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Physiological and Chemical Indicators for Early and Late Stages of Sepsis in Conscious Rats

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Key Words
Conscious rat · Physiological parameters · Endotoxin shock · Multiple organ failure · Chemical factors

Abstract
Endotoxin shock is a major cause of death in patients with septicemia. Endotoxin induces nitric oxide (NO) production and causes tissue damage. In addition, the release of oxygen free radicals has also been observed in endotoxin shock and was found to be responsible for the occurrence of multiple organ failure. The purpose of the present study was to evaluate suitable indicators for early and late stages of endotoxin shock. The experiments were designed to induce endotoxin shock in conscious rats by means of an *Escherichia coli* lipopolysaccharide (LPS) injection. Arterial pressure (AP) and heart rate (HR) were continuously monitored for 72 h after LPS administration. The maximal decrease in AP and increase in HR and nitrate/nitrite level occurred at 9–12 h following LPS administration. The white blood cell (WBC) count had decreased at 3 h. Hydroxyl radical (methyl guanidine, MG) decreased rapidly after LPS administration. Plasma levels of blood urea nitrogen (BUN), creatinine (Cr), lactic dehydrogenase (LDH), creatine phosphokinase (CPK), and glutamic oxaloacetic transaminase increased before the rise of amylase. Our results suggest that changes in AP, HR, WBC, free radicals, and chemical substances (BUN, Cr) can possibly serve as approximate indicators for the early stage of endotoxin shock. Severe multiple organ damage may be caused by amylase release in the late stage of endotoxin shock.

Introduction

In recent years, sepsis has remained one of the major causes of death in intensive care units [13, 20, 32]. Septicemia leading to endotoxin shock is characterized by systemic hypotension, hyporeactivity to vasoconstrictors, generalized tissue damage, and mortality [14, 20, 29, 33]. Animal experiments under anesthesia have demonstrated that endotoxin induces nitric oxide (NO) production in many organs and causes tissue damage [7, 21, 27]. It has also been reported that NO catalyzed by NO synthase (NOS) plays an important role in cardiovascular failure and tissue injury [13, 26, 29, 31]. The inducible NOS (iNOS), one of the isoforms of NOS, can be activated by immunological stimuli, such as endotoxin and cytokines in macrophages and leukocytes [11, 23, 31]. The action of iNOS in endotoxemia enhances NO production, leading to circulatory failure and endotoxin shock [10, 31]. Release of free radicals has been shown to be involved in

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endotoxin shock and multiple organ failure [4–7, 9]. Early sepsis is usually reversible. However, patients in shock status have high mortality [12, 20]. Accordingly, investigating appropriate indicators for early and late stages of septicemia is an important issue in clinical practice.

In the present study, we observed changes in arterial pressure (AP), heart rate (HR) and white blood cell (WBC) count. In addition, free radicals such as nitrate/nitrite and methyl guanidine (MG) were detected. Blood urea nitrogen (BUN), creatinine (Cr), lactic dehydrogenase (LDH), creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT), and amylase (a pancreatic enzyme) were determined in conscious animals before and after the induction of sepsis. We developed a simple technique to maintain the rats in a conscious state without restraints. Rats were placed in Chen's metabolic cage (Shingshieying, Hualien, Taiwan), and their tails were fixed with a piece of tape [8]. The animals were free to move and had access to food and water. However, they never left the cage for as long as 1 week. This new, simple model using conscious, unrestrained rats allowed us to continuously monitor changes in AP and HR for as long as 72 h. Blood samples (1 ml) for measurements of white blood cells (WBC) and biochemical substances were taken before *Escherichia coli* lipopolysaccharide (LPS) administration and at 3, 6, 9, 12, 18, 24, 48, and 72 h after LPS administration. The present study may provide important information for the clinical prevention of sepsis and its therapy in patients. The time course of these changes was used to evaluate organ failure in the early and late stages of sepsis in animals without anesthesia. To our knowledge, this is the first report of animals given LPS and continuously observed for as long as 72 h.

Materials and Methods

Preparation of Animals

Sixteen-week-old male Wistar-Kyoto rats weighing 351–368 g used in the experiments were purchased from the National Animal Center and were housed in the university's animal rooms under a 12-hour light/dark cycle. Food and water were provided ad libitum. The animals were anesthetized with ether inhalation for about 10 min. During the period of anesthesia, a femoral artery was cannulated and connected to a pressure transducer (Gould Instruments, Cleveland, Ohio, USA) to record AP and HR on a polygraph recorder (Power Lab, AD Instruments, Mountain View, Calif., USA). A femoral vein was catheterized for intravenous administration of drugs. The operation was completed within 15 min, and the section wound was as small as possible (less than 0.5 cm^2). After the operation, the animal was placed in Chen's metabolic cage. The rat awakened soon after the operation.

Endotoxin Shock

Endotoxin shock was induced by a slow intravenous infusion of 10 mg/kg of LPS (Sigma Chemical, St. Louis, Mo., USA) in 20 min. The infusion was begun at 12 h after the operation. The drug was dissolved in sterile physiological saline immediately before use. All invasive devices were operated under aseptic conditions. After endotoxin administration, the animals were continuously observed for 72 h.

Blood Sample Analyses

The blood sample for the measurement of WBC (Sysmex K-1000, N.Y., USA) was taken and immediately centrifuged at 3,000 *g* for 10 min. The supernatant was used for nitrate/nitrite measurement with high-performance liquid chromatography (ENO-20, AD Instruments) [15].

As the formation of MG is an index of hydroxyl radical production in the blood [16], this substance was measured using its fluorescence spectrum (Jasco 821-FP, Spectroscopic Co., Tokyo, Japan). The emission maximum was set at 500 nm and the excitation maximum at 395 nm. The assay was calibrated with authentic MG (Sigma M0377).

The plasma samples were diluted 1:100 with distilled water before measurements. Plasma BUN, Cr, LDH, GOT, CPK, and amylase were measured with an autoanalyzer (Vitros 750, Johnson & Johnson, N.Y., USA) for evaluating various organ functions, i.e., BUN and Cr for the kidney, GOT for the liver, CPK and LDH for the heart as well as possibly other organs, and amylase for pancreatic function [19].

Statistical Analysis

All data are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) was used to evaluate differences related to group and time. Student's paired t test was used to compare treatment effects within different times. A p value of less than 0.05 was considered to be significant.

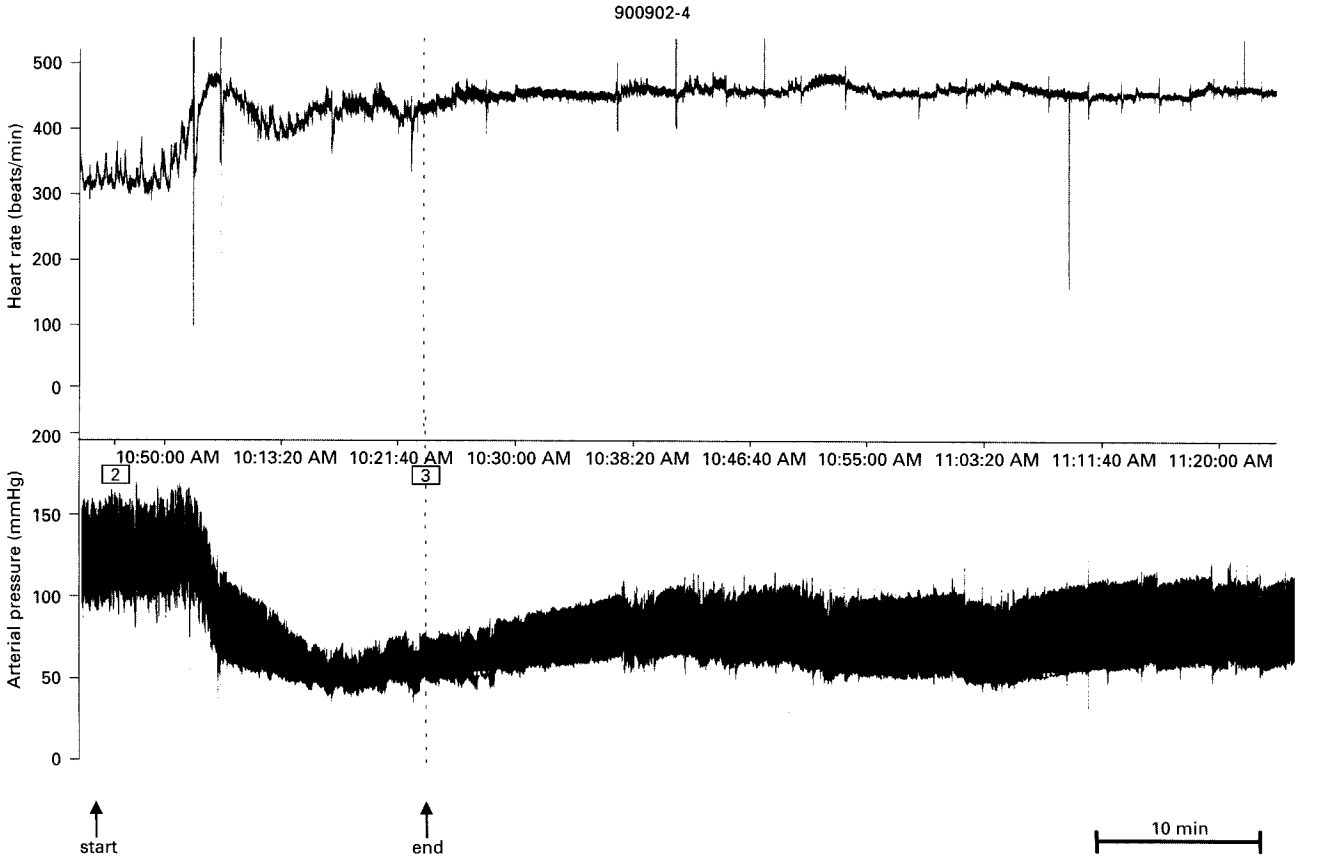
Results

Hemodynamic Profile

Mean lung weight was 1.78 ± 0.22 g. Representative changes in AP and HR in our experiment are shown in figure 1. The first hypotensive phase occurred rapidly after LPS injection. A secondary hypotensive phase was observed at 9 h (from the basal level of 121.5 ± 5.8 to 93.3 ± 7.7 mm Hg) and at 12 h (85.2 ± 7.1 mm Hg) following LPS injection (fig. 2). At these times, the HR

Fig. 1. Example showing the decrease in AP and increase in HR following infusion (from start to end) of LPS.

Fig. 2. Time course of changes in mean AP and HR. Data are the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration (pre) level of LPS.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Heart rate (beats/min)
500
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10:50:00 AM
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10:30:00 AM
10:38:20 AM
10:46:40 AM
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11:03:20 AM
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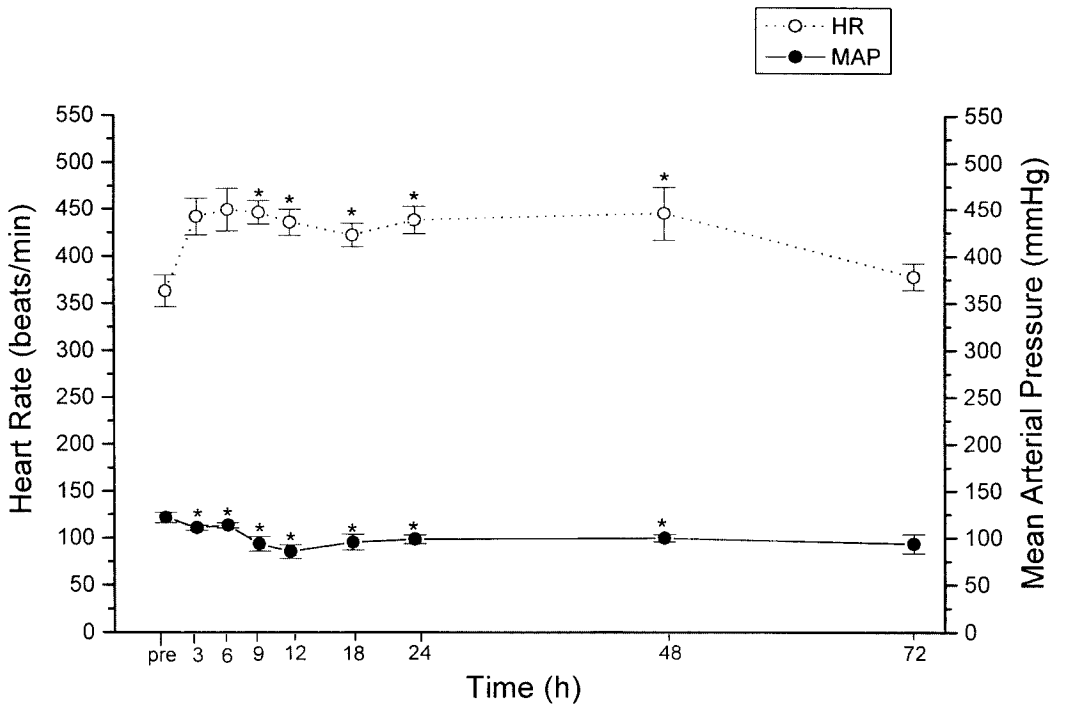
1


HR
MAP
Heart Rate (beats/min)
Mean Arterial Pressure (mmHg)
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24
48
72
Time (h)

2

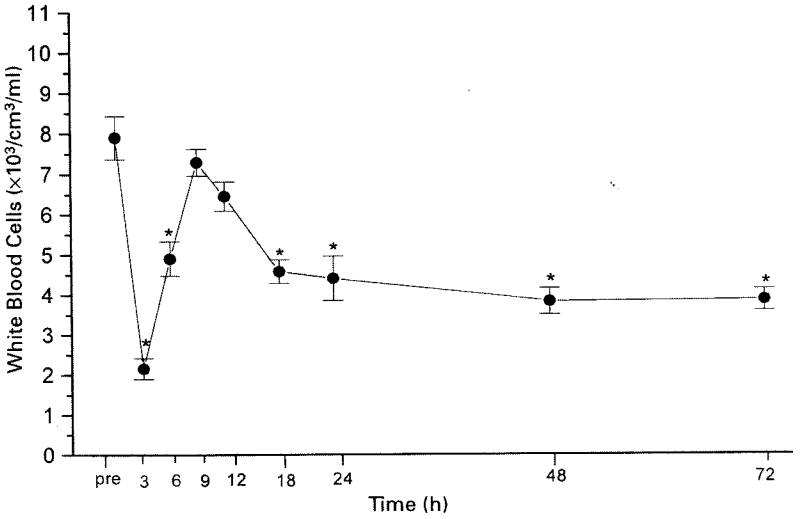
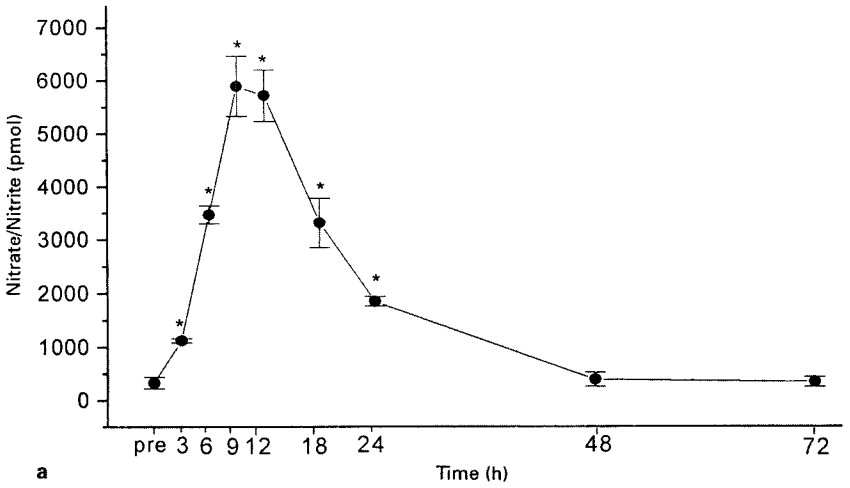


Fig. 3. Changes in WBC level. Bars represent the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration (pre) level of LPS.



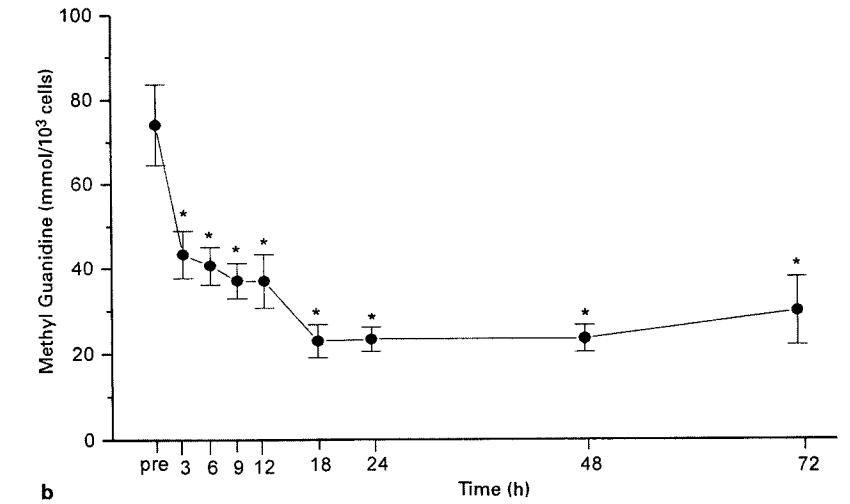


Fig. 4. Time course of changes in nitrate/nitrite (**a**) and MG (**b**). Values are the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration level of LPS.

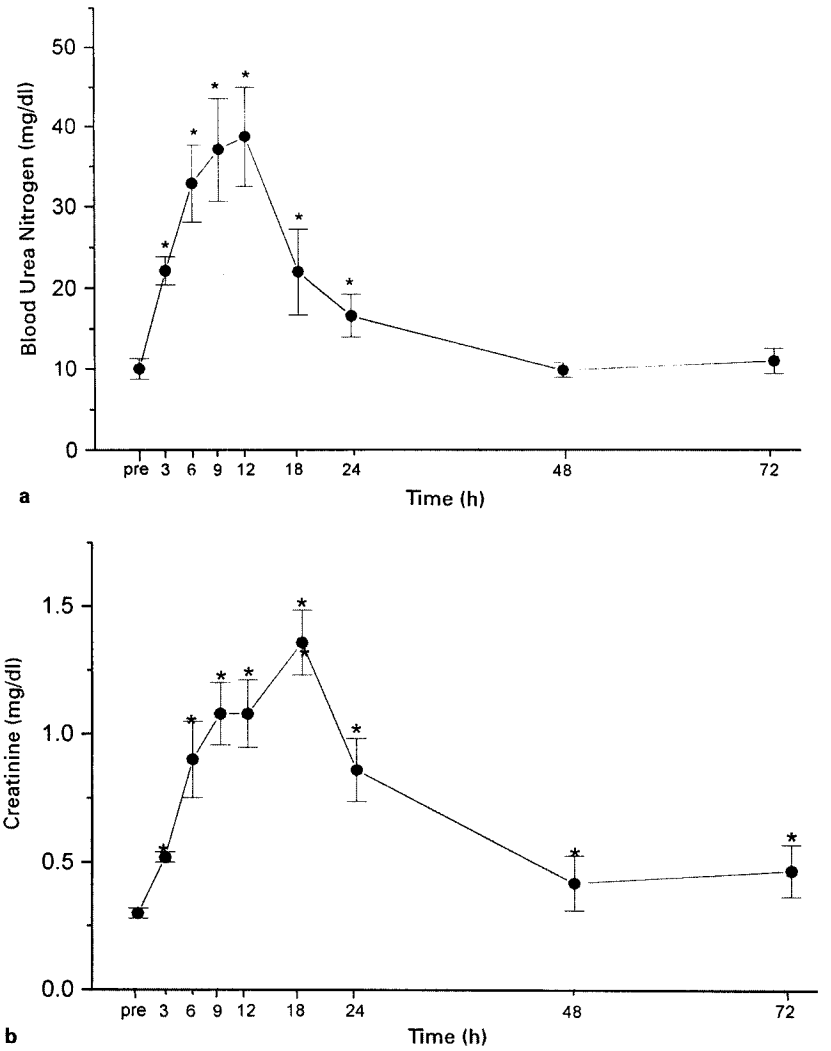


Fig. 5. Blood urea nitrogen (**a**) and creatinine (**b**) levels used to indicate renal function. Data are the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration (pre) level of LPS.

was significantly higher at 446 ± 12 to 436 ± 14 beats/min (bpm) than the basal level of 363 ± 17 bpm ($p < 0.05$).

Blood Cells

WBC counts had dramatically decreased by 3 h [(7.9 ± 0.53) × 10^3 vs. (2.12 ± 0.26) × 10^3/μl] after LPS injection (fig. 3), and had progressively recovered to near the basal level by 9 h [(7.3 ± 0.33) × 10^3/μl)]. After 12 h, a secondary and gradual decrease was observed.

NO and Free Radicals

The basal plasma nitrate/nitrite level was 335.2 ± 109 pmol/l. The level progressively increased up to 9 h (5,892.8 ± 565.5 pmol/l) and then returned to the basal level at 48 h (fig. 4a); however, there was a rapid decrease in MG after LPS injection (fig. 4b).

Biochemical Analyses

BUN increased rapidly from the basal 10 ± 1.24 mg/dl to a peak of 38.75 ± 6.26 mg/dl at 12 h and slowly declined thereafter (fig. 5a). The Cr level increased from the basal 0.3 ± 0.02 mg/dl to a peak of 1.36 ± 0.13 mg/dl at 18 h (fig. 5b). LDH rose from the basal 807.3 ± 135.1 units/l to a peak of 4,782.1 ± 226.2 units/l at 6 h (fig. 6a). The level of CPK had increased by 24 h (from the basal 271 ± 45.73 to 4,422.25 ± 406.97 units/l), and had returned to the basal level by 72 h (fig. 6b). The concentra-

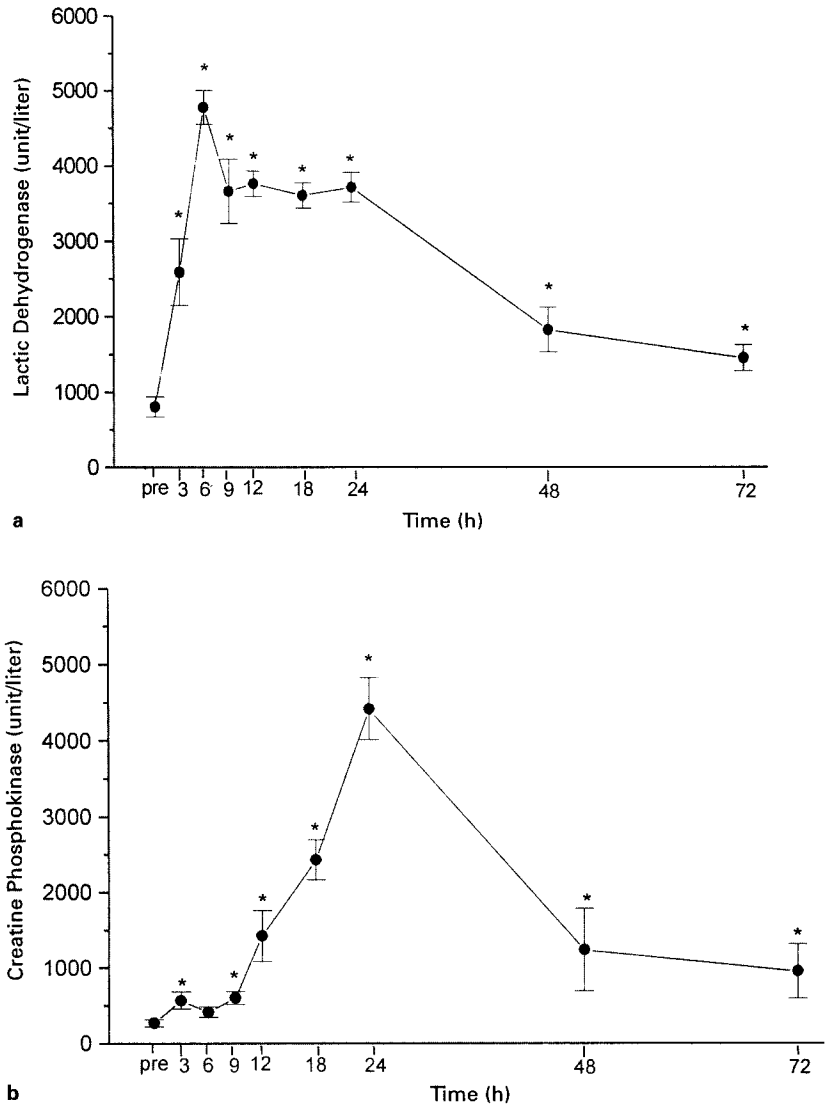


Fig. 6. Alteration in blood lactic dehydrogenase (**a**) and creatine phosphokinase (**b**). Data are the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration (pre) level of LPS.

tion of GOT had two peak phases. The first peak was seen at 9 h (from the basal 280.4 ± 77.8 to 663.9 ± 139.9 units/l), with a secondary phase at 24 h. Then GOT remained at a high level until 48 h, after which it returned to the basal level by 72 h (fig. 7). Amylase reached a peak (from the basal 1,622.6 ± 151.3 to 7,581.3 ± 270.3 units/l) at 24 h after LPS administration and decreased thereafter (fig. 8).

Discussion

Administration of endotoxin resulted in decreased blood pressure. In this study, early hypotension that followed LPS injection was similar to that reported in other studies [10, 20, 29]. Secondary decreases in AP occurred at 9 and 12 h after LPS with a concomitant increase in HR. The nitrate/nitrite level increased and reached a peak at about the same time. This implies that production of NO contributes to the hypotension in septic shock. The time course of WBC counts during endotoxin shock was similar to those reported in previous studies [2, 25]. There

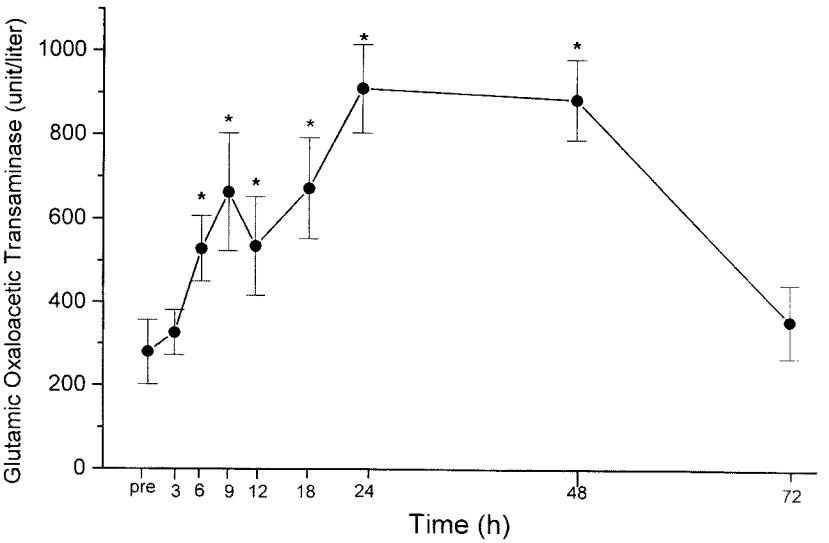


Fig. 7. Evaluation of liver function by GOT. Data are the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration (pre) level of LPS.

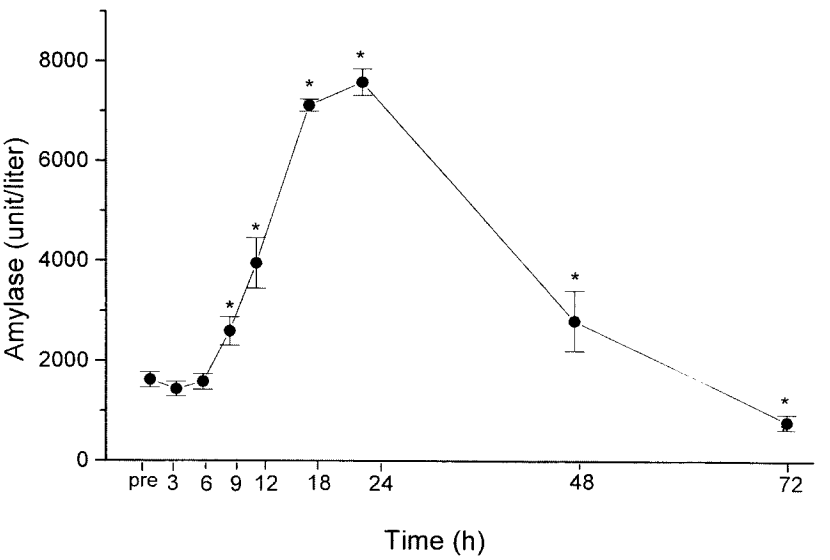


Fig. 8. Changes in the pancreatic enzyme, amylase. Data are the mean ± SEM (n = 8). * $p < 0.05$ compared with the pre-administration (pre) level of LPS.

is a rapid decrease in the WBC count after LPS administration. This leukocytopenia is likely due, at least in part, to the fact that a large number of WBCs are used for fighting the initial invasion of endotoxin, with a concomitant rise in free radicals. In addition, WBC counts increase following kidney and liver damage. As is known, overproduction of the hydroxyl radical can induce acute organ damage. Zurovsky and Haber [34] reported that the decline in renal function was markedly slower in rats given antioxidants. In our study, the hydroxyl radical level decreasing after LPS injection is likely due to the reaction of NO with the superoxide anion to form a potent oxidant such as peroxynitrite [28].

The kidneys and liver have important functions in the excretion of waste products and exchange of metabolites. Nussler et al. [18] and Salzman et al. [24] revealed that endotoxin induces iNOS synthesis in human intestinal and liver cells with functional and morphologic damage. In a previous animal study, polymicrobial sepsis resulted in elevated serum BUN and Cr detected 18 h after surgery

[12]. However, in our study, the plasma level of BUN and Cr increased in the early phase of endotoxin shock at 3 h after LPS injection. In addition, results of our study were similar to those of Ayala et al. [1], in which the liver function deterioration began in early sepsis, while LDH was also concomitantly elevated. Chan et al. [3] showed that plasma endotoxin levels progressively increased as liver function declined. Thus the impaired clearance of endotoxin may play an important role in the progression of hepatic and renal disturbances [17]. Therefore renal and hepatic functions must be measured in the early stage of sepsis, and the plasma levels of BUN, Cr and GOT may also be used as indicators for early sepsis.

Amylase is commonly used as an indicator of pancreatic function [19]. Inflammatory mediators such as endotoxin have been implicated in the pathogenesis of pancreatitis [22]. Vaccaro et al. [30] reported that LPS infusion induced tissue lesions and impaired exocrine protein secretion of the pancreas in rats. Thereafter, endotoxin induced increases in intestinal epithelial permeability and bacterial translocation [24]. However, the present study demonstrated that blood levels of amylase approached peaks at 18 and 24 h after LPS administration, and decreased after 48 h. The level of CPK increased at approximately the same time. These data suggest that pancreatic and heart damage occurs after impairment of renal and hepatic functions. Thus amylase and CPK levels are both useful as indicators for late sepsis.

In conclusion, endotoxin produces renal and hepatic failure before damage to the pancreas occurs. Plasma levels of nitrate/nitrite, hydroxyl radical, BUN, Cr, GOT, and LDH may be suitable indicators for the early stage of endotoxin shock. Subsequent multiple organ failure may be caused by amylase release which is induced by the rise in intestinal permeability in the late stage of endotoxin shock. An anesthetized animal model has been used in a number of studies for experiments on sepsis. The anesthetic drugs may affect sepsis progression and cause differences from the clinical situation of patients with sepsis. On the other hand, previous conscious animal experiments did not continuously measure physiological parameters at different time points for a relatively long period. It also had limitations in taking blood samples because the blood frequently clotted in the catheters. However, the conscious and unrestrained rat model used in this study is a convenient and efficient method for continuously monitoring AP and HR and measuring biochemical substances for as long as 72 h.

Acknowledgements

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