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The Effect of Ketamine Anesthesia on the Immune Function of Mice with Postoperative Septicemia

Tetsuya Takahashi, MD, PhD,* Manabu Kinoshita, MD, PhD,† Satoshi Shono, MD,† Yoshiko Habu, PhD,† Takahiro Ogura, MD,* Shuhji Seki, MD, PhD,† and Tomiei Kazama, MD, PhD*

BACKGROUND: It is unknown how ketamine anesthesia immunologically affects the outcome of patients with postoperative septicemia. We investigated the effects of ketamine anesthesia on mice with an *Escherichia coli* or lipopolysaccharide (LPS) challenge after laparotomy, focusing on phagocytosis by liver macrophages (Kupffer cells) and cytokine production.

METHODS: C57BL/6 mice received ketamine or sevoflurane anesthesia during laparotomy, which was followed by an *E. coli* or LPS challenge; thereafter, mouse survival rates and cytokine secretions were examined. The effects of a β-adrenoceptor antagonist, nadolol, on ketamine anesthesia were also assessed to clarify the mechanisms of ketamine-induced immunosuppressive effects.

RESULTS: Ketamine anesthesia increased the mouse survival rate after LPS challenge after laparotomy compared with sevoflurane anesthesia, whereas such an effect of ketamine was not observed after *E. coli* challenge. Ketamine suppressed tumor necrosis factor (TNF) and interferon (IFN)-γ secretion after LPS and *E. coli* challenge. When bacterial growth was inhibited using an antibiotic, ketamine anesthesia effectively improved mouse survival after *E. coli* challenge compared with sevoflurane anesthesia. Neutralization of TNF also improved survival and decreased IFN-γ secretion after bacterial challenge in antibiotic-treated mice with sevoflurane anesthesia, suggesting that ketamine's suppression of TNF may improve survival. Ketamine also suppressed in vivo phagocytosis of microspheres by Kupffer cells in LPS-challenged mice. Concomitant use of nadolol with an anesthetic dose of ketamine did not restore TNF suppression in LPS-challenged mice, suggesting a mechanism independent of the β-adrenergic pathway. However, it restored TNF secretion under low-dose ketamine (10% anesthetic dose). In contrast, nadolol restored the decrease in phagocytosis by Kupffer cells, which was induced by the anesthetic dose of ketamine via the β-adrenergic pathway, suggesting distinct mechanisms.

CONCLUSION: Ketamine suppresses TNF production and phagocytosis by Kupffer cells/macrophages. Therefore, unless bacterial growth is well controlled (by an antibiotic), postoperative infection might not improve despite reduction of the inflammatory response. (Anesth Analg 2010;111:1051–8)

Anesthesia affects the immune functions of patients. The decision to use one or a combination of anesthetics might affect both patient outcome and prognosis.[1] A combination of anesthetics also might have affected the tumor metastasis in experimental animals.[2] Ketamine is an anesthetic commonly used for patients and laboratory animals with septicemia or trauma. Ketamine reduces proinflammatory cytokine production, and thereby improves the survival rate in lipopolysaccharide (LPS)-induced septicemia models.[3–5] Whereas ketamine reportedly also improves the survival rate of animals after *E. coli* infection,[4] other reports have demonstrated that ketamine does not affect the outcome of sepsis using not only C3H/HeN mice but also C3H/HeJ mice initiated by cecal ligation and puncture.[6] Moreover, it has been reported that ketamine increases mouse mortality after cecal ligation and puncture.[7] In addition, studies of human polymorphonuclear leukocytes have demonstrated that ketamine attenuates not only proinflammatory cytokine secretion but also phagocytosis and bactericidal activity in vitro.[8–11] These equivocal results may be attributed in part to the different septic models used. Nonetheless, a detailed immunological analysis of ketamine anesthesia is required to fully understand the ramifications of its clinical use.

Sepsis is defined as a systemic inflammatory response syndrome (SIRS) induced by infection.[12] If SIRS occurs in the absence of massive bacterial infection, suppressing inflammatory responses and cytokine production may increase host survival.[13,14] In contrast, when the inflammatory response is insufficient and production of proinflammatory cytokines is inadequate, the host cannot eliminate the invading bacteria.[15–17] Thus, inflammatory responses induced in the presence and absence of bacterial infection must be considered separately to fully understand the processes that are critical to survival.

Ketamine stimulates the β-adrenergic pathway in sympathetic nerves and induces the production of catecholamines.[18,19] β-Adrenoceptor agonists such as adrenaline and isoproterenol (catecholamine) suppress LPS-induced

From the *Department of Anesthesiology, National Defense Medical College, 3-2 Namiki Tokorozawa Saitama Japan 359-8513, †Department of Immunology and Microbiology, National Defense Medical College, 3-2 Namiki Tokorozawa Saitama Japan 359-8513.

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Address correspondence to Shuhji Seki, MD, Department of Immunology and Microbiology, National Defense Medical College, 3-2 Namiki, Tokorozawa, 359-8613 Japan. Address e-mail to btraums@ndmc.ac.jp

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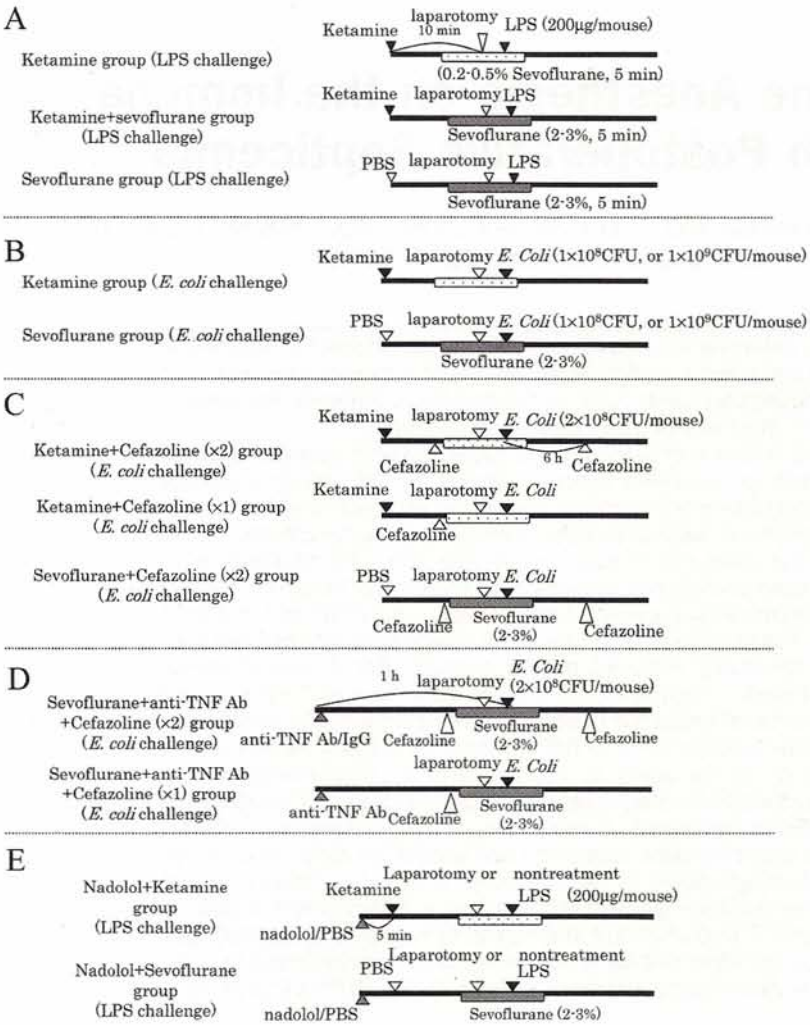

Figure 1. Experimental design of ketamine anesthesia in mice.

tumor necrosis factor (TNF) production[20–22] and natural killer (NK) cell activity.[23–25] Therefore, ketamine-induced sympathetic nerve stimulation may impair the host immune system. Nevertheless, there is only limited understanding of how ketamine anesthesia affects cytokine production and phagocytosis by macrophages and liver Kupffer cells and how the ketamine-stimulated β-adrenergic pathway affects host immune functions. We hypothesized that ketamine anesthesia during surgery may change the proinflammatory cytokine response against LPS and bacteria challenges and may also affect phagocytosis of bacteria by Kupffer cells. Based on this hypothesis, we found the immune-suppressive effect of ketamine on Kupffer cells and its possible mechanism.

METHODS

Animals and Regents

All experiments were approved by the National Defense Medical College Institution Animal Care and Use Committee. Male C57BL/6 mice (10 weeks old, 25 g) were obtained from SLC, Inc. (Shizuoka, Japan). *Escherichia coli* strain B (ATCC11303, Sigma-Aldrich, St. Louis, MO) and LPS (*E. coli* 0111: B4, Sigma-Aldrich) were used for experiments. Ketamine (preservative-free; Daiichi Sankyo Propharma, Kanagawa, Japan) and sevoflurane (Maruishi, Osaka, Japan) were used as anesthetics. α-Galactosylceramide (α-GalCer) was kindly provided by Pharmaceutical Research Laboratory, Kirin Brewery, Takasaki, Japan.

Ketamine Anesthesia and Surgical Intervention

Mice were injected intraperitoneally (IP) with an anesthetic dose of ketamine (100 mg/kg/0.5 mL) or phosphate buffer sodium (PBS) (0.5 mL) 10 minutes before laparotomy. During laparotomy, mice were anesthetized with approximately 0.2% to 0.5% sevoflurane (ketamine group, n = 25) or approximately 2% to 3% sevoflurane (ketamine plus sevoflurane group, n = 25) for 5 minutes after ketamine injection or approximately 2% to 3% sevoflurane for 5 minutes (sevoflurane group, n = 25) in room air via a vaporizer (Fig. 1A). During laparotomy, we must sometimes change the concentration of sevoflurane to regulate the movement and respiratory conditions of mice during surgical maneuvers, as described in our previous study.[2] The 3-cm midline incision was made for the laparotomy and was closed in layers using 4 to 0 silk sutures. An anesthetic dose of ketamine alone cannot satisfactorily sedate mouse movement during laparotomy. Also, ketamine is not clinically used without other anesthetics. We

studied the effects of anesthetic methods using ketamine but not the direct pharmacological effect of ketamine per se. Therefore, a small dose of sevoflurane inhalant (approximately 0.2%–0.5%) was administered to ketamine-treated mice during the laparotomy to obtain the appropriate sedative state.

E. coli or LPS Challenge

Immediately after wound closure, mice were challenged IP with a lethal (1×10^9 colony-forming units (CFU)/mouse) or sublethal (1×10^8 CFU/mouse) dose of *E. coli* (n = 25 in each group) (Fig. 1B). Antibiotic-treated mice were also challenged IP with 2×10^8 CFU/mouse of *E. coli* immediately after laparotomy (Fig. 1C). Similarly, mice were challenged IP with LPS (200 μg/mouse) after laparotomy (Fig. 1A, n = 25 in each group).

Antibiotic Treatment (Double and Single Injections)

Tail vein injections of cefazolin sodium (50 mg/kg) were administered to mice in both the ketamine (with approximately 0.2%–0.5% sevoflurane) and sevoflurane (approximately 2%–3%) groups, one immediately before the laparotomy and the second 6 hours after the *E. coli* challenge (n = 20 in each group). A single injection with cefazolin sodium (50 mg/kg) was also given to ketamine-anesthetized mice just before the laparotomy but not after bacterial challenge (Fig. 1C, n = 25).

Neutralization of TNF with Antibiotic Treatment

Anti-TNF neutralizing antibody (0.5 mg/mouse, MP6-XT3; BD Pharmingen) or rat IgG was injected via IV 1 hour before *E. coli* challenge (2×10^8 CFU/mouse) after laparotomy. The mice received double or single injections with cefazolin sodium, as described above (Fig. 1D, n = 20 in each group).

Pretreatment with Nadolol

Nadolol is a nonselective β-adrenoceptor antagonist. Mice were injected with nadolol (10 mg/kg/0.2 mL) or 0.2 mL PBS via the tail vein 5 minutes before ketamine/PBS treatment. The mice were then laparotomized, followed by LPS challenge (Fig. 1E, n = 5 in each group). To precisely evaluate the relationship between ketamine and nadolol, some mice did not receive laparotomy, thereby avoiding sevoflurane treatment. Ten minutes after ketamine/PBS treatment, these mice were similarly challenged IP with LPS (n = 5 in each group). To examine the affect of nadolol on mice treated with a low dose of ketamine, mice were also injected IP with 10 mg/kg ketamine (10% of the anesthetic induction dose) 5 minutes after the nadolol/PBS treatment (n = 5 in each group).

Cytokine Measurements and Bacterial Counts

Blood samples of individual mice were obtained from the retro-orbital sinus immediately before anesthesia administration and at 1, 3, 6, 12, 24, and 48 hours after *E. coli* or LPS challenge. Sera and culture supernatants of mononuclear cells (MNCs) were detected using Enzyme-Linked Immuno-Sorbent Assay (ELISA) kits (TNF and interferon [IFN]-γ, BD Pharmingen, San Diego, CA; IL-12, Endogen, Woburn, MA). Livers were aseptically removed and homogenized in PBS. Homogenates were serially diluted 10-fold with PBS, plated on brain heart infusion agar, and then incubated at 37°C for 24 hours for colony counting.

Isolation of Peripheral Blood Leukocytes, Liver, and Spleen MNCs, and In Vitro LPS Stimulation

Blood samples were obtained from mice by cardiac puncture, and leukocytes were separated by hemolysis. Liver MNCs were prepared as previously described.[13,26,27] In brief, liver specimens were minced, placed in 0.05% collagenase solution (Wako, Osaka, Japan), shaken for 20 minutes in a 37°C water bath, and passed through steel mesh. After mixing in 33% Percoll, the samples were centrifuged at 500*g* for 20 minutes at room temperature. Liver MNCs were obtained after the red blood cells were lysed. Splenocytes were also obtained after the spleen was passed through a mesh and the red blood cells were lysed. The cells (5×10^5 per well in 200 μL Roswell Park Memorial Institute [RPMI] 1640 supplemented with 10% fetal bovine serum in 96 well flat–bottomed plates) were incubated with ketamine (1×10^{-6} M) at 37°C, in 5% CO_2 for 1 hour. Subsequently, the cells were incubated with LPS (200 ng/mL) for 2 hours. Three individual experiments were performed, with three or four samples per each group.

In Vivo Phagocytosis of Microspheres by Kupffer Cells

Mice were injected with 20 μL of Fluoresbrite YG (FITC) Carboxylate Microspheres (75-nm diameter; Polysciences Europe, Eppelheim, Germany) (hereafter called FITC-microspheres) via tail veins, immediately after LPS/PBS challenge (n = 5 in each group). One hour later, liver MNCs were isolated. After incubation for 10 minutes with an Fc-blocker (2.4 G2; Pharmingen) to prevent nonspecific binding, the MNCs were labeled with PE-conjugated anti-CD11b mAb and PC5-conjugated anti-Gr-1 mAb. Kupffer cells stain positively with CD11b but are negative for Gr-1 staining.[15] $CD11b^+Gr\text{-}1^-$ Kupffer cells were analyzed for in vivo phagocytosis by flow cytometry (EPICS XL, Coulter, Miami, FL).

Statistical Analysis

The data are presented as means ± SE. Statistical analysis was performed using Graphpad-Prism software, version 4.00 (Graphpad Software, Inc., San Diego, CA). Survival frequencies were compared using log rank tests. Changes in serum cytokine concentrations were compared using two-way analysis of variance (ANOVA). Other cytokine levels between groups or among several groups were compared using the Student *t* test or one-way ANOVA followed by the Newman-Keuls test. The survival of antibiotic-treated mice was evaluated using Fisher exact test. The level of significance was set at $P < 0.05$. Sample sizes were chosen based on data in other papers in this field.[3,5,28–30]

RESULTS

Ketamine Anesthesia Improves Survival After Laparotomy with LPS Challenge and Suppresses Elevation of Serum Proinflammatory Cytokines

Both ketamine-treated groups (anesthetized either with 0.2%–0.5% sevoflurane or 2%–3% sevoflurane) showed

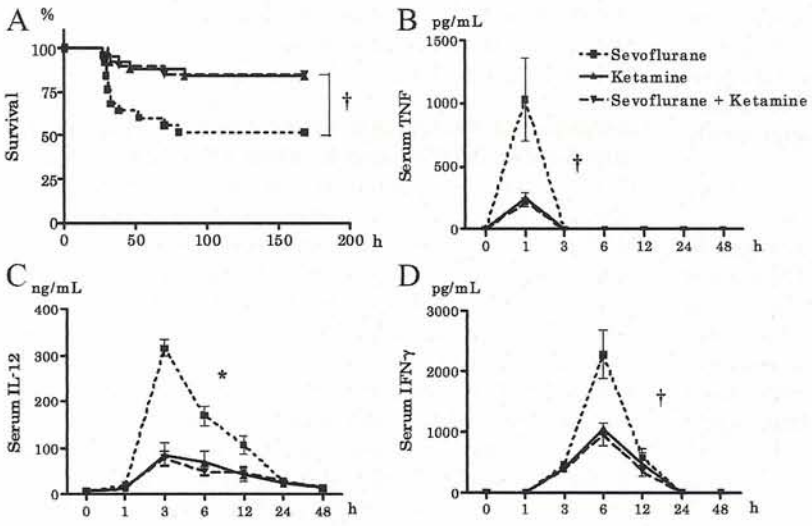

Figure 2. The effect of ketamine anesthesia on survival (A), serum tumor necrosis factor (TNF) (B), interleukin (IL)-12 (C), and interferon (IFN)-γ (D) levels in mice after lipopolysaccharide (LPS) challenge after laparotomy. Mice were anesthetized with ketamine +0.2% to 0.5% sevoflurane (ketamine group), ketamine +2% to 3% sevoflurane (sevoflurane + ketamine group) or 2% to 3% sevoflurane (sevoflurane group). Data are the means ± SE, n = 25 in each group. $*P < 0.01$, $\dagger P < 0.05$ vs other groups.

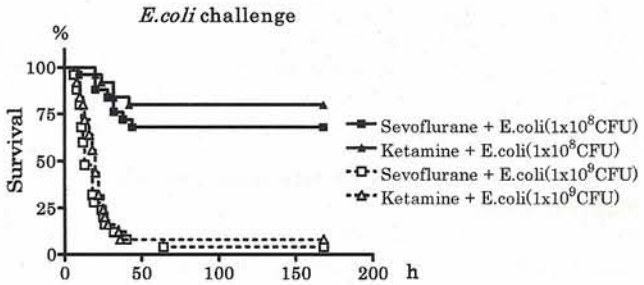

Figure 3. The effects of ketamine anesthesia on mouse survival after *E. coli* challenge after laparotomy. Mice received ketamine anesthesia (+0.2%–0.5% sevoflurane) or sevoflurane (2%–3%) anesthesia. Thereafter, they were challenged IP with sublethal (1×10^8 colony-forming units (CFU)/mouse) or lethal (1×10^9 CFU/mouse) doses of *E. coli* after laparotomy; n = 25 in each group.

Table 1. Serum TNF and IFN-γ Levels in Mice After *E. coli* Challenge Following Laparotomy

	Sevoflurane	Ketamine
Serum TNF at 1 h		
1×10^8 CFU	119 ± 14	66 ± 2*
1×10^9 CFU	404 ± 53	146 ± 19*
Serum IFN-γ at 6 h		
1×10^8 CFU	396 ± 63	145 ± 32*
1×10^9 CFU	1103 ± 181	530 ± 51*

TNF = tumor necrosis factor; IFN = interferon.
Sera were obtained from the mice 1 hour and 6 hours after *E. coli* challenge to measure serum TNF and IFN-γ, respectively. Data are means ± SE (pg/mL) from 25 mice in each group. * $P < 0.01$ vs sevoflurane.

significantly higher survival rates and greater suppression of serum peaks of TNF, interleukin (IL)-12, and IFN-γ compared with the sevoflurane (approximately 2%–3%) group (Fig. 2). However, the two ketamine-treated groups showed similar survival rates and serum cytokine levels, suggesting that ketamine increases mouse survival and suppresses proinflammatory cytokine secretion. The differences observed between the mice treated with ketamine/low-dose sevoflurane (ketamine group) versus high-dose sevoflurane alone (sevoflurane group) might not have been due to the use of a lower dose of sevoflurane but due to the use of ketamine. Therefore, the affect of ketamine anesthesia on the ketamine (with low-dose sevoflurane) and sevoflurane groups was compared in further experiments.

Ketamine Anesthesia Does Not Increase Survival After Laparotomy with *E. coli* Challenge Despite Suppression of Serum TNF and IFN-γ

Mice in the ketamine group did not survive at higher rates after laparotomy with *E. coli* (1×10^8 CFU/mouse) challenge compared with those in the sevoflurane group (Fig. 3), despite significant suppression of TNF and IFN-γ (Table 1). Ketamine anesthesia also failed to increase survival after laparotomy with lethal *E. coli* challenge (1×10^9 CFU/mouse) (Fig. 3), despite suppression of TNF and IFN-γ (Table 1).

Ketamine Anesthesia Plus Antibiotic Therapy Increases Survival After Laparotomy with *E. coli* Challenge

Unlike LPS challenge, bacterial growth and proliferation could affect the survival of *E. coli*–challenged mice after ketamine treatment. Because perioperative septic patients are usually treated with antibiotics, *E. coli*–challenged mice were given two injections of cefazolin. In addition, TNF was depleted in mice receiving sevoflurane anesthesia and cefazolin injections to determine if TNF levels decreased by ketamine might explain the increased survival of cefazolin-treated mice in postoperative infection. Concomitant use of cefazolin with ketamine anesthesia significantly increased survival after *E. coli* challenge with suppression of TNF and IFN-γ compared with sevoflurane anesthesia alone (Fig. 4, Table 2). The TNF-depleted mice also showed a marked increase in survival after postlaparotomy *E. coli* challenge after sevoflurane anesthesia (Fig. 4) with significant suppression of IFN-γ (Table 2).

Suppression of TNF Decreases Survival in *E. coli*–Challenged Mice Under Insufficient Regulation of Bacterial Growth

TNF-neutralizing antibodies (Ab) was administered to single cefazolin-treated mice before laparotomy (but not after *E. coli* challenge) followed by sevoflurane anesthesia.

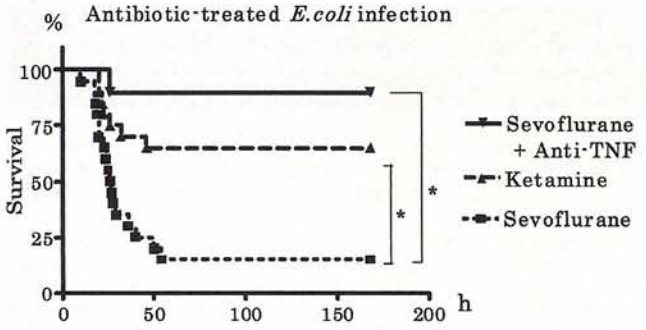

Figure 4. The effect of ketamine anesthesia and neutralizing tumor necrosis factor (TNF) + antibiotic therapy on mouse survival after sublethal *E. coli* challenge after laparotomy. Mice received ketamine anesthesia, sevoflurane anesthesia, or sevoflurane anesthesia after TNF neutralization. They were then treated twice with cefazolin and challenged IP with 2×10^8 *E. coli* colony-forming units (CFU)/mouse after laparotomy; n = 20 in each group, $*P < 0.01$.

Table 2. Serum TNF and IFN-γ Levels in Mice After Antibiotic-Treated *E. coli* Challenge Following Laparotomy

	Sevoflurane	Sevoflurane + Anti-TNF Ab	Ketamine
Serum TNF	172.0 ± 23.2*	Undetected	58.6 ± 2.2*
Serum IFN-γ	649.3 ± 86.7*	124.5 ± 13.1	146.7 ± 18.1

TNF = tumor necrosis factor; IFN = interferon; Ab = antibodies.
Cefazolin (double injections)-treated mice were injected IV with anti-TNF neutralizing Ab or rat IgG, as a control, 1 hour before *E. coli* challenge. Sera were obtained from the mice at 1 hour and 6 hour to measure serum TNF and IFN-γ, respectively. Data are means ± SE (pg/mL) from 20 mice in each group. * $P < 0.01$ vs other groups.

Table 3. The Effect of Neutralizing Anti-TNF Antibody on Survival After *E. coli* Challenge of Mice Treated by a Single Injection of Antibiotic

	Survival	
	Sevoflurane	Sevoflurane + Anti-TNF-Ab
36 h	15/20	17/20
72 h (3 d)	13/20	4/20*
168 h (7 d)	12/20	3/20*

TNF = tumor necrosis factor; Ab = antibodies.
Mice were injected IV with anti-TNF neutralizing antibody or rat IgG 1 hour before *E. coli* challenge following laparotomy. Cefazolin was injected once immediately before laparotomy. * $P < 0.05$ vs sevoflurane.

Most survived the initial 36-hour period but eventually died 7 days after infection (Table 3). Bacteria were then counted in the livers of TNF-depleted mice treated with cefazolin (one or two injections) or without cefazolin 24 hours after *E. coli* challenge. A single injection of cefazolin did not effectively suppress bacterial growth (counts) in the liver compared with double injections (single; 1.1 ± 0.4 versus double; 0.1 ± 0.1 × 10^6 CFU, $P < 0.05$, n = 6 in each group), although growth was significantly inhibited in antibiotic-treated mice (without antibiotic injection; 5.1 ± 0.3 × 10^6 CFU, $P < 0.01$, n = 6). Because ketamine suppressed TNF secretion, survival after *E. coli* challenge was examined in the mice receiving ketamine anesthesia that were treated with a single injection of cefazolin. During the initial 36 hours, these mice exhibited a rate of survival comparable to the mice with two cefazolin injections but showed significantly lower survival at 7 days, although both antibiotic treatments increased mouse survival after bacterial challenge (Table 4). These findings suggest that unless bacterial growth/proliferation is well controlled (by repeated antibiotic injections), suppression of TNF (by neutralizing Ab or ketamine) may adversely affect surgical patients with bacterial infections.

Table 4. The Effect of Antibiotic Treatment on Survival After *E. coli* Challenge Following Laparotomy in Ketamine-Anesthetized Mice

	Survival		
	Without injection	Single injection	Double injections
36 h	10/25*	20/25	21/25
72 h (3d)	5/25*	17/25	20/25
168 h (7d)	4/25*	12/25§	19/25

Ketamine-anesthetized mice were treated with a single injection of cefazolin, double injections, or without injection. * $P < 0.01$ vs other groups, § $P < 0.05$ vs double injections.

Table 5. The Effect of In Vitro Ketamine Treatment on LPS-Stimulated TNF Production

	Ketamine	
	Without ketamine	With ketamine (10^{-6} M)
Circulating leukocytes	240 ± 20	111 ± 20*
Liver MNCs	548 ± 31	278 ± 20*
Spleen MNCs	200 ± 12	82 ± 5*

LPS = lipopolysaccharide; MNCs = mononuclear cells.
Data are means ± SE (pg/mL) from three individual experiments with two samples per each group. * $P < 0.05$ vs without ketamine.

Table 6. The Effect of Ketamine Treatment on Phagocytosis by Kupffer Cells in LPS-Challenged Mice

Without laparotomy				
Treatment	PBS	Ketamine	PBS	Ketamine
Challenge	PBS	PBS	LPS	LPS
Phagocytosis (%)	13.6 ± 1	7.5 ± 0.8*	20.7 ± 1.1*	14.02 ± 1.1
Laparotomy				
Anesthesia	Sevoflurane	Ketamine	Sevoflurane	Ketamine
Challenge	PBS	PBS	LPS	LPS
Phagocytosis (%)	14.9 ± 0.5	9.4 ± 0.8*	21.4 ± 0.7*	14.6 ± 0.6

PBS = phosphate buffer sodium; LPS = lipopolysaccharide.
Proportion of microsphere phagocytosis by Kupffer cells are shown as means ± SE from 5 mice in each group. * $P < 0.05$ vs other groups.

Ketamine Suppresses LPS-Induced TNF Production by Cultured Lymphocytes In Vitro

Coculturing with ketamine significantly suppressed TNF production from blood leukocytes, liver, or spleen MNCs stimulated by LPS in vitro, suggesting that ketamine directly suppresses LPS-induced TNF production by these cells (Table 5).

Ketamine Attenuates In Vivo Phagocytic Activity of Kupffer Cells in LPS-Challenged Mice

Ketamine treatment significantly decreased phagocytosis by Kupffer cells in mice challenged with PBS (Table 6).

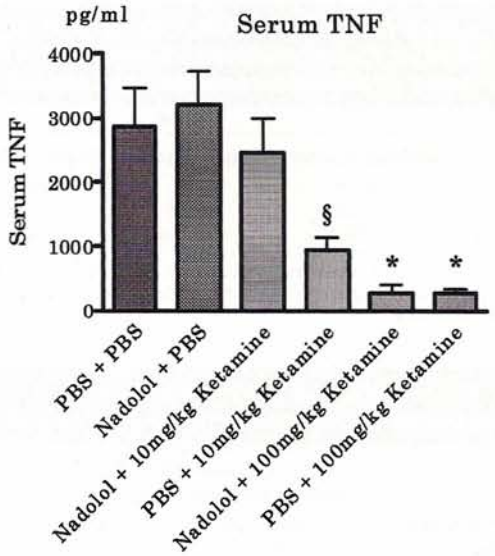

Figure 5. The effect of nadolol on serum tumor necrosis factor (TNF) levels after lipopolysaccharide (LPS) challenge in mice given the anesthetic or low dose of ketamine. After pretreatment with nadolol or phosphate buffer sodium (PBS), mice were treated with ketamine (100 mg kg^{-1} = anesthetic induction dose; 10 mg kg^{-1} = 10% anesthetic induction dose) or PBS followed by LPS challenge. Sera were obtained from the mice 1 hour after LPS challenge. Data are the means ± SE from five mice in each group. §$P < 0.05$, *$P < 0.01$ vs other groups.

Table 7. The Effect of Nadolol on the Serum TNF Levels After LPS Challenge Following Laparotomy

Pretreatment	PBS	PBS	Nadolol	Nadolol
Anesthesia	Sevoflurane	Ketamine	Sevoflurane	Ketamine
Serum TNF levels at 1 h	989 ± 74	248 ± 47*	1020 ± 109	239 ± 34*

TNF = tumor necrosis factor; LPS = lipopolysaccharide;
Data are means ± SE (pg/mL) from 5 mice in each group. * $P < 0.01$ vs sevoflurane.

Although LPS challenge increased phagocytosis by Kupffer cells, ketamine treatment also significantly decreased their phagocytosis (Table 6). Although laparotomy alone did not significantly affect the phagocytic activity by Kupffer cells, ketamine anesthesia significantly decreased the phagocytosis after laparotomy (Table 6).

The Effect of Nadolol Pretreatment on Ketamine-Suppressed Proinflammatory Cytokine Response or Phagocytosis in LPS-Challenged Mice

Although the anesthetic dose of ketamine (100 mg/kg) markedly suppressed serum TNF levels 1 hour after LPS challenge in mice (without laparotomy), nadolol pretreatment did not abolish the ketamine-induced suppression of TNF (Fig. 5). A low dose of ketamine (10 mg/kg, 10% of the anesthetic dose) also suppressed serum TNF levels after LPS challenge; however, nadolol pretreatment significantly restored TNF levels in mice (Fig. 5). Further examination of LPS-induced TNF secretion after laparotomy revealed that laparotomy obviously suppressed LPS-induced TNF elevation in sevoflurane-anesthetized mice (Table 7, Fig. 5). Many investigators have also demonstrated that surgical

Table 8. The Effect of Nadolol on Phagocytosis by Kupffer Cells in With or Without Ketamine-Treated Mice

Without laparotomy				
Pretreatment	PBS	PBS	Nadolol	Nadolol
Anesthesia	PBS	Ketamine	PBS	Ketamine
Phagocytosis (%)	14.5 ± 0.7	8.4 ± 0.5*	15.8 ± 1.3	15.8 ± 1.5
Laparotomy				
Pretreatment	PBS	PBS	Nadolol	Nadolol
Anesthesia	Sevoflurane	Ketamine	Sevoflurane	Ketamine
Phagocytosis (%)	15.8 ± 0.9	9.6 ± 0.8*	16.7 ± 1.0	15.0 ± 0.9

PBS = phosphate buffer sodium.
Proportion of microsphere phagocytosis by Kupffer cells are shown as means ± SE from 5 mice in each group. * $P < 0.05$ vs other groups.

stress such as surgery or traumatic injury induces hyporesponsiveness of TNF secretion to LPS, in particular, the early phase after surgery,[31–34] but TNF suppression by ketamine anesthesia (100 mg/kg) did not significantly differ in mice with (Table 7) or without laparotomy (Fig. 5).

Phagocytosis by Kupffer cells was examined in LPS-challenged mice (without laparotomy) that received an anesthetic dose of ketamine. Unlike the TNF response, nadolol almost completely restored the ketamine-suppressed phagocytic activity of Kupffer cells (Table 8). This restoration by nadolol was also observed in laparotomized mice (Table 8).

DISCUSSION

Ketamine anesthesia improved survival in LPS-challenged mice but not in mice challenged with viable *E. coli*. Nevertheless, the survival rate after *E. coli* challenge was improved by the concomitant use of an antibiotic and ketamine anesthesia, suggesting that inhibition of bacterial growth critically influences the ketamine-induced beneficial effect on survival.

Since the septic condition induced by LPS is merely a result of SIRS without infection, suppression of proinflammatory cytokines by ketamine improves mouse survival. In contrast, septicemia after *E. coli* challenge is a result of infection accompanied by SIRS. TNF and IFN-γ are important for the elimination of invading bacteria, although they also induce shock and organ injury.[13,14,16] The suppression of proinflammatory cytokines by ketamine may thereby attenuate bacterial elimination. Ketamine also decreased the phagocytic activity of Kupffer cells, indicating further impairment of bacterial clearance. These findings may explain why ketamine (without antibiotic) could not improve the survival of *E. coli*–infected mice. A subanesthetic dose of ketamine reportedly suppressed LPS-stimulated TNF production by peripheral blood MNCs in patients,[11] suggesting that ketamine, even at a low dose, suppresses LPS-induced TNF production in humans and mice.

IL-12 and α-GalCer, a synthetic NKT cell ligand, were used to determine whether ketamine directly affects IFN-γ production from NK and/or NKT cells. Exogenous IL-12 directly induces IFN-γ production by mouse NKT cells.[35–37] α-GalCer also directly stimulates IFN-γ production from NKT cells and subsequently from NK cells.[2,28,38–41] Ketamine anesthesia did not suppress serum IFN-γ levels after IL-12 or α-GalCer challenge in mice (data not shown), suggesting that the IFN-γ

producing capacities of NK/NKT cells are not directly affected by ketamine anesthesia but are suppressed primarily through reduced IL-12 production from macrophages/Kupffer cells.

Sevoflurane anesthesia for 15 to 30 minutes reportedly suppresses LPS-induced TNF secretion in mice.[42,43] In this study, sevoflurane was only administered for 5 minutes. The TNF elevation observed in the ketamine plus high-dose sevoflurane group was similar to that seen after LPS challenge to the ketamine plus low-dose sevoflurane group, suggesting that ketamine-induced suppression of TNF is independent of sevoflurane dose.

Nadolol did not inhibit suppression of TNF secretion by an anesthetic dose of ketamine (100 mg/kg), although it significantly restored TNF secretion under low-dose ketamine (10 mg/kg). Low-dose ketamine also reportedly increased serum adenosine levels (0.4 μM) and thereby inhibited secretion of proinflammatory cytokines after LPS challenge,[5] as evidenced by use of an adenosine receptor antagonist. However, this study did not examine how the anesthetic dose of ketamine affects serum adenosine levels or proinflammatory cytokine levels. Propranolol (another β-adrenoceptor blocker) inhibited the secretion of adenosine from the kidneys after electric stimulation of the periarterial sympathetic nerve.[44] Therefore, adenosine and, presumably, adrenaline appear to be responsible for the reduction of TNF production by activated-macrophages after a low dose of ketamine. However, the direct effect (non-β-adrenergic pathway) of an anesthetic dose of ketamine may overcome or overwhelm the inhibitory effect via the β-adrenergic pathway by nadolol (as seen with low-dose ketamine) and further suppress TNF production. The addition of ketamine to cultures consistently suppressed LPS-induced TNF production from isolated MNCs in vitro, suggesting a direct suppression of TNF by ketamine.

The results of this study also demonstrated for the first time that ketamine anesthesia significantly inhibited in vivo phagocytic activity by Kupffer cells. Previous in vitro studies had shown that ketamine did not inhibit phagocytosis by either polymorphonuclear leukocytes or macrophages at clinical concentrations, although it could suppress at higher concentrations.[9,10,45] Interestingly, inhibiting the β-adrenergic pathway by nadolol restored ketamine-suppressed phagocytosis by Kupffer cells. The anesthetic dose of ketamine might directly suppress TNF secretion in LPS-challenged mice independent of the β-adrenergic pathway, while also indirectly suppressing phagocytosis by Kupffer cells via the β-adrenergic pathway. Inhibiting the β-adrenergic pathway by nadolol may be effective in ketamine anesthesia for septic patients because it might abrogate the enhanced catecholamine effect. However, when nadolol was administered to *E. coli*–challenged mice under ketamine anesthesia without using an antibiotic, survival was not improved (unpublished observations). Nadolol may induce heart failure and severe hypotension in mice with septicemia under ketamine anesthesia and thereby mask restoration of the phagocytic function of macrophages.

Ketamine anesthesia may be preferred for septic patients because of its suppressive effects of proinflammatory cytokines. However, ketamine also might decrease bacterial phagocytosis by macrophages/Kupffer cells, which presumably enhance bacterial growth/proliferation in perioperative septic patients. Therefore, antibiotic treatment could be important for septic patients who received ketamine anesthesia. It should also be noted that ketamine reportedly injures Kupffer cells and endothelial cells and may affect liver functions.[46]

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