

Primary research

Heat stress is associated with decreased lactic acidemia in rat sepsis

Girish G Deshpande, Sabrina M Heidemann and Ashok P Sarnaik

Wayne State University School of Medicine and Children's Hospital of Michigan, Detroit, Michigan, USA

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Abstract

Background: Elevated plasma lactate has been shown to correlate with mortality in patients with septic shock. Heat stress prior to sepsis has resulted in reduction in acute lung injury and mortality. We investigated whether heat stress resulted in decreased plasma lactate concentration and protected the lung by decreasing the inflammatory response to sepsis.

Results: Plasma lactate concentration was elevated in septic rats without prior heat stress. Lactic acid levels were significantly lower in heat-treated septic rats ($P < 0.05$) and were not significantly different when compared with control rats. Septic rats with or without heat pretreatment had significantly higher myeloperoxidase activity in the lung than did control groups. Heat pretreatment did not prevent neutrophil infiltration or inflammatory mediator production in the lung.

Conclusion: Prior heat stress ameliorates lactic acidemia in rat sepsis. Heat stress did not attenuate the pulmonary inflammatory process. The mechanism of heat-induced protection from lactic acidemia in sepsis needs to be further explored.

Keywords: acute lung injury, heat stress, lactate, septic shock, tumor necrosis factor

Introduction

Septic shock continues to have a high mortality rate in spite of earlier recognition, newer antibiotics, and improved supportive care [1]. Severe sepsis is associated with organ dysfunction, hypoperfusion, and hypotension. Clinically, hypoperfusion manifests as lactic acidosis, oliguria, or altered mental status. The arterial lactate is a reliable marker of tissue perfusion and correlates with survival

[2–6]. A lactate of 2.0 mmol/l or greater is considered indicative of inadequate tissue perfusion [4].

Sepsis is characterized by the inflammatory response to bacteria, which begins when endotoxin induces macrophages and monocytes to produce cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β [7]. TNF- α and IL-1 β then promote the release of numer-

ous other mediators, such as IL-6, IL-8, eicosanoids, and macrophage inflammatory protein (MIP)-2 [8]. Acute lung injury/acute respiratory distress syndrome (ARDS) is one of the most common consequences of sepsis [9,10]. Neutrophil migration and sequestration within the lung is a histologic hallmark of ARDS [11].

Heat stress response is an evolutionarily highly conserved response, which is characterized by the production of heat shock proteins [12,13]. These proteins are proposed to have a protective role by taking part in the cellular repair mechanism, by degradation of proteins beyond repair, and by altering inflammatory cellular functions [12,13]. Heat stress administered before sepsis has resulted in cardiac protection, reduction in sepsis-induced acute lung injury, and decreased mortality in animals [14–17]. The mechanism of cardiopulmonary protection by heat in sepsis is unknown but may be related to a decrease in proinflammatory cytokine production.

The objectives of this study were to determine whether heat stress resulted in decreased plasma lactate concentration and protected the lung by decreasing the inflammatory response to sepsis. Our hypotheses were that heat exposure prior to sepsis results in attenuation of lactic acidemia and that heat-exposed rats have less sepsis-induced lung injury from suppression of inflammatory response. We showed that heat stress ameliorates lactic acidemia in rat sepsis but does not attenuate the pulmonary inflammatory process.

Materials and methods

This study was approved by the Animal Investigation Committee at Wayne State University and performed in accordance with US National Institutes of Health guidelines for the use of animals in research. Thirty-two male Sprague–Dawley rats (Charles Rivers Laboratories, Wilmington, MA, USA) weighing 250–350 g, were assigned to one of four groups: (1) heated with cecal ligation and perforation (CLP; $n=10$); (2) no heat and CLP ($n=10$); (3) heat and sham CLP ($n=6$); and (4) no heat and sham CLP ($n=6$).

Day 1

Rats were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg). A rectal probe was placed to monitor temperature.

Heat stress groups

Rats were placed in a small heating chamber, which was previously warmed to 42°C. After 40–45 min, the rats' temperatures reached 41°C, at which time the rats were removed from the chamber and allowed to cool passively. The temperature remained above 41°C for 10–15 min. Rats were then returned to their cages and allowed free access to food and water.

Normothermia groups

Rats were placed at room temperature for 45 min in the same chamber that was previously used to heat the rats. Rats were then returned to their cages and allowed free access to food and water.

Day 2

All rats were again anesthetized with ketamine and xylazine.

Cecal ligation and perforation (groups 1 and 2)

CLP was performed as previously described [18]. Briefly, a 2-cm midline incision was made and the cecum was isolated. Stool was milked back from the ascending colon into the cecum. The cecum was ligated just inferior to the ileocecal valve to maintain the continuity between ileum and colon. The antimesenteric surface was punctured twice using an 18-gauge needle. The cecum was returned to the abdomen and the abdomen was closed in two layers. Rats were then given 0.9% saline (5 ml/kg body weight) by intraperitoneal injection, and 0.1 ml buprenorphine subcutaneously.

Sham cecal ligation and perforation (groups 3 and 4)

A 2-cm midline incision was made and cecum was isolated. It was returned to the abdomen without ligation or perforation. The abdomen was closed in two layers. Rats were then given 0.9% saline (5 ml/kg body weight) by intraperitoneal injection, and 0.1 ml buprenorphine subcutaneously.

Day 3

Arterial blood was obtained for blood gas, lactate and determination of cytokines. The rats were then killed and their cecums were inspected for evidence of ischemia. The pulmonary artery was cannulated, abdominal aorta was transected, and cold phosphate-buffered saline was infused until the wash fluid from the severed aorta was clear. The trachea was then cannulated and the lungs were lavaged with 50 ml warmed phosphate-buffered saline (37°C). The lavage fluid was then centrifuged and the supernatant was saved at –70°C until further analysis.

Tumor necrosis factor- α , interleukin-1 β , and macrophage inflammatory protein-2 assays

All assays were performed using commercially available kits (Biosource International, Camarillo, CA, USA). Previously coated microtiter plates containing the primary antibodies specific for rat TNF- α , IL-1 β , and MIP-2 were used. After the addition of standard concentrations of the respective cytokine and unknown samples of plasma or lavage fluid, secondary cytokine specific antibodies were placed into these wells. After removing excess antibody, streptavidin–peroxidase was added, which binds to the secondary biotinylated antibody. After washing the plate, a substrate solution was added, which binds to streptavidin–peroxidase to produce a color. The intensity of this color is proportional to the TNF- α , IL-1 β , or MIP-2 concen-

trations, respectively. The sensitivities of these assays are less than 4 pg/ml, less than 20 pg/ml and less than 1 pg/ml for TNF- α , IL-1 β , and MIP-2, respectively.

Myeloperoxidase assays

Myeloperoxidase activity was determined in the lung tissue as previously described [19]. Lung tissue was homogenized and suspended in a potassium phosphate (50 mmol/l) buffer solution (reagent 1). It was centrifuged at 40 000 \times g for 30 min. The pellet was resuspended in hexadecyltrimethyl-ammonium bromide buffer (reagent 2). The tube contents were then sonicated with an ultrasonic dismembrator for 10 s at 40% power, and three cycles of freezing and thawing were performed, after which the sonication was repeated. The tubes were centrifuged again for 30 min. Supernatant (100 μ l) was added to reagent 1 with 0.167 mg/ml of *O*-dianisidine dihydrochloride (Sigma Chemical, St Louis, Missouri, USA) and 0.0005% hydrogen peroxide. Myeloperoxidase activity in each sample (in triplicate) was determined by measuring the change in absorbency per minute at 460 nm using a spectrophotometer (Shimadzu, Wood Dale, Illinois, USA) capable of performing a kinetic scan over 120 s. The protein content in 100 μ l of sample supernatant was determined (bicinchoninic acid kit Sigma). Myeloperoxidase activity was expressed as change in optical density/min/g/l protein.

6-Keto-prostaglandin F_{1 α} and thromboxane B₂ assays

Alveolar lavage fluid was analyzed for 6-keto-prostaglandin F_{1 α} and thromboxane B₂ using commercially available enzyme immunoassay systems (Biotrak, Amersham International plc, Buckinghamshire, UK). Peroxidase labeled thromboxane B₂ and 6-keto-prostaglandin F_{1 α} competes with the thromboxane B₂ and 6-keto-prostaglandin F_{1 α} in the unknown sample for a limited number of binding sites on the respective specific antibody. Tetramethyl benzidine with hydrogen peroxide single pot substrate was added, and after 15 min the reaction was stopped with sulfuric acid. The optical density was determined at 450 nm using a microtiter plate photometer. Concentrations of thromboxane B₂ and 6-keto-prostaglandin F_{1 α} were determined in lavage fluid samples from a standard curve. The sensitivities of the thromboxane B₂ and 6-keto-prostaglandin F_{1 α} assays are 3.6 and 3.0 pg/ml, respectively.

Statistics

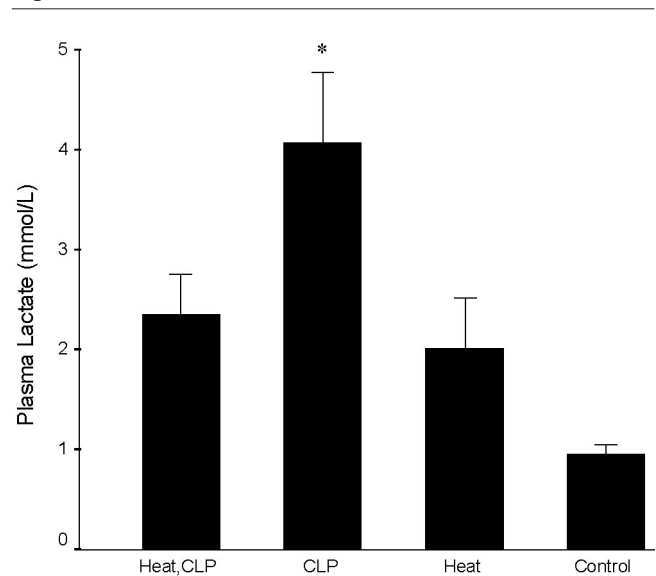
All variables were analyzed using a one-way analysis of variance with *post hoc* analysis using a Student Neuman-Keuhl test when appropriate. Values are expressed as mean \pm standard error of the mean.

Results

Survival

Among rats with CLP, two rats in the heated group and one rat in the unheated group died before sample collec-

Figure 1



Plasma lactate concentrations. * $P \leq 0.05$, versus all other groups. CLP, cecal ligation and perforation.

tion. All rats receiving a sham operation survived regardless of administration of heat.

Cecum

Rats with CLP had ischemia of the cecum. The cecums of sham rats appeared normal.

Plasma lactate

Plasma lactate concentration was significantly elevated in rats with CLP without prior heat treatment (group 2; $P < 0.05$; Fig. 1). Pretreatment with heat resulted in prevention of lactic acidemia in septic rats (group 1). Plasma lactate concentrations in group 1 were similar to those in control rats (groups 3 and 4).

Arterial blood gases

There was no difference in pH, partial carbon dioxide or oxygen tensions, or alveolar-arterial oxygen gradient on room air among the four groups (Table 1). When only the rats with CLP were compared (groups 1 and 2), the alveolar-arterial oxygen gradient was significantly lower in rats receiving heat pretreatment ($P < 0.03$; see Table 1).

Pulmonary myeloperoxidase activity

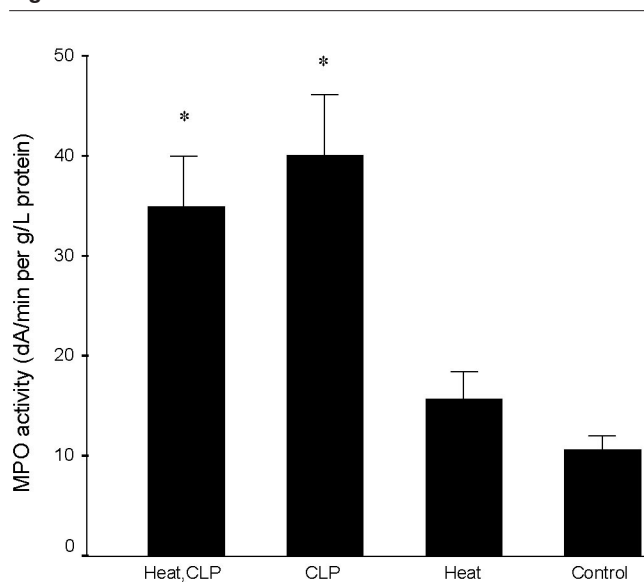
Pulmonary myeloperoxidase activity, an indicator of neutrophil aggregation, was significantly elevated in rats with cecal ligation and perforation. Heat pretreatment did not prevent the increase in myeloperoxidase activity in septic rats (Fig. 2).

Table 1

Blood gas values for various groups				
Groups	pH	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	A-aDO ₂ (mmHg)
Heat, CLP	7.34 ± 0.01	71 ± 5	53 ± 1	10 ± 3
No heat, CLP	7.37 ± 0.01	61 ± 5	53 ± 4	20 ± 2*
Heat alone	7.32 ± 0.03	70 ± 5	56 ± 3	6 ± 2
Control	7.34 ± 0.01	61 ± 6	55 ± 3	16 ± 6

Values are expressed as mean ± standard error of the mean. **P* < 0.03 compared with the heat, cecal ligation and perforation (CLP) group. A-aDO₂, alveolar-arterial oxygen difference; PaCO₂, arterial carbon dioxide tension; PaO₂, arterial oxygen tension.

Figure 2



Pulmonary myeloperoxidase activity. **P* ≤ 0.05, versus sham rats. MPO, myeloperoxidase; CLP, cecal ligation and perforation.

Cytokines and eicosanoids in plasma and lung lavage

TNF-α, IL-1β, and MIP-2 were not detected in the plasma. Lavage fluid of the rats that had CLP contained TNF-α and MIP-2 but no IL-1β. Pretreatment of the rats with heat did not influence the production of these mediators.

Table 2

Eicosanoids	Groups			
	Heat, CLP	No heat, CLP	Heat alone	Control
TXB ₂ (pg/ml)	777 ± 281	627 ± 160	559 ± 161	595 ± 174
PGF _{2α} (pg/ml)	680 ± 208	677 ± 182	334 ± 59	415 ± 65

Values are expressed as mean ± standard error of the mean. CLP, cecal ligation and perforation; PG, prostaglandin; TX, thromboxane.

Eicosanoids were present in the lavage but concentrations did not differ among the groups (Table 2).

Discussion

In severe sepsis, lactic acidemia results from many different mechanisms, such as supply dependency of oxygen utilization favoring anaerobic metabolism [3], poor organ perfusion [4], or metabolic consequences of reduced activity of pyruvate dehydrogenase in mitochondria [20]. In the present rat model of endotoxemia, hyperlactatemia could have resulted from shock, metabolic dysfunction, sepsis-induced liver damage, and/or ARDS. Heat stress caused attenuation of an increase in serum lactate in rats with CLP. The mechanism of prevention of lactic acidemia by heat stress in sepsis is unclear, but it may be related to decreased lactate production or increased lactate clearance. Decreased lactate production could be a result of either increased oxygen delivery or improved mitochondrial oxygen utilization. A lower lactic acid level in the heat-stressed septic rats could also reflect improved organ perfusion, and therefore increased lactate clearance and utilization.

Previous studies [14] have demonstrated that heat stress protects against pulmonary injury in sepsis. Studies [16,17] show that pretreatment of septic animals with heat resulted in decreased endothelial damage and less pulmonary edema than septic animals without prior exposure to heat. Proposed mechanisms include the downregulation of the inflammatory response, and attenuation of TNF-α and IL-1β production, thus reducing acute lung injury [14,15,21]. In the present study, septic rats had

inflammation and acute lung injury as shown by sequestration of neutrophils in the lung indicated by pulmonary myeloperoxidase activity. The myeloperoxidase activity in the lungs was similar in septic rats with or without prior heat stress. Attenuation of increase in lactic acid in the heated, septic group does not appear to be related to decreased pulmonary inflammation by heat stress.

Pulmonary protection from heat stress may depend on duration of temperature exposure and peak body temperature reached. We have previously shown [22,23] that alveolar macrophages, and lung and spleen cells produced heat shock protein when rats were heated to 41°C for 10–15 min according to the protocol utilized in the present study. In cell culture studies, the inflammatory mediator production in response to lipopolysaccharide exposure has been shown to vary depending on the degree and duration of heat stress [24,25]. Longer periods of heat exposure or higher temperatures than used in the present study might be more effective in attenuation of the pulmonary inflammatory response in sepsis.

In conclusion, we observed that heat stress ameliorated lactic acidemia in a rat model of sepsis, possibly by preserving the organ perfusion, improving oxygen delivery or preventing cellular dysfunction. We were unable to demonstrate attenuation of acute lung injury by heat stress in endotoxemia. Further studies are required to determine the optimal heat stress conditions for cardiopulmonary protection of endotoxemic rats and regulation of the inflammatory response.

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Authors' affiliation: Wayne State University School of Medicine and Children's Hospital of Michigan, Detroit, Michigan, USA

Correspondence: Sabrina M Heidemann, MD, Children's Hospital of MI, 3901 Beaubien, Detroit, MI 48201, USA. Tel: +1 313 745 5629; fax: +1 313 966 0105; e-mail: Sheidemann@med.wayne.edu