行政院國家科學委員會補助專題研究計畫成果報告

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計畫主持人：戴裕庭

共同主持人：陳瑞明

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行政院國家科學委員會專題研究計畫成果報告

麻醉藥物之免疫調控作用研究： 探討靜脈麻醉藥物 2 ,6-雙異丙烷酚調控巨噬細胞功能之機制

- MODULATORY EFFECTS OF ANESTHETIC AGENTS: STUDY OF SUPPRESSIVE MECHANISM - OF 2,6-DIISOPROPYLPHENOL - ON MACROPHAGE FUNCTIONS

計畫編號：NSC91－2314－B－038－026－
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Abstract

Background: Propofol is an intravenous anesthetic agent that may impair host defense system. This study was aimed 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, chemotaxis, phagocytosis, 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 hour-treated macrophages, propofol significantly reduced macrophage functions of chemotaxis and oxidative ability in a concentration-dependent manner. However, the suppressive effects were partially or completely reversed after 6 and 24 hours. Propofol could reduce phagocytotic 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 mitochondria of macrophages, 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 through inhibiting their mitochondrial membrane potential and adenosine triphosphate synthesis instead of direct cellular toxicity.

Keywords: 2,6-diisopropylphenol, macrophages, lipopolysaccharide, cytokines, chemotaxis, phagocytosis

Introduction

Propofol (PPF; 2,6-diisopropylphenol) is one of widely used intravenous anesthetic agents for induction and maintenance of anesthesia in surgical procedures (1). PPF 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 PPF might have immunomodulating effects (5-7).

Macrophages play a critical role in cellular host defense to 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 non-specific cell mediated immunity (10). An ex vivo study revealed that anesthesia with PPF or isoflurane time-dependently decreased phagocytosis and microbicidal activities of alveolar macrophages intraoperatively (11). PPF anesthesia has also been reported to induce pro-inflammatory cytokines, including interleukin-1β, interleukin-8, interferon-γ (IFN-γ) and tumor necrosis factor-α in orthopedic surgery patients (12). However, there are multiple factors in the surgical procedures contributing to the modulation of macrophage functions (13). Therefore, an in vitro study will be needed to validate the role of PPF 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 (ATP), synthesized from the mitochondria respiratory chain reaction, can enhance chemotaxic migration and phagocytotic 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 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 PPF might impair mitochondrial electron transport chain and ATP production in rat brain synaptosomes, human platelets and guinea pig cardiomyocytes (21-25). However, there is a paucity of studies evaluating the effects of PPF on macrophage mitochondria. This study is aimed at evaluating the effects of PPF on the modulation of macrophage functions from the aspects of cytotoxicity, chemotaxis, phagocytosis, oxidative ability and IFN-γ mRNA production and 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, 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 ^oC in a humidified atmosphere of 5 % CO_2. The cells were grown to a confluence prior to PPF administration.

PPF donated by Zeneca Limited (Macclesfield, Cheshire, UK) was stored under nitrogen, protected from light and freshly prepared by dissolving it in dimethyl sufoxide (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, PPF at 3, 30 and 300 μM, which are corresponded to 0.1, 1 and 10 times the clinical plasma concentration (26), were chosen to be the administered dosage in this study. Control macrophages were treated with DMSO only.

Assay of Cytotoxicity

To determine the toxicity of PPF 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 (MTT) into an insoluble dark blue formazan as described previously (27). The amounts of lactate dehydrogenase in the culture medium were quantified using a model 7450 automatic autoanalyzer system of 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 PPF. After administration of PPF, 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 ^oC for 30 min and resuspended with 20 μg/ml propidium iodide. The stained macrophages were analyzed by a flow cytometer (FACS Calibur, Becton Dickinson, San Jose, CA, USA).

Assay of Chemotaxic Activity

The migrating capacity of macrophages was determined by using the Costar Transwell cell culture chamber inserts, pore size 8 μm, according to the application guide provided by Corning Costar Corporation (Cambridge, MA, USA). The rich RPMI-1640 medium (1.5 ml) was first added to 12-well tissue cluster plates (Corning Costar Corporation, Cambridge, MA, USA), and the Transwell was inserted in the plates. Macrophages (1 x 10^5) suspended with PPF in 0.5 ml rich medium was added to the inside of Transwell and cultured at 37 ^oC for 1, 6 and 24 hours 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 3 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 as described previously (28). Briefly, 1 x 10^5 macrophages were cultured in 12-well tissue culture clusters for overnight, and then co-treated with PPF and 2,7-dichlorofluorescein 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, Becton Dickinson, San Jose, CA, USA).

Assay of Phagocytotic 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, Inc., Eugene, OR, USA), 0.5 μm 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 Corporation, Tokyo, Japan).

Reverse Transcription – Polymerase Chain Reaction Assay

Messenger RNA from macrophages exposed to PPF for one hour was prepared for reverse transcriptase-polymerase chain reaction (RT-PCR) analyses of interferon-γ (IFN-γ) and β-actin mRNA following the instruction of the ExpressDirect™ mRNA Capture and RT System for RT-PCR kit (Pierce, Rockford, IL, USA). Oligonucleotides for PCR analyses of mouse IFN-γ and β-actin mRNA were designed and synthesized by the CLONTECH Laboratories, Inc. (Palo Altc, CA, USA). The primer sequences for IFN-γ mRNA analysis are 5'-TGAACGCTACACACTGCATCTTGG-3' and 5'-CGACTCCTTTTCCGCTTCCTGAG-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'-CTCTTTGATGTCACGCACGATTTC-3', respectively. The PCR reaction was carried out using 35 cycles including 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 a 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 (1988) (29). Briefly, 1×10^5 macrophages were seeded in 12-well tissue culture clusters for overnight and then treated with PPF for 1, 6 and 24 hours. 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 x PBS buffer, and the cellular fluorescent intensities were analyzed by a flow cytometer (FACS Calibur, Becton Dickinson, San Jose, CA, USA).

Detection of Cellular Adenosine Triphosphate Levels

The levels of cellular ATP in macrophages were determined by a bioluminescence assay, which was based on luciferase's requirement for ATP in producing emission light, according to the protocol of Molecular Probes' ATP Determination kit (Molecular Probes, Inc., Eugene, OR, USA). The luminent light (560 nm) emitted by the luciferase-mediated reaction of ATP and lucifenin was detected by a WALLAC $VICTOR^2{}_{TM}$ 1420 multilabel counter (Welch Allyn Inc., Turku, Finland).

Statistical Analysis

The statistical difference between groups was considered significant when the *P* value of the Duncan's multiple rang test was less than 0.05. Statistical analysis between groups over time was carried out by the two-way ANOVA.

Results

The toxicity of PPF to macrophages was analyzed according to its influence to cell viability, lactate dehydrogenase release and cell cycle. Exposure of macrophages to 3 and 30 μM PPF for 1, 6 and 24 hours did not affect cell viability (Table 1). However, PPF at 300 μM caused 28 % and 44 % cell death in the 6 and 24 hour PPF-treated macrophages, respectively. Administration of 3 and 30 μM PPF for 1, 6 and 24 hours 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 % following treatment of 300 μM PPF for 6 and 24 hours, respectively. Analysis of cell cycle revealed that PPF at 3 and 30 μM did not affect the growth of macrophages (Table 1). However, PPF at 300 μM significantly caused a 26 % increase in G1/S phase and a 38 % decrease in G2/M phase after 24 hours (Table 1).

The cell number that migrated to the bottom membrane of Transwell was counted to determine the chemotaxic activities of macrophages (Fig. 1). In the 1 hour-treated macrophages, PPF at 30 and 300 μM significantly reduced chemotaxic activity by 29 % and 47 %, respectively (*top panel*). When the treated time intervals reached 6 hours, 30 and 300 μM PPF decreased chemotaxic activity by 17 % and 40 % (*middle panel*). After 24 hours, PPF only at 300 μM significantly caused a 32 % decrease in chemotaxic

activities (*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 hour-treated macrophages, PPF at 30 and 300 μM decreased oxidative ability by 47 % and 73 %, respectively (*top panel*). After 6 hours, 30 and 300 μM PPF reduced oxidative ability by 20 % and 65 %, respectively (*middle panel*). PPF did not affect oxidative ability when exposing to this anesthetic for 24 hours (*bottom panel*).

The fractions of macrophages that ingested at least one fluorescent particle were counted in order to determine the phagocytotic activities of these immune cells. Exposure of macrophages to 3 μM PPF for 1, 6 and 24 hours did not affect phagocytotic activity (Table 2). Treatment of 30 μM PPF for 6 and 24 hours caused 54 % and 64 % decreases in the fractions of macrophages that ingested fluorescent particles. PPF at 300 μM decreased phagocytotic activity by 61 % and 72 % in the 6 and 24 hour-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 (lane 2). Exposure of macrophages to 30 μM PPF did not affect the expression of IFN-γ mRNA (lane 3). Co-treatment of PPF and lipopolysaccharide apparently inhibited lipopolysaccharide-induced IFN-γ mRNA (lane 4). The levels of β-actin mRNA was detected as the internal standard (Fig. 3A, *bottom panel*). Digital analysis of image revealed that PPF significantly inhibited about 80 % lipopolysaccharide-induced IFN-γ mRNA level (Fig.. 3B).

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

Exposure of macrophages to a clinically relevant concentration of PPF, 30 μM, for 1 hour significantly led to a 43 % decrease in cellular ATP levels (Table 3). The levels of cellular ATP in macrophages exposed to PPF for 6 hours were decreased about 30 %. Treatment of PPF for 24 hours caused a 29 % decrease in cellular ATP levels.

Discussion

The present study has shown that PPF could impair macrophage functions. PPF could suppress macrophage capacities for migration, particle ingestion and oxidant production. In response to lipopolysaccharide stimulation, PPF 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 cytokines release (8,9). Dysfunction of these activities will affect host macrophage-mediated immunity (10). The concentration of PPF used in this study, 30 μM, was within the range of clinical relevance (26). Therefore, PPF at a therapeutic concentration, 30 μM, was able to suppress macrophage functions of chemotaxis, phagocytosis, oxidant production and IFN-γ mRNA synthesis. Report from Kotani et al. (1998, 1999) revealed that during operation, PPF anesthesia caused cell aggregation, decreased phagocytotic and microbicidal activities and modulated cytokine expression in alveolar macrophages (11,12). Because a variety of factors can be involved in the surgical procedures, the ex vivo studies did not clarify if PPF alone could modulate macrophage activities (11). After administration of PPF for 1, and 6 hours, the concentrations of this anesthetic agent in the cells and culture medium remained half and one-third. However, after 24 hours, the concentration of PPF was non-detectable in macrophages and culture medium. The present study has provided in vitro data to identify the suppressive effects of PPF on macrophage functions.

In parallel to the macrophage dysfunction, this study demonstrated that therapeutic concentrations of PPF could reduce the membrane potential of mitochondria. Hare and Atchison (1992) showed that DMSO had voltage-dependent effects on mitochondrial membrane potential in synaptosomes. 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. The levels of cellular ATP were also significantly decreased following administration of PPF. Mitochondria play a critical role in the maintenance of microphage functions (14,15).. For example, the mitochondrial ATP synthesis has been reported to contribute to the activities of chemotaxis and phagocytosis in macrophages and neutrophils (17,18). Ayala and Chaudry (1996) reported that the reduction of cellular ATP levels decreased the functions of lymphocytes and macrophages (20). Thus, the suppression of macrophage functions induced by PPF might be caused through its impact to the mitochondrion function and the reduction of cellular ATP levels. Previous studies had shown that PPF 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 PPF on mitochondrial membrane potential and cellular ATP levels in macrophages. Exposure of macrophages to therapeutic concentrations of PPF did not affect cell viability. Thus, the PPF-caused

suppression of macrophage functions may be not due to the cytotoxic effect.. From the present data, we suggest that the mechanism of PPF-caused suppression on macrophage functions is possibly through its modulation to mitochondrial membrane potential and cellular ATP synthesis.

PPF could decrease chemotaxic activities of macrophages. A therapeutic concentration of PPF, 30 μM, reduced the migration of macrophages. Cellular ATP is involved in the modulation of chemotaxic activities (16,19). This study showed that PPF at therapeutic concentrations inhibited ATP synthesis. Thus, one of possible reasons to explain the inhibitory effects of PPF on chemotaxis is that this anesthetic agent could decrease ATP synthesis and then led to the reduction of chemotaxic activities. Administration of 300 μM PPF for 24 hours caused 44 % cell death. However, when macrophages were exposed to 300 μM for 24 hours, 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 their sensitivity.

The PPF-caused suppression of chemotaxis in macrophages was descended with time. In the 1 hour PPF-treated group, therapeutic concentrations of PPF significantly decreased chemotaxic activities of macrophages. The suppressive effects were partially and completely recovered after 6 and 24 hours. PPF could be progressively decomposed after exposure to visible light and in aerobic condition (1). This characteristic might explain why the PPF-caused suppression of macrophage chemotaxis is decreased with time. However, the time-dependent inhibition of chemotaxic suppression was not observed in macrophages exposed to the high concentration, 300 μM, of PPF. Exposure of macrophages to 300 μM PPF for 6 and 24 hours significantly caused cell death. Thus, PPF at the high concentration could not result in time-dependent recovery of chemotaxic suppression possibly because of its death effect under the long-term treatment.

The phagocytotic activities of macrophages were determined by assaying the fractions of cell ingesting fluorescent particles. This study demonstrated that PPF decreased phagocytotic activities of macrophages in concentration- and time-dependent manners. Previous studies had shown that the integrity of mitochondrion activities, including ATP synthesis, was involved in macrophage phagocytosis (17,18). PPF could reduce mitochondrial membrane potential and ATP synthesis. The PPF-caused mitochondria dysfunction might be one of possible reasons for explaining the suppression of phagocytotic activities. After administration of macrophages with 300 μM PPF for 24 hours resulted in 44 % cell death. The inhibitory effects of a high concentration PPF (300 μM) on macrophage phagocytosis might be partially due to the cytotoxicity of this anesthetic agent to the cells.

PPF could reduce oxidative ability of macrophages, but the inhibitory effect was recovered with time. In the 1 hour-treated group, PPF reduced the levels of intracellular reactive oxygen species in macrophages in a concentration-dependent manner. After administration of PPF for 6 and 24 hours, the PPF-caused reduction of intracellular oxidant production were partially and completely descended. Hydrogen peroxide, superoxide and nitric oxide are three typical oxidants in macrophages for killing infected pathogens (33).. Being similar to phenol-containing α-tocopherol and butylated hydroxytoluene in structure, PPF has been shown to be able to directly scavenging hydrogen peroxide and superoxide (34). Our previous study has further shown that therapeutic concentrations of PPF could protect macrophages from nitric oxide-induced cell death 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 PPF mediated suppression of oxidative ability in macrophages. The reason to explain the time-dependent decrease in PPF-caused suppression of macrophage chemotaxis might be suitable to explain why the suppression of phagocytosis was recovered with time.

This study demonstrated that PPF at a therapeutic concentration, 30 μM, could inhibit IFN-γ mRNA synthesis in lipopolysaccharide-activated macrophages. In response to lipopolysaccharide stimulation, the levels of IFN-γ mRNA were significantly induced. However, the induction was blocked by PPF. Massion et al. (1996) reported that DMSO (1 %) could inhibit inflammatory cytokine IL-8 production in human bronchial epithelial cells (36). Although the concentration of DMSO used in this study was less than 0.1 %, the possibly suppressive effect of DMSO on lipopolysaccharide-induced IFN-γ mRNA was not ruled out. During inflammation, IFN-γ has a critical role in the chemotaxic migration and microorganism ingestion of macrophages (30,32). The PPF-caused inhibition of IFN-γ mRNA in macrophages could decrease the inflammatory responses. Lechleitner et al. (1998) reported that interferons could regulate adhesion molecule-1 and influence cell migration (30). The expression of adhesion molecule-1 has been identified in murine macrophage-like Raw 264.7 cells (31). Our present data revealed that PPF could inhibit IFN-γ mRNA in macrophages. Therefore, another possible mechanism of PPF-caused suppression on macrophage chemotaxis might be due to the inhibition of IFN-γ mRNA. Weinshank et al. (1988) showed that the IFN-γ stimulation could increase the levels of receptor Fc gamma Rs in Raw 264.7 cells, and then induced cell phagocytosis (32). Suppression of IFN-γ response to lipopolysaccharide by PPF provides a possible mechanism for its inhibition to phagocytotic activity of macrophages.

Our present data reveals that PPF caused significant decreases in the mitochondrial membrane

potential of macrophages. Mitochondria are the target organelle for synthesizing ATP in macrophages (14,15). In parallel to the inhibition of the membrane potential, PPF suppressed the levels of cellular ATP. ATP, which is an important energy source, contributes to the maintenance of macrophage functions (16,18,19). The PPF-caused reduction of mitochondrial membrane potential could directly affect ATP synthesis and further suppresses macrophage functions. The inhibitory role of PPF on the membrane potential of mitochondria decreased with time. The major explanation for the time-dependent decrease of the inhibitory effect of PPF might be that this anesthetic agent could be progressively decomposed after exposure to visible light and in aerobic condition (1). Another possible reason might be due to the metabolism of PPF by cytochrome P450-dependent monooxygenases and uridine diphosphate glucoronosyltransfeases in macrophages (37).

In conclusion, the present study has shown that therapeutic concentrations of PPF could suppress macrophage functions of chemotaxis, phagocytosis, oxidative ability and IFN-γ mRNA production. Our results also demonstrated that PPF at therapeutic concentrations could reduce mitochondrial membrane potential and cellular ATP synthesis but did not affect cell viability. According to the present data, we suggest that the mechanism of PPF-caused suppression of macrophage functions might be through the inhibition of mitochondrial membrane potential and ATP synthesis but not through a reduction in cell viability.

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Table 1. Cytotoxic effects of propofol on macrophages

Treatment	Cell viability (% of control)	LDH (U/L)	Cell cycle, % G1/S Phase	Cell cycle, % G2/M phase
1 hour				
PPF, 0 μM	100	65 ± 18	56 ± 3	28 ± 3
PPF, 3 μM	100 ± 11	70 ± 25	55 ± 3	28 ± 2
PPF, 30 μM	104 ± 22	58 ± 11	54 ± 3	30 ± 5
PPF, 300 μM	101 ± 15	80 ± 22	55 ± 3	28 ± 3
6 hours				
PPF, 0 μM	100	74 ± 16	55 ± 3	28 ± 3
PPF, 3 μM	96 ± 25	71 ± 15	55 ± 2	29 ± 3
PPF, 30 μM	108 ± 33	74 ± 20	56 ± 3	28 ± 3
PPF, 300 μM	72 ± 16*	113 ± 10*	54 ± 3	29 ± 3
24 hours				
PPF, 0 μM	100	78 ± 22	54 ± 2	29 ± 3
PPF, 3 μM	101 ± 11	77 ± 21	55 ± 4	29 ± 2
PPF, 30 μM	103 ± 13	82 ± 15	57 ± 5	29 ± 6
PPF, 300 μM	56 ± 18*	175 ± 18*	68 ± 6*	18 ± 6*

Macrophages were treated with 3, 30 and 300 μM propofol (PPF) for 1, 6 and 24 hours. Cell viability, lactate dehydrogenase (LDH) release and cell cycle were analyzed to determine the toxicity of PPF to macrophages. Each value was expressed as Mean ± SD for n = 9. *Values were considered to be statistically different from the respective control when the *P* value was less than 0.05.

Table 2. Concentration- and time-dependent effects of propofol on phagocytotic activities of macrophages

PPF, μM	Phagocytosis, cell number x 10^2 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*

Macrophages were treated with 3, 30 and 300 μM propofol (PPF) for 1, 6 and 24 hours. Phagocytotic activities were determined by counting the fractions of macrophages that digested at least one fluorescent particle. Each value was expressed as 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.

Table 3. Effects of propofol on cellular ATP levels

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

Macrophages were exposed to 30 μM propofol (PPF) for 1, 6 and 24 hours. The amounts of cellular adenosine triphosphate (ATP) were detected by a bioluminescence assay. Each value was 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.

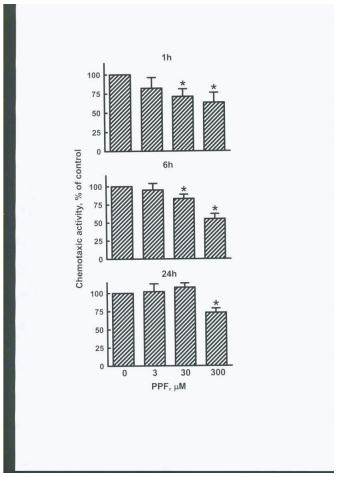
Legends to figures

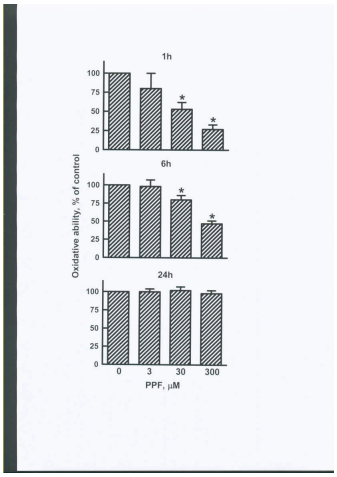
Fig. 1. Concentration- and time-dependent effects of propofol (PPF) on chemotaxic activities of macrophages. Macrophages were exposed to 3, 30 and 300 μM PPF for 1, 6 and 24 hours, respectively. Chemotaxic activities were assayed by the Transwell cell culture chamber inserts as described in Materials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

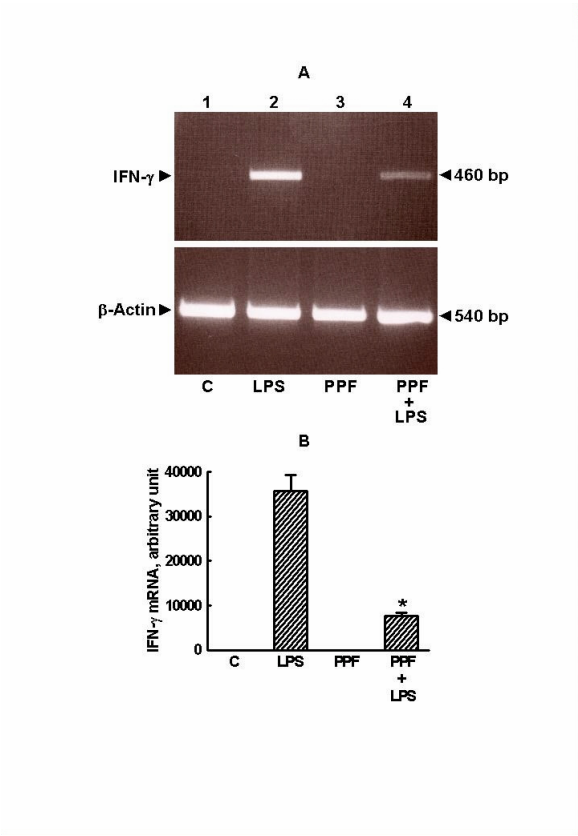
Fig. 2. Concentration- and time-dependent effects of propofol (PPF) on oxidative ability of macrophages. Macrophages were exposed to 3, 30 and 300 μM PPF for 1, 6 and 24 hours. The levels of intracellular reactive oxygen species were determined by the flow cytometric method. Each value was expressed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

Fig. 3. Effect of propofol (PPF) on 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 RT-PCR 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 was expressed as Mean ± SEM for 3 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 hours. Mitochondrial membrane potential were determined by the flow cytometric method. Each value was expressed as Mean ± SEM for n > 6. *Values were considered to be statistically different from the respective control when the *P* value was less than 0.05.






A
1
2
3
4
IFN-γ ▶
◀460 bp
β-Actin ▶
◀540 bp
C
LPS
PPF
PPF + LPS
B
IFN-γ mRNA, arbitrary unit
40000
30000
20000
10000
0
C
LPS
PPF
PPF + LPS
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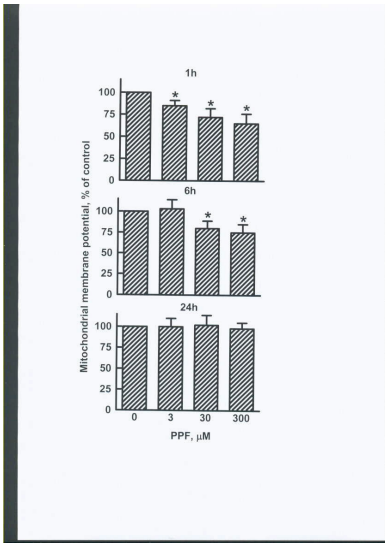
1h
6h
24h
Mitochondrial membrane potential, % of control
100
75
50
25
0
*
0
3
30
300
PPF, μM