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Nafamostat prevents hypothermia and improves survival time after administration of lipopolysaccharide in a mouse surgical model

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Abstract
Lipopolysaccharide (LPS) is an endotoxin known to induce disseminated intravascular coagulation and multiple organ failure followed by septic shock in animals. Nafamostat is a synthetic protease inhibitor with anticoagulant effects. This study investigated the effect of systemic administration of nafamostat on thermogenic homeostasis and survival time in a mouse surgical model. Male C57Bl/6 mice were anesthetized with sevoflurane and implanted with intraabdominal telemetry transmitters. Following the surgery, three groups of animals were administered *Escherichia coli* LPS (0127: B8) subcutaneously at doses of 0.3, 1.0, or 3.0 $mg \cdot kg^{-1}$, and one group received saline without LPS. Three other groups received 3 $mg \cdot kg^{-1}$ LPS with 1, 3, or 10 $mg \cdot kg^{-1}$ of nafamostat. In another group, 10 $mg \cdot kg^{-1}$ of nafamostat only was administered. The times to the onset of hypothermia (body temperature < 30°C) and death were determined. LPS significantly shortened the duration of both normothermia and survival, and nafamostat prolonged the normothermic periods that were reduced by 3 $mg \cdot kg^{-1}$ LPS. Survival time was significantly correlated with the duration of normothermia ($n = 48$; $r^2 = 0.79$; $P < 0.000001$). The results demonstrated the effect of systemic administration of nafamostat against LPS-induced hypothermia. Nafamostat prevented hypothermia, and the consequent normal thermoregulation may have prolonged the survival period.

Key words Nafamostat · Lipopolysaccharide · Hypothermia · Survival

Systemic inflammation is accompanied by changes in body temperature, leading to either fever or hypothermia [1,2]. Fever is one of the most apparent physiological responses to infection, trauma, and injury. The highly regulated nature of fever might serve to support the argument that it has evolved as a host defense response [3]. A decrease in body temperature during the perioperative period causes immune system dysfunction and exacerbates pathophysiological abnormalities [4–6], but recently it has been reported that induced mild hypothermia reduces mortality during acute and severe inflammatory status in animals [7,8] and in humans [9]. The high fevers seen in septic patients may be maladaptive [3].

The administration of lipopolysaccharide (LPS) induces disseminated intravascular coagulation (DIC) as a fatal pathological phenomenon [10], and LPS-induced DIC and subsequent septic shock in experimental animals are widely used models for laboratory investigations of endotoxemia. Sepsis is often accompanied by changes in the regulation of body temperature, i.e., fever or hypothermia, which are key symptoms in all definitions of septic shock and related syndromes [11,12].

Nafamostat is a synthetic protease inhibitor, developed in Japan, which induces anticoagulative effects [13]. It is widely used during continuous hemodialysis and filtration [14], and may show effects against sepsis similar to those shown by other anticoagulants [10]. In murine models, low-molecular-weight heparin attenuates multiple organ failure following LPS-induced DIC and septic shock [10]. However, the protective value of nafamostat against septic shock has not been established, and the effect of systemic administration of nafamostat on changes in body temperature and the survival of experimental animals has never been assessed.

This study investigated the effect of nafamostat on thermal homeostasis and survival after LPS administration in mouse surgical models.

The institutional animal investigation committee of Hamamatsu University School of Medicine approved the present investigation. On the first day of the experiment, 8 week-old male C57Bl/6 mice (weight range, 28–30 g) were anesthetized with sevoflurane under voluntary respiration, and intraabdominal telemetry transmitters (TA10TA-F20; Data Sciences International, St.

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Paul, MN, USA) were implanted in the lower abdominal compartment. Electrodes for electrocardiography were placed subcutaneously in the region of the right axilla and left thorax. After implantation, the wound was gently sutured with silk string. Surgery was performed under aseptic conditions and completed within 15 min.

Mice (n = 48) were divided into eight treatment groups of six animals each. Following surgery, three groups were subcutaneously administered 0.3, 1.0, or 3.0 mg·kg^{-1} of *Escherichia coli* LPS (0127: B8; Sigma, St. Louis, MO, USA), and a fourth group received saline solution only. Three other groups received 3.0 mg·kg^{-1} LPS with 1, 3, or 10 mg·kg^{-1} of nafamostat. One other group received 10 mg·kg^{-1} of nafamostat only. All injections were adjusted to 0.3 ml throughout the experiment.

Sevoflurane inhalation was discontinued after surgery and the mice were placed in a cage where they recovered within 5 min. The ambient temperature was kept at 24°C and the animals could move freely in the cage with access to food and water. The radio signal from the telemetry transmitter, which included body temperature and electrocardiography, was acquired by a flat receiver (RPC-1; Data Sciences International) set under the cage. Real-time data were recorded by a personal computer using Dataquest A.R.T. software (Data Sciences International). Body temperature and heart rate were monitored for 72 h after surgery. The times taken for the onset of hypothermia (body temperature <30°C) and cardiac arrest were determined. When normothermia and survival were observed at the end of experiments, the results were recorded as 72 h.

Analysis of variance (ANOVA) was carried out to determine whether there were significant differences (P < 0.05) between the treatment groups. Treatment means were compared post hoc using the Newman-Keuls multiple comparison test. Data are presented as means ± SD.

Typical changes in body temperature and heart rate are shown in Fig. 1. The average time to the beginning of hypothermia was 68.4 ± 7.3 h in the control group, and 53.4 ± 19.7 h, 34.8 ± 19.2 h, and 27.3 ± 25.4 h in the LPS 0.3, 1.0, and 3.0 mg·kg^{-1} groups, respectively (Fig. 2). Significant differences were found between the control group and the LPS 1.0 and 3.0 mg·kg^{-1} groups (P < 0.05). When nafamostat, at doses of 3 or 10 mg·kg^{-1} mg, was administered with 3.0 mg·kg^{-1} LPS, the time to hypothermia was significantly prolonged, to 32.4 ± 27.6 h and 53.3 ± 24.7 h, respectively (Fig. 2). Administration of LPS (1 mg·kg^{-1} and 3 mg·kg^{-1}) significantly shortened the time to death (57.4 ± 14.0 h and 46.3 ± 20.5 h, respectively, vs 71.9 ± 0.2 h in the control group; Fig. 3). No other significant differences were found with regard to the time to death. The survival period after the surgery was significantly correlated with the duration of normothermia (n = 48; r^2 = 0.79; P < 0.000001).

The results of this preliminary investigation demonstrated the protective effect of the systemic administra-

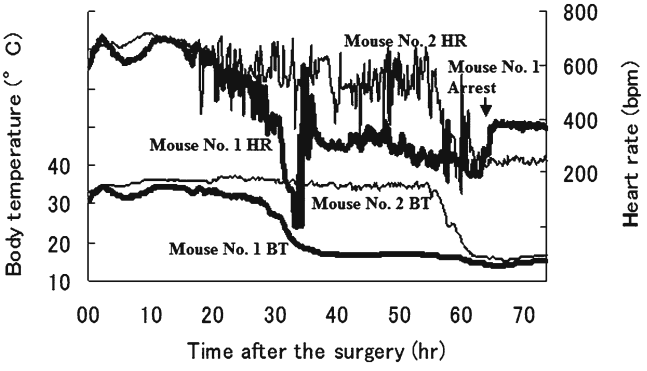

Fig. 1. Typical changes in body temperature (*BT*) and heart rate (*HR*) are shown. Both mice were administered 0.3 mg·kg^{-1} of lipopolysaccharide (LPS). Sudden decreases in BT and HR were followed, after an interval, by cardiac arrest. *bpm*, Beats·min^{-1}

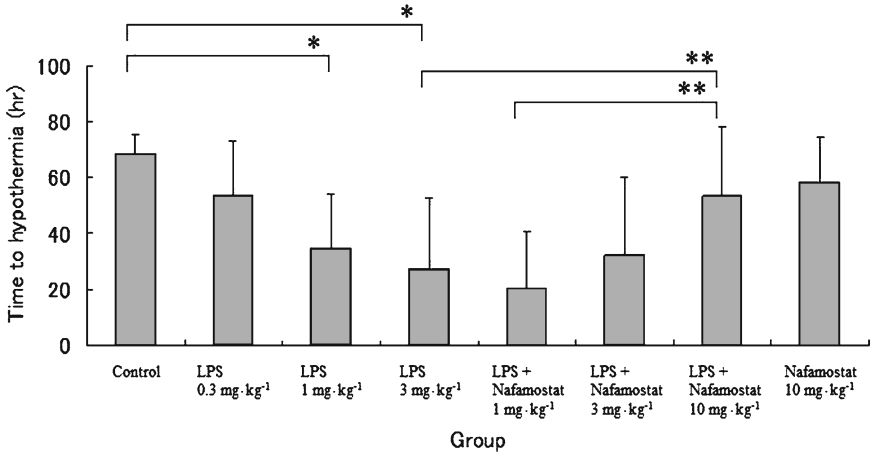

Fig. 2. The effect of lipopolysaccharide (*LPS*) on the time to the onset of hypothermia, and the antagonistic effect of nafamostat. Values are expressed as means ± SD. *P < 0.05 among the first four groups. **P < 0.05 among the next four groups. No significant difference was found between the control and 10 mg·kg^{-1} nafamostat groups

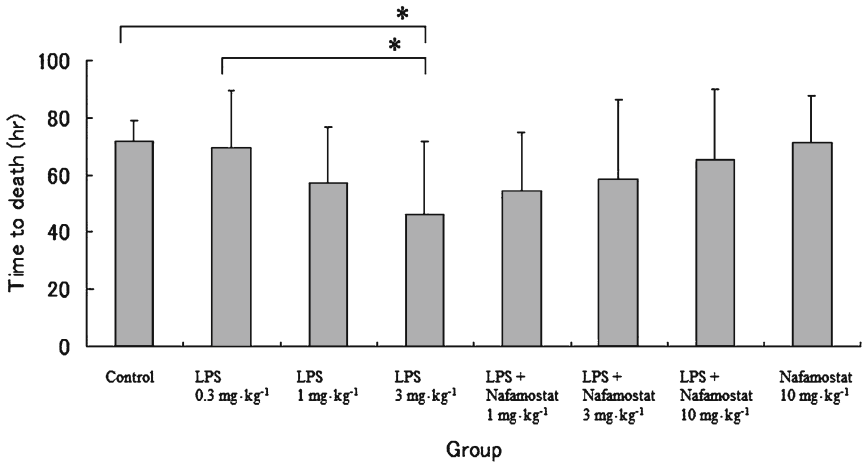

Fig. 3. The effect of lipopolysaccharide (*LPS*) on the time to death, and the antagonistic effect of nafamostat (not significant). Values are expressed as means ± SD. $*P < 0.05$ among the first four groups. No significant difference was found between the control and 10 mg·kg^{-1} nafamostat groups

tion of nafamostat against LPS-induced toxic changes in animals. Although the pathological, immunological, and physiological effects of nafamostat have been extensively investigated in vitro [15,16], in vivo studies of the effects of nafamostat on mortality and morbidity have been limited. Our subacute post-surgery infection model, with the implantation of a foreign body and the administration of LPS, is intended to represent the recovery period of postoperative patients in clinical settings, and may be appropriate for the evaluation of agents that protect against septic shock. However, in the present study, a few animals without LPS administration also died; the surgical stress itself may have interfered with the results. Other strains of mice could possibly show different results.

Anticoagulants improve the outcome of septic shock [17], and low-molecular-weight heparin attenuates multiple organ failure in a murine model of DIC and septic shock [10]. Activated protein C, a vitamin K-dependent serine protease, protects against septic shock [18]. Nafamostat is a trypsin-like serine protease inhibitor, which has an anticoagulant effect [13]. In Japan, nafamostat is used as an alternative to heparin as an anticoagulant during continuous hemodialysis and filtration [14]. It is possible that nafamostat, like heparin, may have a protective effect against LPS-induced DIC and subsequent septic shock.

Change in body temperature was the focus of the present investigation. In our study, the administration of LPS shortened the duration of normothermia and the survival time. The duration of normothermia was significantly correlated with the time to death. Two explanations are possible: (1) hypothermia could be induced initially as a response to sepsis, and the hypothermia could prolong the survival time by reducing confrontational inflammation, (2) the maintenance of thermogenesis and normothermia could have been a protective response of the animals to sepsis, and the breakdown of thermo homeostasis caused the demise of the animals. It is possible that even though hyperthermia is detrimental in the septic state, profound hypothermia may be more deleterious, and a thermogenetic response during septic shock could have protective consequences for animals [19]. Also, the anticoagulant effect of nafamostat may improve the microcirculation and retain thermo homeostasis [20].

In the present study, the duration of survival was not increased by the administration of nafamostat. We simulated subacute post-surgery infection by reducing the dose of LPS. The average survival time was higher than that in a previous investigation [19]; however, the technical limitations of the experiment reported here led to the observations being terminated 72 h after the surgical implantation. This may be why no significant difference in survival time was found among the groups in this preliminary investigation.

In summary, the systemic administration of nafamostat had a protective effect against septic shock in an animal surgical model. Nafamostat maintained the thermogenesis which had been impaired by LPS administration in vivo.

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