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Dose- and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxin-induced shock in rats

Takumi Taniguchi[1], Akihide Kurita[2], Kyoko Kobayashi[2], Ken Yamamoto[2], and Hideo Inaba[1]

[1] Department of Emergency and Critical Care Medicine, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan
[2] Department of Anesthesiology and Intensive Care Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

Abstract
Purpose. Our previous study demonstrated that dexmedetomidine drastically reduced mortality and inhibited the inflammatory response during endotoxemia in rats. The aim of this study was to clarify the dose- and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxemia in rats.
Methods. Male Wistar rats ($n = 96$) were anesthetized intraperitoneally with pentobarbital sodium and assigned to one of two protocols: one representing the dose-related effects of dexmedetomidine, and the other, the time-related effects of dexmedetomidine. To evaluate the dose-related effects, the animals were randomly assigned to one of four groups ($n = 15$ each): endotoxemic group (group E), receiving intravenous *Escherichia coli* endotoxin (15 mg·kg^{-1} over 2 min); small-dose group (group S), treated with a small dose of dexmedetomidine (2.5 μg·kg^{-1}·h^{-1}, IV); medium-dose group (group M), treated with a medium dose (5 μg·kg^{-1}·h^{-1}, IV); and large-dose group (group L), treated with a large dose (10 μg·kg^{-1}·h^{-1}, IV). To evaluate the time-related effects, the animals were randomly assigned to one of three groups ($n = 12$ per group): endotoxemic group; early posttreatment group, treated with 10 μg·kg^{-1}·h^{-1} dexmedetomidine at 1 h after endotoxin injection; and late posttreatment group, treated with 10 μg·kg^{-1}·h^{-1} at 2 h after endotoxin injection. Hemodynamics and arterial blood gases were recorded and plasma cytokine concentrations were measured throughout the observation period. The mortality rate was assessed up to 8 h after endotoxin injection.
Results. In the dose-related study, the mortality rates at 8 h after endotoxin injection were 81%, 26%, 32%, and 20% for groups E, S, M, and L, respectively. Plasma tumor necrosis factor-alpha (TNF) concentrations were lower in groups M and L than in group E at 2 h after endotoxin injection. Plasma interleukin-6 (IL-6) concentrations were lower in groups M and L than in group E at 4 and 5 h after endotoxin injection. In the time-related study, the mortality rates at 8 h after the endotoxin injection were 83%, 33%, and 58% for the endotoxemic, early posttreatment, and late posttreatment groups, respectively. The TNF concentration was lower in the early posttreatment group than in the endotoxemic group at 2 h after endotoxin injection, and the IL-6 concentration was lower in the early posttreatment group than in the endotoxemic group at 5 h after endotoxin injection.
Conclusion. Dexmedetomidine dose-dependently attenuated extremely high mortality rates and increases in plasma cytokine concentrations after endotoxin injection. Moreover, the early administration of dexmedetomidine drastically reduced the high mortality rate and inhibited cytokine responses in endotoxin-exposed rats. These findings suggest that dexmedetomidine administration may be effective during sepsis.

Key words Cytokine · Dexmedetomidine · Dose · Endotoxin · Mortality · Time

Introduction

Endotoxemia and endotoxin shock are common problems in the intensive care unit and carry a very high mortality rate. Cardiovascular dysfunction is common among patients with endotoxemia and is often resistant to aggressive interventions. Endotoxemia increases the production of endogenous cytokines, including tumor necrosis factor-alpha (TNF), interleukin (IL) -6, and IL-8 [1–4]. Not only endotoxin but also cytokines have been implicated in the pathophysiology of endotoxin shock and the development of cardiovascular dysfunction in endotoxemia [1–3]. Patients with endotoxemia often require drugs for sedation and analgesia in the intensive care unit (ICU), and several investigators have reported on the effects of certain anesthetics upon endotoxemia [4,5].

Our previous study demonstrated that dexmedetomidine, a new sedative agent, drastically reduced mortality and inhibited the inflammatory responses during endotoxemia in rats [6]. However, we did not determine

Address correspondence to: T. Taniguchi
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whether these beneficial effects were dose-dependent or whether dexmedetomidine would have different beneficial effects when given at different times after endotoxin injection. The aim of this study was to clarify the dose-related and time-related effects of dexmedetomidine on mortality and inflammatory responses during endotoxin-induced shock in rats.

Materials and methods

Ninety-six male, Wistar rats, 12 ± 1 weeks old, weighing 369 ± 14 g, were used in this study. The Animal Care Committee of our institute approved the experimental protocol, and the care and handling of the animals were in accordance with the National Institutes of Health guidelines.

General procedure

The method used to prepare the animals has been reported previously [6–8]. Briefly, after receiving an intraperitoneal injection of pentobarbital sodium (30 $mg \cdot kg^{-1}$), the animals were ventilated through a tracheotomy. The femoral artery was cannulated to monitor the blood pressure and to draw blood samples. Lactated Ringer's solution, containing a muscle relaxant (pancuronium bromide, 0.02 $mg \cdot ml^{-1}$) and pentobarbital sodium (0.5 $mg \cdot ml^{-1}$) was infused continuously at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$ through the femoral vein cannula. The rats were connected to a pressure-controlled ventilator (Servo 900C; Siemens-Elema, Solna, Sweden), that delivered 100% oxygen at a frequency of 30 $breaths \cdot min^{-1}$ with an inspiratory/expiratory ratio of 1:1. After this procedure, the animals were allowed to rest for more than 30 min to allow their blood gases and hemodynamic parameters to stabilize; baseline heart rate (HR) and systolic arterial pressure (SAP) readings were then taken.

Study 1: dose-related effects of dexmedetomidine

After the baseline measurements, 60 rats were randomly allocated to one of four groups (n = 15 per group).

Endotoxemic group (group E)
Endotoxemia was induced by a bolus injection of *Escherichia coli* lipopolysaccharide (LPS) derived from *E. coli* 0111:B4 (Difco Laboratories, Detroit, MI, USA), which was injected intravenously at a rate of 15 $mg \cdot kg^{-1}$ over 2 min.

Small-dose treatment group (group S)
Endotoxemia was induced as in group E, and dexmedetomidine was administered intravenously (infusion, 2.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) immediately before the injection of the endotoxin.

Medium-dose treatment group (group M)
Endotoxemia was induced as in group E, and dexmedetomidine was administered intravenously (infusion; 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) immediately before the injection of the endotoxin.

Large-dose treatment group (group L)
Endotoxemia was induced as in group E, and dexmedetomidine was administered intravenously (infusion; 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) immediately before the injection of the endotoxin.

Rectal body temperature was maintained between 36°C and 38°C with the aid of a heating pad. Arterial blood samples (0.25 ml) were drawn 1, 3, and 5 h after endotoxin and used to measure the arterial pH (pH_a), arterial carbon dioxide pressure (Pa_{CO_2}), and arterial oxygen pressure (Pa_{O_2}). Additionally, arterial blood samples (1.5 ml) were drawn, to measure plasma cytokine concentrations, at 2, 4, and 5 h after endotoxin. A total amount of 5.0 ml of blood was drawn from each animal over the 8-h observation period.

Study 2: time-related effects of dexmedetomidine

After the baseline measurements, 36 rats were allocated randomly to one of three groups (n = 12 per group).

Endotoxemic group
Endotoxemia was induced by a bolus injection of LPS, which was injected intravenously at 15 $mg \cdot kg^{-1}$ over 2 min.

Early posttreatment group
Endotoxemia was induced as in the endotoxemic group, and dexmedetomidine was administered intravenously (infusion; 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) 1 h after the injection of the endotoxin.

Late posttreatment group
Endotoxemia was induced as in the endotoxemic group, and dexmedetomidine was administered intravenously (infusion; 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) 2 h after the injection of the endotoxin.

Body temperature and blood sampling were performed in each group as in the study 1 protocol.

Sample analysis (both studies)

The blood samples used to determine the cytokine concentrations were centrifuged for 10 min at 3000 g and 4°C. The plasma was then decanted and stored at –70°C

until analysis. All cytokine (TNF and IL-6) concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (BioSource, Camarillo, CA, USA).

Statistical analysis (both studies)

Data values are presented as means ± SDs. Differences between groups at baseline were analyzed using an unpaired Student's *t*-test. Hemodynamic and cytokine changes during the study were analyzed using two-way analysis of variance with repeated measures, followed by a post-hoc test (Bonferroni's method). The mortality rates of the groups were compared using the Kaplan Meier and the Mantel-Cox methods. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using StatView software (Version 5.0, Macintosh; Abacus Concepts, Berkeley, CA, USA).

Results

Study 1: dose-related effects of dexmedetomidine

Hemodynamics and mortality rate

No significant differences in baseline HR or SAP were noted among the four groups (Fig. 1). The SAP decreased in group E, but not in the other groups. SAP in group L was significantly lower than that in groups S and M 8 h after the endotoxin injection. The mortality rates at 8 h after the endotoxin injection were 81%, 26%, 32%, and 20% for groups E, S, M, and L, respectively (Fig. 2). The mortality rates in the groups that received dexmedetomidine were significantly lower than that in group E.

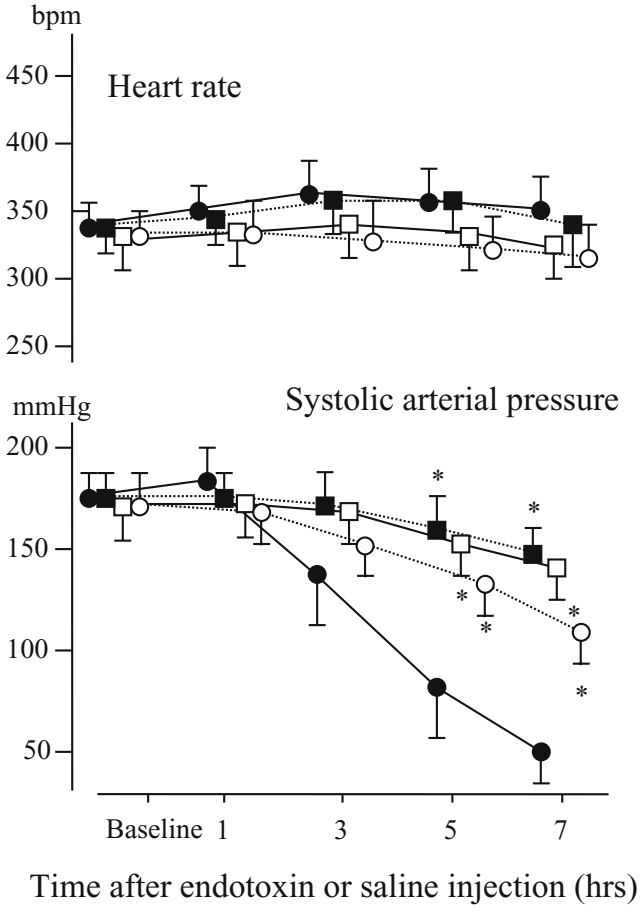

Fig. 1. Heart rate (*top*) and systolic arterial pressure (*bottom*) at baseline and after injection of endotoxin (means ± SD). *Closed circles*, Endotoxemic group; *closed squares*, small-dose treatment group; *open squares*, medium-dose treatment group; *open circles*, large-dose treatment group. *$P < 0.05$ vs endotoxemic group

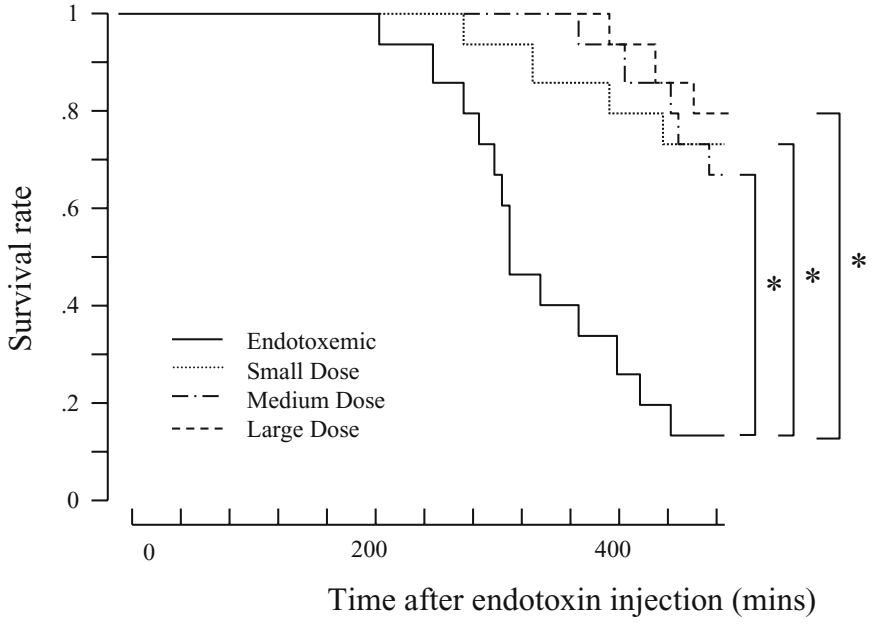

Fig. 2. Survival curves for endotoxemic, small-dose treatment, medium-dose treatment, and large-dose treatment groups. *$P < 0.05$ vs endotoxemic group

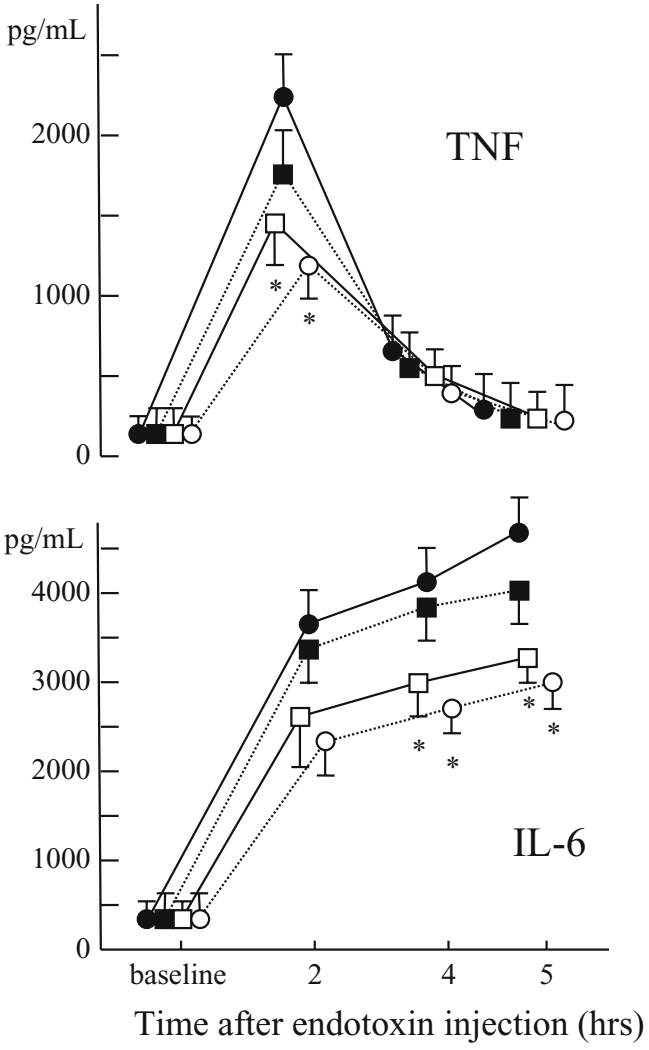

Fig. 3. Levels of plasma tumor necrosis factor α (*TNF*; *top*) and interleukin-6 (*IL-6*; *bottom*) at baseline and after injection of endotoxin (means ± SD). *Closed circles*, Endotoxemic group; *closed squares*, small-dose treatment group; *open squares*, medium-dose treatment group; *open circles*, large-dose treatment group. **P* < 0.05 vs endotoxemic group

Plasma cytokine concentrations

The baseline cytokine values in the four groups were similar. The TNF concentration increased in all four groups, but the concentrations in groups M and L were lower than that in group E (Fig. 3; top). The plasma IL-6 concentrations also increased in all four groups (Fig. 3; bottom), but those in groups M and L were significantly lower than that in group E.

Blood gases

The Pa_{CO_2} and Pa_{O_2} values in the four groups were not significantly different at any time point during the experimental period (Table 1). The pHa decreased in group E, but did not decrease in the other groups.

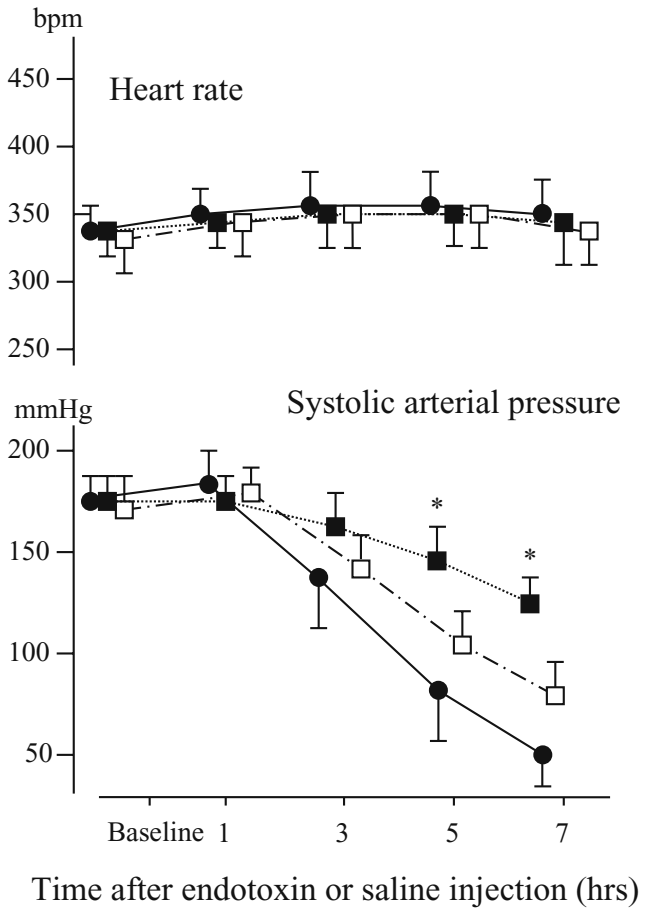

Fig. 4. Heart rate (*top*) and systolic arterial pressure (*bottom*) at baseline and after injection of endotoxin (means ± SD). *Closed circles*, Endotoxemic group; *closed squares*, early posttreatment group; *open squares*, late posttreatment group. **P* < 0.05 vs endotoxemic group

Study 2: time-related effects of dexmedetomidine

Hemodynamics and mortality rate

No significant differences were noted in baseline HR or SAP among the groups (Fig. 4). Endotoxin injection reduced SAP in the endotoxemic and late posttreatment groups, but not in the early posttreatment group. Mortality rates 8 h after endotoxin injection were 83%, 33%, and 58% for the endotoxemic, early posttreatment, and late posttreatment groups, respectively (Fig. 5). The mortality rate for the early posttreatment group was significantly lower than that for the other two groups.

Plasma cytokine concentrations

All baseline values were similar for the three groups (Fig. 6). Endotoxin injection increased the TNF concentration in all groups, but the concentration was lower in the early posttreatment group than in the other two

Table 1. Study 1: arterial blood gas values at baseline and after endotoxin injection

	Time after endotoxin injection (h)			
	Baseline	1	3	5
pH_a				
Endotoxemic group	7.43 ± 0.07	7.33 ± 0.08	7.28 ± 0.10	7.19 ± 0.10
Small-dose treatment group	7.47 ± 0.08	7.39 ± 0.08	7.35 ± 0.07	7.33 ± 0.08*
Medium-dose treatment group	7.46 ± 0.09	7.39 ± 0.09	7.37 ± 0.10	7.35 ± 0.09*
Large-dose Treatment group	7.46 ± 0.08	7.40 ± 0.10	7.40 ± 0.10	7.37 ± 0.09*
Pa_{O_2} (mmHg)				
Endotoxemic group	515 ± 38	495 ± 57	474 ± 61	468 ± 70
Small-dose treatment group	518 ± 45	503 ± 47	489 ± 52	480 ± 74
Medium-dose treatment group	524 ± 44	506 ± 46	498 ± 63	482 ± 82
Large-dose Treatment group	520 ± 44	509 ± 48	504 ± 64	495 ± 76
Pa_{CO_2} (mmHg)				
Endotoxemic group	34 ± 9	36 ± 13	36 ± 12	34 ± 12
Small-dose treatment group	33 ± 8	34 ± 10	34 ± 11	35 ± 9
Medium-dose treatment group	33 ± 9	32 ± 11	33 ± 13	34 ± 14
Large-dose Treatment group	33 ± 9	33 ± 12	34 ± 13	34 ± 13

* $P < 0.05$ vs endotoxemic group
All data values are expressed as means ± SD
pH_a, Arterial pH; Pa_{O_2}, arterial oxygen pressure; Pa_{CO_2}, arterial carbon dioxide pressure

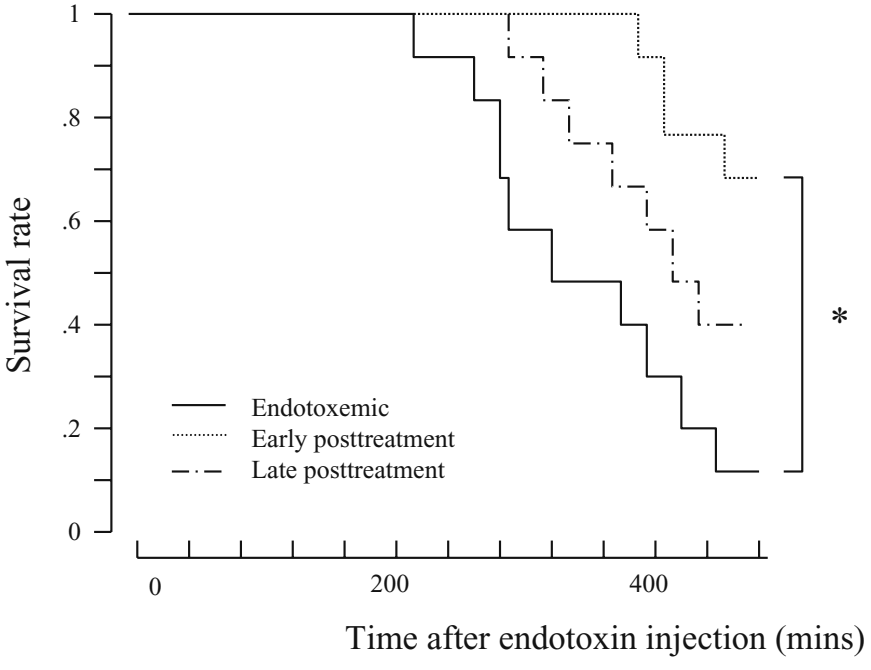

Fig. 5. Survival curves for endotoxemic, early posttreatment, and late posttreatment groups. *$P < 0.05$ vs endotoxemic group

groups (Fig. 6; top). Plasma IL-6 concentration became elevated in all groups (Fig. 6; bottom), but the IL-6 concentration in the early posttreatment group was significantly lower than those in the other two groups.

Blood gases

The Pa_{CO_2} and Pa_{O_2} values in the four groups were not significantly different at any time point during the experimental period (Table 2). The pH_a decreased in the endotoxemic and late posttreatment groups, but did not decrease in the early posttreatment group.

Discussion

Injection of endotoxin alone in our experiments produced a high mortality rate and a large increase in inflammatory cytokine concentrations. Dexmedetomidine dose-dependently reduce the high mortality rate and attenuated the increase in cytokine concentrations after the endotoxin injection. Moreover, the early administration of dexmedetomidine drastically reduced the high mortality rate and inhibited cytokine responses in endotoxin-exposed rats. These are the most important findings of this study.

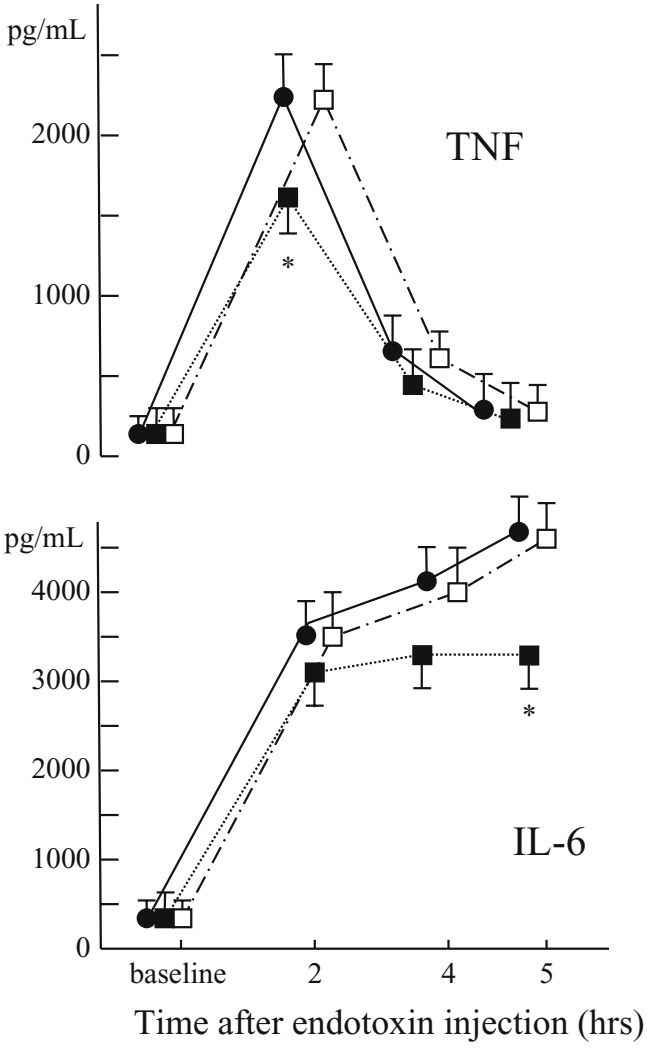

Fig. 6. Levels of plasma tumor necrosis factor α (*TNF*; *top*) and interleukin-6 (*IL-6*; *bottom*) at baseline and after injection of endotoxin (means ± SD). *Closed circles*, Endotoxemic group; *closed squares*, early posttreatment group; *open squares*, late posttreatment group. *$P < 0.05$ vs endotoxemic group

Our previous study demonstrated that dexmedetomidine remarkably reduced mortality and inhibited the inflammatory responses during endotoxemia in rats [6]. However, we did not determine whether these beneficial effects were dose-dependent or whether effects varied when dexmedetomidine was given at different times after endotoxin injection. Of note, there are no reports about the dose-related and time-related effects of dexmedetomidine on endotoxemia in vitro or in vivo. The present study demonstrated that even a small dose of dexmedetomidine drastically reduced the high mortality rate of endotoxemia in vivo, and also showed that dexmedetomidine dose-dependently inhibited the elevation of inflammatory cytokine responses. Moreover, the present study showed that dexmedetomidine reduced the high mortality rate when it was initiated 1 h after endotoxin injection in vivo.

The dose-dependent effect of dexmedetomidine on the production of inflammatory cytokines after endotoxin injection is very interesting. Circulating endotoxin induces the release of cytokines such as TNF and IL-6, which can lead to hypotension and metabolic acidosis [4,5]. Our study found that dexmedetomidine dose-dependently inhibited increases in the plasma concentrations of TNF and IL-6 induced by endotoxemia in vivo. Several investigators have described the effects of dexmedetomidine and alpha 2-adrenoreceptor agonists on cytokines [9–12]. In vitro, paminoclonidine, an alpha 2-adrenoceptor agonist, suppressed IL-6 production [9], and clonidine suppressed TNF production in monocytes [10]. Moreover, alpha 2-adrenoceptor agonists modulated the LPS-induced production of TNF in macrophages [11]. In a clinical study, dexmedetomidine attenuated elevated IL-6 levels in postoperative patients [12]. However, these previous studies were insufficient to evaluate the dose-related effects of dexmedetomidine and alpha 2-adrenoreceptor agonists. Our findings demonstrated that, even in an in vivo experiment, dexmedetomidine dose-dependently inhibited cytokine responses to endotoxemia. These findings suggest that one of the mechanisms responsible for the anti-inflammatory effects of dexmedetomidine may involve the modulation of cytokine production by macrophages and monocytes.

In our present time-related effect study, we evaluated the effect of dexmedetomidine on mortality and cytokine responses in response to endotoxin-induced shock in vivo. We found that when dexmedetomidine administration was initiated 1 h after endotoxin injection, the responses were remarkably suppressed. This finding suggests that early dexmedetomidine administration may prevent inflammatory effects in patients with sepsis and patients with septic shock and that dexmedetomidine would not harm patients with sepsis.

In our late posttreatment group, we evaluated whether the administration of dexmedetomidine could inhibit the high mortality and cytokine responses during endotoxemia if the plasma TNF concentration increased as markedly as it did in the endotoxemic group. Our study showed that, in the late posttreatment group, the mortality rate and marked increase in plasma IL-6 concentration were similar to those in the endotoxemic group. IL-6 is, thus, a potent chemotactic and activating factor in response to proinflammatory stimuli such as TNF and endotoxin [13]. A comparison of the findings for the late posttreatment group with those for the early posttreatment group indicates that dexmedetomidine may inhibit only the TNF response and that it cannot attenuate the IL-6 response if the plasma TNF concentration shows a

Table 2. Study 1: arterial blood gas values at baseline and after endotoxin injection

	Time after endotoxin injection (h)			
	Baseline	1	3	5
pH_a				
Endotoxemic group	7.45 ± 0.08	7.35 ± 0.08	7.29 ± 0.09	7.18 ± 0.10
Early posttreatment group	7.46 ± 0.08	7.36 ± 0.08	7.34 ± 0.08	7.32 ± 0.08*
Late posttreatment group	7.45 ± 0.09	7.35 ± 0.09	7.31 ± 0.10	7.24 ± 0.09
Pa_{O_2} (mmHg)				
Endotoxemic group	517 ± 42	490 ± 52	478 ± 65	455 ± 88
Early posttreatment group	511 ± 48	500 ± 49	481 ± 62	479 ± 64
Late posttreatment group	512 ± 47	501 ± 56	472 ± 68	462 ± 82
Pa_{CO_2} (mmHg)				
Endotoxemic group	34 ± 9	35 ± 11	34 ± 10	34 ± 12
Early posttreatment group	34 ± 8	34 ± 10	34 ± 11	35 ± 9
Late posttreatment group	34 ± 9	33 ± 11	36 ± 11	34 ± 11

* $P < 0.05$ vs endotoxemic group
All data values are expressed as means ± SD
pH_a, Arterial pH; Pa_{O_2}, arterial oxygen pressure; Pa_{CO_2}, arterial carbon dioxide pressure

marked increase. There are few reports on the relationship between dexmedetomidine and cytokine responses during endotoxemia. Further investigations are needed on this point.

In the present in vivo study, we injected 15 $mg \cdot kg^{-1}$ of LPS endotoxin (approximately 2× lethal dose for 50% of animals [LD50]) and observed hypotension 4 h after injection. This dose of endotoxin produced a high mortality rate (81% in Study 1 and 83% in Study 2) at 8 h after injection, enabling the beneficial effects of dexmedetomidine on endotoxin-induced shock to be evaluated in vivo. In the present study, the dose of dexmedetomidine we used was relatively high compared with the doses required to produce anesthesia in humans. Further investigation is needed on this point, but the required drug dose is known to be species-dependent.

Critically ill patients with sepsis and septic shock suffer a high degree of stress because of pain, anxiety, and organ-specific responses to sepsis. An important objective in the management of these patients is to achieve an adequate level of sedation and analgesia. Though further investigations are needed in a clinical environment, our findings suggest that dexmedetomidine may dose-dependently prevent inflammatory effects in patients with sepsis and those with septic shock during sedation and analgesia.

In summary, extremely high mortality rates and increases in plasma cytokine concentrations after endotoxin injection were dose-dependently attenuated by the administration of dexmedetomidine. Moreover, the early administration of dexmedetomidine drastically reduced the high mortality rate and inhibited the cytokine responses in endotoxin-exposed rats. These findings suggest that dexmedetomidine administration may be effective during sepsis.

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