J Anesth (2007) 21:354–360
DOI 10.1007/s00540-007-0533-y

Journal of
Anesthesia

The antiinflammatory effects of propofol in endotoxemic rats during moderate and mild hypothermia

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Abstract
Purpose. We previously found that propofol attenuated the mortality rate and inflammatory responses during endotoxemia in rats; however, whether propofol retains its antiinflammatory effects during hypothermia has not been determined. We investigated the effects of propofol on endotoxemic rats subjected to moderate or mild hypothermia.
Methods. Male Wistar rats ($n = 88$) were anesthetized intraperitoneally with pentobarbital sodium and assigned to one of two protocols: one representing moderate hypothermia (30°–32°C) and the other representing mild hypothermia (33°–35°C). Each protocol included four equal-sized groups: group A, *Escherichia coli* endotoxin (15 mg·kg^{-1}, i.v.) and normothermia; group B, propofol (10 mg·kg^{-1}·h^{-1}, i.v.) and normothermia after endotoxin injection; group C, endotoxin (15 mg·kg^{-1}, i.v.) and hypothermia; and group D, propofol (10 mg·kg^{-1}·h^{-1}, i.v.) and hypothermia after endotoxin injection. Rats then were warmed or cooled to maintain rectal temperatures as above for 6 h. The mortality rate was assessed up to 6 h after endotoxin injection. In addition, we assessed hemodynamics, acid–base status, and plasma cytokine concentrations.
Results. Endotoxemic rats developed hypotension and metabolic acidosis as well as increased plasma cytokine concentrations. Mortality rates 6 h after endotoxin injection were 70%, 40%, 10%, and 0% for groups A–D, respectively, at moderate hypothermia. Propofol administration to endotoxemic rats with hypothermia, whether moderate or mild, also attenuated the high mortality rate, metabolic acidosis, and elevation of cytokines, but these effects were not superior to those of hypothermia alone.
Conclusion. During hypothermia, propofol administration does not have additive beneficial antiinflammatory effects.

Key words Propofol · Endotoxemia · Hemodynamics · Hypothermia · Cytokine

Introduction

Endotoxemia and endotoxin shock are common problems in the intensive care unit (ICU) and carry a very high mortality rate. Cardiovascular dysfunction is common among patients with endotoxemia and is often resistant to aggressive interventions. Endotoxemia increases production of endogenous cytokines, including tumor necrosis factor alpha (TNF-alpha), interleukin-6 (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 ICU, and several investigators have reported on the effects of certain anesthetics on endotoxemia [4,5]. In a previous study, we found that administration of propofol to endotoxemic rats decreased the mortality rate and inhibited metabolic acidosis and elevations of plasma cytokines such as TNF-alpha and IL-6 [6,7].

Recently, therapeutic moderate and mild hypothermia has become an issue in the ICU; several reports have shown that such treatment after cardiac arrest improves the likelihood of a favorable neurologic outcome [8] as well as reducing mortality [9,10]. Moreover, critically ill patients with endotoxemia often experience hypothermia, and perioperative hypothermia also is a common complication of anesthesia and surgery. Thus, the relationships between anesthetics and hypothermia in endotoxemia pose very important unsolved questions. However, whether propofol retains its antiinflammatory effects during moderate and mild hypothermia has not been determined. We therefore investigated the effects of propofol on endotoxin-induced shock in rats during moderate and mild hypothermia.

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Received: August 4, 2006 / Accepted: April 11, 2007

Methods

All experimental procedures were approved by the Animal Care Committee of Kanazawa University and were in accordance with the US National Institutes of Health guidelines for animal use. The method of animal preparation has been reported previously [6,7]. Briefly, male Wistar rats ($n = 88$) weighing 370 ± 10 g (mean ± SD) were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg·kg^{-1}). Ventilation was performed via 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·ml^{-1}) and pentobarbital sodium (0.5 mg·ml^{-1}) was infused continuously at a rate of 10 ml·kg^{-1}·h^{-1} through a femoral vein cannula. The heart rate (HR) was recorded from lead II of the electrocardiogram. The rats were ventilated by a pressure-controlled ventilator (Servo 900C, Siemens-Elema, Solna, Sweden) delivering 100% oxygen at a frequency of 30 breaths min^{-1} with an inspiratory : expiratory ratio of 1 : 1. The animals were then rested for at least 30 min to allow stabilization of hemodynamic variables, followed by baseline recordings of HR and systolic arterial pressure (SAP) as well as arterial blood gas sampling and acid–base analyses (ABL-520, Radiometer, Copenhagen, Denmark).

Moderate hypothermia

After baseline measurements, 40 rats were allocated randomly to one of four groups ($n = 10$ per group). In group A, endotoxin shock was induced with a bolus injection of endotoxin (15 mg·kg^{-1}) and normothermia was maintained. We used lipopolysaccharide prepared from *Escherichia coli* (0111:B4; Difco, Detroit, MI, USA) as the endotoxin. Group B received an infusion of propofol (10 mg·kg^{-1}·h^{-1}). After initiation of the infusion, a bolus of endotoxin (15 mg·kg^{-1}) was given. Normothermia was maintained. Group C was exposed to moderate hypothermia and endotoxin shock was induced with a bolus injection of endotoxin (15 mg·kg^{-1}). Group D was exposed to moderate hypothermia and received an infusion of propofol (10 mg·kg^{-1}·h^{-1}). After initiation of the infusion, a bolus of endotoxin (15 mg·kg^{-1}) was given.

Rectal temperatures in the normothermia groups were maintained between 36° and 38°C with the aid of a heating pad. Immediately after administration of saline or endotoxin, animals in the moderate hypothermia groups were cooled externally so that by 1 h after initiation of cooling, the rectal temperature was between 30° and 32°C. The mortality rate was assessed up to 6 h after endotoxin injection. Arterial blood samples (0.25 ml) were drawn 1, 3, and 5 h after endotoxin injection for measurement of arterial pH (pHa), O_2 tension (Pa_{O_2}), and CO_2 tension (Pa_{CO_2}). Additional arterial blood samples (1.5 ml) were drawn for the measurement of plasma cytokine concentrations 2 and 4 h after endotoxin injection. The total amount of blood drawn from each animal was 4.0 ml over 6 h.

Mild hypothermia

After baseline measurements, 48 animals were allocated randomly to one of four groups ($n = 12$ per group). Injections, infusions, and blood sampling were performed in each group as was done in the moderate hypothermia protocol. Immediately after the administration of endotoxin, animals in the mild hypothermia groups were cooled externally so that by 1 h after initiation of cooling, the rectal temperature was between 33° and 35°C.

Sample analysis (all experiments)

Blood used to measure the cytokine concentrations was centrifuged for 10 min at 3000 × *g* at 4°C. Plasma was decanted and stored at −70°C until analysis. Cytokine concentrations (TNF-alpha and IL-6) were measured by enzyme-linked immunosorbent assays (BioSource, Camarillo, CA, USA).

Statistical analysis

Data are presented as the mean ± SD. Differences between groups at baseline were analyzed with unpaired Student *t* tests and the Mann-Whitney *U* test. Comparisons among mortality rates of the groups were made with the Kaplan-Meier and the Mantel-Cox methods. During the study, hemodynamic variables as well as cytokine concentrations were analyzed using two-way analyses of variance for repeated measures, followed by a post hoc test (Bonferroni's method). Statistical significance was defined at $P < 0.05$. Statistical analyses were performed using StatView software (version 5.0 for Macintosh; Abacus Concepts, Berkeley, CA, USA).

Results

Moderate hypothermia

Mortality rate and hemodynamics

No significant differences were noted in baseline HR or SAP values between the four groups (Fig. 1). Endotoxin injection significantly reduced the SAP in group A, but not in the other groups. The SAP for group A was significantly lower than that for the other groups at 6 h

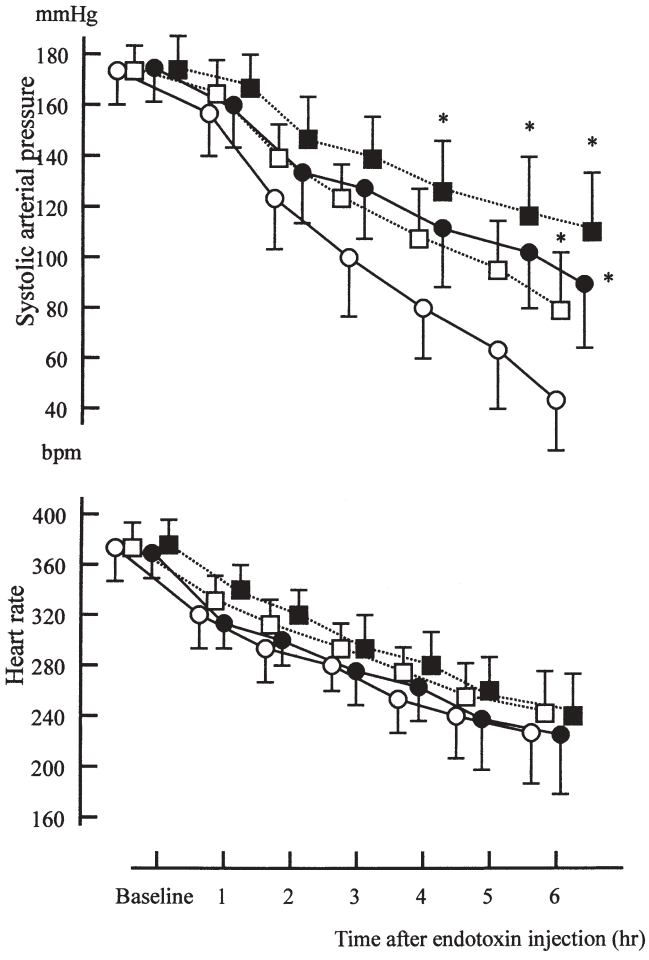

Fig. 1. Systolic arterial pressure (*top*) and heart rate (*bottom*) at the baseline and after endotoxin or saline injection during normothermia or moderate hypothermia (mean ± SD). *Open circles*, saline/normothermia group; *open squares*, propofol/normothermia group; *solid circles*, saline/moderate hypothermia group; *solid squares*, propofol/moderate hypothermia group. *$P < 0.05$ vs saline/normothermia group

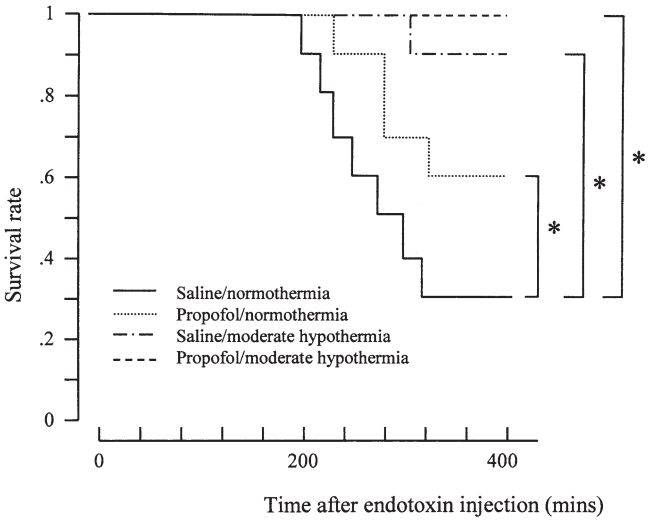

Fig. 2. Survival curves for the saline/normothermia, propofol/normothermia, saline/moderate hypothermia, and propofol/moderate hypothermia groups. *$P < 0.05$ vs saline/normothermia group

after the endotoxin injection. Mortality rates 6 h after endotoxin injection were 70%, 40%, 10%, and 0% for groups A–D, respectively (Fig. 2). The HR in group D did not differ from that in group B and group C.

Blood gases

Pa_{CO_2} and Pa_{O_2} showed no significant differences between the four groups at any point during the experimental period (Table 1). The pHa in group A alone decreased significantly. The pHa in group D did not differ significantly from that in group B and group C.

Plasma cytokine concentrations

All baseline values were similar for the four groups (Fig. 3). Endotoxin injection increased the TNF-alpha concentrations 2 h after endotoxin injection, but the TNF-alpha concentrations in group B, group C, and group D were significantly lower than that in group A. The TNF-alpha concentrations in group D did not differ significantly from that in group B and group C. Endotoxin injection increased the IL-6 concentrations, but the IL-6 concentrations in group B, group C, and group D were significantly lower than that in group A. The IL-6 concentrations in group D did not differ significantly from that in group B and group C.

Mild hypothermia

Mortality rate and hemodynamics

No significant differences were noted in baseline HR or SAP between the four groups (Fig. 4). Endotoxin injection significantly reduced the SAP in group A, but not in the other groups. The SAP for group A was significantly lower than that for the other groups at 6 h after the endotoxin injection. Mortality rates 6 h after endotoxin injection were 83%, 34%, 17%, and 8% for groups A–D, respectively (Fig. 5).

Blood gases

Pa_{CO_2} and Pa_{O_2} showed no significant differences between the four groups at any point during the experimental period (Table 2). The pHa in group A alone decreased significantly. The pHa in group D in mild hypothermia did not differ significantly from that in group B and group C.

Table 1. Baseline arterial blood gas values and those after endotoxin injection during normothermia or moderate hypothermia

		Time after endotoxin injection (h)		
	Baseline	1	3	5
Saline/normothermia group (n = 10)				
pHa	7.40 ± 0.10	7.30 ± 0.09	7.20 ± 0.09#	7.11 ± 0.07#
Pa_{CO_2} (mmHg)	35 ± 11	34 ± 10	35 ± 12	36 ± 13
Pa_{O_2} (mmHg)	585 ± 49	589 ± 64	577 ± 61	579 ± 70
Propofol/normothermia group (n = 10)				
pHa	7.41 ± 0.10	7.35 ± 0.10	7.30 ± 0.10	7.25 ± 0.09*
Pa_{CO_2} (mmHg)	34 ± 10	36 ± 11	34 ± 10	33 ± 10
Pa_{O_2} (mmHg)	568 ± 74	589 ± 81	578 ± 68	591 ± 64
Saline/moderate hypothermia group (n = 10)				
pHa	7.46 ± 0.11	7.35 ± 0.09	7.32 ± 0.08	7.29 ± 0.09*
Pa_{CO_2} (mmHg)	34 ± 10	34 ± 10	33 ± 10	31 ± 13
Pa_{O_2} (mmHg)	573 ± 47	579 ± 78	590 ± 66	588 ± 81
Propofol/moderate hypothermia group (n = 10)				
pHa	7.43 ± 0.10	7.36 ± 0.10	7.34 ± 0.10	7.31 ± 0.10*
Pa_{CO_2} (mmHg)	35 ± 10	36 ± 10	35 ± 9	34 ± 10
Pa_{O_2} (mmHg)	578 ± 57	581 ± 65	591 ± 58	590 ± 66

All data are expressed as mean ± SD
pHa, arterial pH; Pa_{O_2}, arterial oxygen pressure; Pa_{CO_2}, arterial carbon dioxide pressure
* $P < 0.05$ vs saline/normothermia group
# $P < 0.05$ vs baseline within group

Table 2. Baseline arterial blood gas values and those after endotoxin injection during normothermia or mild hypothermia

		Time after endotoxin or saline injection (h)		
	Baseline	1	3	5
Saline/normothermia group (n = 12)				
pHa	7.43 ± 0.10	7.32 ± 0.09	7.21 ± 0.08#	7.12 ± 0.08#
Pa_{CO_2} (mmHg)	36 ± 10	38 ± 11	36 ± 11	35 ± 13
Pa_{O_2} (mmHg)	579 ± 57	588 ± 70	591 ± 65	578 ± 75
Propofol/normothermia group (n = 12)				
pHa	7.42 ± 0.10	7.33 ± 0.10	7.31 ± 0.10	7.29 ± 0.10*
Pa_{CO_2} (mmHg)	35 ± 10	37 ± 10	36 ± 9	36 ± 10
Pa_{O_2} (mmHg)	584 ± 70	588 ± 67	565 ± 65	594 ± 60
Saline/mild hypothermia group (n = 12)				
pHa	7.44 ± 0.11	7.34 ± 0.10	7.28 ± 0.09	7.27 ± 0.10*
Pa_{CO_2} (mmHg)	36 ± 10	36 ± 11	35 ± 10	34 ± 11
Pa_{O_2} (mmHg)	570 ± 47	591 ± 77	588 ± 64	591 ± 68
Propofol/mild hypothermia group (n = 12)				
pHa	7.44 ± 0.12	7.34 ± 0.11	7.30 ± 0.11	7.29 ± 0.10*
Pa_{CO_2} (mmHg)	36 ± 9	35 ± 10	36 ± 7	35 ± 8
Pa_{O_2} (mmHg)	595 ± 58	598 ± 64	569 ± 69	589 ± 69

All data are expressed as mean ± SD
pHa, arterial pH; Pa_{O_2}, arterial oxygen pressure; Pa_{CO_2}, arterial carbon dioxide pressure
* $P < 0.05$ vs saline/normothermia group
# $P < 0.05$ vs baseline within group

Plasma cytokine concentrations

All baseline values were similar for the four groups (Fig. 6). Endotoxin injection increased the TNF-alpha concentrations 2 h after endotoxin injection, but the TNF-alpha concentrations in group B, group C, and group D were significantly lower than that in group A. The TNF-alpha concentrations in group D did not differ significantly from that in group B and group C. Endotoxin injection increased the IL-6 concentrations, but the IL-6 concentrations in group B, group C, and group D were significantly lower than that in group A. The IL-6 concentrations in group D in mild hypothermia did not differ significantly from that in group B and group C.

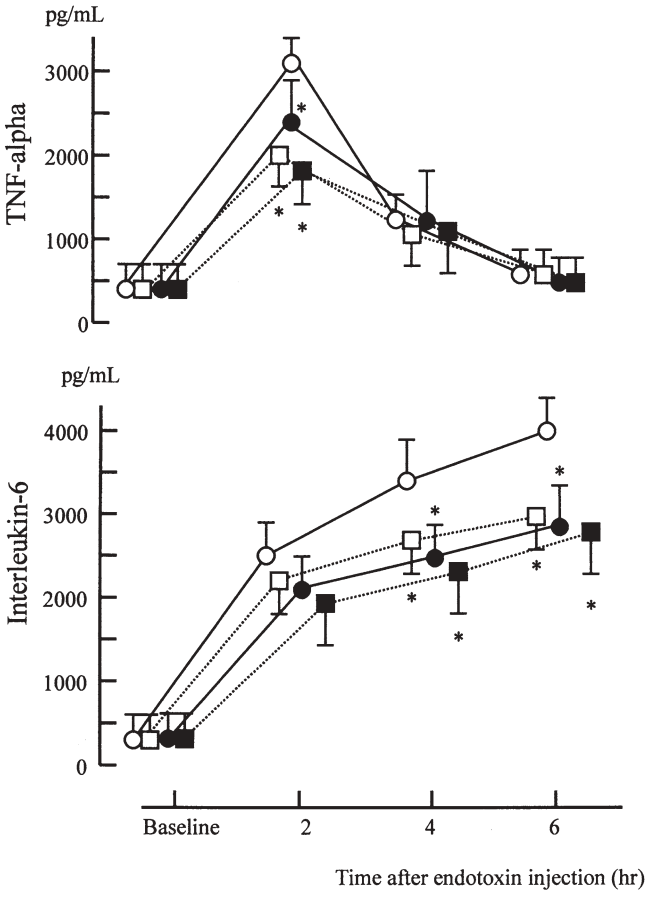

Fig. 3. Changes of plasma tumor necrosis factor-alpha (*TNF-alpha*) (*top*) and interleukin-6 (IL-6) (*bottom*) at the baseline and after endotoxin or saline injection during normothermia or moderate hypothermia (mean ± SD). *Open circles*, saline/normothermia group; *open squares*, propofol/normothermia group; *solid circles*, saline/moderate hypothermia group; *solid squares*, propofol/moderate hypothermia group. *$P < 0.05$ vs saline/normothermia group

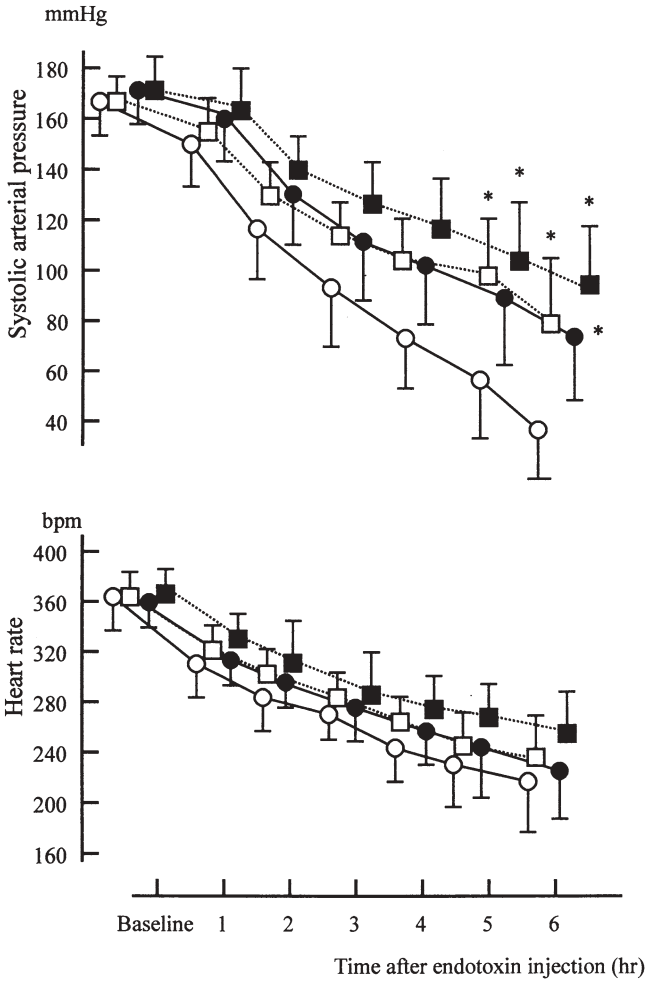

Fig. 4. Systolic arterial pressure (*top*) and heart rate (*bottom*) at the baseline and after endotoxin or saline injection during normothermia or mild hypothermia (mean ± SD). *Open circles*, saline/normothermia group; *open squares*, propofol/normothermia group; *solid circles*, saline/mild hypothermia group; *solid squares*, propofol/mild hypothermia group. *$P < 0.05$ vs saline/normothermia group

Discussion

Endotoxemia in rats is associated with a high mortality rate and metabolic acidosis as well as increases in plasma cytokine concentrations in normothermic rats; propofol reduces these changes in normothermic rats. However, after administration of propofol, hypothermic endotoxemic rats, both under moderate and mild hypothermia, continued to show hypotension, metabolic acidosis, and increased plasma cytokine in the same way that the endotoxemic control groups did. During hypothermia, therefore, propofol administration in our rat endotoxin model did not exhibit significant additive inhibitory effects on inflammatory responses. This fact was the most important finding of the present study.

In previous studies, propofol has been reported to have inhibitory effects on inflammatory responses such as metabolic acidosis, cytokine elevations, and neutrophil activations during endotoxemia [6,7, and 11]. Our previous reports, as well as the present one, demonstrated that propofol administration reduces the mortality rate, the elevation of TNF-alpha and IL-6 levels, and metabolic acidosis during normothermic endotoxemia [6,7]. However, these previous studies did not assess the additive antiinflammatory effects of propofol on endotoxemia accompanied by hypothermia. Our study showed that propofol did not exhibit any significant additional effects during hypothermia.

Several other relationships between propofol and hypothermia have been studied. Kahveci et al. [12] found that propofol administration during hypothermia reduced intracranial pressure and local cerebral blood flow after brain injury in rats. Leslie et al. [13] reported

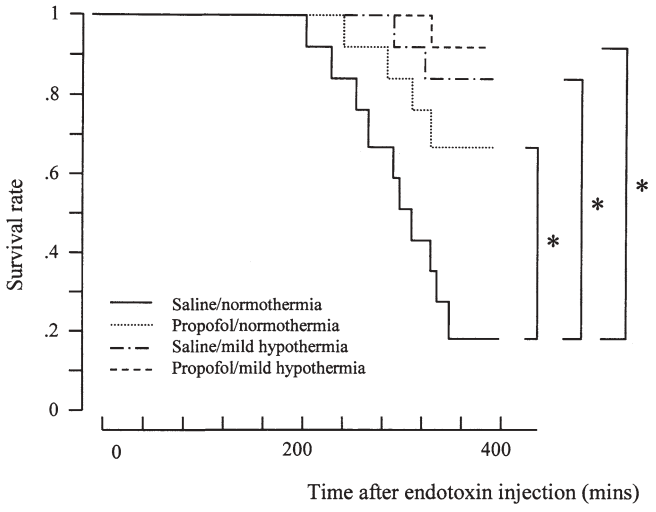

Fig. 5. Survival curves for the saline/normothermia, propofol/normothermia, saline/mild hypothermia, and propofol/mild hypothermia groups. *$P < 0.05$ vs saline/normothermia group

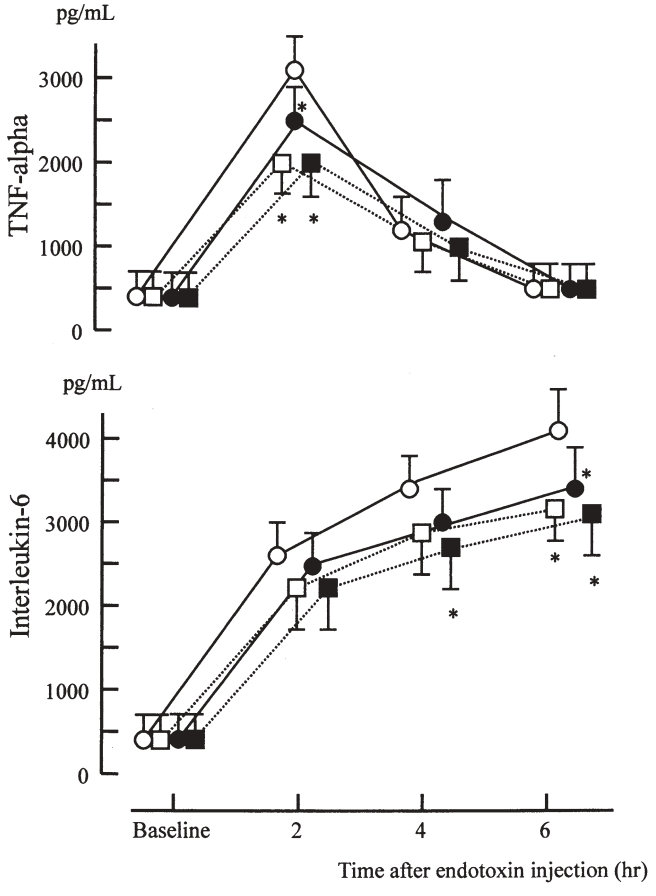

Fig. 6. Changes of plasma TNF-alpha (*top*) and IL-6 (*bottom*) at the baseline and after endotoxin or saline injection during normothermia or mild hypothermia (mean ± SD). *Open circles*, saline/normothermia group; *open squares*, propofol/normothermia group; *solid circles*, saline/mild hypothermia group; *solid squares*, propofol/mild hypothermia group. *$P < 0.05$ vs saline/normothermia group

that mild hypothermia altered propofol's pharmacokinetics. However, few reports have addressed the relationship between propofol and hypothermia during endotoxemia. Our findings suggest that propofol may not be useful as an antiinflammatory drug during hypothermia, because it did not significantly reduce the mortality rate or cytokine responses during hypothermia in endotoxemic rats.

In the present study, propofol administration was less effective under hypothermia than under normothermia. Two reasons for this are possible: maybe there was an actual decrease in the drug's antiinflammatory effects, or maybe the antiinflammatory effects of hypothermia overshadowed the effects of propofol. Few reports have analyzed these mechanisms, and further investigations are needed.

The important question of whether the lipids used as a solvent for propofol have antiinflammatory effects remains unanswered. Heine et al. [14] documented that lipids inhibited the respiratory burst of neutrophil, and Gordon et al. [15] showed that phospholipids reduced the increase of TNF-alpha and IL-6 on exposure to endotoxin. However, Mikawa et al. [16] demonstrated that the amount of lipids contained in propofol formulations had no effect on the reactive oxygen species generated by neutrophils. Our preliminary study has shown that the lipids present in propofol formulations had no effects on hemodynamics or cytokine responses.

Another important question remains concerning the dose–response relationships between propofol and antiinflammatory effects during hypothermia. In the present study, we used a propofol dose of $10\,mg \cdot kg^{-1} \cdot h^{-1}$ because Takemoto [17] showed that this dose is the maximally effective dose in endotoxemic rats during normothermia. Leslie et al. [13] showed that propofol blood concentrations were approximately 28% higher on average at 34°C than at 37°C. More generally, several investigations have shown that hypothermia alters the pharmacokinetics of intravenous anesthetics [8]. However, few reports have examined propofol pharmacokinetics during hypothermia. Thus, the pharmacokinetics of propofol during hypothermia in endotoxemic rats requires further investigation.

The present study has evaluated the antiinflammatory effects of propofol for moderate hypothermia and mild hypothermia: because we thought that the influences of propofol during moderate hypothermia are markedly different from those during mild hypothermia, such as in the cytokine and hemodynamic responses. In clinical studies, several reports have shown that the hemodynamics during moderate hypothermia deteriorated more than they did during mild hypothermia [18]. In our experiments, the effects of propofol under mild and moderate hypothermia have not been different. Further investigations are needed on this point.

In summary, the study presented here shows that although propofol administration decreased the mortality rate and acidosis in endotoxemic rats during normothermia, during moderate and mild hypothermia, propofol administration did not have significant additive beneficial antiinflammatory effects.

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