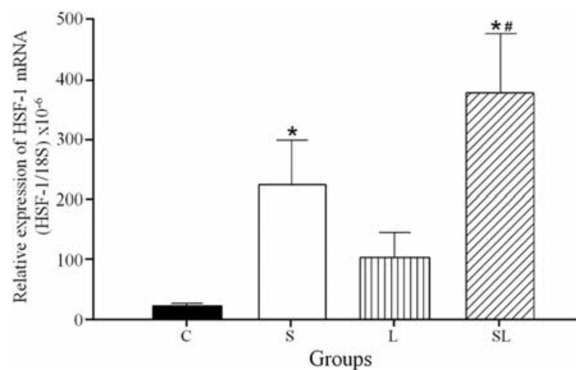

Fig. 7 HSF-1 mRNA level in the lung of rats by qRT-PCR. C: control group, S: steam-aerosolized group, SR: steam-recovery group, L: LPS group, SL: steam-LPS group. All values are mean ± SEM; * $p<0.05$ compared to C group; # $p<0.05$ compared to L group.

A single injection of LPS, an outer membrane of gram-negative bacteria, is a most commonly used for sepsis and septic shock model which is mimic human sepsis syndrome[12]. Previous studies have shown that an intraperitoneal injection of LPS at a dose more than 0.5 mg/kg causes severe septic conditions and increases in systemic cytokine levels in animal models[12,16]. Chaidee et al[13] demonstrated that LPS at a dose of 10 mg/kg could induce a shock-liked state in rats within 6 hours. The same dosage of LPS was used in the present study and the similar results were found. Six hours after 10 mg/kg of LPS injection, the rats showed fallen blood pressure, increased heart rate and impaired vascular response to vasoconstrictor.

HSPs are crucial to cellular self-protection and repair. HSP70 is the most important protein in HSP family and has been reported in several studies that the induction of HSP70 protects organs against sepsis and reduces mortality rates in several animal models[4-6]. It is a temperature-sensitive protein which can be activated by external stress such as whole body hyperthermia or local heating. Heat shock pretreatment by whole body hyperthermia protected circulatory failure in LPS-induced septic rat[17]. In rats heated at 42°C for 15 min, HSP70 was expressed in the serum and could reduce tissues injury caused by LPS[18]. Warming the skin of pigs by raising the surface temperature to 43°C for 2-3 hours before surgery could reduce rates of necrosis and apoptosis of skin flap via HSP70 induction[19]. In clinical situation, inhalation of thermal water has been used empirically in the treatment of chronic diseases of upper and lower respiratory tract[9]. Chaidee et al[13] indicated that steam aerosolization at 40°C for 1 hour induced the maximum level of HSP70 expression without any effect on blood pressure and heart rate. In the present study, the authors pretreated the rats with steam aerosolization at 40°C for 1 hour before 10 mg/kg LPS injection and performed a vascular response to vasoconstrictor at the sixth hour after LPS administration. Consistent with previous studies, the current data showed that steam aerosolization at 40°C for 1 hour induced HSP70 expression in rat lungs and its level remained stable for six hours as shown by no significance difference of HSP70 expression between steam aerosolization and steam aerosolization-recovery groups (Fig. 6). HSP70 can be also induced by endotoxin like LPS. The expression of HSP70 in LPS-treated rats and steam aerosolization prior to LPS-treated rats were at the same level. It indicates that steam aerosolization at 40°C for 1 hour induces maximum expression of HSP70 and no more additional expression by either LPS or other inducers. The possible mechanism may be an autoregulation or self-limiting expression of HSP70. Once cell is fully activated and recovers from stress, free HSP70 protein binds directly to HSP70 mRNA resulting in its degradation and reduction of further HSP70 production[20]. It has been reported that the transcription of HSP70 is mainly controlled by HSF-1[21]. It is phosphorylated and then translocated to the nucleus in cells exposed to heat stress leading to induce HSPs expression in the cells[21]. A significantly increase in both HSP70 and HSF-1 mRNA expression in rat lung by thermal steam aerosolization were found in the experiment. Unexpectedly, LPS induced only HSP70 expression but did not influence to significant increase in HSF-1 mRNA expression. An induction of HSP70 by LPS may be mediated through activation of another pathway like MAP kinase cascades[22].

Hypotension and vascular hyporeactiveness to vasoconstrictor catecholamine are hallmarks of circulatory failure in sepsis[3]. Adrenaline, a vasoconstrictor used in clinically to increases arterial pressure during shock[23], was used to test vascular contraction in this study. We found that LPS-treated rats exhibited hypotension and vasoplagia by showing a diminished contractile response of aortic rings to adrenaline but it was partially restored by giving steam aerosolization pretreatment. Nitric oxide is well document to associate with hypotension and vascular hyporeactivity to vasoconstrictor in sepsis. The relevance of overproduction of NO to the development of endotoxic shock has been observed in several

studies. Inhibition of NO synthesis has been demonstrated to attenuate hypotension and vascular hyporeactivity in both animal septic shock model and patient with sepsis[24]. Thus, we investigated the effect of steam aerosolization on plasma nitrate/nitrite levels which referred to a total plasma nitric oxide. As expect, administration of LPS induced significantly increase in plasma nitrate/nitrite levels compared with the control rats and steam-aerosolized rats ($p<0.05$). It is clear that systemic pro-inflammatory cytokines such as TNF-alpha, IL-1beta, and IL-6 are implicated in septic response both human and animal models[25]. Patients with severe sepsis show high levels of IL-1beta and IL-6[25]. It is evident that pro-inflammatory cytokines stimulate NO production by activating inducible nitric oxide synthase (iNOS) pathway[24]. The present study showed that LPS could induce massive production of pro-inflammatory cytokines, IL-1beta and IL-6, which might response to overproduction of NO, hypotension, tissue injuries and shock. Interestingly, we found that steam aerosolization pretreatment could reduce serum IL-1beta and IL-6 in LPS-treated rats. Restoration of blood pressure and vasoresponse to adrenaline in LPS-treated rats by steam aerosolization observed in this study may result from a decrease in serum IL-1beta and IL-6 which leads to diminish NO synthesis.

Activation of NF-kappa B plays a crucial role in the pathogenesis of septic shock and multiple organ failure. NF-kappa B is a transcription factors found in almost animal cell types and has a key role in inducing the expression of various genes including pro-inflammatory cytokines[7]. A decrease of serum IL-1beta and IL-6 in LPS-treated rats by steam aerosolization in the present study may be possible to relate to NF-kappa B activity. To evaluate NF-kappa B activity, we monitored NF-kappa B p65, a subunit of NF-kappa B transcription complex, in rat lung lysate. LPS-treated rats showed a significant increase in NF-kappa B p65 activity compared with the control group and steam aerosolized group. However, pretreatment with steam aerosolization reduced NF-kappa B p65 activity in LPS-treated rats. These results suggested that steam aerosolization could attenuate NF-kappa B p65 translocation in rat lungs. Similar results have been reported by Chan JY and et al[17] that hyperthermia heat shock (42°C for 15 minutes) preconditioning in LPS-treated rats decreased activity of NF-kappa B by blocking the degradation of inhibitory-kappa B (I-kappa B) in the rostral ventrolateral medulla through HSP70 expression. It is postulated that HSP70 can suppress NF-kappa B activity. Once NF-kappa B activity is diminished, the activity of IL-1beta and IL-6 is decreased resulting in decreased plasma nitric oxide.

Conclusion

The present study revealed that thermal steam aerosolization could improve the stage of shock in LPS-induced septic rats. The improvement may occur through HSP70 expression. It showed the protective role on hemodynamic instability by improving vascular hyporeactivity in a parallel to decrease serum pro-inflammatory cytokines and plasma nitric oxide levels. Thermal steam aerosolization may be a beneficial therapeutic effect and may be applied to the clinical approach for septic shock patient.

What is already known on this topic?

Heat shock proteins (HSPs) are induced by various stresses, such as elevated temperature, hypoxia, oxidative stress, physical injury or chemical stimuli. They play a crucial role to maintain cell homeostasis against these stressors. The previous studies have reported that thermal pretreatment-induced heat shock protein 70 (HSP70) can ameliorate lung damage and increase animal survival in experimental sepsis. Steam aerosolization at 40°C for 1 hour could induce HSP70 expression in rats without any effect on blood pressure and heart rate.

What this study adds?

We found that steam aerosolization at 40°C for 1 hour could delay the stage of shock in LPS-induced septic rats. The mechanism may be mediated by HSP70 expression which involves with a reduction of pro-inflammatory cytokines and nitric oxide levels and a suppression of NF-kappa B activity. Thermal steam aerosolization may be a beneficial therapeutic effect and may be applied to the clinical approach for septic shock patient.

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Potential conflicts of interest

None.

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ละอองไอน้ำอุ่นป้องกันภาวะพิษจากการติดเชื้อในกระแสเลือดในหนูขาวที่ถูกชักนำด้วยไลโปโพลีแซคคาไรด์

อุดมลักษณ์ มาตย์สถิตย์, จันทนา เฆมสีประหลาด, อรพิณ วงศ์สวัสดิ์กุล, ปิยานี รัตนชำนอง, ประสิทธิ์ อุพาพรรณ, ลัดดาวัลย์ ผิวทองงาม

ภูมิหลัง: *ภาวะติดเชื้อในกระแสเลือด คือ ภาวะที่ร่างกายตอบสนองต่อการติดเชื้อด้วยการเกิดกลุ่มอาการอักเสบอย่างรุนแรงทั่วร่างกาย การชักนำให้เกิด Heat shock protein 70 (HSP70) โดยภาวะเครียดจากความร้อนสามารถต้านอันตรายต่อชีวิตจากการติดเชื้อในกระแสเลือดได้ในทางคลีนิค การสูดดมน้ำที่อุณหภูมิสูง ถูกนำมาใช้ในการรักษาโรคเรื้อรังของระบบทางเดินหายใจ ดังนั้นละอองไอน้ำอุ่น อาจสามารถป้องกันภาวะพิษจากการติดเชื้อในกระแสเลือดโดยผ่านการชักนำให้เกิด HSP70 ได้*

วัตถุประสงค์: *การศึกษานี้ทดสอบสมมุติฐานว่า ละอองไอน้ำอุ่นสามารถป้องกันภาวะพิษจากการติดเชื้อในกระแสเลือดในหนูขาวที่ถูกชักนำ ด้วยสารไลโปโพลีแซคคาไรด์ (LPS)*

วัสดุและวิธีการ: *ในการศึกษาครั้งนี้ได้ทำการศึกษาในหนูขาวเพศผู้สายพันธุ์ Sprague-Dawley โดยให้ละอองไอน้ำอุ่นที่ 40 องศาเซลเซียส เป็นเวลา 1 ชั่วโมง ก่อนที่จะฉีดสาร LPS หลังจากนั้นทำการบันทึก ความดันโลหิต อัตราการเต้นของหัวใจ และอุณหภูมิร่างกาย ทุก ๆ 2 ชั่วโมง เมื่อสิ้นสุดการทดลองในแต่ละช่วงได้นำหลอดเลือดแดงมาทดสอบการตอบสนองต่อสารที่ชักนำให้เกิดการหดตัว นอกจากนี้ยังได้วัดปริมาณของ pro-inflammatory cytokines (IL-1beta และ IL-6) ในซีรัม โดยใช้เทคนิค ELISA วัดปริมาณ nitric oxide ในพลาสม่าโดยใช้ nitrate/nitrite fluorometric assay kit และวัดการแสดงออกของ HSP70, heat shock factor-1 mRNA (HSF-1), nuclear factor-kappa B (NF-kappa B) activity ในเนื้อเยื่อปอด โดยใช้ western blot analysis, quantitative real-time PCR และ transcription factor kits สำหรับ NF-kappa B p65 ตามลำดับ*

ผลการศึกษา: *การให้ละอองไอน้ำอุ่นสามารถป้องกันการลดลงของ systolic, diastolic และ mean arterial blood pressure และคืนการตอบสนองต่อการหดตัวของหลอดเลือดแดงต่อสาร adrenaline จากการชักนำของ LPS นอกจากนี้พบว่าการให้ละอองไอน้ำอุ่นสามารถลดระดับ nitrate/nitrite ในพลาสมาลดระดับ IL-1beta และ IL-6 ในซีรัม และลด NF-kappa B activity ในเนื้อเยื่อปอด ขณะเดียวกันละอองไอน้ำอุ่นยังสามารถชักนำให้เกิดการแสดงออก HSP70 และ HSF-1 mRNA*

สรุป: *การศึกษาครั้งนี้แสดงให้เห็นว่าละอองไอน้ำอุ่นสามารถชะลอสภาวะช็อคในหนูขาวที่ติดเชื้อในกระแสเลือดจากการชักนำด้วยสาร LPS ได้ ละอองไอน้ำอุ่นมีประโยชน์ในการบำบัดภาวะช็อคจากการติดเชื้อและอาจจะนำสู่การประยุกต์ใช้ทางคลีนิคสำหรับผู้ป่วยภาวะช็อคจากการติดเชื้อ*