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Suppressive effects of ketamine on macrophage functions

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Abstract

Ketamine is an intravenous anesthetic agent. Clinically, induction of anesthesia with ketamine can cause immunosuppression. Macrophages play important roles in host defense. In this study, we attempted to evaluate the effects of ketamine on macrophage functions and its possible mechanism using mouse macrophage-like Raw 264.7 cells as the experimental model. Exposure of macrophages to 10 and 100 μM ketamine, which correspond to 0.1 and 1 times the clinically relevant concentration, for 1, 6, and 24 h had no effect on cell viability or lactate dehydrogenase release. When the administered concentration reached 1000 μM, ketamine caused a release of lactate dehydrogenase and cell death. Ketamine, at 10 and 100 μM, did not affect the chemotactic activity of macrophages. Administration of 1000 μM ketamine in macrophages resulted in a decrease in cell migration. Treatment of macrophages with ketamine reduced phagocytic activities. The oxidative ability of macrophages was suppressed by ketamine. Treatment with lipopolysaccharide induced TNF-α, IL-1β, and IL-6 mRNA in macrophages. Administration of ketamine alone did not influence TNF-α, IL-1β, or IL-6 mRNA production. Meanwhile, cotreatment with ketamine and lipopolysaccharide significantly inhibited lipopolysaccharide-induced TNF-α, IL-1β, and IL-6 mRNA levels. Exposure to ketamine led to a decrease in the mitochondrial membrane potential. However, the activity of mitochondrial complex I NADH dehydrogenase was not affected by ketamine. This study shows that a clinically relevant concentration of ketamine (100 μM) can suppress macrophage function of phagocytosis, its oxidative ability, and inflammatory cytokine production possibly via reduction of the mitochondrial membrane potential instead of direct cellular toxicity.

Keywords: Ketamine; Macrophages; Chemotactic activity; Phagocytic activity; Oxidative ability; Inflammatory cytokines; Mitochondrial membrane potential

Introduction

Ketamine is an intravenous anesthetic agent used for induction and maintenance of anesthesia during surgical procedures. Because ketamine has more stable hemodynamics than barbiturates or inhaled anesthetic agents, this anesthetic agent is widely applied in critically ill patients as an inducer of anesthesia (White et al., 1982). Ketamine has relatively high clearance, which accounts for the short elimination half-life of less than 3 h in adults (Clements and Nimmo, 1981). Clinically, induction of anesthesia with ketamine may be associated with increases in cardiac output, arterial blood pressure, and heart rate (Reich and Silvay, 1989; Traber et al., 1971). In lipopolysaccharide (LPS)-stimulated leukocytes, ketamine was reported to attenuate cell adherence and migration (Hofbauer et al., 1998; Schmidt et al., 1995). In neutrophils, ketamine was shown to suppress oxidant production and chemotactic activity (Weigand et al., 2000; Zahler et al., 1999). Thus, those studies using leukocytes and neutrophils demonstrate that ketamine has possible immunomodulating effects.

Macrophages play critical roles in cellular host defense against infection and tissue injury (Nathan, 1987; Valledor and Ricote, 2004). In response to stimuli, macrophages undergo a series of inflammatory processes, including chemotaxis, phagocytosis, intracellular killing, and release

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of inflammatory cytokines (Aderem, 2001; Nathan, 1987). Dysfunction of macrophages may decrease host nonspecific cell-mediated immunity (Lander, 1997). In our previous study, we showed that propofol, another popular intravenous anesthetic agent, can suppress macrophage chemotactic activity, phagocytic activity, oxidative ability, and inflammatory cytokine production (Chen et al., 2003a, 2003b). However, few studies have evaluated the effects of ketamine on macrophages. In LPS-activated macrophages, ketamine was reported to reduce TNF-α and nitric oxide production (Sakai et al., 2000; Shimaoka et al., 1996; Takenaka et al., 1994). Effects of ketamine on other macrophage functions are still unknown.

Mitochondria are important energy-producing organelles and participate in macrophage activation (Brown and Borutaite, 1999; Diehl and Hoek, 1999). Previous studies showed that adenosine triphosphate (ATP), synthesized from the mitochondrial respiratory chain reaction, can enhance chemotactic migration and phagocytic ingestion of macrophages and neutrophils through the purinergic P2 receptor pathway or by elevation of intracellular Ca^{2+} (Di Virgilio et al., 2001; Fredholm, 1997; Lammas et al., 1997; Oshimi et al., 1999). In murine polymicrobial sepsis, a decrease in cellular ATP levels was reported to be associated with marked suppression of the functions of lymphocytes and macrophages (Ayala and Chaudry, 1996). Maintenance of the mitochondrial membrane potential and complex I NADH dehydrogenase activity is critical for mitochondrial functions and ATP synthesis (Papucci et al., 2003; Pearce et al., 2001). However, there is a paucity of studies evaluating the effects of ketamine on macrophage mitochondria. In this study, we attempted to evaluate the effects of ketamine on macrophage functions from the aspects of cell viability, chemotaxis, phagocytosis, oxidative ability, and inflammatory cytokine (TNF-α, IL-1β, and IL-6) mRNA production and its possible mechanism from the viewpoint of mitochondrial membrane potential and complex I NADH dehydrogenase activity.

Materials and methods

Cell culture and drug treatment. A murine macrophage cell line Raw 264.7 was purchased from American Type Culture Collection (Rockville, MD, USA). Macrophages were cultured in RPMI 1640 medium (Gibco-BRL, Grand Island, NY, USA) supplemented with 10% fetal calf serum, L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml) in 75-cm^2 flasks at 37 °C in a humidified atmosphere of 5% CO_2. Cells grew to confluence prior to ketamine administration.

Ketamine was dissolved in phosphate-buffered saline (PBS) (0.14 M NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4). According to the clinical application, concentrations of ketamine at 10, 100, and 1000 μM, which correspond to 0.1, 1, and 10 times the clinical plasma concentration (Domino et al., 1982; Grant et al., 1983), were chosen as the administered dosages in this study. Prior to the addition of ketamine, macrophages were washed with PBS buffer, and nonadherent cells were removed. Control macrophages received PBS only.

Cytotoxic assay. Analyses of cell viability and lactate dehydrogenase release were carried out to determine the toxicity of ketamine to macrophages. Cell viability was analyzed using a trypan blue exclusion method. Briefly, macrophages (2×10^5 cells) were cultured in 24-well tissue culture plates (Corning-Costar, Cambridge, MA, USA) overnight. After administration of 10, 100, and 1000 μM ketamine for 1, 6, and 24 h, cells were trypsinized by 0.1% trypsin-EDTA (Gibco-BRL). Following centrifugation and washing, macrophages were suspended in PBS and stained with an equal volume of trypan blue dye (Sigma, St. Louis, MO, USA). Fractions of dead cells with a blue signal were determined using a reverse-phase microscope. The amounts of lactate dehydrogenase in the culture medium were quantified using a model 7450 automatic autoanalyzer system from Hitachi (Tokyo, Japan).

Chemotactic activity. The migrating capacity of macrophages was determined by using Costar Transwell cell culture chamber inserts, with a pore size of 8 μm, according to the application guide provided by Corning-Costar. The bottom layers of the Transwell membranes have been commercially coated with collagen. The RPMI 1640-rich medium (1.5 ml) was first added to 12-well tissue cluster plates, and the Transwell was inserted in the plates. Macrophages (1×10^5 cells) suspended with 10, 100, and 1000 μM ketamine in 0.5-ml-rich medium was added to the inside of the Transwell and cultured at 37 °C for 1, 6, and 24 h in an atmosphere of 5% CO_2. Macrophages that migrated to the bottom surface of polycarbonate filters were counted in each field and averaged for three fields with the aid of a crosshair micrometer (Nikon, Tokyo, Japan). The data were normalized to the numbers of living cells.

Phagocytic activity. The macrophage function of phagocytosis was assayed by detecting the number of cells that ingested at least one fluorescent particle according to the method of Kotani et al. (1998). Macrophages (1×10^6 cells) were cultured in 12-well tissue culture plates overnight. After exposure to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h, macrophages were trypsinized and suspended in PBS. Red fluorescent FluoSphere carboxylate-modified microspheres (Molecular Probes, Eugene, OR, USA), with a diameter of 0.5 μm, were added to the cell suspensions and incubated at 37 °C on a shaking platform for 20 min. The ratio of particles-to-cells was 15:1. The reaction was stopped by immersion in an ice-cold saline solution. Proportions of macrophages that ingested at least one particle were counted with the aid of a crosshair micrometer (Nikon). The data were normalized to the numbers of living cells.

Oxidative ability. Amounts of intracellular reactive oxygen species were quantified to determine the oxidative ability of macrophages according to a previously described method (Simizu et al., 1997). Briefly, 1×10^5 macrophages were cultured in 12-well tissue culture clusters overnight, and then cotreated with 10, 100, and 1000 μM ketamine and 2,7-dichlorofluorescin diacetate, a reactive oxygen species-sensitive dye, for 1, 6, and 24 h. After drug treatment, macrophages were harvested and suspended in PBS. The fluorescence intensities in cells were quantified using a flow cytometer (FACS Calibur, Becton Dickinson, San Jose, CA, USA). Only living cells were analyzed in this assay.

Reverse-transcription polymerase chain reaction assay. Messenger RNA from macrophages exposed to 100 μM ketamine, 1 ng/ml LPS, and a combination of ketamine and LPS for 1 h was prepared for reverse-transcriptase polymerase chain reaction (RT-PCR) analyses of TNF-α, IL-1β, IL-6, and β-actin following instructions of the ExpressDirect mRNA Capture and RT system for the RT-PCR kit (Pierce, Rockford, IL, USA). Oligonucleotides for PCR analyses of TNF-α, IL-1β, IL-6, and β-actin were designed and synthesized by Clontech Laboratories (Palo Alto, CA, USA). The oligonucleotide sequences of these primers were 5′-ATGAGCACAGAAAGCATGATCCGC-3′ and 3′-CTCAGGCCCGTCCAGATGAAACC-5′ for TNF-α; 5′-ATGGCAACTGTTCCTGAACTCAACT-3′ and 3′-TTTCCTTTCTTAGATATGGACAGGAC-5′ for IL-1β; 5′-ATGAAGTTCCTCTCTGCAAGAGACT-3′ and 3′-CACTAGGTTTGCCGAGTAGATCTC-5′ for IL-6; and 5′-GTGGGCCGCTCTAGGCACCAA-3′ and 3′-CTTTAG-CACGCACTGTAGTTTCTC-5′ for β-actin.

The PCR reaction was carried out using 35 cycles including 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 2 min. The PCR products were loaded onto a 1.8% agarose gel containing 0.1 μg/ml ethidium bromide and electrophoretically separated. DNA bands were visualized and photographed under UV-light exposure. The intensities of the DNA bands in the agarose gel were quantified with the aid of the UVIDOCMW version 99.03 digital imaging system (UVtec, Cambridge, England, UK).

Quantification of the mitochondrial membrane potential. The membrane potential of macrophage mitochondria was determined according to the method of Chen (1988). Briefly, 1×10^5 macrophages were seeded in 12-well tissue culture clusters overnight and then treated with 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. Macrophages were harvested and incubated with 3,3′-dihexyloxacarbocyanine ($DiOC_6(3)$), a positively charged dye, at 37 °C in a humidified atmosphere of 5% CO_2 for 30 min. After a process of washing and centrifuging, cell pellets were resuspended with PBS and the fluorescent intensities were analyzed by flow cytometry (FACS Calibur). Only living cells were analyzed in this assay.

Mitochondrial complex I NADH dehydrogenase activity. NADH dehydrogenase activity was determined by a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay following the method of Wu et al. (2003). Briefly, macrophages (5×10^5 cells) were seeded in 96-well tissue culture clusters overnight. After treatment with 10, 100, and 1000 μM ketamine for 1, 6, and 24 h, cells were cultured with new medium containing 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for another 3 h. The blue formazan products in cells were dissolved in dimethyl sulfoxide and spectrophotometrically measured at a wavelength of 570 nm. The data were normalized to the numbers of living cells.

Statistical analysis. The statistical significance of differences between control and ketamine-treated groups was evaluated using Student's *t* test, and differences were considered statistically significant at *P* values of less than 0.05. Differences between ketamine- and LPS-treated groups were considered significant when the *P* value of Duncan's multiple-range test was less than 0.05. Statistical analysis between groups over time was carried out using two-way ANOVA.

Results

Cell viability and lactate dehydrogenase release were evaluated to determine the toxicity of ketamine to macrophages (Table 1). The differences between the ketamine-free control and ketamine-treated groups were considered statistically significant at *P* values of less than 0.05.

Table 1
Effects of ketamine on macrophage viability

Treatment	Cell viability (cell number × 10^2)	LDH (U/L)
1 h		
Ketamine, 0 μM	332 ± 35	71 ± 19
Ketamine, 10 μM	309 ± 41	68 ± 23
Ketamine, 100 μM	329 ± 34	78 ± 21
Ketamine, 1000 μM	344 ± 27	83 ± 23
6 h		
Ketamine, 0 μM	349 ± 22	64 ± 26
Ketamine, 10 μM	313 ± 30	78 ± 17
Ketamine, 100 μM	322 ± 46	74 ± 22
Ketamine, 1000 μM	288 ± 28*	121 ± 18*
24 h		
Ketamine, 0 μM	489 ± 56	77 ± 22
Ketamine, 10 μM	503 ± 47	79 ± 20
Ketamine, 100 μM	487 ± 34	83 ± 25
Ketamine, 1000 μM	336 ± 40*	203 ± 30*

Macrophages were exposed to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. Cell viability was assayed using a trypan blue exclusion method. Levels of lactate dehydrogenase (LDH) in the culture medium were determined by an automatic analyzer. Each value represents the mean ± SEM for $n = 12$.

* Values significantly differ from the respective control, $P < 0.05$.

Exposure of macrophages to 10 and 100 μM ketamine for 1, 6, and 24 h did not affect cell viability. However, treatment with 1000 μM ketamine for 6 and 24 h caused significant 13% and 31% cell death, respectively. Treatment with 10 and 100 μM ketamine had no effect on the release of lactate dehydrogenase from macrophages to the culture medium (Table 1). The amounts of lactate dehydrogenase in the culture medium were significantly increased by 89% and 164% following administration of 1000 μM ketamine for 6 and 24 h, respectively.

Chemotactic activity was assayed to evaluate the effect of ketamine on the migrating capacity of macrophages (Table 2). The differences between the ketamine-free control and ketamine-treated groups were considered statistically significant at P values of less than 0.05. Administration of 10, 100, and 1000 μM ketamine in macrophages for 1, 6, and 24 h did not influence chemotactic activity.

Proportions of macrophages that ingested at least one fluorescent particle were counted in order to determine the effect of ketamine on phagocytic activities of macrophages (Fig. 1). The differences between the ketamine-free control and ketamine-treated groups were considered statistically significant at P values of less than 0.05. In 1-h-treated macrophages, treatment with 10, 100, and 1000 μM ketamine had no effect on phagocytic activity (Fig. 1, top panel). After administration for 6 h, 10 μM ketamine still did not influence the phagocytic activity of macrophages (Fig. 1, middle panel). However, when the administered concentrations reached 100 and 1000 μM, ketamine significantly decreased phagocytic activities by 23% and 27%, respectively. In 24-h-treated macrophages, the phagocytic activity was not affected by 10 μM ketamine (Fig. 1, bottom panel). Meanwhile, exposure to 100 and 1000 μM ketamine caused significant 29% and 31% decreases in phagocytic activities of macrophages, respectively.

In order to determine the effect of ketamine on the oxidative ability of macrophages, levels of intracellular reactive oxygen species were quantified (Fig. 2). The differences between the ketamine-free control and ketamine-treated groups were considered statistically significant at P values of less than 0.05. In 1-h-treated macrophages, treatment with 10, 100, and 1000 μM ketamine did not affect the levels of intracellular reactive oxygen species (Fig. 2, top panel). After administration for 6 h, ketamine at 10 μM still did not affect oxidative ability (Fig. 2, middle panel). Meanwhile, when the administered concentrations reached 100 and 1000 μM, ketamine significantly reduced the oxidative ability by 18% and 25%, respectively. In 24-h-treated macrophages, the oxidative ability was not affected by 10 μM ketamine (Fig. 2, bottom panel). However, treatment with 100 and 1000 μM ketamine led to significant 32% and 37% decreases in the oxidative ability of macrophages.

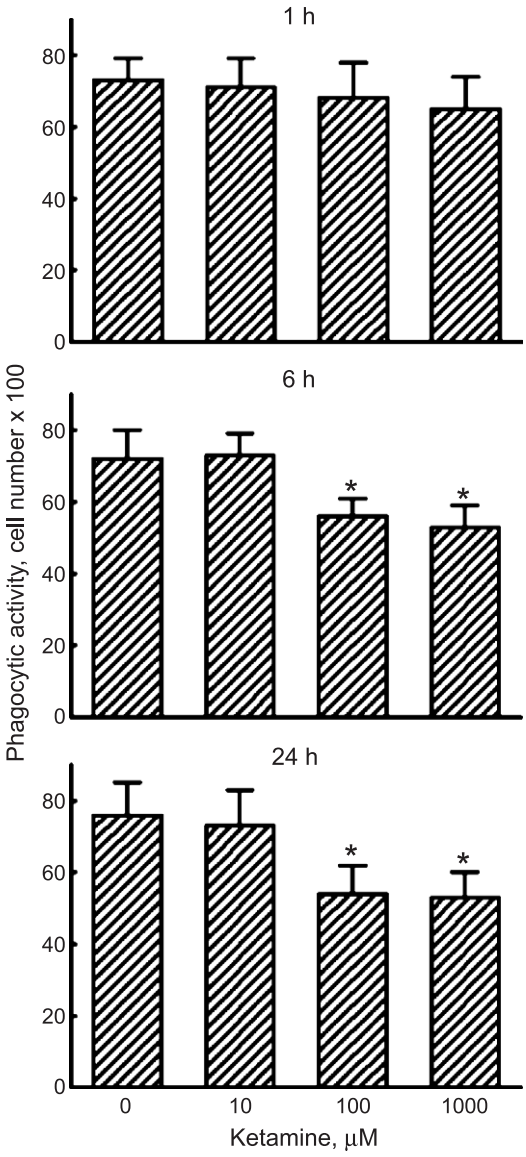

Fig. 1. Concentration- and time-dependent effects of ketamine on phagocytic activities of macrophages. Macrophages were exposed to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. Phagocytotic activities were determined by counting the proportions of macrophages that had digested at least one fluorescent particle as described in Materials and methods. Each value represents the mean ± SEM for $n = 12$. *Values significantly differ from the respective control, $P < 0.05$.

Table 2
Effects of ketamine on chemotactic activities of macrophages

Time (h)	Chemotactic activity (cell number × 10^2)		
	10 μM	100 μM	1000 μM
0	161 ± 28	158 ± 38	169 ± 25
1	152 ± 29	145 ± 33	157 ± 29
6	149 ± 29	134 ± 28	134 ± 22
24	148 ± 34	129 ± 33	130 ± 28

Macrophages were exposed to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. Chemotactic activity was assayed using Transwell cell culture chamber inserts as described in Materials and methods. Each value represents the mean ± SEM for $n = 6$. *Values significantly differ from the respective control, $P < 0.05$.

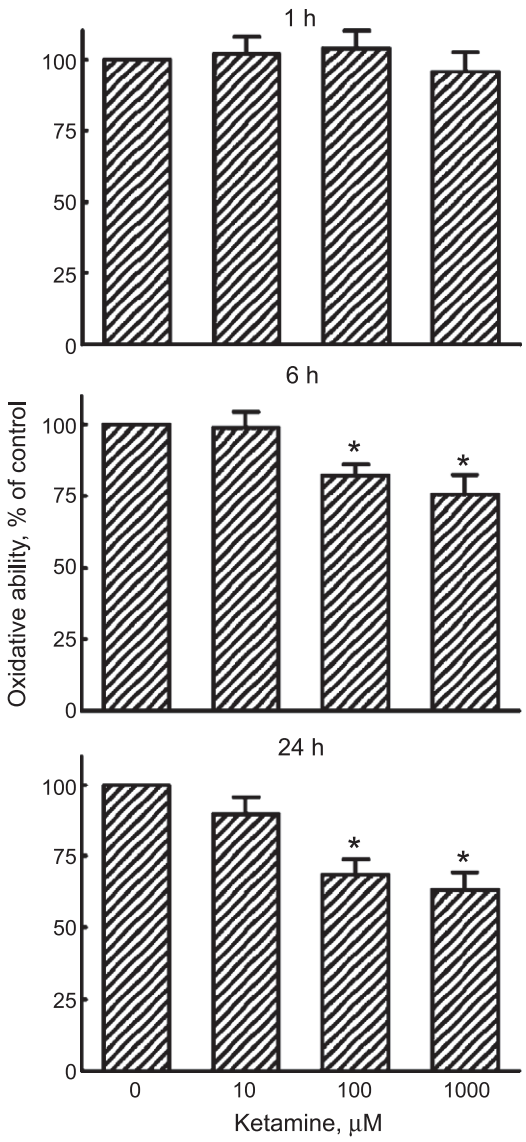

Fig. 2. Concentration- and time-dependent effects of ketamine on the oxidative ability of macrophages. Macrophages were exposed to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. Levels of intracellular reactive oxygen species were determined by a flow cytometric method. Each value represents the mean ± SEM for $n = 12$. *Values significantly differ from the respective control, $P < 0.05$.

RT-PCR analyses were carried out to determine the effects of ketamine on TNF-α, IL-1β, and IL-6 mRNA production in LPS-activated macrophages (Fig. 3). In untreated macrophages, low levels of TNF-α, IL-1β, and IL-6 mRNA were detected (Fig. 3, lane 2). Treatment with a clinically relevant concentration of ketamine (100 μM) did not affect TNF-α, IL-1β, or IL-6 mRNA production (Fig. 3, lane 3). LPS obviously induced TNF-α, IL-1β, and IL-6 mRNA (Fig. 3, lane 4). Cotreatment with ketamine and LPS significantly inhibited LPS-induced TNF-α, IL-1β, and IL-6 mRNA (Fig. 3, lane 5). Intensities of cDNA bands were quantified using a digital imaging system, and the data are shown in Table 3. Exposure to a therapeutic concentration

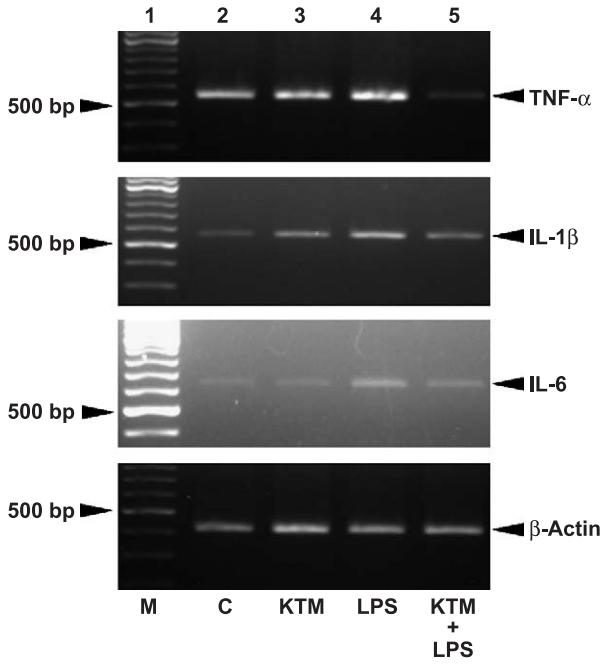

Fig. 3. Effects of ketamine (KTM) on TNF-α, IL-1β, and IL-6 mRNA production in lipopolysaccharide (LPS)-activated macrophages. Messenger RNAs from macrophages exposed to 100 μM KTM, 1 ng/ml LPS, and a combination of KTM and LPS were prepared for RT-PCR analysis of TNF-α, IL-1β, and IL-6 mRNA. Amounts of β-actin mRNA were determined as an internal standard (bottom panel). M, DNA 100-bp marker; C, control.

of ketamine had no effect on TNF-α, IL-1β, or IL-6 mRNA production. Administration of LPS in macrophages significantly induced levels of TNF-α, IL-1β, and IL-6 mRNA by 2-, 3-, and 4-fold, respectively. Ketamine significantly inhibited LPS-induced TNF-α, IL-1β, and IL-6 mRNA by 76%, 63%, and 65%, respectively (Table 3).

To validate the role of mitochondria in ketamine-caused suppression of macrophage functions, the mitochondrial membrane potential was determined (Fig. 4). The differences between the ketamine-free control and ketamine-treated groups were considered statistically significant at P values of less than 0.05. In 1-h-treated macrophages,

Table 3
Effects of ketamine on TNF-α, IL-1β, and IL-6 mRNA levels

Treatment	TNF-α mRNA (arbitrary units)	IL-1β mRNA (arbitrary units)	IL-6 mRNA (arbitrary units)
Control	2578 ± 626	862 ± 150	247 ± 53
Ketamine	1826 ± 488	936 ± 174	268 ± 74
LPS	5271 ± 934*	2785 ± 445*	991 ± 269*
Ketamine + LPS	1261 ± 355†	1026 ± 258†	344 ± 102†

Macrophages were exposed to 100 μM ketamine, 1 ng/ml lipopolysaccharide (LPS) and a combination of ketamine and LPS for 1, 6, and 24 h. Levels of TNF-α, IL-1β, and IL-6 mRNA were determined using RT-PCR analyses. Amounts of β-actin mRNA were detected as an internal standard. Intensities of RNA bands were quantified using a digital imaging system. Each value represents the mean ± SEM for $n = 3$.

* Values significantly differ from the respective control, $P < 0.05$.

† Values significantly differ from the LPS-treated groups, $P < 0.05$.

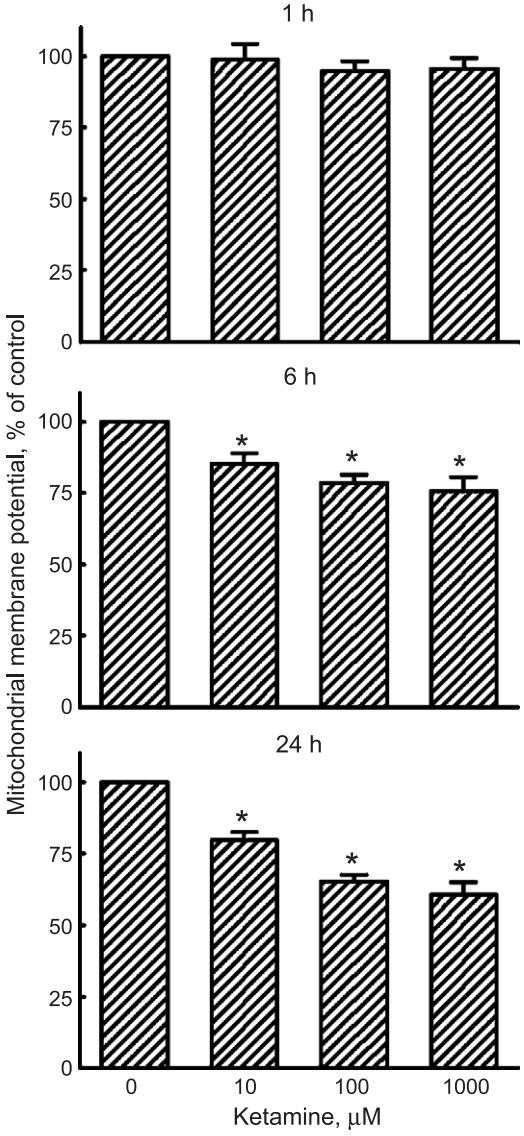

Fig. 4. Concentration- and time-dependent effects of ketamine on the membrane potential of macrophage mitochondria. Macrophages were exposed to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. The mitochondrial membrane potential was determined by a flow cytometric method. Each value represents the mean ± SEM for $n = 12$. *Values significantly differ from the respective control, $P < 0.05$.

treatment with 10, 100, and 1000 μM ketamine did not affect the membrane potential of macrophage mitochondria (Fig. 4, top panel). After 6 h, exposure to 10, 100, and 1000 μM ketamine caused significant 15%, 22%, and 25% decreases in the mitochondrial membrane potential (Fig. 4, middle panel). In 24-h-treated macrophages, the mitochondrial membrane potential was significantly decreased by 20%, 35%, and 40% after administration of 10, 100, and 1000 μM ketamine, respectively (Fig. 4, bottom panel).

Activity of mitochondrial complex I NADH dehydrogenase was assayed to evaluate if ketamine modulated this metabolizing enzyme (Table 4). The differences between the ketamine-free control and ketamine-treated groups were considered statistically significant at P values of less than 0.05. Administration of 10, 100, and 1000 μM ketamine in macrophages for 1, 6, and 24 h did not affect NADH dehydrogenase activity (Table 4).

Discussion

This study shows that ketamine at a therapeutic concentration (100 μM) can suppress macrophage functions. The concentration of ketamine used in this study, 100 μM, is within the range of clinical relevance (Domino et al., 1982; Grant et al., 1983). Ketamine at 100 μM was not cytotoxic to macrophages, but significantly suppressed macrophage capacities for particle ingestion and oxidant production. This study used LPS as a common inducer to increase inflammatory cytokine mRNA in macrophages. In response to LPS stimulation, 100 μM ketamine was also shown to inhibit TNF-α, IL-1β, and IL-6 mRNA syntheses in macrophages. During inflammation, macrophages destroy invading microorganisms or abnormal tumor cells through a series of reactions, including chemotaxis, phagocytosis, oxidant production, and inflammatory cytokine release (Aderem, 2001; Nathan, 1987). Dysfunction of these activities can affect host macrophage-mediated immunity (Lander, 1997). Therefore, this study provides in vitro data to identify that a clinically relevant concentration of ketamine, 100 μM, has suppressive effects on macrophage functions via inhibition of phagocytic activities, oxidative ability, as well as TNF-α, IL-1β, and IL-6 mRNA syntheses.

In parallel to macrophage dysfunction, this study demonstrated that a therapeutic concentration of ketamine can reduce the mitochondrial membrane potential. Depolarization of the mitochondrial membrane can lead to mitochondrial dysfunction or even cell insult (Pearce et al., 2001). This is the first study to identify the suppressive effects of ketamine on the mitochondrial membrane potential. Exposure of macrophages to a therapeutic concentration of ketamine did not affect cell viability. Thus, the ketamine-caused suppression of macrophage functions is

Table 4
Effects of ketamine on mitochondrial complex I NADH dehydrogenase activity

Time (h)	NADH dehydrogenase activity (OD values at 570 nm)		
	10 μM	100 μM	1000 μM
0	1.05 ± 0.24	1.03 ± 0.24	1.24 ± 0.18
1	1.01 ± 0.26	0.97 ± 0.22	1.15 ± 0.22
6	0.99 ± 0.21	1.09 ± 0.24	0.99 ± 0.21
24	0.98 ± 0.25	0.97 ± 0.20	0.93 ± 0.17

Macrophages were exposed to 10, 100, and 1000 μM ketamine for 1, 6, and 24 h. The activity of mitochondrial complex I NADH dehydrogenase was assayed using a colorimetric method. Each value represents the mean ± SEM for $n = 12$.

not due to the cytotoxic effect of this intravenous anesthetic agent. Macrophage functions are dependent on the maintenance of the mitochondrial membrane potential (Ayala and Chaudry, 1996; Brown and Borutaite, 1999). Therefore, the ketamine-caused reduction in the mitochondrial membrane potential may be one of the possible mechanisms to explain the suppression of macrophage functions by this anesthetic agent.

Ketamine can decrease the phagocytic activities of macrophages. Previous studies revealed that cellular ATP contributes to phagocytic activities of macrophages (Lammas et al., 1997; Oshimi et al., 1999). Modulation of the mitochondrial membrane potential can disturb ATP synthesis (Yoon et al., 2003). This study showed that ketamine at a therapeutic concentration (100 μM) decreased the mitochondrial membrane potential. Thus, the inhibitory effects of ketamine on macrophage phagocytosis may be attributed to the decrease in ATP synthesis because of the reduction of the mitochondrial membrane potential induced by this anesthetic agent. Weinshank et al. (1988) showed that stimulation of inflammatory cytokines, including TNF-α and IFN-γ, increases the levels of receptor FcγRα in Raw 264.7 cells and thus enhances phagocytic activity. The present study shows that a clinically relevant concentration of ketamine can inhibit TNF-α mRNA production. Therefore, the ketamine-caused suppression of inflammatory cytokine production provides another possible mechanism to explain the inhibition of phagocytic activities by this anesthetic agent. A previous study reported that ketamine can reduce phagocytic activity of polymorphonuclear leucocytes (Krumholz et al., 1995). This study further demonstrated that ketamine at a clinical concentration has a suppressive effect on the phagocytic activity of macrophages.

Ketamine can reduce the oxidative ability of macrophages. Administration of ketamine in macrophages concentration- and time-dependently decreased the levels of intracellular reactive oxygen species. Activated macrophages can produce and secrete large amounts of hydrogen peroxide, superoxide, and nitric oxide for killing infective pathogens (Tripathi and Maiti, 2003). This study used DCFH-DA dye to stain and determine the levels of intracellular reactive oxygen species. DCFH-DA dye has been reported to stain hydrogen peroxide, superoxide, and nitric oxide (Chen et al., 2003; Rao et al., 1992; Simizu et al., 1997). A decrease in the levels of intracellular reactive oxygen species corresponds to a reduction in hydrogen peroxide, superoxide, and nitric oxide production. Thus, ketamine is able to decrease oxidant production in macrophages. In human neutrophils, ketamine can suppress the release of reactive oxygen species (Weigand et al., 2000). The present data further reveal that a therapeutic concentration of ketamine can suppress the oxidative ability of macrophages.

Ketamine significantly inhibited TNF-α, Il-1β, and IL-6 mRNA production in LPS-activated macrophages. In response to LPS stimuli, TNF-α, IL-1β, and IL-6 are massively produced by activated macrophages and involved in immune responses and host defense (Carswell et al., 1975). Suppression of inflammatory cytokine production can decrease the host immune response. Previous studies have shown that ketamine can reduce TNF-α production in LPS-activated macrophages and mice (Takenaka et al., 1994; Shimaoka et al., 1996). In addition to TNF-α, this study further demonstrated that ketamine can also inhibit LPS-induced IL-1β and IL-6 mRNA expression in macrophages. LPS is a common tool to activate macrophages. This study specifically showed the effects of ketamine on inflammatory cytokine expression in LPS-activated macrophages. However, the effects of ketamine on inflammatory cytokine expression in macrophages in response to other generic triggers, such as interferon-γ, were not determined in this study.

Sakai et al. (2000) reported that ketamine suppresses endotoxin-induced NF-κB expression. NF-κB is one of the common transcriptional factors that induce TNF-α, Il-1β, and IL-6 in macrophages in response to the ketamine-induced suppression of TNF-α, IL-1β, and IL-6 expression in LPS-stimulated macrophages via inhibition of NF-κB activation. Our previous study showed that a decrease in nitric oxide synthesis can significantly inhibit LPS-induced TNF-α, Il-1β, and IL-6 expressions in macrophages (Wu et al., 2003). Data from this study revealed that ketamine at a therapeutic concentration (100 μM) decreased the levels of intracellular reactive oxygen species, including nitric oxide. Shimaoka et al. (1996) also reported that ketamine decreased nitric oxide synthesis in activated macrophages. Therefore, another possible mechanism to explain the suppression of TNF-α, IL-1β, and IL-6 expressions by ketamine is through reduction of intracellular reactive oxygen species.

Our present data reveal that ketamine caused a significant decrease in the mitochondrial membrane potential of macrophages. Mitochondria are the target organelles for synthesizing ATP in macrophages (Papucci et al., 2003). The ketamine-caused reduction in the mitochondrial membrane potential can directly affect ATP synthesis and further suppress macrophage functions. Ketamine at a clinically relevant concentration had no effect on mitochondrial complex I NADH dehydrogenase activity. NADH dehydrogenase is a critical enzyme contributing to the mitochondrial respiratory chain reaction and ATP synthesis (Pearce et al., 2001). Therefore, a therapeutic concentration of ketamine selectively reduces the mitochondrial membrane potential without affecting NADH dehydrogenase activity. The ketamine-caused suppression of the mitochondrial membrane potential may be one of major mechanisms that induce mitochondrial dysfunction and further suppress macrophage functions.

Treatment with 10 and 100 μM ketamine did not affect macrophage migration. However, administration of 1000 μM ketamine in macrophages potentially decreased the migration capacity of macrophages. Results from the analysis of cell viability revealed that ketamine at 1000 μM induced macrophage death in a time-dependent manner.

Thus, suppression of chemotactic activity induced by a high concentration of ketamine may be due to the cytotoxic effects of this intravenous anesthetic agent on macrophages. Kress and Segmuller (1987) reported that neither ketamine nor etomidate affected the mobility of polymorphonuclear leukocytes. However, in LPS-stimulated leukocytes, ketamine was shown to attenuate cell adherence and cell migration (Hofbauer et al., 1998; Schmidt et al., 1995). In neutrophils, ketamine can suppress oxidant production and chemotactic activity (Weigand et al., 2000; Zahler et al., 1999). In this study, we further demonstrated that therapeutic concentrations of ketamine (<100 μM) did not affect macrophage migration.

In conclusion, the present study shows that a therapeutic concentration of ketamine (100 μM) can suppress macrophage functions of phagocytosis, oxidative ability, as well as TNF-α, IL-1β, and IL-6 mRNA production. Our results also demonstrated that ketamine at a clinically relevant concentration can lead to mitochondrial dysfunction specifically via reduction in the mitochondrial membrane potential but not through modulation of mitochondrial complex I NADH-dehydrogenase activity. Ketamine at clinical concentrations is not cytotoxic to macrophages. Therefore, according to the present data, we suggest that the mechanism of ketamine-caused suppression of macrophage functions may be possibly via inhibition of the mitochondrial membrane potential but not through a reduction in cell viability. There are certain limitations to this study. Although Raw 264.7 cells have a variety of characteristics of normal macrophages, these cells were derived from murine tumor tissues. In our lab, the effects of ketamine on functions of normal macrophages are being determined using murine peritoneal cells as the experimental model. In addition, because this in vitro study did not evaluate ketamine's effects on other humoral and tissue factors, we cannot draw definite, clinically relevant conclusions about the possible effects of ketamine on macrophage functions.

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References

Aderem, A., 2001. Role of Toll-like receptors in inflammatory response in macrophages. Crit. Care Med. 29, S16–S18.

Ayala, A., Chaudry, I.H., 1996. Immune dysfunction in murine polymicrobial sepsis: mediators, macrophages, lymphocytes and apoptosis. Shock 6, S27–S38.

Brown, G.C., Borutaite, V., 1999. Nitric oxide, cytochrome *c* and mitochondria. Biochem. Soc. Symp. 66, 17–25.

Carswell, E.A., Old, L.J., Kassel, R.L., 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. U.S.A. 72, 3666–3670.

Chen, L.B., 1988. Mitochondria membrane potential in living cells. Annu. Rev. Cell Biol. 4, 155–181.

Chen, R.M., Wu, C.H., Chang, H.C., Wu, G.J., Lin, Y.L., Sheu, J.R., Chen, T.L., 2003a. Propofol suppresses macrophage functions and modulates mitochondrial membrane potential and cellular adenosine triphosphate levels. Anesthesiology 98, 1178–1185.

Chen, R.M., Wu, G.J., Tai, Y.T., Lin, Y.L., Jean, W.C., Chen, T.L., 2003b. Propofol downregulates nitric oxide biosynthesis through inhibiting inducible nitric oxide synthase in lipopolysaccharide-activated macrophages. Arch. Toxicol. 77, 418–423.

Clements, J.A., Nimmo, W.S., 1981. Pharmacokinetics and analgesic effect of ketamine in man. Br. J. Anaesth. 53, 27–30.

Diehl, A.M., Hoek, J.B., 1999. Mitochondrial uncoupling: role of uncoupling protein anion carriers and relationship to thermogenesis and weight control "the benefits of losing control". J. Bioenerg. Biomembranes 31, 493–506.

Di Virgilio, F., Chiozzi, P., Ferrari, D., Falzoni, S., Sanz, J.M., Morelli, A., Torboli, M., Bolognesi, G., Baricordi, O.R., 2001. Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 97, 587–600.

Domino, E.F., Zsigmond, E.K., Domino, L.E., Domino, K.E., Kothary, S.P., Domino, S.E., 1982. Plasma levels of ketamine and two of its metabolites in surgical patients using a gas chromatographic mass fragmentographic assay. Anesth. Analg. 61, 87–92.

Fredholm, B.B., 1997. Purines and neutrophil leukocytes. Gen. Pharmacol. 28, 345–350.

Grant, I.S., Nimmo, W.S., McNicol, L.R., Clements, J.A., 1983. Ketamine disposition in children and adults. Br. J. Anaesth. 55, 1107–1110.

Hofbauer, R., Moser, D., Hammerschmidt, V., Kapiotis, S., Frass, M., 1998. Ketamine significantly reduces the migration of leukocytes through endothelial cell monolayers. Crit. Care Med. 26, 1545–1549.

Kotani, N., Hashimoto, H., Sessler, D.I., Kikuchi, A., Suzuki, A., Takahashi, S., Muraoka, M., Matsuki, A., 1998. Intraoperative modulation of alveolar macrophage function during isoflurane and propofol anesthesia. Anesthesiology 89, 1125–1132.

Kress, H.G., Segmuller, R., 1987. Intravenous anesthetics and human neutrophil granulocyte motility in vitro. Anaesthesist 36, 356–361.

Krumholz, W., Endrass, J., Hempelmann, G., 1995. Inhibition of phagocytosis and killing of bacteria by anaesthetic agents in vitro. Br. J. Anaesth. 75, 66–70.

Lammas, D.A., Stober, C., Harvey, C.J., Kendrick, N., Panchalingam, S., Kumararatne, D.S., 1997. ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic P2Z(P2X7) receptors. Immunity 7, 433–444.

Lander, H.M., 1997. An essential role for free radicals and derived species in signal transduction. FASEB J. 11, 118–124.

Nathan, C.F., 1987. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. J. Clin. Invest. 80, 1550–1560.

Oshimi, Y., Miyazaki, S., Oda, S., 1999. ATP-induced Ca2+ response mediated by P2U and P2Y purinoceptors in human macrophages: signalling from dying cells to macrophages. Immunology 98, 220–227.

Papucci, L., Schiavone, N., Witort, E., Donnini, M., Lapucci, A., Tempestini, A., Formigli, L., Zecchi-Orlandini, S., Orlandini, G., Carella, G., Brancato, R., Capaccioli, S., 2003. Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property. J. Biol. Chem. 278, 28220–28228.

Pearce, L.L., Epperly, M.W., Greenberger, J.S., Pitt, B.R., Peterson, J., 2001. Identification of respiratory complexes I and III as mitochondrial sites of damage following exposure to ionizing radiation and nitric oxide. Nitric Oxide 5, 128–136.

Rao, K.M., Padmanabhan, J., Kilby, D.L., Cohen, H.J., Currie, M.S., Weinberg, J.B., 1992. Flow cytometric analysis of nitric oxide

production in human neutrophils using dichlorofluorescein diacetate in the presence of a calmodulin inhibitor. J. Leukocyte Biol. 51, 496–500.

Reich, D.L., Silvay, G., 1989. Ketamine: an update on the first twenty-five years of clinical experience. Can. J. Anaesth. 36, 186–197.

Sakai, T., Ichiyama, T., Whitten, C.W., Giesecke, A.H., Lipton, J.M., 2000. Ketamine suppresses endotoxin-induced NF-κB expression. Can. J. Anaesth. 47, 1019–1024.

Schmidt, H., Ebeling, D., Bauer, H., Bach, A., Bohrer, H., Gebhard, M.M., Martin, E., 1995. Ketamine attenuates endotoxin-induced leukocyte adherence in rat mesenteric venules. Crit. Care Med. 23, 2008–2014.

Shimaoka, M., Iida, T., Ohara, A., Taenaka, N., Mashimo, T., Honda, T., Yoshiya, I., 1996. Ketamine inhibits nitric oxide production in mouse-activated macrophage-like cells. Br. J. Anaesth. 77, 238–242.

Simizu, S., Imoto, M., Masuda, N., Takada, M., Umezawa, K., 1997. Involvement of hydrogen peroxide production in erbstain-induced apoptosis in human small cell lung carcinoma cells. Cancer Res. 56, 4978–4982.

Takenaka, I., Ogata, M., Koga, K., Matsumoto, T., Shigematsu, A., 1994. Ketamine suppresses endotoxin-induced tumor necrosis factor alpha production in mice. Anesthesiology 80, 402–408.

Traber, D.L., Wilson, R.D., Priano, L.L., 1971. The effect of alphaadrenergic blockade on the cardiopulmonary response to ketamine. Anesth. Analg. 50, 737–742.

Tripathi, S., Maiti, T.K., 2003. Stimulation of murine macrophages by native and heat-denatured lectin from *Abrus precatorius*. Int. J. Immunopharmacol. 3, 375–381.

Valledor, A.F., Ricote, M., 2004. Nuclear receptor signaling in macrophages. Biochem. Pharmacol. 67, 201–212.

Weigand, M.A., Schmidt, H., Zhao, Q., Plaschke, K., Martin, E., Bardenheuer, H.J., 2000. Ketamine modulates the stimulated adhesion molecule expression on human neutrophils in vitro. Anesth. Analg. 90, 206–212.

Weinshank, R.L., Luster, A.D., Ravetch, J.V., 1988. Function and regulation of a murine macrophage-specific IgG Fc receptor, FcγRα. J. Exp. Med. 167, 1909–1925.

White, P.F., Way, W.L., Trevor, A.J., 1982. Ketamine: its pharmacology and therapeutic uses. Anesthesiology 56, 119–136.

Wu, C.H., Chen, T.L., Chen, T.G., Ho, W.P., Chiu, W.T., Chen, R.M., 2003. Nitric oxide modulates pro- and anti-inflammatory cytokines in lipopolysaccharide-activated macrophages. J. Trauma 55, 540–545.

Yoon, Y.S., Byun, H.O., Cho, H., Kim, B.K., Yoon, G., 2003. Complex II defect via down-regulation of iron-sulfur subunit induces mitochondrial dysfunction and cell cycle delay in iron chelation-induced senescence-associated growth arrest. J. Biol. Chem. 278, 51577–51586.

Zahler, S., Heindl, B., Becker, B.F., 1999. Ketamine does not inhibit inflammatory responses of cultured human endothelial cells but reduces chemotactic activation of neutrophils. Acta Anaesthesiol. Scand. 43, 1011–1016.