Capsaicin Improves Survival Rate in Mice with Lipopolysaccharide–Induced Endotoxin Shock

Yuri Tsukura*, Yuko Takabatake*, Hanako Nakazato*, Ryuji Kato*, Yoshio Ijiri*, Kazuhiko Tanaka*

Abstract

Capsaicin (Cap) has been reported to inhibit endotoxin-induced production of cytokines and NOx in vitro. In this study, we investigated the effects of Cap on endotoxin-induced shock mice. Lipopolysaccharide (LPS) from Escherichia coli was administered to male BALB/c mice intraperitoneally (i.p.) and Cap was administered subcutaneously (s.c.). Survival of mice was monitored at intervals of 24 hours for 7 days and serum levels of tumor necrosis factor-α (TNF-α), interleukin-1beta (IL-1β), and nitrite/nitrate (NOx) were measured after LPS administration (20mg/kg, i.p.). Treatment with Cap (4mg/kg, s.c.) at 5 min after LPS administration to the mice significantly improved the rate of 7-day survival rate from 0.0 to 91.7% (p < 0.001). However, treatment with Cap at one hour before LPS administration did not improve the survival rate. Cap reduced area under the response curve (AUC) by 41% for TNF-α (p < 0.01), 61% for IL-1β (p < 0.05), and 76.1% for NOx (p < 0.05) in endotoxemic mice. These findings suggest that Cap administration may be beneficial during sepsis.

Key words; capsaicin, lipopolysaccharide, tumor necrosis factor-alpha, endotoxin shock, interleukin-1beta, nitric oxide

Introduction

Endotoxemia is a major cause of the systemic inflammatory response syndrome1,2. Lipopolysaccharide (LPS) is one of endotoxins, derived from a Gram-negative bacterial outer membrane3, mediates cellular activation in human and animals, and is strongly associated with sepsis and septic shock4. LPS administration has been used for the experimental induction of endotoxemia in laboratory animals5–7. LPS induces activation of monocytes (macrophages), neutrophils, and endothelial cells through toll-like receptor 4 on the cell membrane8,9. As a consequence, LPS increases the release of pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6, IL-10), and nitric oxide (NO)10. These biochemical effects result in systemic hypotension, multiple organ dysfunction, and high mortality4.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, Cap), a major ingredient of hot pepper, has been used for several therapeutic indications including amelioration of neuropathic pain or itching, and inhibition of neurogenic inflammation11–13, and has a pain-specific local anesthesia14. A receptor of Cap is a transient receptor potential vanilloid subtype 1 (TRPV1), and it is assumed that Cap exerts a physiological function through TRPV115. Cap had an anti-inflammatory effect and reduced pro-inflammatory mediators by LPS-stimulated macrophage16–18. In a rat sepsis model by cecal ligation and puncture (CLP), treat-
ment with Cap also exhibited the decrease of pro-inflammatory mediators. Thus, in the present study, we examined whether administration of Cap prevents LPS-induced mortality and inhibits LPS-induced overproduction of inflammatory mediators.

Materials and Methods

A. Animals

Male BALB/c mice (Japan SLC, Inc., Shizuoka, Japan) were given a standard laboratory diet and water ad libitum and housed under controlled environmental conditions. They were 7 to 9 weeks of age (weighing; 21~26g) at the start of experiments after a minimum 7-day acclimation period.

B. Reagents

LPS (Escherichia coli, serotype O55:B5 (Lot.No. 114K4107)) used in this study was manufactured by Sigma-Aldrich Co. (St. Louis, MO, USA), and was dissolved in endotoxin-free physiological saline. Cap purified highly (more than 98%) was kindly provided by Maruishi Pharmaceutical, Co., Ltd. (Osaka, Japan), and was dissolved in 1% ethanol and 1% Tween 20/physiological saline. The solutions were prepared immediately before experiments. Each control animal received the vehicle in these experiments. However, we used the data obtained with physiological saline as a representative control in the present study, because results in control experiments using physiological saline were not significantly different from those obtained with use of physiological saline (data not shown).

C. Treatment of mice

LPS was administered to mice intraperitoneally (i.p.) and Cap was administered subcutaneously (s.c.). All mice received 0.02mL/g as a total volume. The models of endotoxemia were performed in mice, using LPS administration (dosage of 10, 15, and 20mg/kg, i.p.) to study survival rate. The mice were given without/with Cap (dosage of 2, 3, 4, 5, and 10mg/kg, s.c.) at one hour before LPS administration, 5 minutes and one hour after LPS administration. Survival rate of mice was monitored at intervals of 24 hours for 7 days. To investigate serum cytokines and stable NO metabolites (nitrite/nitrate, NOx) levels, after mice were anesthetized with ether, they were exsanguinated via cardiac puncture. Blood samples were collected before LPS administration, 1, 3, 6, and 12 hours after LPS administration. Mice had almost lived by 12 hours after 20mg/kg of LPS administration and then they died one after another. Therefore, we measured mediators of endotoxemic mice until 12 hours after LPS administration. All experiments were performed in accordance with the Declaration of Helsinki and were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences.

D. Measurement of serum TNF-α, IL-1β and NOx

Serum levels of TNF-α and IL-1β were determined by an enzyme-linked immunosorbent assay kit (R&D systems, Inc, Minneapolis, USA). The lower limits of quantification of TNF-α and IL-1β were 5.1 and 3.0pg/mL, respectively. To measure serum levels of NOx, the serum samples were deproteinized by filtration through vivaspin 500, molecular cut-off filters (Sartorius AG, Goettingen, Germany), and assayed by the fluorometric method with a commercial kit (Calbiochem, Darmstadt, Germany).

E. Data Analysis

Survival rate data were plotted as Kaplan-Meier curves and examined for significant differences among the various treatment groups with use of the log rank test. Area under the response curve (AUC) was calculated by trapezoidal analysis, and differences in TNF-α, IL-1β, and NOx levels among saline + saline-treated, saline + Cap-treated, LPS + saline-treated, and LPS + Cap-treated mice were determined with ANOVA followed by Scheffe’s F test. Values are presented as mean±SD, with significance recognized at p<0.05. Statistical analysis was performed with StatMate III (ATMS Co., Ltd., Tokyo, Japan).

Results

A. Dosage of LPS for preparation of endotoxin shock-model mice

The survival rate of BALB/c mice treated with LPS...
was decreased in a dose-dependent manner (Fig. 1a). All of the 10 mice treated with physiological saline and LPS (10mg/kg, i.p.) survived the same as in the control group treated with physiological saline alone. However, 6 of the 10 mice treated with LPS (15mg/kg, i.p.) died, and all of the 12 mice treated with LPS (20mg/kg, i.p.) died within 1 to 3 days. We therefore prepared endotoxin shock model mice by administrating LPS (from E. coli) i.p. at a dosage of 20mg/kg.

B. Dosage of Cap for treatment of endotoxin shock-model mice

Treatment with Cap (2mg/kg, s.c.) at 5 min after LPS (20mg/kg, i.p.) administration improved the rate of 7-day survival from 0.0% to 50.0% and corresponding rates for higher doses were as follows; 70.0% for 3mg/kg, 91.7% for 4mg/kg, 90.0% for 5mg/kg, and 70.0% for 10mg/kg (Fig. 1b). The differences in survival rate among groups without/with Cap (3, 4, and 5mg/kg) were significant. The optimal dosage of Cap treatment with endotoxin shock-model mice was therefore set at 4mg/kg.

C. Timing of Cap administration to mice with LPS-induced endotoxin shock

In mice treated with Cap (4mg/kg, s.c.) at one hour before LPS administration, survival rate was only 25.0%. In mice treated with Cap (4mg/kg, s.c.) at one hour after LPS administration, survival rate was 50.0%, while in mice treated with Cap (4mg/kg, s.c.) at 5 minutes after LPS administration, survival rate was 91.7%. There was a significant difference in survival rate between the groups of mice treated without Cap and with Cap not only at 5 minutes after LPS administration but also at one hour after LPS administration (p<0.01). However, there was not a significant difference in survival rate between the groups of mice treated without and with Cap at one hour before LPS administration. There was a significant difference in survival rate between the groups of mice treated with Cap at one hour before LPS administration and 5 minutes after LPS administration (p<0.01) (Fig. 1c).

D. Effects of Cap on serum TNF-α IL-1β and NOx levels in LPS-treated Mice

Serum TNF-α level was nearly undetectable in the Cap and control groups. However, the serum TNF-α level peaked at one hour after LPS administration and returned to near basal levels until 12 hours (Fig. 2a, Table 1). Cap significantly reduced the peak level of TNF-α to 19% of that without Cap. There were no significant differences in TNF-α levels among the control, LPS, and LPS+Cap groups at 3 hours after LPS administration. As compared with the AUC for TNF-α in LPS group, that in LPS+Cap group was decreased by 41% (p<0.01). In contrast, serum IL-1β level peaked at 3 hours after LPS administration and tended to decrease (Fig. 2b, Table 1). In addition, IL-1β levels without and with Cap returned to near basal levels within 24 hours after LPS administration (data not shown). There were no significant differences in the IL-1β levels between without and with Cap after LPS administration. However, as compared with the AUC for IL-1β in LPS group, that in LPS+Cap group was decreased by 61% (p<0.05). In addition, Cap itself did not increase serum levels of TNF-α nor IL-1β. The serum level of NOx increased time-dependently in BALB/c mice administered LPS (20mg/kg, i.p.), and approximately 12 times basal level at 12 hours after LPS administration. The LPS+Cap group exhibited significant reduction of serum NOx level by 18.7% at 3 hours and 62.0% at 6 hours after LPS administration compared with the LPS group (p<0.05) (Fig. 2c, Table 1). As compared with the AUC in LPS group, that in LPS+Cap group was decreased by 76.1% (p<0.05). In addition, Cap itself did not increase serum level of NOx.

Discussion

In this study, we compared the survival rate after administration of LPS (20mg/kg ip) from E. coli with that in the control group of BALB/c mice. Cap significantly improved the survival rate of mice with LPS-induced endotoxin shock with a bell-shaped curve effect (Fig. 1b). Thus, 4mg/kg of Cap was the
Survival rate of the mice was monitored for 7 days after LPS administration.

(a) Dosage of LPS for preparation of endotoxin shock-model mice. Mice of the control group were given physiological saline (0.01mL/g, s.c.) at 5 minutes after physiological saline (0.01mL/g, i.p.). Mice of 10mg/kg LPS group were given physiological saline (0.01mL/g, s.c.) at 5 minutes after LPS (10mg/kg, i.p.). Mice of 15mg/kg LPS group were given physiological saline (0.01mL/g, s.c.) at 5 minutes after LPS (15mg/kg, i.p.). Mice of 20mg/kg LPS group were given physiological saline (0.01mL/g, s.c.) at 5 minutes after LPS (20mg/kg, i.p.). The difference in survival rate between the each group was significant. **p<0.01 versus Control; ††p<0.01, versus LPS 10mg/kg.

(b) Dosage of Cap for treatment of endotoxin shock-model mice. Mice were given various concentration of Cap (2, 3, 4, 5, 10mg/kg, s.c.) at 5 minutes after LPS (20mg/kg, i.p.) at day 0. The overall difference in survival rate between the each group was significant. *p<0.05, **p<0.01 versus LPS 20mg/kg + saline, s.c.; †p<0.05 versus LPS 20mg/kg, i.p. + Cap 4mg/kg, s.c.

(c) Timing of Cap administration to mice with LPS-induced endotoxin shock. Mice were given saline (s.c.) at 5 minutes after LPS (20mg/kg, i.p.) and mice were given Cap (4mg/kg, s.c.) at one hour before LPS (20mg/kg, i.p.), 5 minutes and one hour after LPS (20mg/kg, i.p.) at day 0. The difference in survival rate between the each group was significant. **p<0.01 versus LPS 20mg/kg, i.p. + saline, s.c. 5 minutes after LPS.; ††p<0.01 versus LPS 20mg/kg, i.p. + Cap 4mg, s.c. one hour before LPS.

Figure 1 Effect of Cap on survival rate of endotoxemic mice.
Capsaicin Improves Endotoxin Shock in Mice

Figure 2  Effects of Cap on serum TNF-α, IL-1β levels (b) and NOx levels (c) in endotoxemic mice.
Mice of the control group were given physiological saline (0.01mL/g, s.c.) at 5 minutes after physiological saline (0.01mL/g, i.p.), mice of the LPS group were given physiological saline (0.01mL/g, s.c.) at 5 minutes after LPS (20mg/kg, i.p.), mice of the Cap group were given Cap (4mg/kg, s.c.) at 5 minutes after physiological saline (0.01mL/g, i.p.) and mice of the LPS + Cap group were given Cap (4mg/kg, s.c.) at 5 minutes after LPS (20mg/kg, i.p.). Each value represents the mean ± SD. *p < 0.05, **p < 0.01 for the LPS + Cap group versus the LPS group.

Table 1  Effects of capsaicin on areas under the response curves of TNF-α, IL-1β, and NOx in lipopolysaccharide-treated mice

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<thead>
<tr>
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<th>Control</th>
<th>Cap</th>
<th>LPS</th>
<th>LPS + Cap</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td>462 ± 90</td>
<td>490 ± 73</td>
<td>10037 ± 1695</td>
<td>4158 ± 1033**</td>
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<tr>
<td>IL-1β</td>
<td>31 ± 19</td>
<td>48 ± 13</td>
<td>1960 ± 398</td>
<td>1379 ± 265*</td>
</tr>
<tr>
<td>NOx</td>
<td>318 ± 95</td>
<td>367 ± 65</td>
<td>4836 ± 869</td>
<td>3633 ± 807*</td>
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Cap, capsaicin; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; IL-1β, Interleukin-1beta; NOx, nitrite/nitrate; Control group, physiological saline (0.01mL/g s.c.) administration at 5 minutes after physiological saline (0.01mL/g i.p.); Cap group, Cap (4mg/kg s.c.) administration at 5 minutes after physiological saline (0.01mL/g i.p.); LPS group, physiological saline (0.01mL/g s.c.) administration at 5 minutes after LPS (20mg/kg i.p.); LPS + Cap group, Cap (4mg/kg s.c.) administration at 5 minutes after LPS (20mg/kg i.p.).

Data are shown as mean ± SD (n = 6). *p < 0.05, **p < 0.01 versus LPS group.
optimal dosage for treatment of endotoxin shock in this study.

Treatment with Cap at 5 minutes after LPS administration prevents LPS-induced death, with a survival rate of 91.7%. Opposing to this result, treatment with Cap at one hour before LPS administration exhibited almost no efficacy in preventing LPS-induced death, with a survival rate of only 25.0%. However, treatment with Cap one hour after LPS administration improved significantly the survival rate to 50.0%. The above findings indicated that not pre-treatment but post-treatment with Cap prevents LPS-induced death. In the 1980s, it was reported that Cap did not protect mice from endotoxic shock. Subsequently, in the 2000s, it was reported that treatment with Cap before CLP inhibited inflammatory responses (TNF-α, NOx) and enhanced IL-10 production in a rat model of sepsis. And pre-treatment of capcitate, Cap related analogues, improved the survival rate of LPS-induced lethal shock by D-galactosamine and LPS administration. Both reports showed that the anti-inflammatory effect were observed with Cap and related analogues administration before induction of sepsis or LPS challenge. Our study is the first report to show that Cap is effective even when administered after LPS challenge. Cap might thus be very useful not for prophylaxis but instead for treatment of severe endotoxemia.

In this study, we showed that Cap suppressed the overproduction of TNF-α, IL-1β, and NOx and furthermore Cap improved survival rate in LPS-induced endotoxin shock-model mice. It is known that in response to endotoxemia, release of pro-inflammatory cytokines (TNF-α, IL-1β) and NOx occurs from activated monocytes (macrophages), neutrophils, and other immune cells into surrounding tissues, producing tissue damage and organ failure in vitro. In vivo, release of pro-inflammatory cytokines (TNF-α, IL-1β etc.) and NOx is induced by LPS. Several studies have found that Cap and related analogues inhibit the production of TNF-α, NOx, and prostaglandin E2 and the expression of inducible nitric oxide synthase and cyclooxygenase 2 in LPS-stimulated macrophages independent of TRPV1. They have also demonstrated that Cap and related analogues inhibit LPS-stimulated nuclear factor kappa B (NF-κB) activation in macrophages. It has also been shown that Cap and related analogues inhibit LPS-stimulated NF-κB activation in T cells and myeloid cells.

The role of TRPV1 in inflammation responses is somehow controversial. Cap and related analogues inhibited the inflammatory responses independent of TRPV1. Meanwhile, other researchers showed that treatment with high dose of Cap before CLP resulted in the increase of the inflammatory responses in a rat model of sepsis because the capsaicin-sensitive sensory neuron was ablated by high dose of Cap. Moreover, other researchers showed that activation of sensory neurons might contribute to reduction of endotoxin-induced hypotension and inflammatory responses by releasing calcitonin-gene related peptide (CGRP) that promotes endothelial production of prostacyclin. CGRP, a potent vasodilatory peptide localized in the central and peripheral nerve system neurons, is released by TRPV1 activation on the sensory neurons and has the regulatory effects on the immune system. Administration of CGRP also protects mice against lethal endotoxemia. Further studies need to focus on this question, whether TRPV1 involves to prevent mice from LPS-induced lethality.

**Conclusion**

Cap inhibited overproduction of TNF-α, IL-1β, and NOx levels and improved the survival rate of mice with LPS-induced endotoxin shock. Moreover, these effects were effective after LPS administration. These findings suggest that Cap administration may be beneficial during sepsis.

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References


