

Changes in Reticuloendothelial Function in Dogs with Endotoxin-Induced Shock

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ABSTRACT. A shock model was experimentally produced by intravenous injection of a lethal dose (3 mg/kg) of endotoxin under general anesthesia induced by pentobarbital sodium using 7 beagles. The effect of this endotoxic shock on the reticuloendothelial function was investigated. The blood endotoxin concentration peaked immediately after administration and decreased subsequently. However, the value still remained on an increased level (1,051 pg/ml) even at 360 min after endotoxin treatment. The lipid emulsion test as an index of reticuloendothelial phagocytotic activity and the arterial ketone body ratio as an index of the energy charge in the liver decreased after endotoxin treatment and failed to recover during the experiment. Fibronectin, one of opsonic proteins, tended to decrease after injection of the endotoxin and was significantly ($p < 0.01$) low at 180 and 360 min compared with the value before injection of the endotoxin. These results suggested the depression of the reticuloendothelial function during endotoxin-induced shock.—**KEY WORDS:** arterial ketone body ratio, canine, endotoxic shock, lipid emulsion test, reticuloendothelial function.

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In consideration of the recent increase in wound infection or infection with gram-negative microorganisms such as *Escherichia coli* and pyocyanic bacteria after administration of antibiotics or immunodepressant drugs, endotoxin shock induced by gram-negative bacteria has become one of the serious problems not only in the field of human medicine but also in the field of veterinary medicine, because of its very poor prognosis and a high rate of mortality resulting from endotoxic shock [5, 6, 14]. Following the onset of depression of the circulatory function and other shock symptoms, endotoxic shock has a risk of causing multiple organ failure involving simultaneously more than two organs such as liver, lung, kidney, heart, digestive mucous membrane and central nervous system [4, 8]. While a large number of studies have been undertaken on the pathogenesis of multiple organs' failure appearing after endotoxin shock, the depression of the reticuloendothelial function has been pointed out as one of the important factors associated with multiple organ failure [3]. The liver plays a central role in reticuloendothelial function in human body. The reticuloendothelial function of the liver is injured at the early stage by sensitization to endotoxin. When the phagocytotic activity of the reticuloendothelial system which is the central site of defense mechanism is affected by endotoxin, then bacteria, endotoxin and other foreign matter partly escape the phagocytosis and are circulated in the body, inducing the development of so-called spill-over phenomenon, which results in damages to various organs [8].

In the clinical field of veterinary medicine, multiple

organ failure secondary to pyometra or various infections are also frequently encountered in dogs. However, its pathology has not yet been unveiled. In this study, a canine model of endotoxic shock was prepared by rapid intravenous injection of endotoxin to determine the changes in hemodynamics and the function of the reticuloendothelial system during shock and to investigate the response of the reticuloendothelial system to the endotoxin.

MATERIALS AND METHODS

1. Experimental dogs and methods

Seven Beagles were used. They ranged in age from 2 to 5 years and in weight from 7 to 13 kg (mean 9.7 kg). They consisted of 5 males and 2 females. They were all free from *Dirofilaria*-infection and were clinically healthy.

General anesthesia was introduced with atropine sulfate (0.05 mg/kg, i.m.) and pentobarbital sodium (30 mg/kg, i.v.). After endotracheal intubation, the animals were immobilized by administration of pancuronium bromide (0.1 mg/kg, i.v.). The respiration was regulated with room air so as to control the arterial carbon dioxide tension (P_{aCO_2}) at 30–40 mmHg. To maintain the depth of anesthesia during the experiment, anesthetics and muscle relaxant were administered as necessary.

For monitoring of the circulation and sampling of blood from the dogs in general anesthesia, a 7 french size catheter was inserted in the dogs placed in a right lateral recumbent position from the right femoral artery into the aorta and a 5 french size Swan-Ganz catheter from the right femoral vein into the pulmonary artery. After confirming the stabilization of circulation, hemodynamic parameters prior to endotoxin treatment were measured.

Endotoxin (*Escherichia coli* 055:B5, 3 mg/kg, Difco,

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Detroit, U.S.A.) was diluted with physiological saline to a total volume of 10 ml and injected over 5 min via the antebrachiocephalic vein. Parameters were measured regularly until 360 min after administration of the endotoxin.

2. Items and methods of examination

Hemodynamics: The heart rate (HR), mean aortic pressure (MAOP), mean pulmonary arterial pressure (MPAP) and cardiac output (CO) were simultaneously recorded. From the values obtained, the stroke volume (SV), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated.

The hemodynamic parameters were measured using a transducer (GOULD, Model P231D) and recorded using a Life-Scope 11 (Model OMP-7201, Nihon Koden, Tokyo). For measurement of CO, a thermal dilution cardiometer (Model MTC-6210, Nihon Koden, Tokyo) was used. The data were measured after rapid administration of 3 ml of cooled physiological saline. This procedure was repeated 3 times and the mean values were used.

Measurement of plasma endotoxin concentration: In determination of the endotoxin concentration in plasma, venous blood was collected aseptically using a γ -ray-treated disposal syringe. Platelet-rich plasma was separated by centrifugation at $1,600 \times G$ for 40 sec. The plasma was pretreated with perchloric acid. The treated plasma was allowed to react to a Toxin-Color test (Seikagaku Kogyo, Tokyo) using a synthetic stroma for 30 min at 37°C. This was followed by diazo-coupling for colorimetric determination of the optical density at 545 nm (Model U-1000, Hitachi, Tokyo). The instruments were all dry-sterilized (more than 2 hr at 250°C) before use.

Examination of reticuloendothelial function: The phagocytotic activity of the reticuloendothelial system was assessed on the basis of the lipid emulsion test, the arterial ketone body ratio (aceto-acetic acid/ β -hydroxybutyric acid ratio, colorimetry) which represents the energy charge of the liver and the concentration of fibronectin [16] which is one of opsonic proteins.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) (UV method) and the blood glucose level (glucose-oxidase method) were examined as other indicators of the hepatic function.

The lipid emulsion test, which examines the phagocytotic activity of the reticuloendothelial system, was carried out by modification of the method of Kim and Pfeifer [11]. A fixed volume (1 ml/kg) of a lipid emulsion (10% Intralipid, Otsuka Pharmaceutical Co., Tokyo) was intravenously infused over 2 min. Blood was collected in 1 ml portions at regular intervals (2, 4 and 6 min after injection), mixed with 3.8% sodium citrate and centrifuged at $400 \times G$ for 15 min. The supernate was diluted 1:4 with distilled water, and the optical density was colorimetrically examined at 580 nm. K value, a phagocytic index, was then calculated.

For calculation of the arterial ketone body ratio, the blood concentrations of aceto-acetic acid and β -hydroxybutyric acid were determined colorimetrically and the ratio of aceto-acetic acid to β -hydroxybutyric acid was calculated.

Fibronectin was measured by immunonephelometry using the venous blood collected into a special container (EDTA-2Na, trasyrol).

Statistical analysis: The above-mentioned parameters were measured regularly until 360 min after administration of endotoxin.

The data were expressed as mean \pm SD, and were statistically analyzed by paired Student *t* test and $p < 0.05$ was defined statistically significant.

RESULTS

1. Changes in hemodynamics

As shown in Table 1, the hemodynamic parameters began to undergo rapid changes from immediately after administration of the endotoxin, and the dogs fell into a state of shock. HR tended to increase from immediately after administration of the endotoxin and stayed at increased levels throughout the course of experiment, compared with the value before endotoxin treatment.

Mean aortic pressure decreased significantly from immediately after administration of the endotoxin. Even at 180 min, the mean value was critically low (below 65 mmHg) but tended to return slightly higher subsequently.

Mean pulmonary arterial pressure decreased compared with the pre-endotoxin-treatment level after a transient increase. It tended to return higher with time.

Table 1. Changes in hemodynamics following intravenous administration of endotoxin in dogs

	Pre	30 min	60 min	180 min	360 min
HR ^{a)} (beat/min)	128.0 \pm 31.1	157.3 \pm 21.2	158.2 \pm 23.8	163.5 \pm 17.2 ^{b)}	148.5 \pm 10.8
MAOP ^{b)} (mmHg)	109.8 \pm 21.6	52.2 \pm 9.8 ^{h)}	58.2 \pm 16.9 ^{h)}	60.7 \pm 14.6 ^{h)}	87.1 \pm 27.7
MPAP ^{c)} (mmHg)	10.9 \pm 2.5	13.9 \pm 6.6	8.1 \pm 1.3	9.7 \pm 2.3	12.9 \pm 3.9
CO ^{d)} (l/min)	1.6 \pm 0.6	0.5 \pm 0.3 ^{h)}	0.9 \pm 0.4 ^{h)}	1.1 \pm 0.2	1.0 \pm 0.2
SV ^{e)} (beat/ml)	11.9 \pm 2.9	3.5 \pm 1.2 ^{h)}	5.8 \pm 1.9 ^{h)}	6.2 \pm 1.0 ^{h)}	6.4 \pm 1.3 ^{h)}
SVR ^{f)} (dyne·sec·cm ⁻⁵)	4,670 \pm 1661	9,500 \pm 3681	5,627 \pm 3966	5,149 \pm 1685	7,373 \pm 1482
PVR ^{g)} (dyne·sec·cm ⁻⁵)	459 \pm 100	2,212 \pm 843 ^{h)}	762 \pm 346	797 \pm 288	1,301 \pm 732

a) Heart rate, b) Mean aortic pressure, c) Mean pulmonary arterial pressure, d) Cardiac output, e) Stroke volume f) Systemic vascular resistance, g) Pulmonary vascular resistance, h) $p < 0.01$ versus pre-administration values.

Cardiac output decreased rapidly and significantly at immediately after administration of the endotoxin. Although it returned slightly higher, it stayed low throughout the course of experiment.

Stroke volume rapidly reduced immediately after administration of the endotoxin, as was CO. It stayed significantly low throughout the course of experiment, compared with the value before administration of the endotoxin.

Systemic vascular resistance and PVR increased transiently rapidly but at 60 min, they returned almost to the values before endotoxin treatment. The increase of PVR was larger.

2.Changes in plasma concentration of endotoxin

The plasma concentration of the endotoxin, which had been in a normal value (7.6 pg/ml) before endotoxin treatment, increased to 693×10^3 pg/ml immediately after administration of the endotoxin. Although it tended to decrease exponentially thereafter, the value was still high (1,051 pg/ml) even at 360 min after administration of the endotoxin (Fig. 1).

3.Reticuloendothelial function

The phagocytic index determined in the lipid emulsion test showed no significant changes. It decreased at 60 min after endotoxin treatment and failed to recover subsequently (Fig. 2).

The arterial ketone body ratio before administration of the endotoxin could not be determined in some of the

dogs, because the volume of ketone body was below the measurable limit. It decreased at 60 min after endotoxin treatment and failed to recover subsequently (Fig. 3).

Fibronectin tended to decrease after administration of the endotoxin. The values at 180 and 360 min reduced significantly compared with the value before endotoxin treatment (Fig. 4).

4.Other parameters of hepatic function

The blood glucose level tended to gradually decrease after a transient increase. AST increased significantly from after administration of the endotoxin, ALT underwent a slighter degree of change (Fig. 5).

DISCUSSION

The importance of the reticuloendothelial function as biological defense mechanism during endotoxin-induced shock is recently recognized [1, 3, 7, 10]. Since 80% of reticuloendothelial system exists in the liver to deal with bacteria, endotoxin and other foreign matter that invaded the body, the hepatic reticuloendothelial system is the center of the whole reticuloendothelial system. The depression of the reticuloendothelial function by shock delays the removal of harmful substance from blood and induces the long retention of harmful substance in blood.

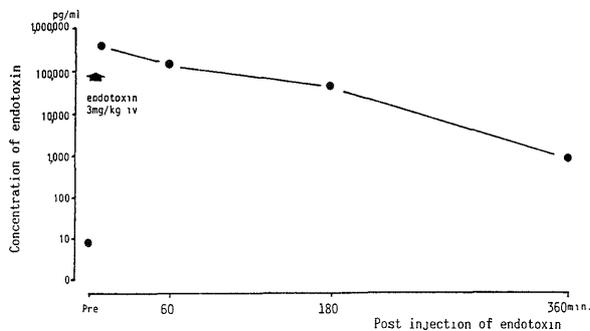


Fig. 1. Changes in plasma endotoxin level following intravenous administration (iv) of endotoxin in dogs.

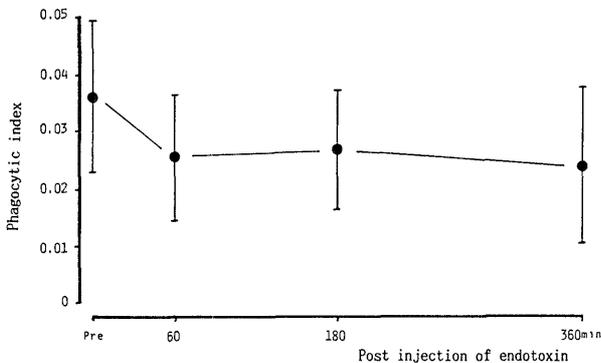


Fig. 2. Changes in phagocytic index following intravenous administration of endotoxin in dogs.

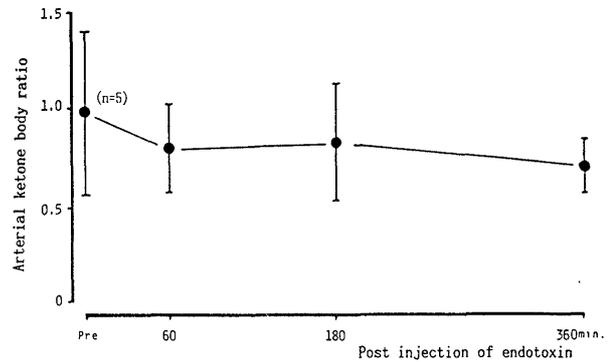


Fig. 3. Changes in arterial ketone body ratio following intravenous administration of endotoxin in dogs.

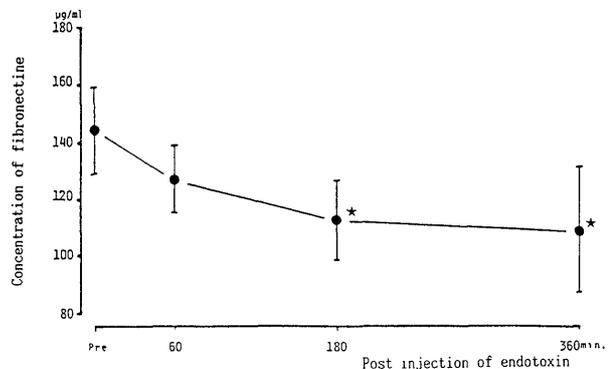


Fig. 4. Changes in fibronectine following intravenous administration of endotoxin in dogs.

*: Significantly different (p<0.01) compared with the pre-administration values.

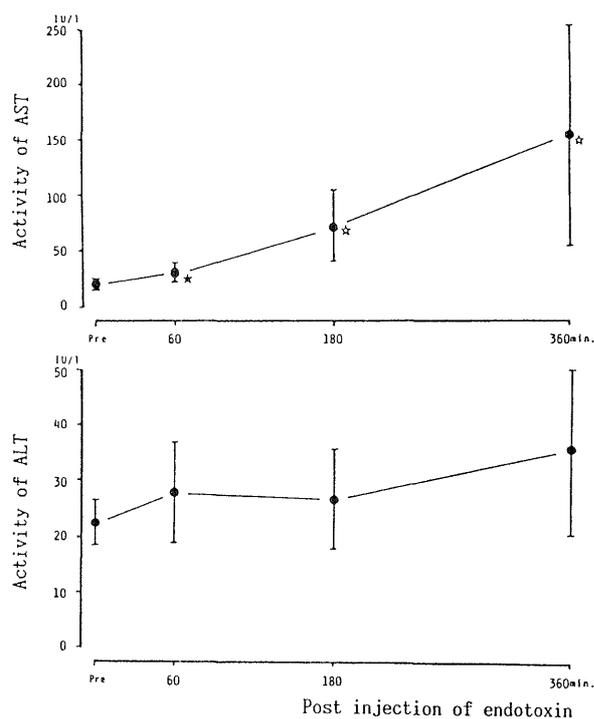


Fig. 5. Changes in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following intravenous administration of endotoxin in dogs. Significantly different (☆: $p < 0.05$; ★: $p < 0.01$) compared with the pre-administration values.

It triggers the onset of a so-called spill-over phenomenon to result eventually in the development of multiple organ failure [8].

Various factors are associated with the depression of the reticuloendothelial function. They may include the reduction of the activity of hepatic kupffer cells and blood opsonin, decrease in hepatic blood flow, direct action of endotoxin and production of reticuloendothelial depressing substance (RDS) [3, 9, 10].

On investigating the changes in the reticuloendothelial function during endotoxic shock in this experiment, it was confirmed that the phagocytotic activity, which is one of the most important function of the reticuloendothelial system, was depressed. The depressed phagocytotic activity results partly from the decreased energy level of the liver which appears in the form of decreased arterial ketone body ratio, because the arterial ketone body ratio (aceto-acetic acid/ β -hydroxybutyric acid ratio) represents the redox state of mitochondria; in other words, NAD^+ / $NADH$ is proportional to the ketone body ratio in hepatic tissue [21] and this ratio reflects the energy charge of the liver [19]. When the arterial ketone body ratio reduces or mitochondria are in a redox state, the migration of citric acid is inhibited and the production of ATP decreases. Consequently, the function of hepatic kupffer cells is disturbed to affect their phagocytotic activity [13]. When the arterial ketone body ratio in man is below 0.4, recovery from shock is difficult [13]. In the present

experiment, the ratio reduced only to approximately 0.7. This may be attributed to the acute nature of shock produced in 6 hr of experiment time.

The blood glucose level was found to decrease in relation to the energy production. This seemed to be attributable to the development of hypoglycemia [20] which occurred because the blood supply to hepatic cells decreased and enzymes of the hepatic glycometabolic system were directly injured [20].

The endotoxin-induced depression of the reticuloendothelial function is also considered to be associated with the decrease in fibronectin, an opsonic protein. Decrease in plasma fibronectin concentration in patients with infection or depressed reticuloendothelial function has been described. Administration of fibronectin augments the phagocytotic activity of the reticuloendothelial system. This may indicate a close relation between the reticuloendothelial phagocytotic activity and the plasma level of fibronectin [15, 17]. A significant decrease in fibronectin was demonstrated in this experiment. This seems to be due to the consumption of fibronectin in dealing with foreign matter such as the endotoxin and microthrombi.

The hepatic blood flow which is another factor associated with the depression of the reticuloendothelial function was not examined by direct measurement in this study. However, CO decreased significantly by administration of the endotoxin. The ratio of total hepatic blood flow to CO is low during shock as reported by Sugiura [18]. When this is combinedly taken into consideration, the decreased blood flow in the liver is considered to constitute an important factor associated with the depressed reticuloendothelial function seen in endotoxic shock.

Endotoxin in blood is mostly disposed of by the reticuloendothelial system. It rapidly disappears from blood immediately after administration. Subsequent disappearance is gradual. Nakao and Shinohara [12] have found that 99.8–99.9% of endotoxin disappeared within 5 min after administration. When endotoxin is used at massive dose such as used in this experiment, it seems that the endotoxin fails due to the depressed reticuloendothelial function to disappear completely from blood and the residual endotoxin causes a so-called spill-over phenomenon.

The depression of the reticuloendothelial function is related to the lung which serves as a secondary filter next to the liver. Although the uptake of endotoxin by the liver decreases in shock, the uptake in the lung is increased as if by compensation and the lung is subjected to invasion of endotoxin [7]. Such a pathological change in the lung is considered to become one of the etiological factors for induction of post-shock adult respiratory distress syndrome, a respiratory insufficiency, manifesting pulmonary edema as its main symptom.

The depression of the reticuloendothelial function observed in this experiment. Thus, endotoxic shock first causes the depression of the reticuloendothelial function and exerts influence on other organs. It is, therefore, suggested that prevention of the functional depression of

the reticuloendothelial system is one of the important management in the control of endotoxin-induced shock.

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