Body Temperature Control in Sepsis-induced Acute Lung Injury

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Abstract

Body temperature is precisely regulated to maintain homeostasis in homeothermic animals. Although it remains unproved whether change of body temperature constitutes a beneficial or a detrimental component of the septic response, temperature control should be an important entity in septic experiments. We investigated the effect of body temperature control on the lipopolysaccharide (LPS)-induced lung injury. Acute lung injury in rats was induced by intratracheal spray of LPS and body temperature was either clamped at 37°C for 5 hours or not controlled. The severity of lung injury was evaluated at the end of the experiment. Intratracheal administration of aerosolized LPS caused a persistent decline in body temperature and a significant lung injury as indicated by an elevation of protein concentration and LDH activity in the bronchoalveolar lavage (BAL) fluid and wet/dry weight (W/D) ratio of lungs. Administration of LPS also caused neutrophil sequestration and lipid peroxidation in the lung tissue as indicated by increase in myeloperoxidase (MPO) activity and malondialdehyde (MDA) production, respectively. Control of body temperature at 37°C after LPS (LPS/BT37, n=11) significantly reduced acute lung injury as evidenced by decreases in BAL fluid protein concentration (983±189 vs. 1403±155 mg/L) and LDH activity (56±10 vs. 123±17 ΔmAbs/min) compared with the LPS group (n=11). Although the W/D ratio of lung and MDA level were lower in the rats received temperature control compared with those received LPS only, the differences were not statistically significant. Our results demonstrated that intratracheal administration of aerosolized LPS induced a hypothermic response and acute lung injury in rats and controlling body temperature at a normal range may alleviate the LPS-induced lung injury.

Key Words: lipopolysaccharide, acute lung injury, body temperature

Introduction

Body temperature is precisely regulated to maintain homeostasis in homeothermic animals. Change of body temperature is a common response to infection and other challenges to host defense. The American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (1) defined the abnormal body temperature in systemic inflammatory response syndrome as either fever to 38°C or above or hypothermia lower than 36°C. Attractive as the definition may be, elevation or decrease in body temperature may by itself alter the pathophysiology of diseases (20, 7, 22) and hence modulate the interactions between invading microorganism and the host (16, 9, 27). Body temperature control is therefore an important issue in biological studies *in vivo*.

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Received: May 29, 2003; Revised: June 24, 2003; Accepted: June 27, 2003.

In many situations, the elevation of body temperature increases chances for animal survival and inhibition of fever in septic animals increases mortality rate (9). Elevation of temperature increases the phagocytic and bactericidal activity of white blood cells (23, 27) and inhibits the growth and virulence of several bacterial species (16). In contrast to the protective effects, it has been reported in non-infectious inflammation that elevation of body temperature may exacerbate tissue injury during inflammatory process (10, 12). Fever elevates metabolic rate and increases generation of toxic fatty acids in human subjects (17) as well as in several animal models of tissue injury (10, 20, 22). Elevation of body temperature increases the neutrophil-endothelium interactions and is associated with a poor prognosis in patients after cardiopulmonary bypass surgery (21) and in patients of intracerebral hemorrhage (29). These studies with conflicting results suggest that elevation of body temperature may constitute a beneficial component of effective host defense against an infectious disease, but may play a detrimental role in enhancing tissue injury during inflammation.

Lowering body temperature may reduce metabolic rate and hence the release of the toxic mediators. Induced hypothermia protects against oxygen radical-mediated lung injury in rats with endotoxemia (11, 28) as well as in patients with septic lung injury (32). Topic hypothermia alleviates organ damage after ischemia and reperfusion in the liver (24) and in the lungs (3). Although it seems that hypothermia protects against tissue damage during acute inflammation, Clemmer *et al*. (4) reported in patients with sepsis syndrome that hypothermia has a significant relationship to the poor clinical outcome. Hypothermia developed during hemodialysis has been shown to alter the oxygen diffusion and transport to the cells (26), which may exacerbate conditions with tissue hypoxia. Furthermore, Dede *et al*. (6) demonstrated in rats with immersion stress that lower body temperature augmented the production of lipid peroxidation. Therefore, in either physiological or pathological point of views, the effects of lowering body temperature on inflammatory tissue injury remains to be elucidated.

Although it remains unproved whether change of body temperature constitutes beneficial or detrimental component of the inflammatory response, temperature control is obviously an important entity in experimental tissue injury. Sepsis, an infectious inflammation manifesting significant changes in body temperature, remains the leading cause of multiple organ dysfunction in critical patients (18, 13). Clinically, severe sepsis is the most common risk factor for the development of acute lung injury and the acute respiratory distress syndrome in the intensive care units (15, 18). While the pathogenesis of sepsis-induced organ damage has been widely investigated and the cellular mechanism has been well determined, the importance of body temperature control was rarely reported. In this study, we investigated the effects of body temperature control on the lung injury induced by intratracheal administration of aerosolized bacterial endotoxin.

Material and Methods

Animals

Male Sprague-Dawely rats weigh 300 to 350g were used in this study. All the experimental procedures were in accordance with the *Guiding Principle in the Care and Use of Animals* approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center, Taiwan, ROC.

Experimental Protocols and Grouping

Rat was anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg, i.p.), followed by tracheal intubation. The rat received intratracheal spray of aerosolized endotoxin at a dosage of 1 mg lipopolysaccharide (Sigma, St. Louise) in 100 μl of saline. Another spray of endotoxin was given at the same dosage 1 hour later. Animals were then placed on a heating pad of a temperature control device (Homeothermic PT-100, DR instruments Co., Taiwan) and the core temperature was monitored continuously *via* a rectal probe. The body temperature was either uncontrolled (the LPS group, n=11) or maintained at 37°C (the LPS/BT37 group, n=11) for 5 hours. Midline thoracotomy was done and the right lung was clamped. Bronchoalveolar lavage (BAL) was done to the left lung for determinations of protein concentration and LDH activity. The right lung was excised for measurements of myeloperoxidase (MPO) and malondialdehyde (MDA) and for wet/dry weight ratio determination. In 3 rats of each group, the right lung was inflated by injection of 10% paraformaldehyde to maintain the intratracheal pressure at 20 cmH_2O and then the lung was excised for pathological exam. The control group (n=8) received intratracheal spray of saline (100 μl) and the body temperature was controlled at 37°C.

Bronchoalveolar Lavage

BAL was performed to the left lung with 5 ml phosphate balanced saline in 2.5 ml aliquots after cannulation of the left trachea. The recovered BAL fluid was centrifuged at 250x g for 10 minutes.

Activity of LDH was measured by the method described by Vassault (31). In brief, part of the supernatant was incubated with 0.24 mM NADH in a Tris/NaCl pH 7.2 buffer at room temperature for 5 minutes. The reaction was then started by the addition of 9.8 mM pyruvate and followed by using a spectrophotometer at 340 nm for 2 minutes. The protein concentration of the supernatant was determined using BCA protein assay reagents (Pierce, Rockford, IL).

Malondialdehyde Assay

Malondialdehyde (MDA) formation was used to quantify the lipid peroxidation in the lung tissues and was measured as thiobarbituric acid-reactive substances (TBARS) by a modification of the Yagi method (33). Lung tissues were homogenized (100 mg/ml) in 1.15% KCl buffer and were centrifuged at 16,000 × g for 20 min at 4°C. A total of 200 µl of the homogenate was then added to a reaction mixture consisting of 1.7 ml of 0.67% thiobarbituric acid, 400 µl of 8.1% sodium dodecyl sulfate, and 1.7 ml of 20% trichloroacetic acid. The mixture was then heated at boiling water for 30 minutes. After cooling to room temperature, 4 ml of butanol was added to the mixture and was mixed well by vigorously shaking for 45 min. The samples were cleared by centrifugation (10,000 × g, 10 min) and their absorbance was measured at 532 nm, using 1,1,3,3-tetramethoxypropane as an external standard. MDA concentrations were expressed as µg/mg of lung tissue.

MPO Determination

A spectrophotometric method (5) was used to determine to myeloperoxidase activity in the lung tissue. In brief, the specimen was freeze-thawed and sonicated three times. Homogenates was centrifuged at 15,000x g for 10 min at 4°C. A 100-µl supernatant was mixed with 900 µl of 50mM phosphate buffer (pH 6.0) containing 0.167 mg/ml of o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. One unit of peroxidase activity equals to the amount of enzyme decomposing 1 µmol of hydrogen peroxide per min at 25°C. Decomposition of hydrogen peroxide is calculated from the oxidation of o-dianisidine using an absorption coefficient of 11.3 /mM/cm at 460 nm.

Lung Histopathology

All the lung tissues were processed using standard histologic methods, sectioned at 5 µm, and stained with haematoxylin and eosin. Lungs were examined from each group and the severity of white blood cell infiltration was assessed by histologic examination. The total number of white blood cells (WBC) in a high power field (HPF) was counted and four HPFs were randomly selected in each slide to calculate the mean cell counts. Statistical Analysis

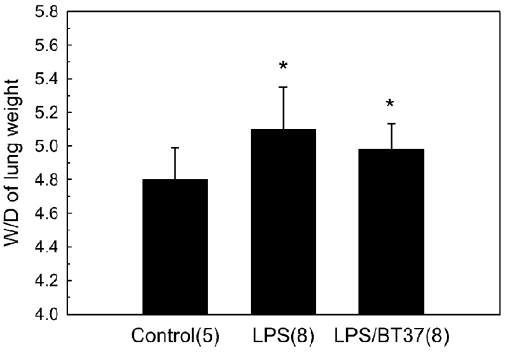

Fig. 1. Wet/dry weight (W/D) ratio of lung after intratracheal spraying of LPS. LPS/BT37, controlling body temperature at normothermia after LPS spraying. *, $P < 0.05$ compared with the control group.

The data were expressed as mean ± SD except the WBC counts in histologic examination, which were expressed as mean ± SEM. The differences among groups were evaluated by using one-way ANOVA. When the variables were found different, a multiple comparison test (Fisher's PLSD) was performed. A value of $P < 0.05$ was accepted as significant.

Results

LPS-induced Hypothermia

The body temperature declined gradually after intratracheal administration of aerosolized LPS and reached a maximal reduction to 34°C in 3 hours. The blood pressure became unstable when the temperature dropped. As the body temperature was maintained at 37°C manually, the blood pressure was maintained well in 5 hours after LPS spraying.

Effects of Temperature Control on LPS-Induced Lung Injury

Intratracheal administration of aerosolized LPS (1 mg) significantly increased the wet-to-dry weight (W/D) ratio of lungs (Fig. 1) and protein concentration and LHD activity in the BAL fluid (Fig. 2). These indicated an acute lung injury induced by LPS. Maintaining body temperature in a normal range (normothermia) after LPS spraying significantly reduced the protein concentration and LDH activity in BAL fluid as compared with the LPS group.

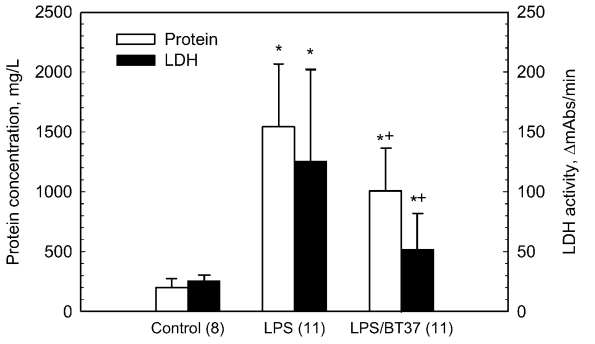

Fig. 2. Effects of body temperature control on protein concentration and LDH activity in the BAL fluid after intratracheal spraying of LPS. *, $P < 0.05$ compared with the control group. +, $P < 0.05$ compared with the LPS group.

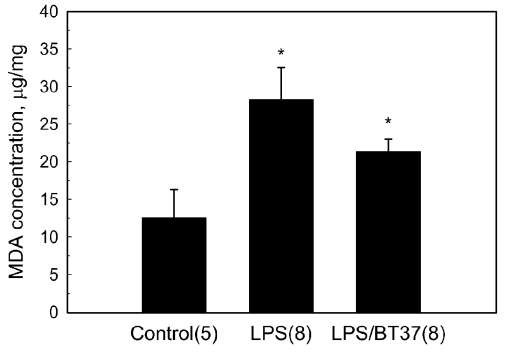

Fig. 3. Intratracheal spraying of LPS increased malondialdehyde (MDA) concentration in the lungs. *, $P < 0.05$ compared with the control group.

Normothermia also reduced the increase of W/D ratio of lungs after LPS although the reduction was not statistically significant (P=0.12).

Effects of Temperature Control on Tissue Lipid Peroxidation

The MDA concentration in the rat lung received intratracheal spraying of LPS was significantly higher than in the control group (Fig. 3), indicating LPS caused lipid peroxidation in lung tissue. Normothermic temperature control slightly reduced the increase in MDA concentration, but it was not statistically significant (P=0.19).

Effects of Temperature Control on Neutrophil Sequestration

Administration of LPS doubled the MPO activity in the lung tissue, compared with the normal lungs (Fig. 4). The lung histologic examination showed significant infiltration of WBC and red blood cells (RBC) in the alveolar space as well as the alveolar

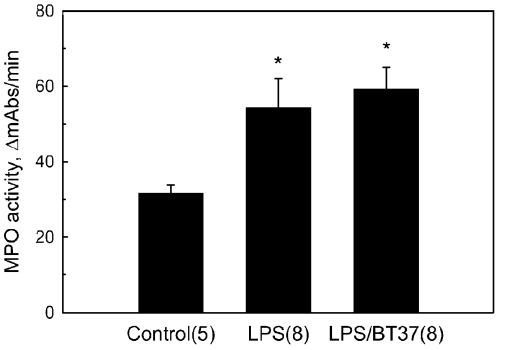

Fig. 4. MPO activity in the lung tissue after intratracheal administration of LPS. *, $P < 0.05$ compared with the control group.

septa (Fig. 5). The total WBC in the lung tissue amounted 261 ± 14 counts per HPF (n=3), which was significantly greater than those in the control group (69 ± 11). These data indicated the WBC sequestration in the lung after intratracheal spray of aerosolized LPS. Maintaining body temperature at normothermia did not reduce the MPO activity in the lung tissue after LPS, but significantly decreased the WBC counts in the lung histopathology (Fig. 5D).

Discussion

Temperature effect is always an important issue and should have been well controlled in biological experiments. However, animal researches in these days seldom reported temperature control in the methodology. It seems that the importance of this factor wanes off in the *in vivo* studies. To examine the effects of temperature control to sepsis-induced acute lung injury, we used a rat model in which the bacterial endotoxin was aerosolized and was administered directly into the lung via intratracheal spray. This insult produced acute lung damage as indicated by an increased W/D ratio of the lung and an elevated LDH activity and protein concentration in the BAL fluid. With this model, our results showed that intratracheal LPS caused gradually decline of body temperature in anesthetized animals. Control of body temperature at normothermia after LPS significantly reduced the increases in BAL protein concentration and LDH activity, tissue MDA concentration, and WBC infiltration in the lungs.

LPS is the major component causing septic responses in the gram-negative bacterial infection. Although sepsis in animals usually manifests elevation of body temperature or fever, decrease in temperature or hypothermia is also a severe sign of sepsis. According to the current definition of sepsis (1), either fever to 38°C or body temperature lower than 36°C can be a major criteria. In septic models with

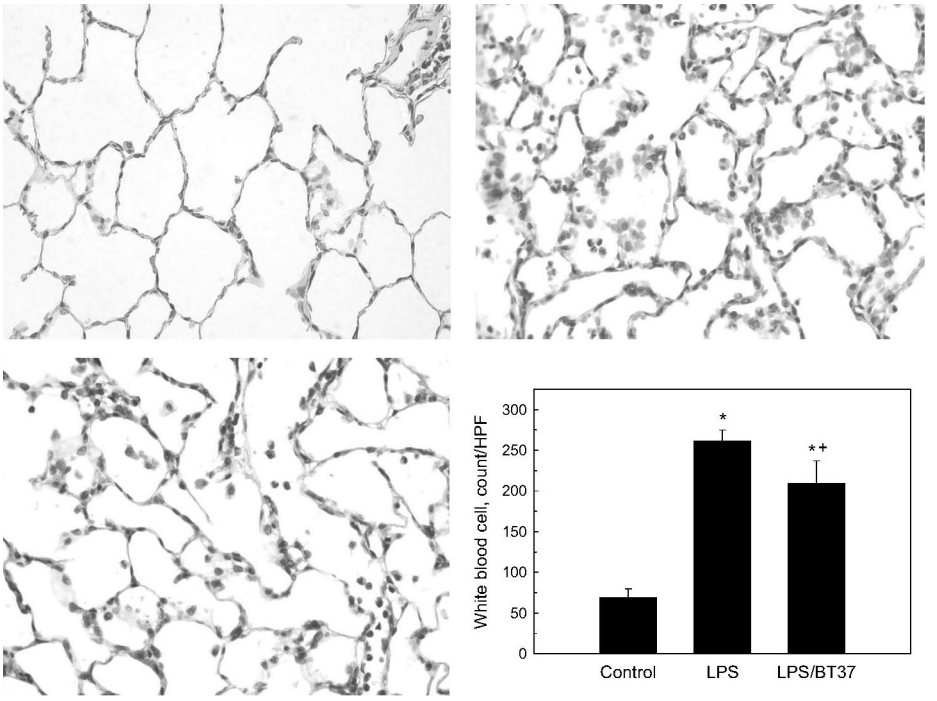

Fig. 5. Effects of body temperature control on sequestration of white blood cells (WBC) in the lungs after intratracheal spraying of LPS. A, the control group receiving saline spraying; B, the body temperature was not controlled after LPS; and C, the body temperature was controlled at 37°C for 5 hours after LPS. The WBC counts in each lung histology were averaged and expressed as bar diagram in panel D. *, $P < 0.05$ compared with the control group. +, $P < 0.05$ compared with the LPS group.

bacterial peritonitis or cecal ligation and puncture (CLP), animals usually show febrile response (14, 19). In contrast, mice, rats, and guinea pigs usually exhibit hypothermia in response to endotoxin challenge under normal laboratory conditions (2). Pedoto *et al*. (25) demonstrated in Sprague-Dawley rats that intravenous infusion of large dose of LPS caused a profound hypothermia (less than 30°C). Our results showed that body temperature of rats declined to 34°C in the first 2 hours after LPS spraying and the hypothermia persisted during a 5-hour experiment course. Our results are compatible with Pedoto's finding (25), but less extent. Although the mechanism is still not known, anesthesia may contribute to the hypothermia by impairing the thermoregulation.

Hypothermia seems to be protective against the damaging effects of endotoxin. Evidences showed that hypothermia reduced oxidative stress (11) and neuron apoptosis (8) and hence protected from tissue injury caused by hypoperfusion or hypoxia/ischemia insults. A therapeutic hypothermia rapidly induced by using cooling device was also proved to be able to improve neurologic outcome and increase survival rate in patients resuscitated from cardiac arrest (32, 30). The beneficial effect of hypothermia has been ascribed to reduction of oxygen consumption at low body temperature. However, hypothermia developed during continuous veno-venous hemofiltration could alter the diffusion of oxygen and then reduce its transport on a cellular basis (26). In addition, a greater lipid peroxidation was found in rats with lower body temperature during immersion stress (32). An explanation of this discrepancy is that the temperature during therapeutic hypothermia was clamped at 34°C rapidly to have a protective effect, whereas a lower body temperature developed slowly as disease deteriorating may cause generation of detrimental mediators.

Elevation of body temperature or fever episode may be associated with increases in neutrophil-endothelium interactions (21), generation of reactive oxygen species (17), and hence the extent of tissue injury in several conditions of inflammatory insults (10, 20, 22, 29). Maintaining of body temperature at normothermia in a septic rat, which is suppose to develop hypothermia, rose the consideration of relatively hyperthermia. Our results, in contrast, showed that controlling body temperature at normothermia alleviated the LPS-induced lung injury. An adequate oxygen transport (26) and less lipid peroxidation (32) at normothermia may explain this protective effect. Our results also demonstrated a less MDA production in lung tissue as the temperature being controlled in normal range, although the difference was not statistically significant compared with the LPS group. Furthermore, the extent of

damage is a consequence of tissue injury balanced by repairing mechanisms. Functions of enzymes including free radical scavengers are temperature-dependent and are usually maintained at normal body temperature. These may explain the findings that controlling body temperature at a normal range alleviated the LPS-induced lung injury and further highlight the importance of body temperature control in biological studies.

In summary, our results showed that hypothermia developed in 3 hours after intratracheal spraying of LPS. Infiltration of white blood cells, lipid peroxidation of lung tissue, and acute lung injury were associated with the change of body temperature. When animal temperature was maintained normothermia, LPS caused less WBC infiltration and lung injury compared with those without temperature control. Although it was not statistically significant, normothermia reduced the lipid peroxidation in the lung. These results indicated that uncontrolled hypothermia may augment the acute lung injury induced by intratracheal administering of LPS. We concluded that temperature control is an important independent factor affecting the experimental results in studies with septic animal model.

Acknowledgment

This study was supported by Grant TSGH-C90-57 from Tri-Service General Hospital, Taiwan, R.O. C.

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