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Effects of Ketamine and Propofol on the Ratio of Interleukin-6 to Interleukin-10 during Endotoxemia in Rats

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TANIGUCHI, T., KANAKURA, H., TAKEMOTO, Y., KIDANI, Y. and YAMAMOTO, Y. *Effects of Ketamine and Propofol on the Ratio of Interleukin-6 to Interleukin-10 during Endotoxemia in Rats.* Tohoku J. Exp. Med., 2003, **200** (2), 85–92 —— Our previous study reported that the change in the ratio of interleukin (IL)-6 to IL-10 influences the severity of sepsis in patients with systemic inflammatory response syndrome. We evaluated the change in the ratio of IL-6 to IL-10 after administration of ketamine or propofol in endotoxin-exposed rats in order to evaluate the relationship of pro-inflammatory and anti-inflammatory cytokines following ketamine or propofol administration during endotoxemia. We randomly assigned 40 rats to one of four equal groups: endotoxin alone, receiving *Escherichia coli* endotoxin (15 mg/kg, i.v.); saline control; ketamine ($10\ mg \cdot kg^{-1} \cdot h^{-1}$, i.v.) before and during exposure to endotoxin; and propofol ($10\ mg \cdot kg^{-1} \cdot h^{-1}$, i.v.) before and during exposure to endotoxin. We measured the plasma concentrations of tumor necrosis factor (TNF)-α, IL-6, and IL-10 and calculated the ratio of IL-6 to IL-10 in each group. The current study showed that ketamine and propofol administration attenuated the increase in TNF-α, IL-6, and IL-10, and ketamine attenuated the increase in the ratio of IL-6 to IL-10, but propofol increased this ratio in rats receiving a single intravenous bolus of endotoxin. While the mechanisms responsible for the inhibitory effects require further investigation, our results suggest that proper use of ketamine as an anesthetic agent may offer certain advantages in the management of patients with endotoxemia. ———— cytokine response; ketamine; lipopolysaccharide; propofol; sepsis

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Endotoxemia is a major cause of the systemic inflammatory response syndrome (SIRS) (Tracey et al. 1986). Circulating endotoxin induces the activation of complement and the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, which in turn can cause infiltration of leukocytes in the lungs (Minghini et al. 1998). Inflammatory mediators from leukocytes can produce hypotension, metabolic acidosis, and tissue damage that may lead to organ dysfunction (Larsen et al. 1998). Our previous study reported that the change in the ratio of IL-6 to IL-10 influences the severity of SIRS patients (Taniguchi et al. 1999a).

Critically ill patients with sepsis suffer a high degree of stress due to pain, anxiety and organ-specific response by sepsis. An important objective in the management of these patients is to achieve an adequate level of sedation. Although the need for adequate sedation in septic patients is generally accepted, there is no consensus regarding the choice of drugs. Intravenous anesthetics such as katamine and propofol are widely used for these purposes.

Ketamine, a phencyclidine derivative, produces dose-related unconsciousness and analgesia, and has been recommended for anesthesia and sedation of septic or severely ill patients because of its stimulating cardiovascular effects. Propofol belongs to a group of alkylphenols that have a hypnotic effect on animals, and because propofol is easily titratable and offers the prospect of rapid recovery for the patient, it is used for sedation of critically ill patients.

Our previous studies have documented that ketamine and propofol attenuate the production and release of pro-inflammatory cytokines in endotoxin-exposed rats (Taniguchi et al. 2000, 2001). However, there are few reports that have studied the relationship of pro-inflammatory and anti-inflammatory cytokines after ketamine or propofol administration during endotoxemia in vivo. We therefore evaluated the change in the relationship of pro-inflammatory and anti-inflammatory cytokines after ketamine or propofol administration in endotoxin-exposed rats.

METHODS

Animal preparation

All experimental procedures were approved by the Animal Care Committee of Kanazawa University School of Medicine, and were in accordance with the National Institute of Health guidelines for animal use. Male Wistar rats ($n=32$), weighing 380 ± 15 g (mean$\pm$S.D.), were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg). Ventilation was performed through a tracheal tube (7 Fr. Internal diameter) after tracheotomy. The femoral artery was cannulated (24 G, Venula Catheter, Top, Tokyo) to monitor the blood pressure and to draw blood samples. Lactated Ringer's solution containing a muscle relaxant (pancuronium bromide, 0.02 mg/ml) and pentobarbital sodium (0.5 mg/ml) was infused continuously at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$ through a femoral vein cannula (24 G, Venula Catheter, Top, Tokyo). The heart rate (HR) was recorded from lead II of the electrocardiogram. The rats were connected to a pressure-controlled ventilator (Servo 900B, Siemens-Elema, Solna, Sweden) delivering 100% oxygen at a frequency of 30 breaths/min. with an inspiratory: expiratory ratio of 1 : 1. The animals then were rested for at least 15 minutes to allow for hemodynamic parameters, followed by baseline readings of HR and systolic arterial pressure (SAP).

Experimental protocols

After baseline measurements, the animals were allocated randomly to one of the following four groups ($n=8$ per group).

Endotoxin group

Endotoxin shock was induced by a bolus injection of endotoxin (15 mg/kg), using

lipopolysaccharide prepared from *Escherichia coli* (0111:B4; Difco Laboratories, Detroit, MI, USA) as endotoxin. After the injection of endotoxin, 0.9% saline was infused continuously ($1\ ml \cdot kg^{-1} \cdot h^{-1}$).

Control group

A bolus injection of 0.9% saline (1.0 ml/kg) was followed by an infusion of 0.9% saline. Animals in this group were not exposed to endotoxin.

Ketamine group

After initiation of the infusion of ketamine ($10\ mg \cdot kg^{-1} \cdot h^{-1}$), a bolus of endotoxin (15 mg/kg) was given.

Propofol group

After initiation of the infusion of propofol ($10\ mg \cdot kg^{-1} \cdot h^{-1}$), a bolus of endotoxin (15 mg/kg) was given.

Rectal temperature was maintained between 36 and 38°C using a heating pad. Arterial blood samples (1.5 ml) were drawn for the measurement of plasma cytokine concentrations at 2, 4, and 5 hours after the endotoxin or saline injection.

Sample analysis

Blood used for determination of cytokine concentrations was centrifuged for 10 minutes at $3000 \times g$ at 4°C. Plasma was decanted and stored at −70°C until analysis. Cytokine concentrations (TNF-α, IL-6, and IL-10) were measured using enzyme-linked immunosorbent assays (BioSource, Camarillo, CA, USA). The lower limits of detection for TNF-α, IL-6, and IL-10 were 4.5 pg/ml, 7.0 pg/ml, and 6.5 pg/ml, respectively.

Statistical analysis

Data are presented as the mean±S.D. Differences between groups at baseline were analyzed by Student unpaired *t*-test and Mann-Whitney's U-test. Hemodynamic and cytokine changes during the study were analyzed using two-way analysis of variance with repeated measures followed by *post hoc* test (Bonferroni's method). Statistical significance was defined at $p < 0.05$. Statistical analyses were performed using the StatView application (version 5.0, Macintosh; Abacus Concepts, Berkeley, CA, USA).

RESULTS

Plasma cytokine concentrations

All baseline values were similar in the four groups (Fig. 1). Endotoxin injection increased the TNF-α concentration in the endotoxin, ketamine, and propofol groups, but the concentration remained significantly higher in the endotoxin group than in the other two groups at 2 hours after injection. There was no significant difference between ketamine and propofol group after injection. Plasma concentrations of IL-6 became elevated after endotoxin injection. Plasma concentrations of IL-6 at 5 hours after injection in endotoxin, control, ketamine, and propofol groups were 3415±645 pg/ml, 125±58 pg/ml, 456±235 pg/ml, and 1578±345 pg/ml, respectively. Ketamine and propofol groups showed significantly lower concentrations than did the endotoxin group at 5 hours after injection. The IL-6 concentration of ketamine group was lower than that of propofol group at 5 hours after injection. Plasma concentrations of IL-10 at 5 hours after injection in endotoxin, control, ketamine, and propofol groups were 688±86 pg/ml, 95±45 pg/ml, 248±82 pg/ml, and 408±56 pg/ml, respectively. The concentrations were significantly lower in the ketamine and propofol groups than those in the endotoxin group at 5 hours after injection. The IL-10 concentration of ketamine group was lower than that of propofol group at 5 hours after injection.

The ratios of IL-6 to IL-10 concentrations in the control and ketamine groups were stable after saline or endotoxin, but the ratios of IL-6 to IL-10 in the endotoxin and propofol groups

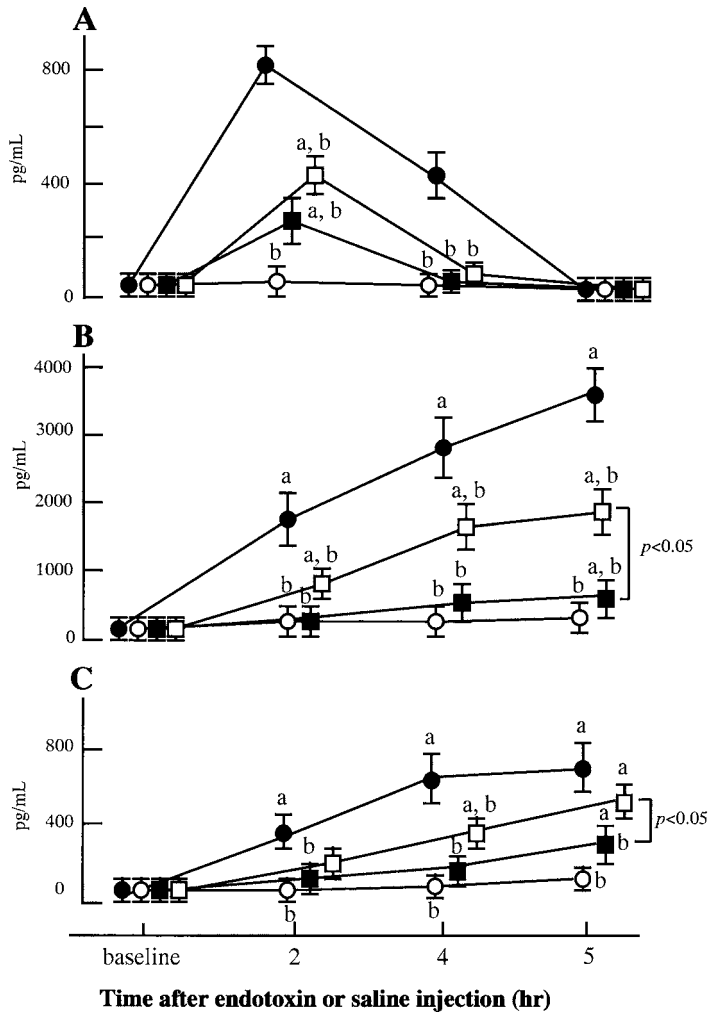

Fig. 1. Changes of plasma cytokine concentrations at baseline and after injection of endotoxin or saline (mean±S.D.).
A: Tumor necrosis factor (TNF)-α; B: interleukin (IL)-6; C: IL-10.
Closed circles, endotoxin group; open circles, control group; closed squares, ketamine group; open squares, propofol group.
[a]$p<0.05$ vs. baseline within group. [b]$p<0.05$ vs. endotoxin group.

increased significantly (Fig. 2). The ratio of IL-6 to IL-10 concentrations in ketamine group was significantly lower than that of propofol group at 5 hours after endotoxin injection (ketamine group; 1.8±0.9 and propofol group; 3.9±1.2).

Hemodynamics

No significant differences were noted in baseline HR or SAP between groups (Fig. 3). Endotoxin injection decreased SAP in the endotoxin and propofol groups, but not in the ketamine group.

DISCUSSION

In the present experiments, injection of endotoxin alone increased pro-inflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokine (IL-10), and the ratio of IL-6 to IL-10. Ketamine administration inhibited the cytokine response and attenuated the increase in the ratio of IL-6 to IL-10. However, propofol administration increased the ratio of IL-6 to IL-10, but nevertheless attenuated the

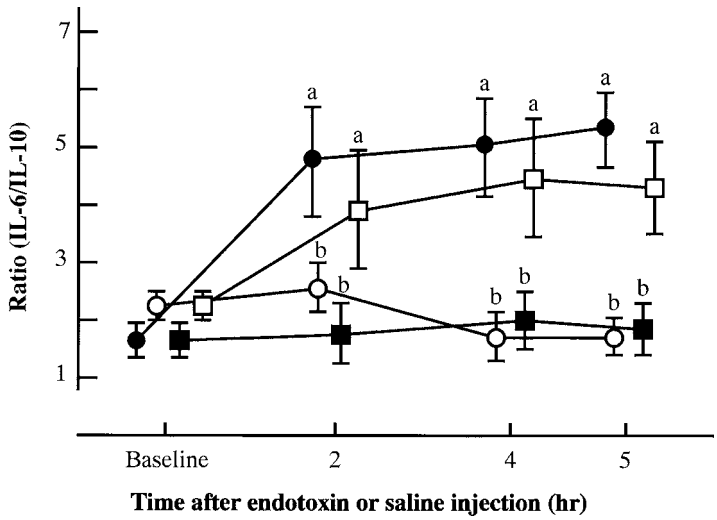

Fig. 2. Changes of the ratios of IL-6 to IL-10 concentrations at baseline and after injection of endotoxin or saline (mean±S.D.).
Closed circles, endotoxin group; open circles, control group; closed squares, ketamine group; open squares, propofol group.
[a]$p<0.05$ vs. baseline within group. [b]$p<0.05$ vs. endotoxin group.

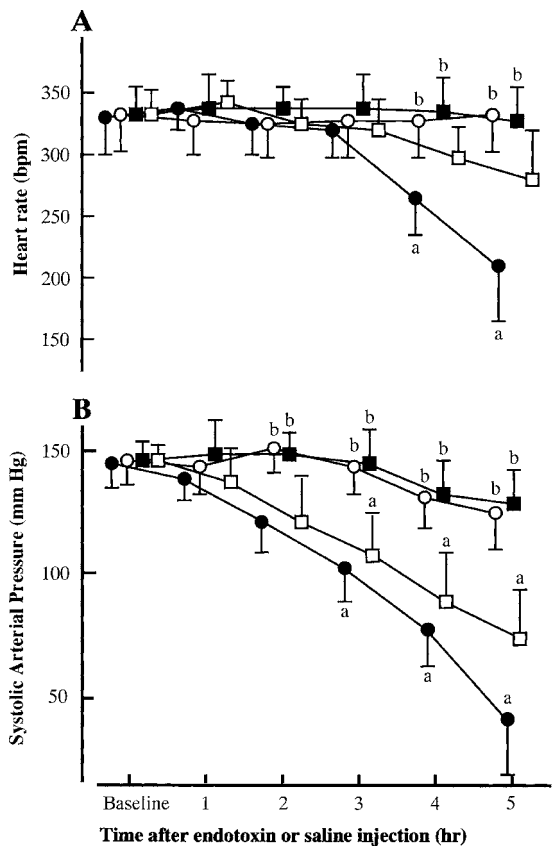

Fig. 3. A: The heart rate, B: and systolic arterial pressure at baseline and after endotoxin or saline (mean±S.D.).
Closed circles, endotoxin group; open circles, control group; closed squares, ketamine group; open squares, propofol group.
[a]$p<0.05$ vs. baseline within group. [b]$p<0.05$ vs. endotoxin group.

cytokine response. Thus, ketamine attenuated the increase in the ratio of IL-6 to IL-10 in endotoxin-exposed rats, representing the most important findings of the current study.

Critically ill patients with sepsis suffer a high degree of stress due to pain, anxiety and organ-specific response by sepsis. An important objective in the management of these patients is to achieve an adequate level of sedation. Although the need for adequate sedation in septic patients is generally accepted, there is no consensus regarding which drugs should be used. Our findings suggest that ketamine may be more effective on preventing inflammatory cytokine responses than propofol for the sedation of septic patients.

Previous reports showed that the increase of plasma IL-6 concentration correlates with the severity of septic patients and that the release of IL-6 from leukocytes can produce hypotension, metabolic acidosis, and tissue damage that may lead to organ dysfunction (Pinsky et al. 1993; Moscovitz et al. 1994). Several investigators reported that IL-10 was induced by TNF-α and IL-6 and diminished the cytokine response in vitro and in vivo (Fiorentino 1991; Gerard et al. 1993). The relationship of pro-inflammatory and anti-inflammatory cytokines is very important in septic patients. The increase in the ratio of IL-6 to IL-10 is thought to signify that the pro-inflammatory response is stronger than the anti-inflammatory response. Our previous reports showed that the increase in the ratio of IL-6 to IL-10 and the outcome correlated with the ratio in SIRS patients (Taniguchi et al. 1999a), and that the ratio of IL-6 to IL-10 correlated with the severity of injury after trauma (Taniguchi et al. 1999b). Moreover, several studies indicated that the relationship between pro-inflammatory and anti-inflammatory cytokines influences the severity of sepsis (Walley et al. 1996; Gogos et al. 2000; Rodringuez-Gaspar et al. 2001). The present study showed that injection of endotoxin alone increased the ratio of IL-6 to IL-10 and caused hypotension in rats.

Of particular interest is the relationship between pro-inflammatory cytokine and anti-inflammatory cytokine under anesthesia. Salo et al. (1997) evaluated the relationship between pro-inflammatory cytokine and anti-inflammatory cytokine with propofol in vitro, and showed that propofol increased the ratio of inflammatory cytokine (interferon-γ) to anti-inflammatory cytokine (IL-4). However, there are few reports on the relationship between pro-inflammatory cytokine and anti-inflammatory cytokine response following ketamine or propofol administration during endotoxemia in vivo. The present study showed that ketamine administration did not increase the ratio of IL-6 to IL-10 during endotoxemia, though propofol administration increased the ratio of IL-6 to IL-10. Our finding suggests that judging from the stable cytokine balance ketamine administration was superior to that of propofol.

Previous reports have described how ketamine and propofol have inhibitory effects on cytokine response in sepsis. Kawasaki et al. (1999) demonstrated that ketamine suppresses endotoxin-induced TNF-α, IL-6 and IL-8 production in human whole blood in vitro. Galley et al. (1998) showed that propofol suppressed IL-8 secretion from human leukocytes in vitro. Our previous studies showed that ketamine and propofol attenuated inflammatory cytokine responses (TNF-α and IL-6) during endotoxemia in vivo (Taniguchi et al. 2000, 2001). The present study also showed that both ketamine and propofol attenuated an increase in TNF-α and IL-6 during endotoxemia in vivo.

We evaluated TNF-α and IL-6 as pro-inflammatory cytokine, and IL-10 as anti-inflammatory cytokine. The present study showed that IL-6 and IL-10 increased continuously after endotoxin injection, but TNF-α increased until 2 hours after endotoxin injection and decreased 2 hours after endotoxin injection. Thus, in order to evaluate the relationship between pro-inflammatory cytokine and anti-

inflammatory cytokine, we used IL-6 and IL-10.

Two important questions, which remain unanswered, are whether a dose-response relationship exists between ketamine and propofol and cytokine responses, and whether a relationship exists between ketamine and propofol administration and outcome. The present study used the dose of $10\ mg \cdot kg^{-1} \cdot h^{-1}$ of ketamine and propofol, because our previous studies demonstrated the anti-inflammatory effects of the same dose of ketamine and propofol (Taniguchi et al. 2000, 2001). In vitro, several studies showed that ketamine and propofol had dose-dependently anti-inflammatory effects (Mikawa et al. 1998; Kawasaki et al. 1999). Further studies need to focus on these questions.

In summary, the current study showed that ketamine and propofol administration attenuated cytokine response while ketamine attenuated the increase in the ratio of IL-6 to IL-10. However, propofol increased this ratio in rats receiving a single intravenous bolus of endotoxin. While the mechanisms responsible for these inhibitory effects require further investigation, our results suggest that judicious use of ketamine as an anesthetic agent may offer certain advantages in the management of patients with endotoxemia.

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