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# Kinetics of HMGB1 level changes in a canine endotoxemia model

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**Abstract :** In this study, we investigated the kinetics of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and high mobility group box 1 (HMGB1) concentrations in a 48-h model of canine endotoxemia by lipopolysaccharide (LPS) injection. Four healthy beagles were slowly administered 1 mg/kg of LPS diluted in normal saline, while two others were administered normal saline as controls. Blood collection was performed at 0 h (baseline), 1 h and 3 h (for TNF-α), 6 h, 12 h, 24 h and 48 h of the experiment, and cytokine levels were determined using the sandwich ELISA method. Early increments of TNF-α and IL-6 were observed (< 3 h), but HMGB1 levels increased the most at 12 h of the experiment and gradually decreased until 48 h. During the whole experiment, IL-6 and HMGB1 were sustained over 12 h of LPS injection, whereas TNF-α decreased within 6 h of LPS injection. Taken together, canine HMGB1 levels increase relatively late (< 12 h) and sustained longer than TNF-α and IL-6 in response to endotoxin. This is the first study to evaluate canine HMGB1 cytokine from endotoxemia in dogs.

**Keywords :** cytokine, dog, endotoxemia, high mobility group box 1, lipopolysaccharide

Canine sepsis is associated with substantial morbidity and mortality in veterinary medicine [1, 5-7, 12]. Because endotoxin plays a central role in sepsis [2], endotoxemia induced by intravenous administration of the endotoxin lipopolysaccharide (LPS), a cell membrane component of Gram-negative bacteria, can emulate sepsis [3]. This endotoxin triggers the synthesis of cytokines, the initial mediators of the inflammatory process.

Around a decade ago, a novel molecule, high mobility group box 1 (HMGB1) had been proposed as a late inflammatory cytokine in human sepsis. Wang and associates identified HMGB1 as a possible cause of the morbidity and mortality that sometimes occurs several hours or days after the injection of LPS in rodents [11]. They found that serum concentrations of HMGB1 were maximally elevated 16~32 h after the administration of LPS in murine models of endotoxemia and sepsis, in contrast to early transient increases in circulating concentrations of tumor necrosis factor (TNF)-α and interleukin (IL)-1. Similarly, we've reported high HMGB1 levels in non-survivors out of 28 dogs with systemic inflammatory response syndrome (SIRS) [14]. We suspected that canine HMGB1 cytokine secretion was up-regulated later than early cytokines such as TNF-α, and that high levels of HMGB1 could be found in dogs with SIRS.

However, the exact kinetics of canine HMGB1 level changes in canine endotoxemia is still unclear, despite of 100% identical protein structure to human HMGB1 [8]. Thus, our objective in this study was to investigate canine cytokine production for 48 h after cytokine induction in endotoxemia model in dogs. Because TNF-α is an initiator of inflammatory response, and release and disappear within several hours, whereas IL-6, one of the septic biomarker [4, 9], appears to be released later than TNF-α, and was detected up to 36 h [10], both of TNF-α and IL-6 levels were also determined to compare kinetics of various pro-inflammatory cytokines.

This study was approved by the committee on Bioethics of Chonbuk National University (CBU 2011-0005). Six healthy beagles (4 female and 2 male, 12~24

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months, 6~8 kg) were included in the study. The dogs were fasted 12 h prior to the experiment and food and water was then provided every 12 h thereafter. Four dogs received high doses (1 mg/kg) of LPS (*E. coli* O111 : B4, Sigma, USA) via slow (over 5 min) intravenous injection, diluted in normal saline to induce endotoxemia [13], while the remaining two dogs received physiologic saline. Fresh whole blood samples were collected before treatment (0 h, baseline) and at 1, 3, 6, 12, 24 and 48 h after the administration of LPS or saline. Sera were obtained and stored at −80℃ until analyzed.

Canine TNF-α and IL-6 concentrations were determined by using commercial matched canine antibody pairs (Duoset ELISA development kit; R&D Systems, USA). To measure canine HMGB-1 concentrations, a commercial antibody test kit (HMGB-1 test kit II; Shino-test Corp., Japan) was used. The lower limits of detection of the cytokine assay were 15.6 pg/mL for TNF-α, 62.5 pg/mL for IL-6, and 313 pg/mL for HMGB-1. Values below these detection limits were assigned default values for statistical analyses. Statistical differences between the control and experimental groups were evaluated using the Student *t*-test. All results are expressed using 95% confidential intervals. Statistical analysis was performed using SPSS software (version 17.0; USA).

Vomiting, hemorrhagic diarrhea, fever and tachycardia were observed after LPS injection (data not shown). Serum concentrations of TNF-α increased rapidly 1 h after LPS injection (0.097~1.360 ng/mL; $p < 0.001$ *vs.* control group), remained high for 3 h (0.438~1.177 ng/mL; $p < 0.05$ *vs.* control group), but then suddenly decreased 6 h after the LPS injection (Fig. 1A). Concentrations of serum IL-6 increased and peaked between 3 h (1.362~2.456 ng/mL; $p < 0.05$ *vs.* control group) and 6 h (0.884~2.681 ng/mL, $p < 0.05$ *vs.* control group) after LPS injection but remained high for 12 h after the experiment (0.141~1.729 ng/mL; $p = 0.05$ *vs.* control group) (Fig. 1B). The mean serum HMGB1 concentrations was the highest at 12 h of the experiment (0.139~4.339 ng/mL, $p > 0.05$), they tended to sustain from 6 to 24 h after the experiment. Serum canine HMGB-1 concentration was the highest at 12 h after the LPS injection (0.863~4.057 ng/mL) (Fig. 1C).

In this study, we were able to observe transient increases in TNF-α concentration after LPS injection while IL-6 and HMGB1 sustained high concentrations over 12 h of canine endotoxemia. Although we could not observe statistically significant increment of HMGB1 level compared to control group, the authors would like to show the kinetics of HMGB1 changes for 48 h of endotoxemia. Larger number of experimental animals should be compared in the future.

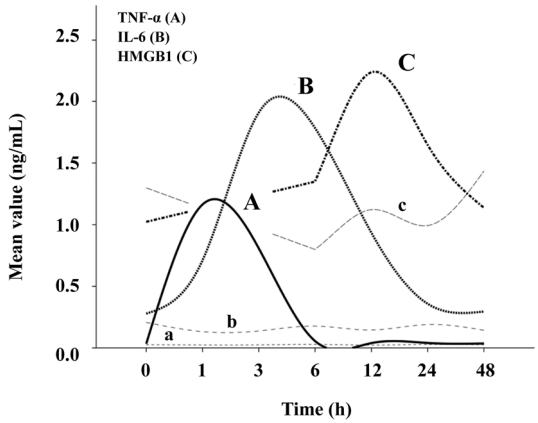


**Fig. 1.** Changes in tumor necrosis factor (TNF)-α (A: endotoxemia, a: control), interleukin (IL)-6 (B: endotoxemia, b: control) and high mobility group box 1 (HMGB1) (C endotoxemia, c: control) concentrations in a canine endotoxemia model over a period of 48 h. Maximum mean TNF-α and IL-6 production was observed during 3~6 h whereas 12 h of HMGB1 production. TNF-α concentration was not detectable after 6 h, but IL-6 and HMGB1 sustained over 12 h.

As biomarkers of cytokines, the elevation of cytokines IL-6 and HMGB1 may be more likely to be found than up-regulated serum TNF-α from the canine clinical patients, since most veterinary septic patients come to the hospital in the late stage of the disease. Higher plasma HMGB1 concentrations were found in not only human septic patients but also rodent septic model [11]. However, it is first reported herein that canine HMGB1 is up-regulated later than onset of IL-6, and sustains over 24 h in septic scenario. This is the first study to evaluate canine HMGB1 cytokine kinetics in endotoxemia model.

Lethal dosage (1 mg/kg) of endotoxin was used in this study in order to emulate more clinically relevant endotoxemia. Selecting LPS dosage should be cautious, and lower dose of endotoxin should be considered. Limited number of experimental design, transient endotoxemia induced by single-dose LPS should be also considered in the further study. Further studies of other cytokines and their actual bioactivities in canine sepsis should be performed as well.

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