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Propofol ameliorates liver dysfunction and inhibits aortic superoxide level in conscious rats with endotoxic shock

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Abstract

Propofol, widely used as a sedative agent, is known to exert antioxidant and anti-inflammatory effects in vitro. We studied the effects of propofol on hemodynamics and the function of several organs in conscious rats with endotoxemia. Intravenous injection of rats with endotoxin (lipopolysaccharide) caused hypotension, vascular hyporeactivity and tachycardia as well as significant lung, liver and kidney damage. Hepatocellular damage caused by lipopolysaccharide for 6 h was significantly attenuated in the lipopolysaccharide + propofol group. Aortic superoxide anion ($O_2^{\cdot -}$) production, but not plasma nitric oxide (NO) and tumor necrosis factor-α (TNF-α) level, was also suppressed by propofol in lipopolysaccharide-injected rats. Light microscopy showed that propofol attenuated the marked infiltration of neutrophils in liver tissues from lipopolysaccharide-injected rats. Moreover, the survival rate of the lipopolysaccharide + propofol group at 16 h was significantly increased when compared with that of the lipopolysaccharide group (53% vs. 12%). These results suggest that inhibition of aortic $O_2^{\cdot -}$ production and amelioration of liver dysfunction contribute to the beneficial effect of propofol in conscious rats with endotoxic shock.

Keywords: Septic shock; Propofol; Organ dysfunction; Nitric oxide (NO); Free radical

1. Introduction

Sepsis-induced multiple organ dysfunction syndrome continues to result in mortality in the vicinity of 40% even with the advent of recent medical technology and more focused therapy (Centers for Disease Control, 1990). Multiple organ dysfunction syndrome has been viewed as the culmination of severe or repetitive tissue damage owing to inappropriate host regulation of the acute inflammatory response induced by many mediators, including endotoxin (e.g. lipopolysaccharide) at a genetic, cellular or organ-specific level. It has been shown that endotoxin stimulates an excessive and systemic release and activation of endogenous inflammatory mediators, mainly produced by mononuclear phagocytes (Morrison and Ulevitch, 1978). Among these endogenous mediators, tumor necrosis factor-α (TNF-α) could directly cause the end-organ dysfunction which occurs in severe sepsis (Beutler and Cerami, 1989) and indirectly activate the inducible isoform of nitric oxide synthase (iNOS), leading to excessive generation of nitric oxide (NO) (Moncada et al., 1991). Overproduction of NO, a potent endogenous vasodilator, has been linked to sepsis-induced hypotension and vascular hyporesponsiveness (Moncada et al., 1991; Thiemermann, 1994). In addition, endotoxin may activate neutrophils to produce a number of short-lived reactive oxygen species, including superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide and hydroxyl radicals, causing inappropriate tissue damage, for example, in the liver (Sugino et al., 1989; Bautista et al., 1990). In particular, $O_2^{\cdot -}$ can react avidly with NO to form peroxynitrite,

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eventually resulting in further damage to nucleic acids and in conformational changes in enzymes (Beckman et al., 1990). Therefore, $O_2^{\cdot -}$ may also play a key role in the pathogenesis of multiple organ dysfunction syndrome during septic shock.

Propofol, 2,6-disopropylphenol, is used as a sedative agent in intensive care units, to attenuate anxiety and irritation. In addition, propofol is chemically similar to phenol-based free radical scavengers, such as butylated hydroxytoluene and the endogenous antioxidant vitamin E (Halliwell and Gutteridge, 1999). Each molecule of propofol, but not intralipid, can scavenge two radicals in a manner similar to vitamin E, with the phenoxyl radical reacting with a lipid peroxyl radical to form a stable non-radical adduct (Murphy et al., 1992). Propofol accumulates in biomembranes far more readily than vitamin E, so that it may be able to boost the antioxidant defense of tissues, and/or specifically lipophilic membrane environments, far more rapidly.

Recent studies have shown that propofol improves mortality in anesthetized rats with endotoxin shock (Taniguchi et al., 2002). Based on the in vitro and in vivo results reported in the literature, therefore, we hypothesized that propofol has protective effects against the circulatory failure and the multiple organ dysfunction syndrome that occur in endotoxic shock in vivo. However, there are no studies evaluating the beneficial effect of propofol in an endotoxin shock using conscious animal model. Thus, in this study, we examined the beneficial effects of propofol in a conscious rat endotoxic shock model induced by *Escherichia coli* lipopolysaccharide to exclude divergent effects of other anesthetics on the results (Schaefer et al., 1987; Imai et al., 1998). In addition, we also attempted to characterize the possible mechanism by which propofol ameliorates the mortality caused by endotoxin.

2. Materials and methods

2.1. Animal model

Male Wistar rats weighing 250–350 g were used in this study. All experiments were approved by the local Institutional Review Board according to the Helsinki recommendations. The experiments were performed in adherence to the National Institutes of Health Guidelines for the treatment of animals. All rats were intraperitoneally anesthetized with sodium pentobarbital (50 mg/kg). Polyethylene catheters were placed in the right internal jugular vein for the administration of drugs and in the left common carotid artery for the measurement of hemodynamics and blood withdrawal. Catheter insertion was performed by cut-down technique and the distal end of the catheter was tunneled under the skin and externalized through an incision in the back of the neck. After the surgery was completed, rats were fasted overnight (about 12 h) during recovery but were allowed water ad libitum.

2.2. Experimental protocols

The experiments were then performed on pairs of rats. The arterial catheter was connected to a pressure transducer (P23ID, Statham, Oxnard, CA, USA) for the measurement of phasic arterial pressure, mean arterial pressure and heart rate, which were recorded continuously on a multichannel recorder (MacLab/4e, AD Instruments, Castle Hill, Australia). After 15 min of hemodynamic stabilization, baseline recording of hemodynamic parameters and blood withdrawal were performed. The animals were randomly allocated to one of four groups and injected intravenously as follows: (1) control, saline 1 ml/kg bolus followed by infusion at 1 ml/kg/h; (2) propofol, propofol 10 mg/kg for 10 min followed by infusion at 10 mg/kg/h; (3) lipopolysaccharide, *E. coli* lipopolysaccharide 10 mg/kg, bolus injection over 10 min; and (4) lipopolysaccharide + propofol, *E. coli* lipopolysaccharide 10 mg/kg and then propofol 10 mg/kg for 10 min followed by infusion at 10 mg/kg/h. These treatments were given over a period of 6 h. The dose of propofol used in this study was that used in the study of Taniguchi et al. (2000).

2.3. TNF-α assay

TNF-α concentrations in plasma were analyzed with enzyme-linked immunoadsorbent assay (ELISA) kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Briefly, plasma samples (50 μl) stored at −20 °C were thawed and diluted up to 1:2 with provided diluents to stay within the linear range of the assay. The samples were then incubated in microwells coated with mouse anti-rat TNF-α for 2 h at room temperature. After repeated the aspiration and washing, 100 μl of the biotinylated goat anti-rat TNF-α was added to each well and incubated for 2 h. After washing, streptavidin conjugated to horseradish peroxidase was added to the wells and incubated for 20 min. Following a wash to remove unbound antibody-enzyme reagent, substrate solution was added. The color intensity of the enzymatic indicator reaction was measured photometrically at 450 nm in an ELISA plate reader.

2.4. Quantification of organ function and injury

The pressor responses to noradrenaline (1 μg/kg, i.v.) were measured at 10 min before and at every hour after vehicle or lipopolysaccharide injection. Arterial blood samples (0.5 ml) were drawn prior to (i.e. at time 0) and at 1, 2, 3 and 6 h after vehicle or lipopolysaccharide injection. Blood (10 μl) was immediately analyzed for glucose (ONE TOUCH test strips, Lifescan, Milpitas, CA, USA) and the remaining blood was immediately centrifuged for 3 min at 16,000 × *g*. The plasma (70 μl) was taken to measure biochemical indicators of multiple organ dysfunction syndrome: (1) liver injury, glutamine-oxaloacetic transaminase,

glutamine-pyruvic transaminase and albumin; (2) renal injury, blood urine nitrogen and creatinine; and (3) cytotoxicity, lactate dehydrogenase (Fuji DRI-CHEM 3030, Fuji Photo Film, Tokyo, Japan). The remaining plasma was stored at −20 °C for subsequent measurement of TNF-α and nitrate/nitrite. Furthermore, extra arterial blood samples (0.2 ml) were drawn at time 0 and 6 h for the measurement of pH, $PaCO_2$, PaO_2, base excess and hematocrit (AVL OPTI Critical Care Analyzer, AVL Scientific, Roswell, GA, USA). Any blood withdrawn was immediately replaced by an equal volume of saline.

The rats were euthanized at the end of each experiment by intravenous administration of an overdose of pentobarbital. The thoracic aortas, lungs, livers and kidneys were rapidly isolated and removed for further studies.

2.5. Histological examination

Parts of the lung, liver and kidney sections were fixed in 10% phosphate-buffered formaldehyde for 3–5 days, and embedded in paraffin. Each paraffin block was processed into 7-μm-thick slices that were stained with hematoxylin and eosin. The infiltration of neutrophils in tissues quantitated as an index on severity of tissue injury was scored 0 (minimal) to 4 (maximal) by a pathologist in a blinded fashion.

2.6. Tissue homogenization and protein quantification

The frozen lungs and livers were thawed and homogenized in lysis buffer (pH 7.8; containing 50 mM Tris–HCl, 1 mM EDTA, 3 mM DL-dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 0.1 mM Nα-*p*-tosyl-L-lysine chloromethyl ketone hydrochloride, 20 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 2 μg/ml leupeptin, 2 μg/ml pepstatin, 2 μg/ml aprotinin) and centrifuged for 25 min at 18,000 × *g* (4 °C). Protein in the supernatant fraction was quantitated with Bio-Rad protein assay reagent (Bio-Rad Laboratories, CA, USA). Aliquots of tissue homogenates were used for NO assay and Western blot analysis.

2.7. Determination of plasma and tissue nitrate/nitrite levels

Nitrate and nitrite are the primary oxidation products of NO and therefore the nitrate/nitrite level in plasma and tissue homogenates can be used as an indicator of NO formation. The sample was analyzed for nitrate/nitrite as previously described (Wu et al., 2001). Briefly, 30 μl of plasma stored at −20 °C and tissue homogenates stored at −80 °C were thawed and de-proteinized by incubation with 95% ethanol (4 °C) for 30 min. The samples were subsequently centrifuged at 16,000 × *g* for 5 min. The supernatant fraction (6 μl) was added to a reducing agent (0.8% vanadium (III) chloride in 1 N HCl) in the purge vessel. Nitrate in the samples was reduced to NO, which was stripped by using helium pure gas. The NO was then drawn into the Nitric Oxide Analyzer (Sievers 280 NOA, Sievers Instruments, Boulder, CO, USA). Nitrate concentrations were calculated by comparison with standard solutions of sodium nitrate as previously described (Wu et al., 2001).

2.8. Western blot analysis of iNOS protein expression

Western blot analysis was performed by separating proteins (200 μg) by 7.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The products were then transferred to a polyvinylidene difluoride membrane by electroblotting overnight at 4 °C. The membranes were blocked with 5% non-fat milk in Tris buffer solution containing 0.1% Tween-20 for 1 h and then incubated for 2 h with the monoclonal anti-mouse iNOS antibody (1:1000 dilution; BD Transduction Laboratories, Lexington, KY, USA). The immunocomplexes were detected with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G secondary antibody (1:7500) for 1.5 h and then developed with the enhanced chemiluminescence reagents (Amersham International, Buckinghamshire, UK) followed by exposure to radiograph film.

2.9. Superoxide anion determination

The thoracic aortas, lungs, and livers were assessed for $O_2^{\cdot-}$ as previously described (Wu et al., 2001). Briefly, the tissues were isolated and removed rapidly after euthanasia. The thoracic aorta was carefully trimmed of extravascular tissues and then cut into rings of 5-mm width. The lung and liver (5 × 5 mm) were taken and cut into pieces. Thereafter, the tissues were incubated with warmed (37 °C) Krebs–HEPES buffer which aerated with 95% O_2/5% CO_2 for 30 min and then transferred to scintillation plates. These scintillation plates containing Krebs–Hepes buffer with 1.25 mM lucigenin (final volume of 250 μl) were placed into a microplate luminometer (Microlumat Plus LB96V, Berthold, Germany). Counts were obtained at 900-s intervals at room temperature. All tissues were then dried in a 90 °C oven for 24 h. The results are expressed as reactive luminescence units (RLU) per 15 min per milligram dry weight (i.e., RLU/15 min/mg dry weight).

2.10. Determination of propofol in blood

The blood was obtained from propofol-treated rats at 6 h after saline or lipopolysaccharide injection and stored at −20 °C until analysis. The assays were performed with the high-performance liquid chromatography (HPLC)-fluorescence method described by Knibbe et al. (1998). Briefly, the frozen plasma (50 μl) was thawed and mixed with 250 μl distilled water. Twenty microliters of methanol was then added to the samples, which were vortexed for 30 s. Five-hundred microliters of internal standard solution (1.0 μg/ml thymol in acetonitrile) was added and the samples were mixed for 30 s. The mixtures were centrifuged for 5 min at

1900 × *g* and 50 μl of the supernatant was injected onto a HPLC column (Keystone Betasil C18 250 × 4.6 mm) for analysis. The fluorescence spectrophotometer had excitation and emission wavelengths of 276 and 310 nm, respectively. The standard curves for propofol concentrations were linear within a range of 0.2–40 μg/ml ($r^2 = 0.99$, $P < 0.01$).

2.11. Survival studies

In rats treated as described above, the lipopolysaccharide-injected groups were studied to determine the effect of propofol on survival. Lipopolysaccharide (10 mg/kg i.v.) was injected in rats of two groups: one was lipopolysaccharide plus saline (1 ml/kg + 1 ml/kg/h) and the other was lipopolysaccharide plus propofol (10 mg/kg + 10 mg/kg/h). The animals were checked hourly for 16 h after lipopolysaccharide administration.

2.12. Chemicals

Bacterial lipopolysaccharide (*E. coli* serotype 0127:B8) and noradrenaline bitartrate were obtained from Sigma, St. Louis, MO. Propofol (Diprivan 1%) was purchased from Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK. Sodium pentobarbital (Somnotol) was obtained from MTC Pharmaceuticals, Cambridge, Ontario, Canada. Vanadium (III) chloride was obtained from Aldrich Chemical (Milwaukee, WI, USA). All solutions were made in saline or distilled water, except vanadium (III) chloride, which was dissolved in 1 N HCl.

2.13. Statistical analysis

The data are expressed as means ± S.E.M. Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by the Bonferroni test as post hoc test. Superoxide products and iNOS expression were analyzed by one-way ANOVA followed by the Bonferroni test. The score for tissue infiltration of neutrophils was compared by the Kruskal–Wallis rank test followed by the Mann–Whitney *U*-test. Survival data were calculated for each time period and compared by a Chi-square test. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of propofol on the circulatory failure caused by endotoxemia

Baseline mean arterial pressure in all groups of animals ranged from 125.2 ± 5.5 to 139.4 ± 4.5 mm Hg and was not significantly different between groups. In the control group, there was no significant change in mean arterial pressure during the experimental period. Infusion of lipopolysaccharide produced a rapid fall in mean arterial pressure from 134.2 ± 4.1 mm Hg (baseline, before infusion of lipopolysaccharide) to 98.6 ± 2.4 mm Hg at 1 h. Thereafter, mean arterial pressure returned to 128.9 ± 2.7 mm Hg at 3 h after lipopolysaccharide injection and subsequently decreased to significantly below control values at 4–6 h (Fig. 1). Treatment of lipopolysaccharide-injected rats with propofol did not affect the early hypotension or the delayed hypotension elicited by lipopolysaccharide.

Baseline heart rate in all groups of animals was not significantly different among groups. In the control group, there was no significant change in heart rate during the experimental period, whereas endotoxemia for 6 h was associated with a significant increase in heart rate (400 ± 15 beats/min at 0 h vs. 486 ± 14 beats/min at 6 h, $n = 11$, $P < 0.05$). Treatment of lipopolysaccharide-injected rats with propofol had no significant effect on tachycardia (at 6 h, 478 ± 12 beats/min, $n = 10$, in the lipopolysaccharide + propofol group vs. 486 ± 14 beats/min in the lipopolysaccharide group, $P > 0.05$). It was noted that infusion of control animals with propofol alone had no significant effect on mean arterial pressure or heart rate during the experimental period.

The mean baseline pressor response to noradrenaline (1 μg/kg, i.v.) was not significantly different between any of the experimental groups studied. In the control group, the injection of saline had no significant effect on the noradrenaline-induced pressor responses during the 6-h experimental period. In contrast, the injection of lipopolysaccharide resulted in a substantial, time-dependent attenuation of the pressor responses elicited by noradrenaline (142 ± 3 mm Hg min at 0 h vs. 106 ± 6 mm Hg min at 6 h, $n = 11$, $P < 0.05$). Treatment of lipopolysaccharide-injected rats

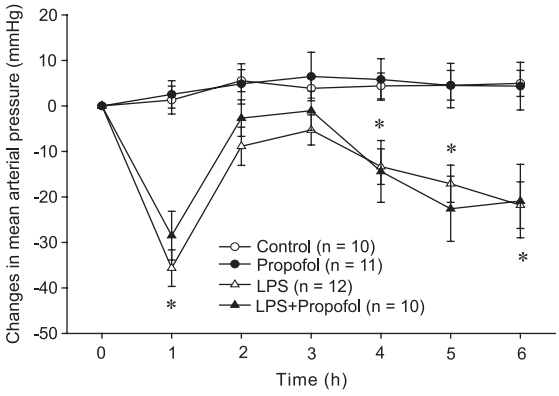

Fig. 1. Effects of propofol on mean arterial pressure in rats treated with endotoxin. Depicted are the changes in mean arterial pressure during the experimental period in different groups of animals which received injections of saline (Control), propofol (Propofol, 10 mg/kg + 10 mg/kg/h), *E. coli* lipopolysaccharide (LPS, 10 mg/kg), or *E. coli* lipopolysaccharide followed by propofol (LPS + Propofol). Data are expressed as means ± S.E.M. of number (*n*) of observations. *$P < 0.05$ represents significant differences between LPS and Control groups at the same time point.

Table 1
Effects of propofol on changes in plasma TNF-α and NO as well as blood glucose levels in rats treated with endotoxin

Variable	Group (*n*)	0 h	1 h	2 h	3 h	6 h
TNF-α (pg/ml)	Control (9)	109 ± 12	1295 ± 967	195 ± 35	195 ± 35	125 ± 16
	Propofol (7)	117 ± 23	2112 ± 284	439 ± 106	303 ± 54	147 ± 19
	LPS (7)	109 ± 16	4010 ± 829[a]	2013 ± 614[a]	716 ± 95[a]	301 ± 110
	LPS + Propofol (8)	118 ± 16	4959 ± 1262	1355 ± 255	711 ± 131	325 ± 41
NO (μM)	Control (7)	25 ± 5	–	–	29 ± 8	53 ± 17
	Propofol (7)	39 ± 7	–	–	63 ± 13	140 ± 33
	LPS (15)	61 ± 18	–	–	358 ± 32[a]	1402 ± 91[a]
	LPS + Propofol (13)	60 ± 24	–	–	411 ± 39	1725 ± 229
Blood glucose (mg/dl)	Control (10)	98 ± 4	89 ± 2	88 ± 2	90 ± 3	88 ± 3
	Propofol (9)	96 ± 4	86 ± 4	78 ± 2	87 ± 2	84 ± 4
	LPS (10)	100 ± 3	159 ± 10[a]	92 ± 4	82 ± 4	86 ± 8
	LPS + Propofol (9)	97 ± 3	134 ± 9	87 ± 3	88 ± 4	94 ± 4

Control (saline), Propofol (propofol, 10 mg/kg + 10 mg/kg/h), LPS (*E. coli* lipopolysaccharide, 10 mg/kg), LPS + Propofol (*E. coli* lipopolysaccharide followed by propofol), tumor necrosis factor-α (TNF-α), nitric oxide (NO).
Data are expressed as means ± S.E.M. of number (*n*) of observations.
It is noted that the plasma NO levels at 1 and 2 h after LPS were not determined.
[a] $P < 0.05$ represents significant differences between LPS and Control groups at the same time point.

with propofol did not significantly attenuate the vascular hyporeactivity to noradrenaline during the experimental period (at 6 h, 107 ± 5 mm Hg min, $n = 10$, in the lipopolysaccharide + propofol group vs. 106 ± 6 mm Hg min in the lipopolysaccharide group, $P > 0.05$). Infusion of normal control animals with propofol alone had no significant effect on the pressor responses to noradrenaline in vivo.

3.2. Effects of propofol on blood glucose during endotoxemia

Baseline blood glucose level was not significantly different among groups. In the control group, there was no significant change in blood glucose during the experimental period. Infusion of lipopolysaccharide produced a significant increase in blood glucose, which reached a peak at 1 h and subsequently decreased to a pre-lipopolysaccharide level after 2 h (Table 1). Treatment of lipopolysaccharide-injected rats with propofol slightly, but not significantly, attenuated the early hyperglycemia. However, infusion of control animals with propofol alone had no significant effect on the blood glucose level during the experimental period.

3.3. Effects of propofol on plasma TNF-α and nitrate/nitrite during endotoxemia

Baseline plasma TNF-α and nitrate/nitrite levels were not significantly different among groups. In the control group, there were no significant changes in TNF-α and nitrate/nitrite levels during the experimental period (Table 1). Infusion of lipopolysaccharide produced a significant increase in TNF-α, which reached a peak at 1 h after lipopolysaccharide injection and subsequently decreased slowly (Table 1). In addition, lipopolysaccharide caused a time-dependent elevation in the plasma nitrate/nitrite level, which reached a 23-fold increase at 6 h (Table 1). Treatment of lipopolysaccharide-injected rats with propofol had no significant effects on the increase in plasma TNF-α and nitrate/nitrite levels. In the control rats treated with propofol alone, there was no significant change in the plasma nitrate/nitrite level. Interestingly, infusion of control animals with propofol

Table 2
Effects of propofol on changes in arterial blood gas levels and hematocrit in rats treated with endotoxin

	pH		PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		Base excess (mmol/l)		Hematocrit (%)	
	0 h	6 h	0 h	6 h	0 h	6 h	0 h	6 h	0 h	6 h
Control ($n = 6$)	7.57 ± 0.03	7.54 ± 0.01	91.2 ± 4.8	93.2 ± 3.7	34.0 ± 2.4	35.7 ± 1.3	7.7 ± 0.8	7.1 ± 0.6	43.8 ± 1.0	38.7 ± 1.0
Propofol ($n = 7$)	7.54 ± 0.01	7.56 ± 0.01	90.8 ± 3.0	96.0 ± 2.0	34.2 ± 2.4	31.8 ± 0.9	6.1 ± 0.7	6.1 ± 0.8	43.8 ± 0.8	37.2 ± 0.8
LPS ($n = 6$)	7.54 ± 0.01	7.50 ± 0.07	93.4 ± 4.6	109.4 ± 13.2	32.6 ± 1.6	23.4 ± 3.9[a]	5.1 ± 0.5	−4.3 ± 2.4[a]	43.9 ± 1.0	40.1 ± 1.1
LPS + Propofol ($n = 6$)	7.56 ± 0.02	7.49 ± 0.02	93.5 ± 1.5	111.2 ± 12.5	32.8 ± 2.5	26.5 ± 2.4	6.3 ± 1.0	−2.0 ± 2.3	41.5 ± 2.1	36.7 ± 2.1

Control (saline), Propofol (propofol, 10 mg/kg + 10 mg/kg/h), LPS (*E. coli* lipopolysaccharide, 10 mg/kg), LPS + Propofol (*E. coli* lipopolysaccharide followed by propofol).
Data are expressed as means ± S.E.M. of number (*n*) of observations.
[a] $P < 0.05$ represents significant differences between LPS and Control groups at the same time point.

alone caused a slight, but not significant, elevation of TNF-α at 1 h.

3.4. Effects of propofol on arterial blood gases during endotoxemia

There were no significant differences in pH, PaO_2, and hematocrit among the groups before endotoxin or saline injection and even between the groups after endotoxin or saline injection. However, infusion of lipopolysaccharide produced a significant decrease in $PaCO_2$ and base excess when compared to levels in the control group (Table 2). Treatment of lipopolysaccharide-injected rats with propofol slightly, but not significantly, attenuated the decrease in $PaCO_2$ and base excess.

3.5. Effects of propofol on liver function during endotoxemia

Glutamine-oxaloacetic transaminase is present in tissues of high metabolic activity, especially in the heart, liver, and skeletal muscle. Glutamine-pyruvic transaminase is primarily present in the liver. These enzymes are released into the circulation following the injury or death of liver cells. Albumin is a protein formed in the liver. Therefore, we used glutamine-oxaloacetic transaminase, glutamine-pyruvic transaminase and albumin levels as indicators of liver function. Baseline levels of glutamine-oxaloacetic transaminase, glutamine-pyruvic transaminase and albumin were not significantly different among groups. In the control group, there were no significant changes in glutamine-oxaloacetic transaminase, glutamine-pyruvic transaminase and albumin levels during the experimental period. Infusion of lipopolysaccharide produced a significant increase in glutamine-oxaloacetic transaminase (Fig. 2A) and glutamine-pyruvic transaminase levels (Fig. 2B), and a significant decrease in albumin level (Fig. 2C). Treatment of lipopolysaccharide-injected rats with propofol significantly attenuated the increase in glutamine-oxaloacetic transaminase and glutamine-pyruvic transaminase levels at 6 h and the decrease in albumin level at 3 h. However, infusion of control animals with propofol alone had no significant effects on these parameters during the experimental period.

3.6. Effects of propofol on renal function during endotoxemia

Urea is carried to the kidneys by the blood to be excreted in the urine. Creatinine is a by-product of energy metabolism and is removed by the kidneys. Measurement of blood urine nitrogen (measuring the nitrogen portion of urea) and creatinine is usually used to diagnose impaired renal function. Baseline levels of blood urine nitrogen and creatinine were not significantly

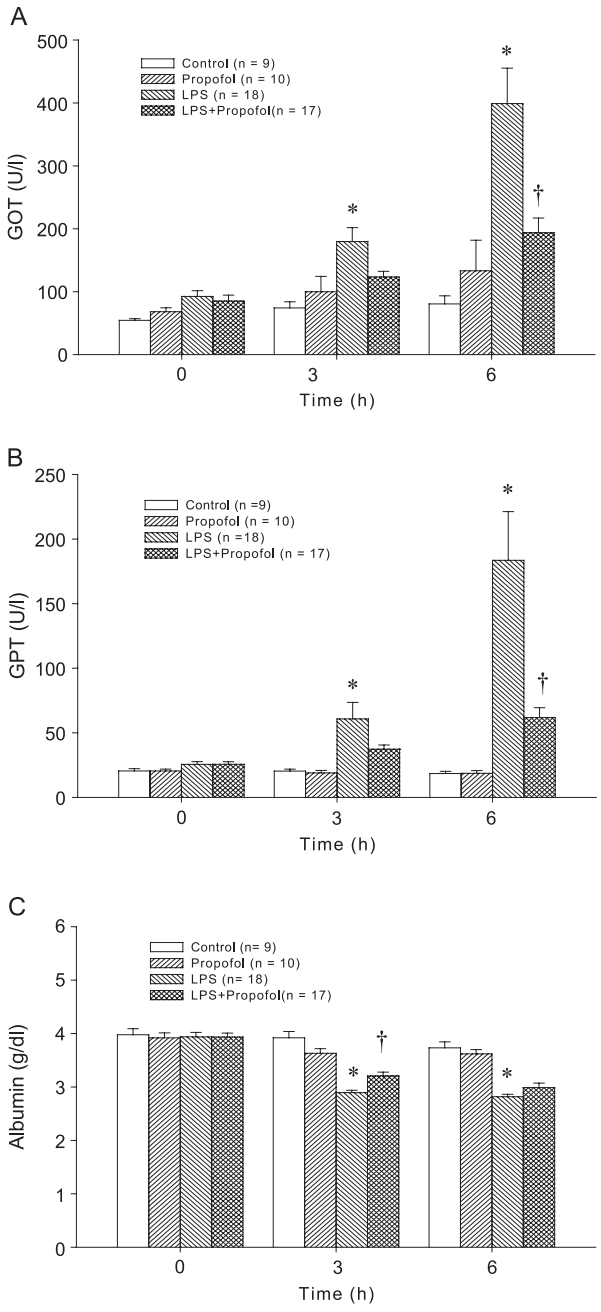

Fig. 2. Effects of propofol on plasma glutamine-oxaloacetatic transaminase (GOT), glutamine-pyruvic transaminase (GPT), and albumin levels in rats treated with endotoxin. Depicted are the changes in plasma GOT (A), GPT (B), and albumin (C) during the experimental period in different groups of animals which received injections of saline (Control), propofol (Propofol, 10 mg/kg + 10 mg/kg/h), *E. coli* lipopolysaccharide (LPS, 10 mg/kg), or *E. coli* lipopolysaccharide followed by propofol (LPS + Propofol). Data are expressed as means ± S.E.M. of number (n) of observations. $^{*}P < 0.05$ represents significant differences between LPS and Control groups at the same time point. $^{\dagger}P < 0.05$ represents significant differences between endotoxemic rats treated with and without propofol.

different between groups. In the control group, there were no significant changes in blood urine nitrogen and creatinine levels during the experimental period. Infusion

of lipopolysaccharide produced a significant increase in blood urine nitrogen (16 ± 1 mg/dl at 0 h vs. 69 ± 3 mg/dl at 6 h, $n = 18$, $P < 0.05$) and creatinine (0.28 ± 0.02 mg/dl at 0 h vs. 0.68 ± 0.07 mg/dl at 6 h, $n = 18$, $P < 0.05$). Treatment of lipopolysaccharide-injected rats with propofol had no significant effect on levels of blood urine nitrogen (at 6 h, 61 ± 4 mg/dl, $n = 16$, in the lipopolysaccharide + propofol group vs. 69 ± 3 mg/dl in the lipopolysaccharide group, $P > 0.05$) and creatinine (at 6 h, 0.56 ± 0.07 mg/dl, $n = 17$, in the lipopolysaccharide + propofol group vs. 0.68 ± 0.07 mg/dl in the lipopolysaccharide group, $P > 0.05$). However, infusion of control animals with propofol alone had no significant effect on blood urine nitrogen and creatinine during the experimental period.

3.7. Effects of propofol on infiltration of polymorphonuclear neutrophils during endotoxemia

In the control group, histopathological sections showed little infiltration of polymorphonuclear neutrophils into the lung and liver (Fig. 3A and D). In contrast, at 6 h after the injection of rats with lipopolysaccharide, there was overt infiltration of polymorphonuclear neutrophils into both the lung and liver (Fig. 3B and E; Table 3). In addition, all of the lipopolysaccharide-treated rats showed marked intersti-

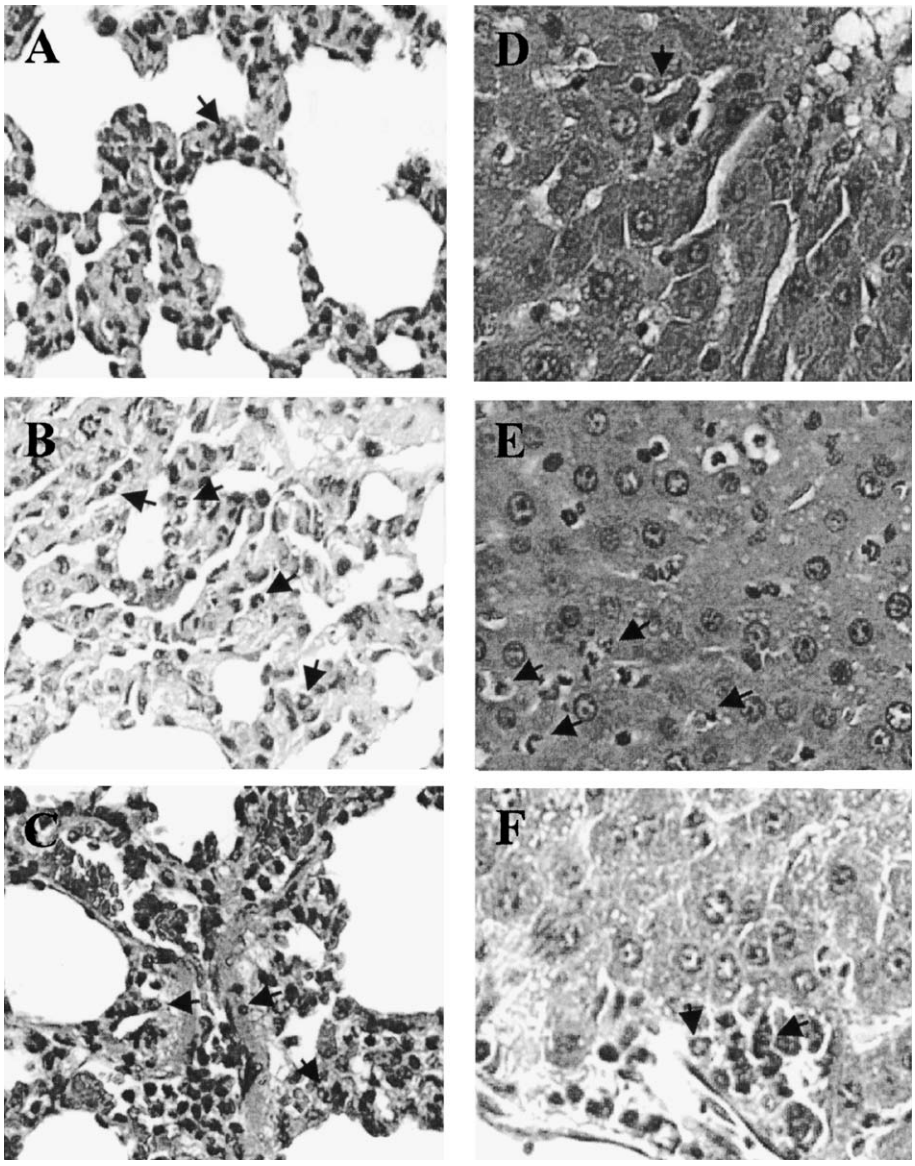

Fig. 3. Histopathological studies. Light microscopy of lung and liver sections from rats in the control group (A and D), in the lipopolysaccharide group (B and E), and lipopolysaccharide + Propofol group (C and F). Arrowheads indicate the infiltration of polymorphonuclear neutrophils. Sections were stained with hematoxylin and eosin stain. Each, 400× (original magnification).

Table 3
Effects of propofol on polymorphonuclear neutrophil infiltration index in lungs, livers and kidneys of rats treated with or without endotoxin

	Lung	Liver	Kidney
Control	1.4 ± 0.3 (n = 8)	1.1 ± 0.3 (n = 8)	0.1 ± 0.1 (n = 9)
Propofol	1.8 ± 0.2 (n = 7)	1.7 ± 0.3 (n = 8)	0.3 ± 0.2 (n = 7)
LPS	2.5 ± 0.2[a] (n = 8)	2.6 ± 0.3[a] (n = 8)	0.5 ± 0.2 (n = 7)
LPS + Propofol	2.2 ± 0.4 (n = 8)	1.5 ± 0.3[b] (n = 7)	0.3 ± 0.2 (n = 8)

Depicted is the neutrophil infiltration index in organs of different groups of animals which received injections of saline (Control), propofol (Propofol, 10 mg/kg + 10 mg/kg/h), *E. coli* lipopolysaccharide (LPS, 10 mg/kg), or *E. coli* lipopolysaccharide followed by propofol (LPS + Propofol). Data are expressed as means ± S.E.M. of number (n) of observations. The neutrophil infiltration index of each organ was scored 0 (minimal) to 4 (maximal).

[a] $P < 0.05$ represents significant differences between LPS and Control groups at the same time point.

[b] $P < 0.05$ represents significant differences between endotoxemic rats treated with and without propofol.

tial edema and/or diffuse congestion of the lung and liver. In lipopolysaccharide-injected rats treated with propofol, the pathological changes were significantly reduced in the liver, but not in the lung (Fig. 3C and F; Table 3). The scores for the infiltration of polymorphonuclear neutrophils into the kidney were not significantly different between the groups (Table 3).

3.8. Effects of propofol on cytotoxicity during endotoxemia

Lactate dehydrogenase is an intracellular enzyme that is widely distributed in the tissues of the body. An increase in the lactate dehydrogenase level usually indicates cell death (i.e. leakage of the enzyme from the cell). Baseline levels of lactate dehydrogenase were not significantly different among groups. In the control group, there was no significant change in lactate dehydrogenase level during the experimental period. Infusion of lipopolysaccharide produced a significant increase in lactate dehydrogenase during the experimental period (Fig. 4). Treatment of lipopolysaccharide-injected rats with propofol significantly attenuated the increase in lactate dehydrogenase level at 1–3 h. Infusion of control animals with propofol alone had no significant effect on the lactate dehydrogenase level during the experimental period.

3.9. Effects of propofol on tissue nitrate/nitrite and iNOS expression during endotoxemia

A detectable level of nitrate/nitrite was measured in the lung and liver of control rats (138 ± 26 μM of lungs, n = 7, and 112 ± 24 μM of livers, n = 7), and this level was not significantly affected by propofol alone (168 ± 30 μM of lungs, n = 6, and 92 ± 13 μM of livers, n = 6). However, a marked increase in the level of nitrate/nitrite was observed in lung and liver homogenates of rats treated with lipopolysaccharide (646 ± 75 μM of lungs, n = 7, and 308 ± 40 μM of livers, n = 7) when compared with that of control rats ($P < 0.05$). Propofol treatment had no significant effect on the increase in the level of nitrate/nitrite in the lung and liver of rats treated with lipopolysaccharide (664 ± 139 μM of lungs, n = 7, and 258 ± 28 μM of livers, n = 7, P>0.05). A marked increase in iNOS expression was also observed in lung and liver homogenates of rats treated with lipopolysaccharide when compared with the expression in control rats. However, propofol treatment had no significant effect on the increased expression of iNOS protein in the lung and liver of rats treated with lipopolysaccharide (data not shown).

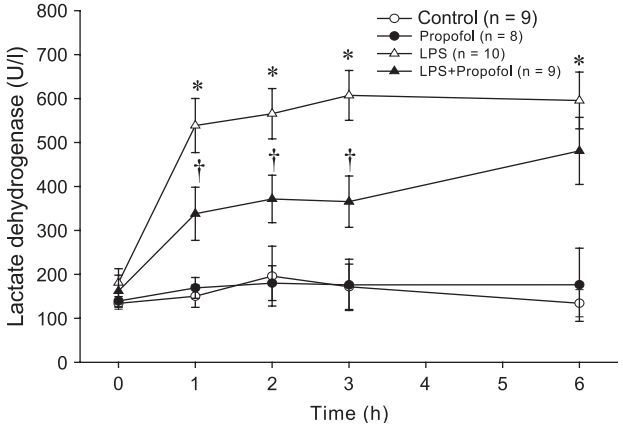

Fig. 4. Effects of propofol on plasma lactate dehydrogenase levels in rats treated with endotoxin. Depicted are the changes in plasma lactate dehydrogenase during the experimental period in different groups of animals which received injections of saline (Control), propofol (Propofol, 10 mg/kg + 10 mg/kg/h), *E. coli* lipopolysaccharide (LPS, 10 mg/kg), or *E. coli* lipopolysaccharide followed by propofol (LPS + Propofol). Data are expressed as means ± S.E.M. of number (n) of observations. *$P < 0.05$ represents significant differences between LPS and Control groups at the same time point. †$P < 0.05$ represents significant differences between endotoxemic rats treated with and without propofol.

Table 4
Effects of propofol on superoxide anion in aorta, lungs, and livers of rats treated with or without endotoxin

	Aorta	Lung	Liver
Control	5147 ± 590 (n = 11)	34,296 ± 8337 (n = 11)	1892 ± 289 (n = 11)
Propofol	6384 ± 1632 (n = 11)	50,231 ± 6435 (n = 11)	1756 ± 493 (n = 9)
LPS	20,181 ± 3163[a] (n = 10)	31,989 ± 5428 (n = 14)	2364 ± 1052 (n = 12)
LPS + Propofol	11,409 ± 2109[b] (n = 8)	46,754 ± 8187 (n = 10)	1829 ± 486 (n = 10)

Control (saline), Propofol (propofol, 10 mg/kg + 10 mg/kg/h), LPS (*E. coli* lipopolysaccharide, 10 mg/kg), LPS + Propofol (*E. coli* lipopolysaccharide followed by propofol).

The results are expressed as reactive luminescence units (RLU) per 15 min per milligram dry weight (i.e., RLU/15 min/mg). Data are expressed as means ± S.E.M. of number (n) of observations.

[a] $P < 0.05$ represents significant differences between LPS and Control groups at the same time point.

[b] $P < 0.05$ represents significant differences between endotoxemic rats treated with and without propofol.

3.10. Effects of propofol on $O_2^{\cdot-}$ in aorta, lung and liver during endotoxemia

The basal production of $O_2^{\cdot-}$ was detectable in the thoracic aorta, lung, and liver obtained from control rats. There were no significant differences in the $O_2^{\cdot-}$ level of aorta, lung and liver between the control rats and rats treated with propofol alone. The injection of lipopolysaccharide caused an about fourfold increase in the aortic $O_2^{\cdot-}$ level (Table 4). The injection of lipopolysaccharide had no significant effect on the $O_2^{\cdot-}$ level in the lung and in liver when compared to the control group. Treatment of lipopolysaccharide rats with propofol significantly inhibited the production of $O_2^{\cdot-}$ in the aorta but not that in the lung or the liver (Table 4).

3.11. Blood propofol concentrations

The blood propofol concentration in control rats treated with propofol alone at 6 h was 3.3±0.2μg/ml, $n=9$, and that in lipopolysaccharide-injected rats treated with propofol was 4.2±0.4 μg/ml, $n=12$, ($P<0.05$).

3.12. Effects of propofol on survival rate during endotoxemia

The injection of rats with lipopolysaccharide (10 mg/kg i.v.) was associated with a 16-h survival rate of about 12% (i.e., 2/17 animals). In contrast, lipopolysaccharide-injected rats treated with propofol had a higher survival rate of 53% (i.e., 8/15 animals) at 16 h (Fig. 5). Thus, propofol significantly ($P=0.011$) increased the survival rate of animals injected with lipopolysaccharide. In the time-control group (i.e., saline instead of lipopolysaccharide), no rats died within 16 h ($n=6$, unpublished data).

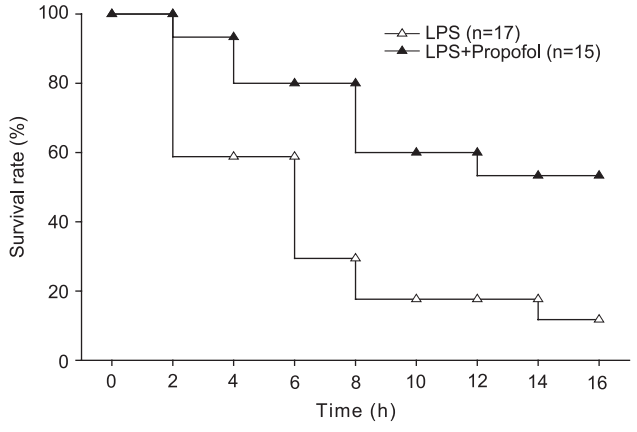

Fig. 5. Effects of propofol on survival of rats treated with endotoxin. Depicted are the changes of survival during the experimental period in different groups of animals which received injections of *E. coli* lipopolysaccharide (LPS, 10 mg/kg), or *E. coli* lipopolysaccharide followed by propofol (LPS + Propofol). Data are expressed as percentage of rats surviving at the observed time point.

4. Discussion

The present in vivo study demonstrates that propofol (i) protects against liver dysfunction and cytotoxicity, (ii) suppresses the increase in polymorphonuclear neutrophils in the liver, (iii) inhibits $O_2^{\cdot-}$ formation in the aorta, and (iv) improves the survival rate of conscious rats treated with endotoxin. Thus, the protective and antioxidant effects of propofol on organ dysfunction may contribute to the increased survival of conscious animals with endotoxic shock. This conscious endotoxic shock model seems to be a useful strategy for animal research, which attempts to extrapolate experimental findings to the clinic. However, propofol had no significant effect on the increased TNF-α and NO levels in the plasma and the expression of iNOS protein in the lung and the liver after lipopolysaccharide exposure, as unlike the situation in anesthetized animal models (Taniguchi et al., 2000, 2002; Mikawa et al., 2001). In addition, propofol neither improved nor worsened hemodynamic changes, lung injury and renal dysfunction in conscious rats with endotoxemia.

Liver Kupffer cells comprise approximately 70% of the total macrophage population of the body, and after its intravenous injection radiolabelled endotoxin is taken up rapidly by the liver (~50% within 5 min) in rabbits (Mathison and Ulevitch, 1979). Hepatectomy (90%) in rats resulted, within 2 h, in bacteriaemia due to bacterial translocation, although the phagocytic function of the lung and the spleen increased immediately (Wang et al., 1993). Therefore, the liver appears to be a potent scavenger of circulating bacteria and their products. Mild hepatic dysfunction might promote systemic endotoxin spillover and the subsequent inflammatory process, suggesting the importance of adequate liver function to patient survival (Chaudry et al., 1986). Our data showed that there is hepatic dysfunction: transaminases (glutamine-oxaloacetic transaminase, glutamine-pyruvic transaminase) were released into the plasma and metabolic functions (e.g. albumin synthesis) were reduced after lipopolysaccharide exposure. The administration of propofol counteracted the lipopolysaccharide-induced liver dysfunction and protected the animals against endotoxemia and death.

In addition, we also demonstrated that endotoxin caused significant infiltration of polymorphonuclear neutrophils in the liver, which was suppressed by propofol administration. This is consistent with in vitro studies showing that propofol inhibits phagocytosis and reactive oxygen species (e.g. $O_2^{\cdot-}$ in this study) production by neutrophils (Mikawa et al., 1998), and that propofol preserves the viability of rat hepatocytes after treatment inducing free radical generation (Navapurkar et al., 1998). Although propofol did not suppress the significant elevation of TNF-α caused by lipopolysaccharide treatment in this conscious animal model, previous studies have shown that endotoxemia increases plasma metabolic products of lipid peroxidation in an anesthetized porcine model, an effect was counteracted by

propofol (Basu et al., 2001). In this study, we demonstrated that endotoxin caused significant elevation of $O_2^{\cdot -}$ levels in intact aortas, an effect suppressed by propofol administration. However, we failed to detect massive $O_2^{\cdot -}$ production in the lung or the liver at 6 h after lipopolysaccharide administration. There are three possibilities for this phenomenon: first, endotoxin could induce endogenous antioxidant enzyme such as superoxide dismutase to lower the $O_2^{\cdot -}$ level (Spolarics, 1996); second, after endotoxin administration $O_2^{\cdot -}$ production could reach a plateau at 4 h in the aorta but return to normal levels at 6 h in the liver (Bautista and Spitzer, 1990; Brovkovych et al., 1997); third, major cellular components of the liver (except large Kupffer cell) from lipopolysaccharide-treated rats are unable to produce detectable $O_2^{\cdot -}$ in the absence of stimulating agents (Bautista et al., 1990). Without experimental evidence, these explanations are speculative. However, our data showed that propofol suppressed the infiltration of polymorphonuclear neutrophils in the liver and reduced $O_2^{\cdot -}$ generation in the aorta of endotoxemic rats, suggesting that the attenuation of hepatic injury by propofol during endotoxemia may be due to the antioxidant effect of propofol in the vascular bed.

Growing evidence shows that administration of lipopolysaccharide reproduces many of the cardiovascular features of septic shock, including the hypotension and the vascular hyporeactivity to vasoconstrictors (e.g. noradrenaline in this study), and that these features are associated with the overproduction of NO, presumably via iNOS (Kosaka et al., 1992). Booke et al. (1996) reported that propofol (10 mg/kg plus 5 mg/kg/h for 30 min) exacerbated the hemodynamic deterioration and the renal blood flow reduction seen in anesthetized sheep with sepsis. Here, we showed that propofol had no significant effects on hypotension and vascular hyporeactivity to noradrenaline 6 h after lipopolysaccharide injection in a conscious rat model. This difference may be related to a loss of compensation (i.e. autonomic nervous system) under anesthesia and the short observation time of experiments in the previous study (i.e. 30 min). In addition, the renal vasculature is very sensitive to NO (Lahera et al., 1991). Although the effects of propofol on constitutive NOS appear to be quite variable (Petros et al., 1993; Horibe et al., 2000), propofol decreases inducible NOS and plasma NO concentration in hamsters with sepsis (Mikawa et al., 2001). Thus, propofol may reduce renal perfusion and then lead to renal dysfunction in septic animals via inhibition of NO production (Booke et al., 1996). However, at the dosage used in the present study, propofol did not have deleterious effects on the kidney. This may be because propofol does not affect NO formation in plasma or iNOS expression in tissues from lipopolysaccharide-treated rats (e.g. lungs and livers in this study), suggesting that propofol has no deleterious effects on renal dysfunction in animals with endotoxic shock. In addition, this may also explain why propofol did not improve hypotension (usually elicited by overproduction of NO) in this study.

Another frequent complication of sepsis is the development of acute lung injury characterized by tachypnea, diffused pulmonary infiltration, reduced pulmonary compliance and severely disordered gas exchange (Bernard et al., 1994). Indeed, in this study we showed a decreased $PaCO_2$ and base excess, and increased infiltration of neutrophils in the lungs from rats treated with lipopolysaccharide. Propofol slightly, but not significantly, attenuated the tachypnea and metabolic acidosis caused by lipopolysaccharide. In addition, there was no significant difference in PaO_2 among all groups during the 6-h observation period. Possible explanations for this finding are that compensatory mechanisms (i.e. autonomic nervous system) were active in this conscious model and that the observation period was not long enough to observe the severe lung dysfunction seen in patients.

Although the induction dose of 10 mg/kg propofol used in this study was relatively high when compared with that required to induce anesthesia in humans, the required induction dose is known to be species-dependent (Simons et al., 1991a). For instance, the required induction dose for the rat is 9.3 mg/kg (Cockshott et al., 1992). The mean blood propofol concentration of 3.3 ± 0.2 μg/ml in normal control rats treated with propofol alone was similar to that in awake rats (Simons et al., 1991b) and it is within the range of 2–5 μg/ml found in clinical anesthesia. Our results showed that the blood propofol concentration (4.2 ± 0.4 μg/ml) was higher in the rats with sepsis, but these animals always responded to tail pinching except during the 10-min propofol-injection period.

In conclusion, we have demonstrated that propofol protects the activity of endogenous hepatic enzymes and decreases the infiltration of neutrophils in the liver of conscious rats with endotoxic shock. In addition, the protective effect of propofol may derive from antioxidative stress in the vascular bed, e.g. $O_2^{\cdot -}$ production in the aorta, distributed in the liver for it is difficult to dissect blood vessels from the liver. These beneficial effects of propofol may contribute to the higher survival rate of conscious rats with endotoxic shock. Thus, we suggest that propofol may be a better choice of sedative agent in patients with sepsis shock during surgery or intensive care.

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