

Propofol Attenuates Diaphragmatic Dysfunction Induced by Septic Peritonitis in Hamsters

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Background: **Sepsis or peritonitis impairs diaphragmatic contractility and endurance capacity. Peroxynitrite, a powerful oxidant formed by superoxide and nitric oxide, has been implicated in the pathogenesis. Propofol scavenges this reactive molecule. The authors conducted the current study to evaluate whether propofol prevents diaphragmatic dysfunction induced by septic peritonitis.**

Methods: **Forty male Golden-Syrian hamsters (120–140 g) were randomly classified into five groups. Groups sham and sham-propofol 50 underwent sham laparotomy alone, whereas groups sepsis, sepsis-propofol 25, and sepsis-propofol 50 underwent cecal ligation with puncture. Groups sham and sepsis received infusion of intralipid, whereas groups sham-propofol 50, sepsis-propofol 25, and sepsis-propofol 50 received propofol at rates of 50, 25, and 50 mg · kg^{-1} · h^{-1}, respectively. Intralipid or propofol was subcutaneously infused from 3 h before surgery until 24 h after operation, when all hamsters were killed. Diaphragmatic contractility and fatigability were assessed *in vitro* using diaphragm muscle strips. Peroxynitrite formation in the diaphragm was assessed by nitrotyrosine immunostaining. Plasma nitrite–nitrate concentrations and diaphragmatic concentrations of malondialdehyde were determined. Using another set of animals, diaphragmatic inducible nitric oxide synthase activity was also measured.**

Results: **Twitch, tetanic tensions, and tensions during fatigue trials were reduced in group sepsis compared with group sham. In group SEPSIS, diaphragm malondialdehyde and inducible nitric oxide synthase activity, and plasma nitrite–nitrate concentrations increased, and positive immunostaining for nitrotyrosine residues was found. Propofol attenuated these changes.**

Conclusions: **Pretreatment with propofol attenuated diaphragmatic dysfunction induced by septic peritonitis in hamsters assessed by contractile profiles and endurance capacity. This beneficial effect of propofol may be caused, in part, by inhibition of lipid peroxidation in the diaphragm caused by the powerful oxidant.**

IT is well-known that endotoxemia or sepsis causes multiple organ injury, including the respiratory muscle (*e.g.,* diaphragm).[1–3] Diaphragmatic dysfunction may contribute to acute respiratory failure in critically ill patients. Although the precise mechanism underlying infection-induced impairment of contractile profile and endurance capacity in the respiratory muscle remains to be elucidated, many mediators are thought to contribut to the pathogenesis of diaphragmatic dysfunction. Oxygen-derived free radicals, which are overproduced in sepsis, have been shown to play a pivotal role in the mechanism.[1–3] The oxygen toxic metabolites impair the diaphragm at least, through lipid peroxidation of the muscle membrane.[1–3] Other plausible mediators causing diaphragmatic dysfunction include peroxynitrite, a powerful oxidant (formed by reaction of superoxide with nitric oxide[NO]).[4–6] In sepsis, endotoxin and inflammatory cytokines induce expression of inducible NO synthase (iNOS) protein, leading to excessive production of NO.[7–9] Large amounts of NO produced by iNOS react readily with the superoxide radical to form peroxynitrite.[7–9] This potent oxidant, freely diffusible, is thought to contribute to membrane peroxidation and protein degeneration of the muscle.[4–6]

Propofol is often used in critically ill patients as a sedative or anesthetic.[10,11] Patients with peritonitis, infection, or sepsis may sometimes receive propofol for sedation. The drug is known to be a peroxynitrite scavenger and an antioxidant.[12,13] An *in vitro* study using a cultured murine macrophage cell line indicates that propofol decreases production of NO, which is important as one of precursors for peroxynitrite, through iNOS.[14] Propofol, therefore, may be able to attenuate sepsis-induced diaphragmatic dysfunction. The aim of the current study was to ascertain this hypothesis. To test this hypothesis, we assessed diaphragmatic contractility and endurance capacity *in vitro* using muscle strips excised from the costal diaphragm of septic hamsters that did or did not receive propofol.

Methods

Group Assignment

The current study was approved by the animal care review board of Kobe University School of Medicine. The care and handling of the animals were in accord with the National Institutes of Health guidelines (IACUC). We used 43 male Golden-Syrian hamsters weighing 120–140 g. Of 43 hamsters, 3 (2 group sepsis, 1 group sepsis-propofol 25) were excluded from analysis because of death during the experiment. Muscle strips obtained from 40 unrestrained hamsters were used. The animals were randomly classified into five groups (groups sham, sham-propofol 50, sepsis, sepsis-propofol 25, and sepsis-propofol 50; n = 8 in each group). Hamsters in groups sham and sham-propofol 50 underwent sham laparotomy, and animals in groups sepsis, sepsis-propofol 25, and sepsis-propofol 50 underwent laparotomy followed by cecal ligation and puncture (CLP).

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Received from the Department of Anesthesiology and the Intensive Care Unit Kobe University School of Medicine, Chuo-ku Kobe, Japan. Submitted for publication February 21, 2000. Accepted for publication November 17, 2000. Support was provided solely from institutional and/or departmental sources.

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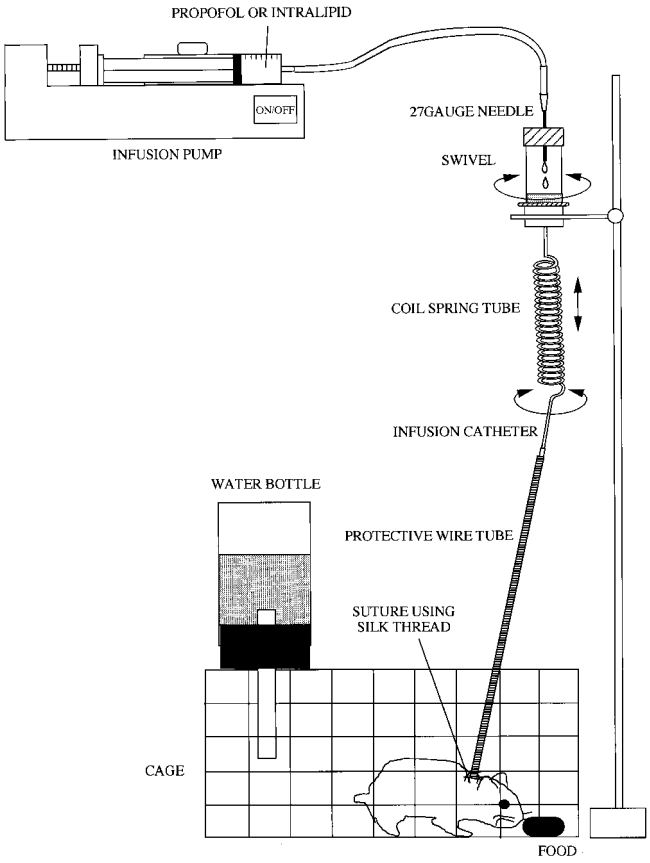

Fig. 1. Schematic diagram of apparatus (Disposwivel Kit, Bioresearch Center) used for continuous subcutaneous infusion in hamsters. A swivel needs less torque for rotation than does the current standard type. A coiled spring tube connecting the swivel and an infusion catheter allow hamsters to move freely in a cage. A protective wire tube prevents animals from biting the infusion catheter. Minimum fixation using No. 4 silk sutures is necessary to secure the catheter.

Groups sham and sepsis received infusion of 10% intralipid (Otsuka, Tokyo, Japan), whereas groups sham-propofol 50, sepsis-propofol 25, and sepsis-propofol 50 received infusion of propofol (Diprivan; Astra-Zeneca, Osaka, Japan) at a rate of 50, 25, and 50 mg · kg^{-1} · h^{-1}, respectively. Intralipid used in the sham or sepsis control has almost the same component as the solvent used in propofol. Propofol and intralipid were subcutaneously administered using the Disposwivel Kit (DS-10; Bioresearch Center, Nagoya, Japan; fig. 1) 3 h before surgery until 24 hours after, when the animals were killed. This device, consisting of a swivel, a coiled spring tube, a protective wire tube, and an infusion catheter, secured continuous infusion in unrestrained hamsters. The animals were permitted to freely move about (*e.g., ad libitum* access to water). All operations were performed using a sterile technique during general anesthesia with sevoflurane.

The CLP technique has served as an intraabdominal sepsis model.[15] In the current study, the cecum was devascularized and ligated tightly at its base with a 3-0 silk thread without obstructing the bowel. Then, the cecum was punctured once with a sterile 18-gauge needle on the antimesenteric border. Gentle pressure was applied to the cecum until a small amount of feces exuded. This procedure ensured that the puncture hole would not close. This regimen produced essentially no early mortality but elicited easily measurable reductions in diaphragmatic contractility. In previous experiments, oral intake was obviously reduced in septic hamsters after laparotomy. Thus, feeding was restricted in all groups after surgery to avoid differences in nutrition supplement among the groups because of difference in oral intake. No analgesics were administered.

Experimental Protocol

At 24 h postsurgery, the hamsters were placed in a bell jar (600 ml) containing sevoflurane (1.5 ml) for 35–40 s. During general anesthesia, they were killed by cervical dislocation. Within 1 min of death, blood samples were obtained by aspiration from the left ventricle to determine the plasma concentrations of endotoxin, nitrite plus nitrate (NOx), and propofol. Thereafter, the left hemidiaphragm was removed and placed in a dissecting dish containing oxygenated (95% O_2 and 5% CO_2) Krebs-Henselheit solution (pH, 7.40, NaCl 135 mM, KCl 5 mM, glucose 11.1 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1 mM, $NaHCO_3$ 14.85 mM, $NaHPO_4$ 1 mM, and insulin 50 U/l). Pancuronium, 2 μM (equivalent to D-tubocurarine 10 μM/l), was added to the solution to eliminate indirect muscle activation mediated by nerves. A part of the right hemidiaphragm was also removed, frozen in liquid nitrogen, and stored at −70 °C for subsequent malondialdehyde analysis. Another part of the right hemidiaphragm was fixed by 10% formaldehyde solution and embedded in paraffin wax for immunohistochemical experiment. An approximately 8-mm muscle strip was dissected from the left hemidiaphragm and mounted in an organ bath containing Krebs-Henselheit solution at 22°C. A previous *in vitro* experiment, in which temperature (15, 25, 37, and 41°C) dependence of diaphragm muscle contractility and fatigue was assessed, revealed that optimal twitch and tetanic tensions were generated at room temperature (25°C).[16] Maximum fatigue resistance was also obtained at the same temperature.[16] Thus, several investigators used solutions at low temperature (21–23°C) to evaluate diaphragmatic function *in vitro*.[2,17,18] The origin of each muscle was secured by a steel hook embeded in the bath, and the tendinous insertion of each strip was secured to another hook tied to silk thread attached to a force transducer (T7–T15, NEC San-ei, Tokyo, Japan). Strips were stimulated with supramaximal currents (1.2 to 1.3 times the current required to elicit a maximal tension) delivered *via* platinum-field electrodes. Current (0.2 ms duration in pulses) was supplied by an electrical stimulator (DPS-1100D; Dia Medical System Co., Tokyo,

Japan). The muscle tension was amplified by an alternating current strain amplifier (AS1202; NEC San-ei).

Strips were equilibrated for 15 min in the organ bath; muscle length was then adjusted to the length at which twitch tension development was maximal. Muscle length was measured using a micrometer. Muscle contractile characteristics were assessed from measurements of twitch kinetics, the diaphragm force frequency relation, and diaphragm fatigability during a series of repetitive rhythmic contractions. Twitch kinetics were assessed by measuring maximum rate of muscle tension development (dp/dtmax) and the time necessary for peak tension to decrease by 50% (half-relaxation time [HRT]) during single muscle twitches. The diaphragm force–frequency relation was assessed by sequentially stimulating muscles at 1, 10, 20, 50, and 100 Hz. Each stimulus train was applied for 800 ms, and adjacent trains were applied at 5-s intervals. After completion of the force–frequency, a 30-s rest period was provided. Muscle fatigability was then assessed by evaluating the rate of decrease of tension over a 5-min record of rhythmic contraction. Rhythmic contraction was induced by applying trains of 20-Hz stimuli (train duration, 500 ms; duty cycle, 0.50) at a 60 train/min rate. At completion of this protocol, the muscle strip was removed from the bath and weighed.

Measurement of Plasma Concentrations of Endotoxin, Propofol, and NOx

The blood taken within 1 min after killing was immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was then stored at −70°C until assayed. Plasma endotoxin concentrations were measured using an endotoxin-specific test (Endospecy; Seikagaku Kogyo, Tokyo, Japan).[19] The lower limit of detection of the method is 5 pg/ml. In groups sham-propofol 50, sepsis-propofol 25, and sepsis-propofol 50, plasma propofol concentrations were also determined with high-performance liquid chromatography using the technique of Plummer.[20] Plasma NOx concentrations were also determined using an automatic analyzer (NOX 1000; Tokyo Kasei, Tokyo, Japan) using the Griess reaction.[21] The lower limit of detection for NOx is 2 μM. The intra- and interassay coefficients of variance were respectively 5.6 and 9.3% for endotoxin, 4.9 and 8.8% for propofol, and 6.1 and 9.6% for NOx.

Nitrotyrosine Immunostaining

Thin sections (5 μm) of each formalin-fixed, paraffin-embedded tissue were cut onto commercially available slides (Superfrost/plus; Fisher Scientific, Pittsburgh, PA), deparaffinized, and cleansed through a series of xylene and alcohol washes. After deparaffinization, endogenous peroxidase was quenched with 0.5% hydrogen peroxide in phosphate-buffered saline for 30 min. Antigen retrieval was performed by heating the sections in citrate buffer using a microwave oven. Nonspecific adsorption was minimized by incubating the section in 5% normal goat serum (Sigma, St. Louis, MO) in phosphate-buffered saline for 20 min. The sections were then incubated overnight at 4°C with 1:200 dilution of primary polyclonal antinitrotyrosine antibody (#06-284; Upstate Biotech, Lake Placid, NY) or goat nonimmune seraum (as an negative control). The slides were probed with biotinylated goat antirabbit secondary antibody (Vector, Burlingame, CA) followed by treatment with the streptavidin–biotin–horseradish peroxidase complex (Amersham-Pharmacia, Uppsala, Sweden). Peroxidase-stained sections were developed with 0.5 mg/ml 3,3'-diaminobenzidine (Sigma) and counterstained with hematoxylin stain. This antinitrotyrosine antibody only recognizes nitrated proteins without cross-reaction with other tyrosine proteins or phosphotyrosine (T. Kasamatsu, Cosmo Bio, Tokyo, Japan, personal communication, June 12, 2000). Immunohistochemical staining was assessed by two independent observers who were unaware of group assignment. The following scores were assigned to each specimen according to the intensity of staining: 0 = none, 1+ = minimal, 2+ = moderate, and 3+ = intense.

Analysis of Malondialdehyde Concentrations

Malondialdehyde was assayed on diaphragmatic samples using a thiobarbituric assay.[22,23] In brief, muscle samples were homogenized with cold 1.15% KCl to make a 10% homogenate. A 0.1-ml aliquot of this homogenate was then added to 0.2 ml sodium dodecyl sulfate, 8.1%, 1.5 ml acetic acid, 20% (pH adjusted to 3.5), and 1.5 ml aqueous thiobarbituric acid, 0.8%. The mixture was made up to 4 ml with distilled water and then heated for 60 min in a water bath at 90°C. After cooling, this solution was mixed with 5 ml butanol and 1 ml distilled water and centrifuged at 2,500 rpm for 20 min. The supernatant was read at 532 nm on a spectrophotometer. Absorbance values were compared with standard curves constructed using malondialdehyde produced in response to known concentrations of tetramethoxypropane (2.5, 5.0, 7.5, and 10 nM). Final malondialdehyde concentrations are reported as nanomoles of malondialdehyde per gram of wet weight of tissue. The intra- and interassay coefficients of variance were respectively 5.1 and 9.8% for malondialdehyde.

Respiration Rate, Arterial Blood Gasses Analysis, and iNOS Activity in the Diaphragm

Another set of hamsters (130–150 g, n = 35) were prepared and classified into five groups (groups sham, sham-propofol 50, sepsis, sepsis-propofol 25, and sepsis-propofol) for determination of arterial tension of oxygen (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}, and diaphragmatic iNOS activity. Respiration rate (RR) were determined by visual inspection immediately before laparotomy alone or CLP, and 10, 18, and 24 h after surgical operation. At

24 h postsurgery, a catheter (24 gauge) was inserted into the left or right carotid artery during anesthesia with sevoflurane. Arterial blood samples were obtained from hamsters in a restrainer to analyze gasses (Pa_{O_2}, Pa_{CO_2}, and pH) using a blood gas analyzer (ABL2; Radiometer, Copenhagen, Denmark) 30 min after discontinuation of sevoflurane to exclude the effect of the anesthetic on respiration.

Immediately after arterial blood gasses analysis, the hamsters were killed by cervical dislocation during general anesthesia with sevoflurane. The diaphragm was quickly excised, cleaned of connected tissue, and frozen at −80°C in liquid nitrogen until assay of iNOS activity determined using a commercial NOS quantitative assay kit (Bioxytech; OXIS International, Portland, OR). Briefly, the frozen diaphragm was homogenized in 20 vol of homogenization buffer (pH 7.4, 25 mM HEPES buffer, 1 mM EDTA, 1 mM EGTA). The crude homogenates were centrifuged at 4°C for 5 min at 15,000 rpm and the supernatants were collected. Diaphragmatic samples 10 μl) were added to reaction buffer (50 μl) of the following composition: pH, 7.4, 25 mM Tris/HCl buffer, 60 mM valine, 1 mM reduced nicotinamide adenine dinucleotide phosphate, 1 μM flavin adenine dinucleotide, 1 μM flavin mononucleotide, 3 μM tetrahydrobiopterin, 1 μl stock L-[^{3}H]-arginine,120 μM (Amersham-Pharmacia), and 2 mM EGTA (except for assay of a positive control). The samples were incubated for 30 min at 25°C and the reaction was discontinued by the addition of ice-cold (2°C) stop buffer (pH, 5.5; 50 mM HEPES, 5 mM EDTA). To obtain free L-[^{3}H]citrulline for the determination of enzyme activity, equilibrated resin was added to eliminate excess L-[^{3}H]-arginine. The supernatant was assayed for L-[^{3}H]citrulline using liquid scintillation counting. Enzyme activity was expressed as counts per minute per milligram of total protein. Protein concentration was measured by the Bradford technique (Protein Assay Kit; Bio-Rad, Hercules, CA) with bovine serum albumin as a standard. NOS activity in the positive control was measured in the presence of 0.6 mM $CaCl_2$ and rat brain homogenate instead of diaphragmatic samples. NOS activity in the presence of 1 mM N^G-nitro-L-arginine methyl ester (L-NAME) served as a negative control. The iNOS activity was calculated as the difference between samples assayed in the presence of EGTA and that measured in the presence of L-NAME.

Data Analysis and Statistical Analysis

Muscle strip cross-sectional areas were calculated by dividing muscle mass by the product of fiber length and muscle density (1.06 g/cm^3).[24] Force generation was normalized as force per unit of cross-sectional area (kg/cm^2). Data are presented as mean ± SD. The data among the groups were analyzed using analysis of variance (ANOVA) with Scheffé *post hoc* testing. The within-group (over time) data were statistically analysed using repeated-measures analysis of variance followed by the Scheffé *post hoc* test. Staining intensity score was analyzed using the Kruskall-Wallis rank test. Two-tailed $P < 0.05$ was deemed statistically significant.

Results

Muscle Strip Characteristics and Plasma Concentrations of Propofol and Endotoxin

Autopsy examination revealed that all hamsters in the three septic groups had panperitonitis. The body weights of the animals before surgery were similar among the groups. There were no significant differences in the body weights immediately before killing among the groups. Mean (± SD) muscle strip length and weights excited were 5.5 ± 0.7 mm, and 34 ± 6 mg, respectively, and were comparable in the five groups. Plasma propofol concentrations (mean ± SD) in groups sham-propofol 50, sepsis-propofol 25, and sepsis-propofol 50 were 7.7 ± 1.7, 2.2 ± 1.1, and 8.4 ± 2.0 μg/ml, respectively. All hamsters receiving propofol were sedated to a greater or lesser degree, but not anesthetized. Plasma endotoxin concentrations (mean ± SD) were less than 5, less than 5, 78 ± 34, 75 ± 40, and 72 ± 37 pg/ml in groups sham, sham-propofol 50, sepsis, sepsis-propofol 25, and sepsis-propofol 50, respectively.

Mechanical Variables

As shown in table 1, dp/dtmax of diaphragmatic contraction was decreased in group sepsis compared with that in group sham. The dp/dtmax was normalized for maximum twitch tension. Normalized dp/dtmax was also decreased in group sepsis, indicating that sepsis-induced decreased in dp/dtmax is not simply reflected by reduction of maximum force (table 1). In diaphragms isolated from propofol-treated hamsters, normalized dp/dtmax was similar to that in the sham control. HRT was also prolonged (table 1). Twitch tensions (by stimulation of 1-Hz frequency) were significantly lower in strips from the septic hamsters than in those from the sham group (table 1). Propofol attenuated impairment of these twitch kinetics. As shown in table 1, intraabdominal sepsis decreased the tensions generated in response to all frequencies of stimulation. Propofol significantly blunted the sepsis-induced reduction of force–frequency relation. This beneficial effect of propofol seemed to be dose-dependent although not significantly.

Figure 2, shows that tensions over time during the figure trial remained lower in the diaphragms isolated from group sepsis than in those from group sham. Propofol slightly increased contraction in the presence of intraabdominal sepsis (fig. 2).

Table 1. dp/dt_{max}, Half Relaxation Time (HRT), and Force (Tension)–Frequency Relations

	Sham	Sham–Propofol 50	Sepsis	Sepsis–Propofol 25	Sepsis–Propofol 50
dp/dt_{max} (mN/s · cm^2)	281 ± 61	237 ± 94	37 ± 22*	106 ± 25*†	144 ± 57*†‡
Normalized dp/dt_{max} (mN/s · cm^2)	30 ± 3	28 ± 3	15 ± 5*	30 ± 4*	29 ± 6†
HRT (ms)	42 ± 11	51 ± 17	92 ± 20*	79 ± 15*	76 ± 11*
Force–frequency relations (mN/cm^2)					
1 Hz	9.5 ± 2.7	8.4 ± 2.8	2.3 ± 0.9*	3.7 ± 1.1*†	4.8 ± 1.4*†
10 Hz	11.5 ± 3.1	11.1 ± 3.9	4.6 ± 1.7*	6.4 ± 1.9*†	9.7 ± 2.1*†‡
20 Hz	18.9 ± 8.4	16.3 ± 6.3	5.9 ± 2.5*	9.2 ± 3.0*†	11.4 ± 3.8*†
50 Hz	22.1 ± 7.4	19.5 ± 7.7	5.4 ± 2.4*	9.4 ± 2.7*†	11.9 ± 3.9*†
100 Hz	19.3 ± 6.3	16.1 ± 6.4	4.2 ± 2.2*	7.1 ± 2.5*†	8.8 ± 4.1*†

Data are expressed as mean ± SD (n = 8 for each group). Group assignment: see text. Normalized dp/dt_{max} was calculated by dividing dp/dt_{max} by twitch tension.

* $P < 0.05$ *versus* group sham. † $P < 0.05$ *versus* group sepsis. ‡ $P < 0.05$ for group sepsis–propofol 50 *versus* group sepsis–propofol 25.

dp/dt_{max} = maximum of the first derivative of developed pressure.

Plasma NOx Concentrations and Diaphragm Malondialdehyde and iNOS Activity

Plasma NOx concentrations were significantly increased in hamsters undergoing CLP, suggesting that intraabdominal sepsis induced NO production. This elevation was significantly attenuated with propofol (table 2). The malondialdehyde concentrations were higher in diaphragmatic samples taken from group sepsis than in those taken from group sham (table 2). Administration of propofol 50 mg · kg^{-1} · h^{-1} significantly attenuated the increase of malondialdehyde observed in the sepsis group. Diaphragmatic iNOS activity was increased in septic hamsters (table 2). This increase was significantly blunted by a higher dose of propofol.

Nitrotyrosine Immunostaining

The κ coefficient between the two observers was 0.556 (data not shown), indicating moderate agreement with their assessment of the data. Immunohistochemical analysis of nitrotyrosine showed that no staining was found in the diaphragms isolated from group sham (fig. 3A, table 3). In contrast, positive immunostaining for nitrotyrosine was observed in inflammatory cells in endomysial and perivascular spaces of the septic group, but the myocytes did not stain perceptibly (fig. 3B). Intensity of nitrotyrosine immunostaining was reduced by 50 mg · kg^{-1} · h^{-1} propofol to concentrations similar to those in group sham (fig. 3C, table 3).

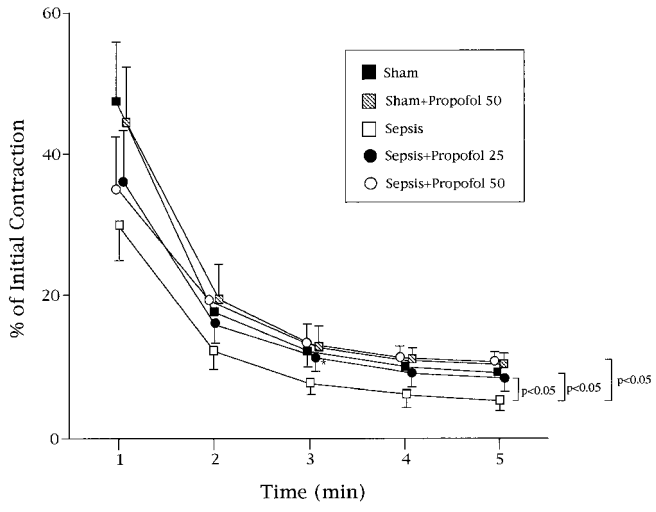

Fig. 2. Tension during fatigue trial. Data (mean ± SD) are expressed as a percentage of initial contraction (at the start of fatigue trial) (n = 8 for each group). Some SD bars are omitted for clarity.

Arterial Blood Gas Analysis

Septic peritonitis markedly increased RR with normocapnia, and impaired oxygenation (table 4). Propofol itself (group sham-propofol 50) caused respiratory depression (RR decrease and hypercapnia). The drug mitigated tachypnea induced by intraabdominal sepsis (table 4). Propofol also attenuated sepsis-induced impairment of oxygenation, but, not significantly.

Discussion

The new findings in the current study include the following: that [1] propofol attenuated intraabdominal sepsis-induced diaphragmatic dysfunction, which is manifested by a reduction of the twitch kinetics and tetanic tensions of the muscle and increased fatigability, and [2] propofol reduced sepsis-induced systemic NO production, diaphragmatic iNOS activity, and nitrotyrosine formation.

Several plausible mechanisms responsible for intraabdominal sepsis-induced diaphragmatic dysfunction are proposed, but the precise one remains to be elucidated. Among many possible mediators for diaphragmatic dysfunction, oxygen free radicals and NO, at least, are thought to contribute to the pathogenesis.[1–3,25,26] One of other possible mediators is peroxynitrite, a highly reactive compound formed by reaction of NO with superoxide anions.[4–6] Peroxynitrite exerts an adverse effect on lipid and protein functions as a result of nitration of tyrosine residues and oxidative modifications, leading to damage of muscle cell membranes and cytoplasmic

Table 2. Plasma NOx (Nitrite + Nitrate) Concentrations, Malondialdehyde (MDA) Concentrations, and iNOS Activity in the Diaphragm

	Sham	Sham–Propofol 50	Sepsis	Sepsis–Propofol 25	Sepsis–Propofol 50
NOx (μM, n = 8 each group)	25 ± 3	24 ± 2	36 ± 5*	31 ± 5*†	28 ± 3†
MDA (nmol/g tissue; n = 8 each group)	55 ± 27	59 ± 21	126 ± 33*	104 ± 25*	94 ± 17*†
iNOS activity (cpm/mg protein; n = 7 each group)	477 ± 208	315 ± 163	5,689 ± 1,176*	3,621 ± 1,352*	3,143 ± 924*†

Data are expressed as mean ± SD. Group assignment: see text.

* $P < 0.05$ *versus* group sham. † $P < 0.05$ *versus* group sepsis.

protein in muscle fiber.[6] Peroxynitrite-mediated injury to membrane channels or pumps could alter action potential propagation and excitation–contraction coupling by causing gradual ionic shifts (leakage of calcium and sodium ion into cells, and potassium ion out of cells). This change may reduce the intracellular calcium ion concentration, thereby decreasing muscle force generation and relaxation. Assault on outer membranes of the muscle is feasible by extracellular peroxynitrite from inflammatory cells surrounding the myocytes. Conversely, high concentrations of peroxynitrite produced in mitochondria may affect intracellular constituents, including contractile proteins and sarcoplasmic reticulum in the muscle cells. In the current study, positive nitrotyrosine immunostaining was mainly shown in surrounding inflammatory cells rather than in the muscle cell itself in septic hamsters. These findings suggest that diaphragmatic dysfunction is predominantly ascribed to extracellular peroxynitrite rather than to a mechanism of intracellular peroxynitrite. However, our findings concerning twitch kinetics suggest that septic peritonitis produces damage of the sarcoplasmic reticulum in the muscle cells because twitch kinetics probably represent function for release and reuptake rate of calcium from this organella.[27]

Although the precise mechanism responsible for the beneficial effects of propofol on sepsis-induced diaphragmatic dysfunction remains unknown, it is reasonable that beneficial effects of the drug are caused, in part, by reduction of lipid peroxidation in the muscle membrane, as assessed by diaphragmatic malondialdehyde concentrations. In the current study, propofol inhibited formation of reactive NO species, including per-

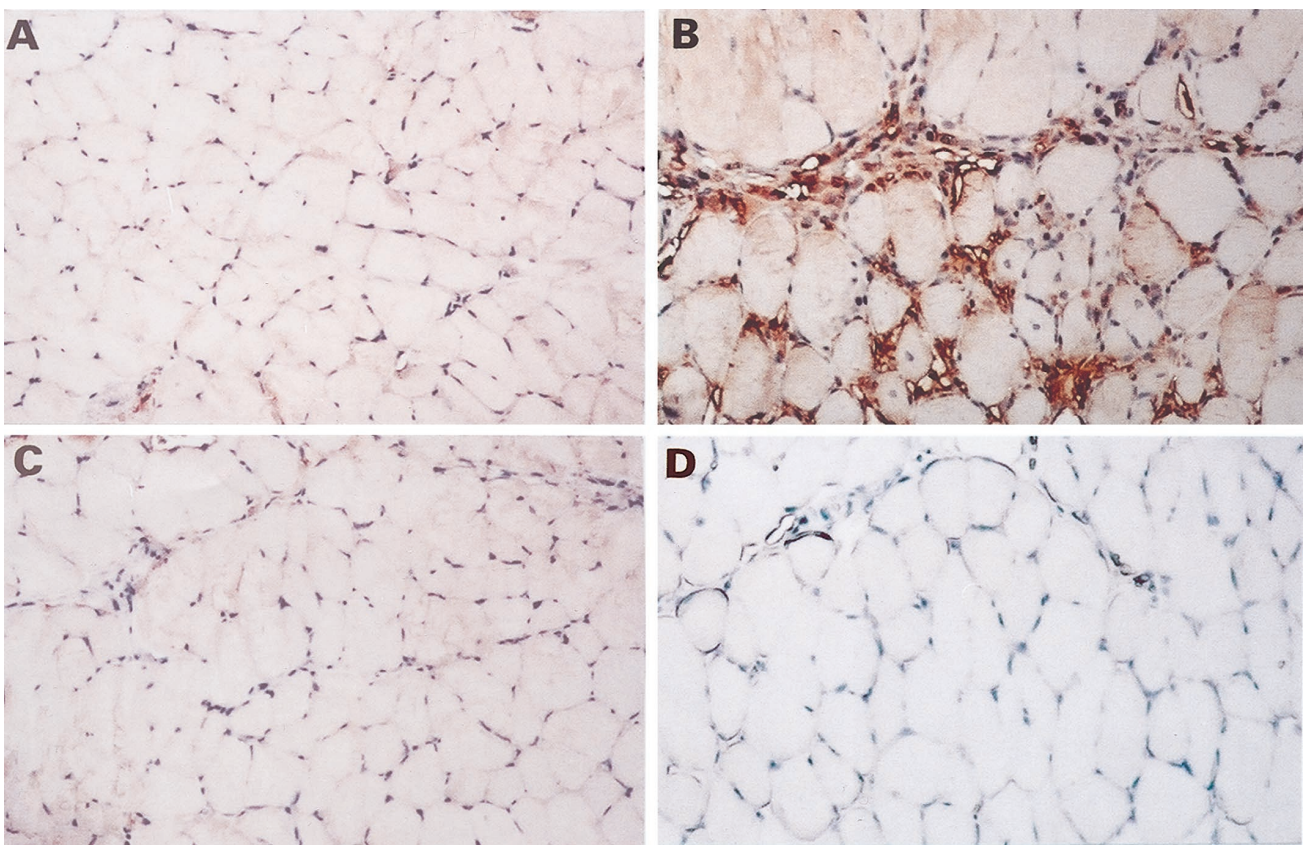

Fig. 3. Representative immunostaining for nitrotyrosine expression in the diaphragm. (*A*) group sham, (*B*) group sepsis, (*C*) group sepsis-propofol 50, (*D*) negative control. Original magnification: ×200.

Table 3. Immunohistochemical Staining Intensity for Nitrotyrosine in the Diaphragm

	Staining Intensity Score (median [range])
Group sham	0 [0–1]
Group sham–propofol 50	0 [0–1]
Group sepsis	3 [1–3]*
Group sepsis–propofol 25	2 [0–3]*†
Group sepsis–propofol 50	1 [0–3]*†
Negative control	0 [0]

n = 8 for each group. Group assignment: see text.

* $P < 0.05$ *versus* group sepsis. † $P < 0.05$ *versus* group sham.

oxynitrite and dinitrogen trioxide, as assessed by nitrotyrosine immunostaining. Our observations suggest that suppression of reactive NO derivatives production with propofol is responsible for attenuation of lipid peroxidation. Propofol at physiologically relevant concentrations has been reported to scavenge peroxynitrite.[12] In agreement with the previous experiments,[14] we have demonstrated systemic suppression of NO production (as assessed by plasma NOx concentration) with propofol. Increase in diaphragmatic iNOS activity (local NO production) was also attenuated with propofol. Reduced NO production cauesd by inhibition of iNOS activity would contribute to a decrease in peroxynitrite generation in the current study. Propofol has also been shown to inhibit reactive oxygen species production.[28] Through this mechanism, lipid peroxidation of the cell membrane may have been attenuated, but the effect of propofol on reactive oxygen species generation themselves was not determined in the current study. Other possible mechanisms underlying the effectiveness of propofol include simple differences in respiratory muscle use as assessed by RR. Sedation with respiratory depression developed in all hamsters receiving propofol, decreasing respiratory activity, and consequent respiratory muscle work. Because contractile activity modulates production of reactive oxygen species by the diaphragm,[29] the protective effect of propofol could be related not only to its antioxidant properties, but also to a decrease in reactive oxygen species production secondary to a reduced activity of the diaphragm.

We used the CLP technique in the current study because this procedure is a representative experimental model of sepsis,[15] and fecal peritonitis caused by anastomotic leakage after colonic or rectal resection often causes sepsis in clinical settings. Unlike simple systemic sepsis models (*e.g.,* intravenous infusion of live bacteria or endotoxin), CLP affects diaphragmatic function through not only the systemic process, but also local action: an intraabdominal inflammatory process that develops in the neighborhood of the diaphragm, in addition to systemic inflammatory process, and directly damages the respiratory muscle. It is likely that activated neutorphils and macrophages directly attached to the abdominal surface of the diaphragm attack the muscle by releasing many inflammatory mediators. Furthermore, the inflammatory medicators are thought to be absorbed by the mesenteric microvasculature and distributed systemically. Aseptic peritonitis induced by intraperitoneal injection of oyster glycogen has been shown to decrease diaphragmatic contractility in rats.[30] Peritonitis has little effect on acute muscle fatigue.[30] In the current study, an increase of plasma NOx and endotoxin concentrations shows systemic septic inflammation. Therefore, the diaphragmatic fatigue observed in our experiment may be caused by systemic sepsis rather than peritonitis.

Sepsis may indirectly affect diaphragmatic contractility and fatigability by depletion of energy because of decreased oral intake. In the current study, the body weights of the hamsters were similar among the groups because of strict dietary control. Therefore, it is unlikely that propofol attenuated the diaphragmatic dysfunction by improving oral ingestion in the hamsters with sepsis. Deterioration of microcirculation in skeletal muscle observed in sepsis may also contribute to indirect impairment.[31] Proinflammatory cytokines (*e.g.,* tumor necrosis factor α, interluekin 1), which are increased in sepsis, promote impairment of diaphragmatic contractility.[32,33] To determine whether successful results of propofol were attained through the direct or indirect mecha-

Table 4. Respiration Rate and Arterial Blood Gases

	Sham	Sham–Propofol 50	Sepsis	Sepsis–Propofol 25	Sepsis–Propofol 50
Respiration rate (breaths/min)					
0 h	82 ± 13	78 ± 12	85 ± 17	74 ± 12	86 ± 15
10 h	77 ± 14	71 ± 11	132 ± 24*	112 ± 23*	99 ± 21†
18 h	84 ± 16	73 ± 15	159 ± 21*	116 ± 22*†	106 ± 25†
24 h	80 ± 13	68 ± 12	176 ± 19*	121 ± 18*†	110 ± 23*†
Arterial blood gases analysis‡					
Pa_{O_2} (mmHg)	92 ± 6	87 ± 7	64 ± 11*	74 ± 10*	78 ± 13
Pa_{CO_2} (mmHg)	39 ± 4	48 ± 6	33 ± 12	38 ± 11	41 ± 9
pH	7.39 ± 0.05	7.32 ± 0.07	7.21 ± 0.11*	7.25 ± 0.13*	7.27 ± 0.11*

n = 7 for each group. Group assignment: see text.

* $P < 0.05$ *versus*, group sham. † $P < 0.05$ *versus*, group sepsis. ‡ Arterial blood gases were analyzed 24 h after surgical operation.

Pa_{O_2} = arterial oxygen tension; Pa_{CO_2} = arterial carbon dioxide tension.

nisms, hemodynamic data and plasma concentrations of the mediators should haven been determined in the current study. Propofol has been shown to reduce cardiac output, heart rate, arterial pressure, and oxygen delivery in septic sheep compared with unanesthetized animals.[34] Systolic arterial pressure did not differ in endotoxemic rats that did and did not receive propofol.[35] These observations indicate that propofol is unlikely to increase diaphragmatic blood flow by improving hemodynamics. Propofol was recently shown to attenuate an endotoxin-induced increase of plasma tumor necrosis facter α concentrations in rats.[34]

Acute ventilatory failure can theoretically contribute to acute respiratory failure by causing alveolar hypoventilation. In the current study, we observed that septic animals exhibited rapid shallow breathing because of diaphragmatic dysfunction. Although we could not measure tidal volume or dead space, normocapnia with extremely increased RR indicates that ventilatory efficiency was decreased in septic hamsters. However, we failed to demonstrate hypoventilation-induced hypoxemia in sepsis. Decreased oxygenation in group sepsis probably reflects pulmonary edema in sepsis-induced lung damage. Therefore, it is difficult to determine the role of diaphragmatic dysfunction in sepsis-related respiratory failure because sepsis simultaneously provokes acute lung injury unrelated to damage of respiratory muscle. To our knowledge, there is no definitive clinical evidence to indicate that sepsis-induced diaphragmatic dysfunction causes acute respiratory failure. We could not analyze CLP-induced inflammation separately into systemic and local inflammatory processes because of its unique nature. These drawbacks lead us to conclude that the clinical relevance of the current study in uncertain.

The current study has other major limitations regarding clinical relevance. There may be species differences in proportions and sizes of diaphragmatic muscle fiber types, blood vessel distribution, immunologic (mediators) responses to sepsis, and diaphragmatic responses to mediators. In the current study, all hamsters in group sepsis did not have hypercapnia, probably because of the short experimental period (only 24 h). A longer observation time may have elicited different effects of propofol. Propofol may aggravate hemodynamic derangement in sepsis. Therefore, we are unable to simply extrapolate our experimental findings to humans.

A potential negative aspect of the antioxidant activity of propofol includes a possible increase in severity of sepsis. Our data in the current study are unable to provide an obvious solution to this problem because we did not assess the effect of propofol on the bactericidal system in this setting. Although a small number of samples in the current study preclude definitive conclusions, propofol does not seem to increase mortality as a result of sepsis. However, further studies are necessary to assess whether propofol would enhance susceptibility to infection or aggravate sepsis.

In conclusion, we have shown that propofol dose-dependently attenuated sepsis-induced impairment of diaphragm function (contractility and fatigability). The attenuation may be caused, in part, by a reduction of diaphragmatic lipid peroxidation. This protection for the muscle cells with propofol is ascribed partly for suppressed reactive NO species formation, such as peroxynitrite and dinitrogen trioxide. A therapeutic effect of propofol on diaphragmatic dysnfunction, once developed, remains to be elucidated.

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