Clinical, Hematological, and Biochemical Analysis of Experimental Endotoxemia in Thoroughbred Horses

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To evaluate the biological response of the horse to endotoxemia, a sublethal amount of lipopolysaccharide (LPS) (10 µg/kg) was given to two Thoroughbreds in two doses 24 hr apart. Each infusion initially produced a significant increase or decrease in rectal temperature, an increase in respiratory rate and heart rate, and a marked decrease in white blood cell count (WBC). The horses subsequently showed signs of shock, characterized by extreme coldness of the skin of lower limbs, as well as cyanosis of the visible mucosae, oliguria accompanied by proteinuria, and abnormalities in gastrointestinal function. The clinical signs of equine endotoxemia may be manifested by the clinical condition of the systemic inflammatory response syndrome (SIRS): two or more of the above signs, such as high or low rectal temperature, increased heart rate and respiratory rate, and leukopenia. Pathobiological responses such as a severe decrease in the number of platelets, hypoglycemia, metabolic acidosis, disorders of hemostasis accompanied by cyanosis of the visible mucosal membranes, proteinuria and oliguria, and abnormal gastrointestinal function, may be manifestations of pathobiological conditions ranging from disseminated intravascular coagulation (DIC) to multiple organ dysfunction syndrome (MODS). Therefore, the clinicopathological responses in equine endotoxemia suggest the nature of SIRS elicited by hypercytokinemia progressing into septic shock.

Key words: endotoxemia, horse, LPS, SIRS, Thoroughbreds

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The clinical syndrome known as endotoxemia is common in horses referred with sepsis, peritonitis, pleuritis, metritis, pneumonia, carbohydrate overload-induced laminitis, and intestinal ischemia caused by intestinal volvulus or incarceration [13]. Because the damaging effects of endotoxemia result from the host's response to endogenous inflammatory mediators, including endotoxins, cytokines, eicosanoids, complements and stress hormones [4], and because the infusion of cytokines causes syndromes clinically indistinguishable from endotoxemia [1, 7, 11, 18], endotoxemia has recently been included in a new disease concept called systemic inflammatory response syndrome (SIRS). SIRS is the massive inflammatory reaction induced by hypercytokinemia; that is, when microbes or their products such as endotoxin or

excessive proinflammatory mediators gain access to the circulation, non-specific defense systems are activated globally, inflicting collateral damage on the host, occasionally progressing into multiple organ dysfunction syndrome (MODS) and a syndrome of shock [2, 3, 5].

Although the clinical signs and clinicopathologic alterations subsequent to endotoxemia in horses have been well described [12, 13], we designed an experiment to reevaluate the biological response of endotoxemia in horses. As infectious complications in horses following surgical and traumatic insults occasionally progress to a syndrome of shock, we employed the second attack theory (also called the 'two-hit phenomenon') in SIRS [6, 16], namely, the intravenous (IV) administration of two spaced (24-hr apart) doses of lipopolysaccharide (LPS), in two Thoroughbred horses to investigate the biological response to two spaced sublethal IV infusions of LPS (10 µg/kg).

Materials and Methods

Two male thoroughbred horses, both 5 years of age (horse 1 and horse 2), were used as the test animals. These horses were withdrawn from training because of locomotor disorders. Despite repeated treatment, the horses failed to return to good health. It was determined that the horses had no chance of recovery. A detailed description of the LPS used and administration of sublethal LPS was given previously [15]. As controls, two male Thoroughbred horses, 3 and 4 years of age (horse 3 and horse 4, respectively), which had been withdrawn from racing because of poor performance, were injected with saline solution on two occasions 24 hr apart. Clinical and hematological examinations were performed 7 times for each infusion: just before infusion, and 1, 2, 3, 4, 5 and 6 hr after infusion. The animals were fed normally until one day before the start of the experiment. On the day of the experiment, they were not fed until the evening, after completion of observations. Drinking water was available *ad libitum* throughout the experiment. The assessed laboratory parameters and the examination methods used are shown in Table 1.

Results

Clinical Findings

After the first LPS administration, rectal temperature declined slightly from immediately after LPS administration to 2 hr after administration. The rectal temperature then began to increase and reached above 39.0°C. In both horses the rectal temperature had returned to normal just before the second infusion of LPS was given (Fig. 1). Transient increases in respiratory rate (>30 breaths/min) were observed in both animals, with peaks 2 and 3 hr after the first infusion. The respiratory rate had returned to normal just before the second infusion was given (Fig. 1). A trend toward tachycardia (>70 beats/min) in both animals were observed after first infusion (Fig. 1). At the second infusion of LPS, respiratory rate and heart rate were higher than those after the first infusion (Fig. 1). The body temperature increased slightly from immediately after the second infusion to 1 hr after, but declined thereafter (approximately 37.0°C) (Fig. 1). Skin, especially in all lower limbs, became cold in both horses after the first and second LPS infusions. Cyanosis was observed in the conjunctivae of both

Table 1. Examination items and examination methods

Items	Methods
WBC Counts	Hitachi Automatic Analyzer*
PCV	Hitachi Automatic Analyzer*
Specific Gravity of Whole Blood	Hitachi Automatic Analyzer*
Blood Sugar Levels	Hitachi Automatic Analyzer*
ALP	Hitachi Automatic Analyzer*
GLDH	Hitachi Automatic Analyzer*
Prothrombin time	Quick first method**
Platelet count	Automated blood counter***
Blood Gas	
pH	Blood Gas Analyzer****
PaCO ₂	Blood Gas Analyzer****
HCO ₃ ⁻	Blood Gas Analyzer****
Base Excess (BE)	Blood Gas Analyzer****

*: Model 747, Hitachi High-Technologies Co., Minato-ku, Tokyo.

** : Clot-timer Model 202 A Type, Lioplastin. ***: Sysmex, K-4500 Type, Tooa Medical Electronics, Tokyo. ****: ABL-510, Radiometer, Copenhagen.

horses after the first and second LPS infusions. After the first LPS infusion, urinary frequency and volume decreased markedly in both horses, and proteinuria (20 to 30 ng/dL) was detected. Just before the second infusion, the excretion, volume and color of urine in both horses returned to normal. After the second LPS infusion, no urine was excreted for the entire 6 hr observation period.

Hematological Findings

After each infusions, an abrupt and marked decrease in the white blood cell (WBC) counts was observed (<2,000 cells/mm³). After the first infusion, there was a trend of the WBC counts to return to baseline value from 3 hr to 6 hr after infusion. The WBC counts just before the second infusion markedly exceeded the baseline value observed before the first infusion (Fig. 2). The decrements observed in the WBC count (<1,000 cells/mm³) after the second infusion were greater than those observed after the first infusion. After each infusion, the packed cell volume (PCV) tended to increase gradually by 3 hr to 4 hr after the infusion, and then proceeded to decrease slowly. The specific gravity of whole blood increased gradually up to 3 hr after the first infusion, and then decreased slowly (Fig. 2). After the second infusion it increased dramatically and exceeded the quantitative limits. It was therefore not measurable from 3 hr after the infusion onward (Fig. 2).

Fig. 1. Clinical findings after the first LPS attack followed 24 hr later by the second LPS attack.

Blood Coagulation Changes

Platelet counts and prothrombin time were measured only for 6 hr after the first infusion. Platelet counts for horse 1 and horse 2 abruptly decreased by 1 hr after the first infusion and remained low (Fig. 3). Prothrombin time remained nearly normal for both horses until 5 hr after the first infusion. At 6 hr after the first infusion, horse 2 showed a slight prolongation (Fig. 3).

Serum Chemical Changes

In both horses, after the first infusion the blood glucose level increased transiently, peaking at 1 to 1.5 hr after infusion. It then, decreased until 3 hr after infusion and tended to increase again thereafter (Fig.

4). The blood glucose level in both horses had returned to nearly normal just before the second infusion. After the second infusion, similar increases in blood glucose levels, with peaks at 1 to 1.5 hr after infusion, were observed, but the decrements observed thereafter were much greater than those detected after the first infusion (Fig. 4).

Serum Enzyme Changes

Glutamate dehydrogenase (GLDH) level in serum increased peaking at 1 hr after the first infusion (Fig. 5). It tended to decrease gradually. GLDH level in both horses returned to near the baseline value observed before the first infusion (Fig. 5). After the

Fig. 2. Hematological findings after the first LPS attack followed 24 hr later by the second LPS attack.

Fig. 3. Blood coagulation changes after the first LPS attack.

Fig. 4. Changes in serum chemistry after the first LPS attack followed 24 hr later by the second LPS attack.

second infusion, similar increases in serum GLDH levels with peaks at 1 hr after the second infusion were observed (Fig. 5). Alkaline phosphatase (ALP) level in serum was measured only after the second infusion. After the second infusion in both cases, ALP levels in serum increased markedly (Fig. 5).

Blood Gas Changes

Similar trends in arterial blood gas values were observed following the first and second infusions (Fig. 6): decreases in pH, base excess (BE) and bicarbonate ion (HCO_3^-) were observed (Fig. 6). The values in pH, BE and HCO_3^- just before the second infusion had nearly returned to baseline values observed before the first infusion.

In summary, the extent of the increments and decrements of each parameter's values described herein were more remarkable after the second infusion than after the first.

Final Outcome

Horse 1 had recovered to near normal clinically by 48 hr after the first infusion. In contrast, horse 2 remained recumbent after the second infusion. At this point, the two horses were sacrificed by an IV overdose of barbiturate, and immediately necropsied [15]. The control animals showed no abnormal signs during the 48 hr period after the first and second infusions of saline solution.

Discussion

The clinical signs following LPS administration to the two horses included significant increase or decrease of rectal temperature; increase of heart rate and respiratory rate; subsequent shock, as characterized by extreme coldness of the skin of the lower limbs; collapse; cyanosis of visible mucosae; oliguria

Fig. 5. Changes in serum enzyme after the first LPS attack followed 24 hr later by the second LPS attack.

accompanied by proteinuria; and abnormalities in gastrointestinal function [15]. The initial clinical signs that follow LPS administration may be manifested by the clinical condition of SIRS: two or more signs such as high or low rectal temperature, increased heart rate and respiratory rate, and leukopenia [2, 3, 5]. These clinical signs may indicate that SIRS is set in motion by LPS administration, and that a cascade of inflammatory mediators is activated within the cardiovascular system, resulting in the onset of clinical signs [9]. Tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1), which are early mediators of equine endotoxemia, can

cause fever [10, 11, 14]. Thromboxane A_2 might act to increase pulmonary vascular resistance, leading to tachypnea [9]. Prostacyclin and bradykinin can cause systemic vasodilatation and reduced cardiac contractility, resulting in tachycardia [9]. The abrupt onset of leukopenia could be due to extravasation of neutrophils and trapping of leukocytes in the expanded capillary bed of the lung, liver, and gastrointestinal tract [9]. All of the clinical findings described above are very similar to those seen after TNF- α infusion in horses [1, 7, 11]. The oliguria accompanied by proteinuria may be the result of renal failure. The alterations of

Fig. 6. Blood gas changes following the first LPS attack and the second LPS attack.

gastrointestinal function [15] might be induced by disturbances in intestinal blood flow [8] and mesenteric vascular damage [15]. The decrease in platelet count and the onset of bleeding tendencies throughout the body suggest the onset of intravascular coagulation [15]. Hemoconcentration, characterized by increases in the PCV and specific gravity of whole blood, might be caused by plasma leakage resulting from the capillary leakiness initiated by leukotriene B₄, bradykinin and C3a [9, 12]. These insults—intravascular coagulation, systemic vasodilatation, and reduced myocardial contractility—may lead to organ dysfunction, such as

hepatic and renal failure and gastrointestinal dysfunction. The hypoglycemia can be caused by hepatic failure. High serum ALP activity might be manifested the osteolysis by TNF- α from activated osteoclasts and liver tissue damage seen at necropsy [15]. The effect of tissue hypoperfusion combined with an endotoxin-induced defect in oxygen extraction by the tissues might induce anerobic glycolysis, thus inducing to metabolic acidosis. As described above, pathobiological responses such as severe decreases in platelet counts, hypoglycemia, metabolic acidosis, disorders of hemostasis accompanied by cyanosis of the

visible mucosal membranes, proteinuria and oliguria, and abnormal gastrointestinal function might be manifested as pathobiological conditions ranging from disseminated intravascular coagulation (DIC) to MODS. Therefore, the clinicopathological responses seen in equine endotoxemia suggest the nature of SIRS elicited by hypercytokinemia progressing into septic shock.

Although the first and second injections produced very similar changes in clinical and laboratory parameters, the intensities of both the clinical symptoms and hematological changes after the second injection tended to be more severe than those observed after the first. Because mediators such as TNF- α and IL-1 are of decisive importance in the intensity of organ damage in SIRS [17], enhancement of the clinical and laboratory parameters might reflect the release of higher quantities of inflammation-producing mediators after the second LPS infusion (triggering neutrophil and macrophage activation) than after the first LPS infusion (priming neutrophils and macrophages) and exacerbation of the MODS state [6, 16]. This phenomenon also suggests that when systemic inflammation is exacerbated by a second inflammatory insult it may develop into MODS, mainly through the neutrophil-endothelial cell interaction [9]. It may be possible to adapt this model of exacerbation of the severity of SIRS in horses to the second attack theory [16].

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