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Propofol Suppresses Macrophage Functions and Modulates Mitochondrial Membrane Potential and Cellular Adenosine Triphosphate Synthesis

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Background: **Propofol is an intravenous anesthetic agent that may impair host defense system. The aim of this study was to evaluate the effects of propofol on macrophage functions and its possible mechanism.**

Methods: **Mouse macrophage-like Raw 264.7 cells were exposed to propofol, at 3, 30 (a clinically relevant concentration), and 300 μM. Cell viability, lactate dehydrogenase, and cell cycle were analyzed to determine the cellular toxicity of propofol to macrophages. After administration of propofol, chemotactic, phagocytic, and oxidative ability and interferon-γ mRNA production were carried out to validate the potential effects of propofol on macrophage functions. Mitochondrial membrane potential and cellular adenosine triphosphate levels were also analyzed to evaluate the role of mitochondria in propofol-induced macrophage dysfunction.**

Results: **Exposure of macrophages to 3 and 30 μM propofol did not affect cell viability. When the administered concentration reached 300 μM, propofol would increase lactate dehydrogenase release, cause arrest of cell cycle in G1/S phase, and lead to cell death. In the 1-h-treated macrophages, propofol significantly reduced macrophage functions of chemotactic and oxidative ability in a concentration-dependent manner. However, the suppressive effects were partially or completely reversed after 6 and 24 h. Propofol could reduce phagocytic activities of macrophages in concentration- and time-dependent manners. Exposure of macrophages to lipopolysaccharide induced the mRNA of interferon-γ, but the induction was significantly blocked by propofol. Propofol concentration-dependently decreased the membrane potential of macrophage mitochondria, but the effects were descended with time. The levels of cellular adenosine triphosphate in macrophages were also reduced by propofol.**

Conclusions: **A clinically relevant concentration of propofol can suppress macrophage functions, possibly through inhibiting their mitochondrial membrane potential and adenosine triphosphate synthesis instead of direct cellular toxicity.**

PROPOFOL (2,6-diisopropylphenol) is a widely used intravenous anesthetic agent for induction and maintenance of anesthesia in surgical procedures.[1] Propofol has the advantages of rapid onset, short duration of action, and rapid elimination.[2] Certain adverse effects such as cardiac depression or hypotension could be observed in the patients anesthetized with this anesthetic agent.[3,4] Studies in human neutrophils and leukocytes have also demonstrated that propofol might have immunomodulating effects.[5–7]

Macrophages play a critical role in cellular host defense against infection or tissue injury.[8] In response to stimuli, macrophages could undergo a series of inflammatory processes, including chemotaxis, phagocytosis, intracellular killing, and release of cytokines.[8,9] Dysfunction of macrophages may decrease host-nonspecific cell-mediated immunity.[10] An *ex vivo* study revealed that anesthesia with propofol or isoflurane time-dependently decreased phagocytosis and microbicidal activities of alveolar macrophages intraoperatively.[11]

Propofol anesthesia has also been reported to induce proinflammatory cytokines, including interleukin-1β, interleukin-8, interferon-γ (IFN-γ), and tumor necrosis factor-α, in orthopedic surgery patients.[12] However, multiple factors could be involved in modulating macrophage functions in the surgical procedures.[13] Therefore, an *in vitro* study will be needed to validate the role of propofol in modulating macrophage functions to rule out the contribution of other factors.

Mitochondria are important energy-producing organelles and participate in macrophage activation.[14,15] Adenosine triphosphate, synthesized from the mitochondria respiratory chain reaction, can enhance chemotactic migration and phagocytic ingestion of macrophages and neutrophils through the purinergic P2 receptor pathway or the elevation of intracellular Ca^{2+}.[16–19] In murine polymicrobial sepsis, a decrease in cellular adenosine triphosphate (ATP) level has been reported to be associated with a marked suppression in the functions of lymphocytes and macrophages.[20] Thus, the integrity of mitochondrial activities, including the membrane potential and ATP synthesis, is crucial to maintain macrophage functions. Previous studies have shown that propofol might impair mitochondrial electron transport chain and ATP production in rat brain synaptosomes, human platelets, and guinea pig cardiomyocytes.[21–25] However, few studies have evaluated the effects of propofol on macrophage mitochondria.

This study was aimed at evaluating the effects of propofol on the modulation of macrophage functions from the aspects of cytotoxicity, chemotaxis, phagocytosis, oxidative ability, and IFN-γ mRNA production and

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its possible mechanism from the viewpoint of mitochondrial membrane potential and cellular ATP production.

Materials and Methods

Cell Culture and Drug Treatment

The murine macrophage cell line Raw 264.7 was purchased from American Type Culture Collection (Rockville, MD). Macrophages were cultured in RPMI 1640 medium (Gibco, BRL, Grand Island, NY) 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. The cells were grown to a confluence prior to propofol administration.

Donated propofol (Zeneca Limited, Macclesfield, Cheshire, UK) was stored under nitrogen, protected from light, and freshly prepared by dissolving it in dimethyl sulfoxide (DMSO) for each independent experiment. DMSO in the medium was less than 0.1% to avoid the toxicity of this solvent to macrophages. According to the clinical application, propofol dosages of 3, 30, and 300 μM (corresponding to 0.1, 1, and 10 times the clinical plasma concentration[26]) were chosen to be administered in this study. Control macrophages were treated with DMSO only.

Assay of Cytotoxicity

To determine the toxicity of propofol to macrophages, analyses of cell viability, lactate dehydrogenase release, and cell cycle were carried out. Cell viability was analyzed by testing the ability of viable cells to convert soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into an insoluble dark blue formazan, as described previously.[27] The amounts of lactate dehydrogenase in the culture medium were quantified with use of a model 7450 automatic autoanalyzer system (Hitachi, Ltd., Tokyo, Japan). Analysis of cell cycle was carried out by determining the percentage of G1/S and G2/M phases in macrophages exposed to propofol.

After administration of propofol, macrophages were harvested and fixed in 80% ethanol. The fixed cells were incubated in a solution containing 3.75 mM sodium citrate, 0.1% Triton X-100 and 30 μg/ml RNase A at 37°C for 30 min and resuspended with 20 μg/ml propidium iodide. The stained macrophages were analyzed with a flow cytometer (FACS Calibur; Becton Dickinson, San Jose, CA).

Assay of Chemotactic Activity

The migrating capacity of macrophages was determined with use of Costar Transwell cell culture chamber inserts (pore size, 8 μm), according to the application guide (Corning Costar, Cambridge, MA). The rich RPMI 1640 medium (1.5 ml) was first added to 12-well tissue cluster plates (Corning Costar), and the Transwell was inserted in the plates. Macrophages (1×10^5) suspended with propofol in 0.5 ml rich medium was added to the inside of 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 the polycarbonate filters were counted in each field and averaged for three fields with the aid of a cross-hair micrometer (Nikon Corporation, Tokyo, Japan).

Assay of Oxidative Ability

The amounts of intracellular reactive oxygen species were quantified to determine the oxidative ability of macrophages according to the method described previously.[28] In brief, 1×10^5 macrophages were cultured in 12-well tissue culture clusters overnight and then cotreated with propofol and 2,7-dichlorofluorescin diacetate, a reactive oxygen species-sensitive dye. After the drug treatment, macrophages were harvested and suspended in phosphate-buffered saline (PBS) buffer (0.14 M NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4). The relative fluorescence intensity in cells was quantified by a flow cytometer (FACS Calibur).

Assay of 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.*[11] Macrophages (1×10^6) were suspended in PBS buffer and incubated at 37°C on a shaking platform. Red fluorescent FluoSphere® carboxylate-modified microspheres (Molecular Probes, Eugene, OR), 0.5 μm in diameter, were added to the cell suspension and incubated for 20 min. The ratios of particle-to-cell were 15:1. The reaction was stopped by an ice-cold saline solution. The fractions of macrophages that ingested at least one particle were counted with the aid of a cross-hair micrometer (Nikon).

Reverse Transcriptase–Polymerase Chain Reaction Assay

The mRNA from macrophages exposed to propofol for 1 h was prepared for reverse transcriptase–polymerase chain reaction (RT-PCR) analyses of IFN-γ and β-actin mRNA according to the manufacturer guidelines (ExpressDirect mRNA Capture and RT System for RT-PCR Kit; Pierce, Rockford, IL). In brief, 1×10^6 cells were seeded in 6-cm tissue culture dishes at 37°C in a humidified atmosphere of 5% CO_2. After administration of propofol, macrophages were lysed and cellular mRNA was prepared by bonding the cell lysates to an oligo(dT) plate. Reverse transcription was carried out by adding 33 μl 1× first-strand complementary DNA mix to the sample and reacting for 1 h at 37°C in a thermocycler block. Complementary DNA (10 μl) was used for PCR amplification of IFN-γ and β-actin. Oligonucleotides for

Table 1. Cytotoxic Effects of Propofol on Macrophages

Propofol, μm	Cell Viability (% of control)	LDH, U/l	Cell Cycle, %	
			G1/S Phase	G2/M Phase
1 h	—	—	—	—
0	100	65 ± 18	56 ± 3	28 ± 3
3	100 ± 11	70 ± 25	55 ± 3	28 ± 2
3	104 ± 22	58 ± 11	54 ± 3	30 ± 5
300	101 ± 15	80 ± 22	55 ± 3	28 ± 3
6 h	—	—	—	—
0	100	74 ± 16	55 ± 3	28 ± 3
3	96 ± 25	71 ± 15	55 ± 2	29 ± 3
30	108 ± 33	74 ± 20	56 ± 3	28 ± 3
300	72 ± 16*	113 ± 10*	54 ± 3	29 ± 3
24 h	—	—	—	—
0	100	78 ± 22	54 ± 2	29 ± 3
3	101 ± 11	77 ± 21	55 ± 4	29 ± 2
30	103 ± 13	82 ± 15	57 ± 5	29 ± 6
300	56 ± 18*	175 ± 18*	68 ± 6*	18 ± 6*

Values shown as mean ± SD for n = 9.

Macrophages were treated with 3, 30, and 300 μm propofol for 1, 6, and 24 h. Cell viability, LDH release, and cell cycle were analyzed to determine the toxicity of propofol to macrophages.

* $P < 0.05$; statistically different from the respective control.

LDH = lactate dehydrogenase.

PCR analyses of mouse IFN-γ and β-actin mRNA were designed and synthesized by CLONTECH Laboratories (Palo Alto, CA).

The primer sequences for IFN-γ mRNA analysis are 5'-TGAACGCTACACACTGCATCTTGG-3' and 5'-CGACT CCTTTTCCGCTTCCTGAG-3'PCR. The RT-PCR analysis of β-actin mRNA was used as an internal standard, and their sequences of upstream and downstream primers are 5'-GTGGGCCGCTCTAGGCACCAA-3' and 5'-CTCTT TGATGTCACGCACGATTTC-3', respectively. The PCR reaction was carried out with 35 cycles of 94°C for 45 sec, 60°C for 45 sec, and 72°C for 2 min. After reaction, the products were loaded and separated in a 1.8% agarose gel containing 0.1 μg/ml ethidium bromide. The intensities of DNA bands in the agarose gel were quantified with the aid of the UVIDOCMW Version 99.03 Digital Imaging System (UVtec Limited, Cambridge, England, UK).

Quantification of Mitochondrial Membrane Potential

The membrane potential of macrophage mitochondria was determined according to the method of Chen.[29] In brief, 1×10^5 macrophages were seeded in 12-well tissue culture clusters overnight and then treated with propofol 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. In a process of washing and centrifuging, the cell pellets were resuspended with 1× PBS buffer, and the cellular fluorescent intensities were analyzed by a flow cytometer (FACS Calibur).

Detection of Cellular Adenosine Triphosphate Levels

The levels of cellular ATP in macrophages were determined by a bioluminescence assay based on the luciferase requirement for ATP in producing emission light, according to the protocol for the Molecular Probes ATP Determination Kit (Molecular Probes, Eugene, OR). The luminant light (560 nm) emitted by the luciferase-mediated reaction of ATP and luciferin was detected by a WALLAC VICTOR® 1420 multilabel counter (Welch Allyn, Turku, Finland).

Statistical Analysis

The statistical difference between control and propofol-treated groups was considered significant when the *P* value of the Duncan multiple range test was less than 0.05. Statistical analysis of values for the groups over time was carried out by means of two-way ANOVA.

Results

The toxicity of propofol to macrophages was analyzed according to its influence on cell viability, lactate dehydrogenase release, and cell cycle. Exposure of macrophages to 3 and 30 μM propofol for 1, 6, and 24 h did not affect cell viability (table 1). However, propofol at 300 μM caused 28% and 44% cell death in the 6- and 24-h propofol-treated macrophages, respectively. Administration of 3 and 30 μM propofol for 1, 6, and 24 h did not affect the release of lactate dehydrogenase from macrophages (table 1).

The amounts of lactate dehydrogenase in the culture medium were significantly increased, by 53% and 220%, after treatment with 300 μM propofol for 6 and 24 h,

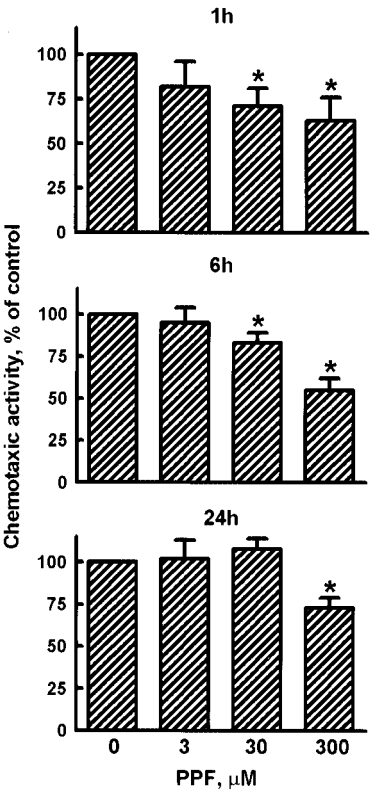

Fig. 1. Concentration- and time-dependent effects of propofol (PPF) on chemotaxic activities of macrophages. Macrophages were exposed to 3, 30, and 300 μM PPF for 1, 6, and 24 h, respectively. Chemotaxic activities were assayed in the Transwell cell culture chamber inserts as described in Materials and Methods. Each value is expressed as mean ± SD for n = 6. *Values were considered to be statistically different from the respective control when the *P* value was <0.05.

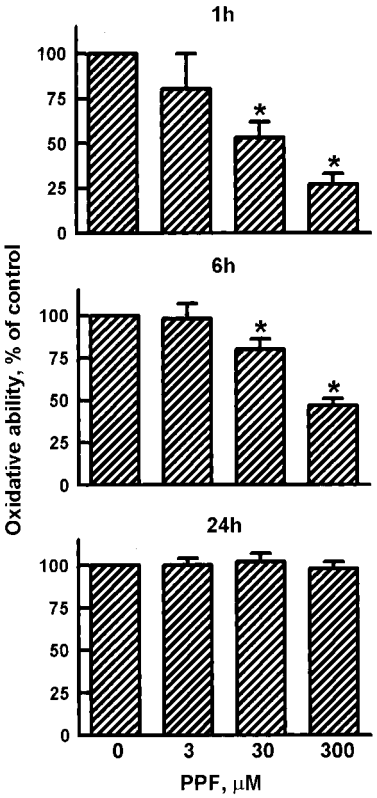

Fig. 2. Concentration- and time-dependent effects of propofol (PPF) on oxidative ability of macrophages. Macrophages were exposed to 3, 30, and 300 μM PPF for 1, 6, and 24 h. The levels of intracellular reactive oxygen species were determined by the flow cytometric method. Each value is expressed as mean ± SD for n = 6. *Values were considered to be statistically different from the respective control when the *P* value was <0.05.

respectively. Analysis of cell cycle revealed that propofol at 3 and 30 μM did not affect the growth of macrophages (table 1). However, propofol at 300 μM caused a significant (26%) increase in G1/S phase and a 38% decrease in G2/M phase after 24 h (table 1).

The cells that migrated to the bottom membrane of Transwell were counted to determine the chemotactic activities of macrophages (fig. 1). In the 1-h-treated macrophages, propofol at 30 and 300 μM significantly reduced chemotactic activity, by 29% and 47%, respectively (fig. 1, *top panel*). When the treated time intervals reached 6 h, 30 and 300 μM propofol decreased chemotactic activity by 17% and 40% (fig. 1, *middle panel*). After 24 h, propofol only at 300 μM significantly caused a 32% decrease in chemotactic activities (fig. 1, *bottom panel*).

In order to determine the oxidative ability of macrophages, the levels of intracellular reactive oxygen species were quantified (fig. 2). In the 1-h-treated macrophages, propofol at 30 and 300 μM decreased oxidative ability by 47% and 73%, respectively (fig. 2, *top panel*). After 6 h, 30 and 300 μM propofol reduced oxidative ability by 20% and 65%, respectively (fig. 2, *middle panel*). Propofol did not affect oxidative ability when exposing to this anesthetic for 24 h (fig. 2, *bottom panel*).

The fractions of macrophages that ingested at least one fluorescent particle were counted in order to determine the phagocytic activities of these immune cells. Exposure of macrophages to 3 μM propofol for 1, 6, and 24 h did not affect phagocytic activity (table 2). Treatment with 30 μM propofol for 6 and 24 h caused 54% and 64% decreases in the fractions of macrophages that ingested

Table 2. Concentration- and Time-dependent Effects of Propofol on Phagocytotic Activities of Macrophages

	Phagocytosis, cell number x 10^2		
Propofol, μm	1 h	6 h	24 h
0	176 ± 43	185 ± 65	204 ± 54
3	194 ± 62	172 ± 58	212 ± 41
30	225 ± 81	86 ± 36*	73 ± 21*
300	174 ± 42	72 ± 22*	58 ± 21*

Values shown as mean ± SD for n = 6. Macrophages were treated with 3, 30, and 300 μm propofol for 1, 6, and 24 h. Phagocytotic activities were determined by counting the fractions of macrophages that digested at least one fluorescent particle.

* $P < 0.05$; statistically different from the respective control.

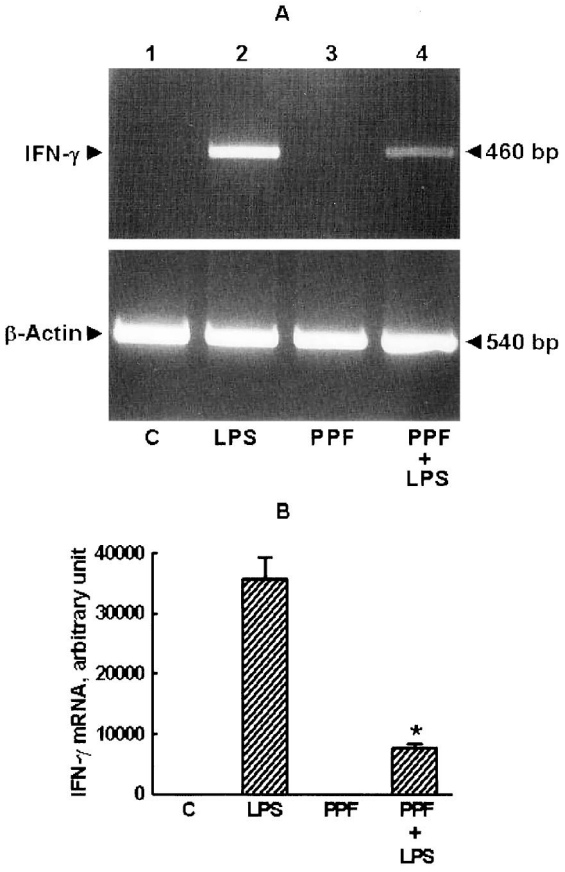

Fig. 3. Effect of propofol (PPF) on interferon (IFN)–γ mRNA in lipopolysaccharide (LPS)–activated macrophages. Messenger RNA from macrophages exposed to 1 ng/ml LPS, 30 μM PPF, and a combination of PPF and LPS were prepared for reverse transcription–polymerase chain reaction analysis of IFN-γ (A, *top panel*) and β-actin (*A*, *bottom panel*). Intensities of DNA bands were quantified by a digital analysis system as described in Materials and Methods (*B*). Each value is expressed as mean ± SD for three determinations. *Values were considered to be statistically different from the respective control when the *P* value was less than 0.05.

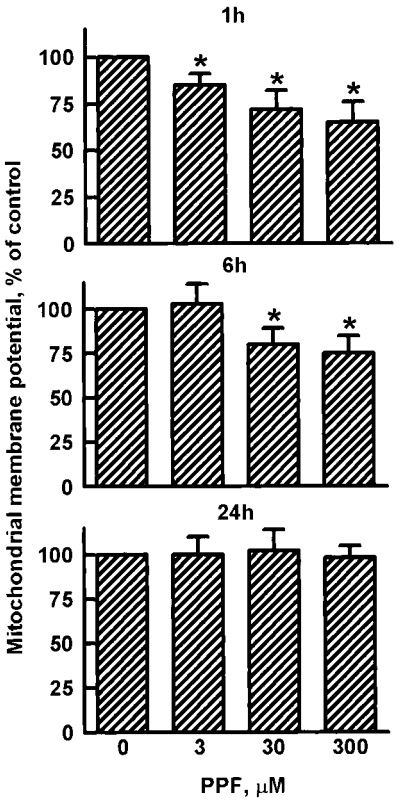

Fig. 4. Concentration- and time-dependent effects of propofol (PPF) on the membrane potential of macrophage mitochondria. Macrophages were exposed to 3, 30, and 300 μM PPF for 1, 6, and 24 h. Mitochondrial membrane potential was determined by the flow cytometric method. Each value is expressed as mean ± SD for n = 6. *Values were considered to be statistically different from the respective control when the *P* value was less than 0.05.

fluorescent particles. Propofol at 300 μM decreased phagocytic activity by 61% and 72% in the 6- and 24-h-treated macrophages, respectively.

In untreated macrophages, IFN-γ mRNA was not detectable (fig. 3A, *top panel*, lane 1). Following lipopolysaccharide stimulation, IFN-γ mRNA was induced in macrophages (fig. 3A, *top panel*, lane 2). Exposure of macrophages to 30 μM propofol did not affect the expression of IFN-γ mRNA (fig. 3A, *top panel*, lane 3). Cotreatment with propofol and lipopolysaccharide apparently inhibited lipopolysaccharide-induced IFN-γ mRNA (fig. 3A, *top panel*, lane 4). The level of β-actin mRNA was detected as the internal standard (fig. 3A, *bottom panel*). Digital analysis of the image revealed that propofol significantly inhibited (approximately 80%) the lipopolysaccharide-induced IFN-γ mRNA level (fig. 3B).

Mitochondrial membrane potential was detected to determine the role of mitochondria in propofol-caused suppression of macrophage functions (fig. 4). In the 1-h-propofol-treated macrophages, propofol at 3, 30, and 300 μM significantly reduced the membrane potential of mitochondria, by 15%, 28%, and 35%, respectively (fig. 4, *top panel*). After 6 h, 30 and 300 μM propofol caused 20% and 25% decreases in the membrane potential of mitochondria (fig. 4, *middle panel*). In the 24-h-treated macrophages, propofol did not influence mitochondrial membrane potential (fig. 4, *bottom panel*).

Exposure of macrophages to a clinically relevant concentration of propofol, 30 μM, for 1 h led to a significant (43%) decrease in cellular ATP levels (table 3). The levels of cellular ATP in macrophages exposed to propofol for 6 h were decreased approximately 30%. Treatment with propofol for 24 h caused a 29% decrease in cellular ATP levels.

Discussion

The present study has shown that propofol could impair macrophage functions. Propofol could suppress macrophage capacities for migration, particle ingestion, and oxidant production. In response to lipopolysaccha-

Table 3. Effects of Propofol on Cellular Adenosine Triphosphate Levels

Time, h	ATP, pmol
0	35 ± 11
1	20 ± 9*
6	24 ± 7*
24	26 ± 9*

Values shown as mean ± SD for n = 6. Macrophages were exposed to 30 μm for 1, 6, and 24 h. The amounts of cellular ATP were detected by a bioluminescence assay.

* $P < 0.05$; statistically different from the respective control.

ATP = adenosine triphosphate.

ride stimulation, propofol has also been shown to inhibit INF-γ mRNA synthesis in macrophages. During inflammation, macrophages destroy invaded microorganisms or abnormal tumor cells through a series of reactions, including chemotaxis, phagocytosis, oxidant synthesis, and cytokine release.[8,9] Dysfunction of these activities will affect host macrophage-mediated immunity.[10]

The concentration of propofol used in this study, 30 μM, was within the range of clinical relevance.[26] Therefore, propofol at a therapeutic concentration, 30 μM, was able to suppress macrophage functions of chemotaxis, phagocytosis, oxidant production, and IFN-γ mRNA synthesis.

A report by Kotani *et al.*[11,12] revealed that during operation, propofol anesthesia caused cell aggregation, decreased phagocytic and microbicidal activities, and modulated cytokine expression in alveolar macrophages. Because a variety of factors can be involved in the surgical procedures, the *ex vivo* studies did not clarify whether propofol alone could modulate macrophage activities.[11] The present study has provided *in vitro* data to identify the suppressive effects of propofol on macrophage functions.

In parallel with the macrophage dysfunction, this study demonstrated that therapeutic concentrations of propofol could reduce the membrane potential of mitochondria. The levels of cellular ATP were also significantly decreased after administration of propofol. Previous studies had shown that propofol could modulate the ATP levels in rat brain synaptosomes, human platelets, or guinea pig cardiomyocytes.[21–25]

This is the first study to identify the suppressive effects of propofol on mitochondrial membrane potential and cellular ATP levels in macrophages. Exposure of macrophages to therapeutic concentrations of propofol did not affect cell viability. Thus, the propofol-caused suppression of macrophage functions may not be due to the cytotoxic effect. Macrophage function is dependent on the maintenance of mitochondrial membrane potential and cellular ATP synthesis.[14,15,20] Therefore, the propofol-caused inhibition of mitochondrial activities could be one possible mechanism of suppression of macrophage functions by this anesthetic agent.

Propofol could decrease chemotactic and phagocytic activities of macrophages. Previous studies revealed that cellular ATP was involved in the modulation of chemotactic and phagocytic activities.[16–19] This study showed that propofol at therapeutic concentrations inhibited ATP synthesis. Thus, one possible reason for the inhibitory effects of propofol on chemotaxis and phagocytosis is that this anesthetic agent could decrease ATP synthesis, which then led to the reduction of chemotactic and phagocytic activities. This study also demonstrated that propofol at a therapeutic concentration, 30 μM, could inhibit IFN-γ mRNA synthesis in lipopolysaccharide-activated macrophages.

Lechleitner *et al.*[30] reported that interferons could regulate adhesion molecule-1 and influence cell migration. The expression of adhesion molecule-1 has been identified in murine macrophage-like Raw 264.7 cells.[31] Weinshank *et al.*[32] showed that IFN-γ stimulation could increase the levels of receptor Fc γ Rs in Raw 264.7 cells and then induce cell phagocytosis. Therefore, the propofol-caused suppression of IFN-γ production is another possible mechanism for the inhibition of chemotactic and phagocytic activities by this anesthetic agent.

The propofol-caused suppression of chemotaxis in macrophages decreased with time. In the 1-h propofol-treated group, therapeutic concentrations of propofol significantly decreased chemotactic activities of macrophages. The suppressive effects were partially and completely recovered after 6 and 24 h, respectively. Propofol progressively decomposes in aerobic conditions or after exposure to visible light.[1] This characteristic might explain why the propofol-caused suppression of macrophage chemotaxis decreases with time.

However, the time-dependent inhibition of chemotactic suppression was not observed in macrophages exposed to the high concentration, 300 μM, of propofol. Exposure of macrophages to 300 μM propofol for 6 and 24 h caused significant cell death. Thus, propofol at the high concentration could not result in time-dependent recovery of chemotactic suppression, possibly because of its death effect with long-term treatment. Administration of 300 μM propofol for 24 h caused 44% cell death. However, when macrophages were exposed to 300 μM for 24 h, the activity of cell migration was suppressed by 32%. The difference in the results of cell viability and chemotaxis could be due to the variation of analytic methods and sensitivities.

This study demonstrated that propofol decreased phagocytic activities of macrophages in concentration- and time-dependent manners. Exposure of macrophages to 300 μM propofol for 24 h resulted in 44% cell death. The inhibitory effect of 300 μM propofol on macrophage phagocytosis might be partially due to the cytotoxic effect of this anesthetic agent on cells.

Propofol could reduce oxidative ability of macrophages, but the inhibitory effect was recovered with time. In the 1-h-treated group, propofol reduced the

levels of intracellular reactive oxygen species in macrophages in a concentration-dependent manner. After administration of propofol for 6 and 24 h, the propofol-caused reduction in intracellular oxidant production was partially and completely reduced. Hydrogen peroxide, superoxide, and nitric oxide are three typical oxidants in macrophages for killing infected pathogens.[33] Similar to phenol-containing α-tocopherol and butylated hydroxytoluene in structure, propofol has been shown to be able to directly scavenge hydrogen peroxide and superoxide.[34]

Our previous study further showed that therapeutic concentrations of propofol could protect macrophages from nitric oxide–induced cell death but not through the direct scavenging of this oxidant.[35] Thus, the direct or indirect decreases in the levels of intracellular oxidants might be the main cause for the propofol-mediated suppression of oxidative ability in macrophages.

Our present data reveal that propofol caused significant decreases in the mitochondrial membrane potential of macrophages. Mitochondria are the target organelles for synthesizing ATP in macrophages.[14,15] The propofol-caused reduction of mitochondrial membrane potential could directly affect ATP synthesis and further suppress macrophage functions. The inhibitory role of propofol on the membrane potential of mitochondria decreased with time.

The major explanation for the time-dependent decrease in the inhibitory effect of propofol might be that this anesthetic agent could be progressively decomposed in aerobic conditions or after exposure to visible light.[1] Another possible reason might be the metabolism of propofol by cytochrome P450–dependent monooxygenases and uridine diphosphate glucuronosyltransferases in macrophages.[36] Because control macrophages were treated with DMSO alone in this study, the possibility of DMSO causing the suppression of macrophage membrane potential could be low.

Our unpublished data reveal that the remaining concentrations of propofol in macrophages and culture medium were one half and one third after administration of this anesthetic agent for 1 and 6 h, respectively. After exposure to propofol for 24 h, propofol was undetectable in cells and culture medium. However, the suppressive effects of propofol on macrophage functions had also been observed in 24-h-treated macrophages. Therefore, propofol could directly result in macrophage dysfunction in a short treatment interval or indirectly impair macrophage activities through activating certain signal-transducing pathways in a long interval.

In this study, propofol was dissolved in DMSO. Masson *et al.*[37] reported that DMSO (1%) could inhibit inflammatory cytokine IL-8 production in human bronchial epithelial cells. Hare and Atchison[38] showed that DMSO had voltage-dependent effects on mitochondrial membrane potential in synaptosomes. Although the concentration of DMSO used in this study was less than 0.1%, the suppression of DMSO on macrophage functions could not be ruled out.

In conclusion, the present study has shown that therapeutic concentrations of propofol could suppress macrophage functions of chemotaxis, phagocytosis, oxidative ability, and IFN-γ mRNA production. Our results also demonstrated that propofol at therapeutic concentrations could reduce mitochondrial membrane potential and cellular ATP synthesis but did not affect cell viability.

On the basis of the present data, we suggest that the mechanism of propofol-caused suppression of macrophage functions might be the inhibition of mitochondrial membrane potential and ATP synthesis but not a reduction in cell viability. There were certain limitations in this study. Because this *in vitro* study did not evaluate the effects of propofol on other humoral and tissue factors, we cannot clinically conclude the possible effects of propofol on macrophage functions.

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