# Anti-Inflammatory and Antioxidative Effects of Propofol on Lipopolysaccharide-Activated Macrophages

RUEI-MING CHEN,[a,b,e] TYNG-GUEY CHEN,[b,e] TA-LIANG CHEN,[c] LI-LING LIN,[a] CHIA-CHEN CHANG,[a] HWA-CHIA CHANG,[b] AND CHIH-HSIUNG WU[d]

[a]*Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan*

[b]*Department of Anesthesiology, Wan-Fang Hospital, College of Medicine, Taipei Medical University, Taipei, Taiwan*

[c]*Taipei City Hospital and Taipei Medical University, Taipei, Taiwan*

[d]*Department of Surgery, Taipei Medical University Hospital, College of Medicine, Taipei Medical University, Taipei, Taiwan*

**ABSTRACT: Sepsis is a serious and life-threatening syndrome that often occurs in intensive care unit (ICU) patients. During sepsis, inflammatory cytokines and nitric oxide (NO) can be overproduced, causing tissue and cell injury. Propofol is an intravenous agent used for sedation of ICU patients. Our previous study showed that propofol has immunosuppressive effects on macrophage functions. This study was designed to evaluate the anti-inflammatory and antioxidative effects of propofol on the biosyntheses of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, and NO in lipopolysaccharide (LPS)-activated macrophages. Exposure to a therapeutic concentration of propofol (50 μM), LPS (1 ng/mL), or a combination of these two drugs for 1, 6, and 24 h was not cytotoxic to the macrophages. ELISA revealed that LPS increased macrophage TNF-α, IL-1β, and IL-6 protein levels in a time-dependent manner, whereas propofol significantly reduced the levels of LPS-enhanced TNF-α, IL-1β, and IL-6 proteins. Data from RT-PCR showed that LPS induced TNF-α, IL-1β, and IL-6 mRNA, but propofol inhibited these effects. LPS also increased NO production and inducible nitric oxide synthase (iNOS) expression in macrophages. Exposure of macrophages to propofol significantly inhibited the LPS-induced NO biosynthesis. The present study shows that propofol, at a therapeutic concentration, has anti-inflammatory and antioxidative effects on the biosyntheses of TNF-α, IL-1β, IL-6, and NO in LPS-activated macro-phages and that the suppressive effects are exerted at the pretranslational level.**

**KEYWORDS: propofol; macrophages; anti-inflammation; TNF-α; IL-1β; IL-6; antioxidation; nitric oxide**

[e] R-M.C. and T-G.C. contributed equally to this paper.

Address for correspondence: Chih-Hsiung Wu, Department of Surgery, Taipei Medical University Hospital, or Ruei-Ming Chen, Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing St., Taipei 110, Taiwan. Voice: +886-2-29307930 ext. 2161; fax: +886-2-86621119.

chwu@tmu.edu.tw or rmchen@tmu.edu.tw

**Ann. N.Y. Acad. Sci. 1042: 262–271 (2005).** **© 2005 New York Academy of Sciences.**
**doi: 10.1196/annals.1338.030**

## INTRODUCTION

Sepsis can cause multiple system organ failure, which often leads to high mortality for intensive care unit (ICU) patients.[1,2] Lipopolysaccharide (LPS), an outer-membrane component of gram-negative bacteria, is one of the critical factors contributing to the pathogenesis of sepsis.[3] *In vivo* studies demonstrated that exposure of animals and human volunteers to LPS can induce inflammatory responses seen in sepsis.[3] Macrophages play important roles in infection-associated tissue injury.[4,5] During inflammation, macrophages can produce and release large amounts of inflammatory cytokines and oxidants into the general circulation to exert the systemic effects that occur in the sepsis syndrome.[6,7] Modulating these inflammatory factors may influence the LPS-induced septic pathogenesis.

Propofol (2,6-diisopropylphenol) is one of the widely used intravenous anesthetic agents for induction and maintenance of anesthesia for surgical procedures.[8] In the ICU, propofol can be used as an effective sedative agent. Propofol has the advantages of rapid onset, short duration of action, and rapid elimination.[9] Studies using human neutrophils and leukocytes demonstrated that propofol might have immunomodulating effects.[10,11] In response to LPS stimuli, tumor necrosis factor $\alpha$ (TNF-$\alpha$), interleukin 1$\beta$ (IL-1$\beta$), IL-6, and nitric oxide (NO) are typical inflammatory factors that are produced by activated macrophages and are involved in immune responses and host defense.[12] Our previous study showed that propofol can modulate macrophage functions via the suppression of migration, phagocytosis, and oxidative ability.[13] Data from another study revealed that therapeutic concentrations of propofol can protect macrophages from NO-induced insults to the cell.[14] The present study was designed to evaluate the anti-inflammatory and antioxidative effects of propofol on the biosyntheses of TNF-$\alpha$, IL-1$\beta$, IL-6, and NO in LPS-activated macrophages.

## MATERIALS AND METHODS

### *Cell Culture and Drug Treatment*

Mouse macrophage-like Raw 264.7 cells were purchased from American Type Culture Collection (Rockville, MD). Macrophages were maintained in RPMI 1640 medium (Gibco-BRL, Grand Island, NY) supplemented with 10% fetal calf serum, 100 IU/mL penicillin, and 100 $\mu$g/mL streptomycin in 75-cm$^2$ flasks at 37°C in a humidified atmosphere of 5% $CO_2$. These cells were grown to confluence before drug treatment.

Propofol donated by 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 kept to <0.1% to avoid the toxicity of this solvent to macrophages. According to the clinical application, propofol at 50 $\mu$M, which corresponds to a clinical plasma concentration,[15] was chosen as the dosage administered in this study. Control macrophages were treated with DMSO only.

### *Assay of Cell Viability*

Macrophage viability was analyzed using a variation of the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is based on the ability of live cells to reduce MTT to a blue formazan product.[16] Macrophages ($1 \times 10^4$) were seeded in 96-well tissue culture plates overnight. After drug treatment, cells were renewed with medium containing 0.5 mg/ml MTT and then cultured for another 3 h. The blue formazan product in cells was dissolved in DMSO and measured spectrophotometrically at a wavelength of 570 nm.

### *Determination of Nitrite*

After drug treatment, the amounts of nitrite, an oxidative product of NO, in the culture medium of macrophages was detected according to the technical bulletin of Promega's Griess reagent system (Promega, Madison, WI).

### *Enzyme-Linked Immunosorbent Assay*

The amounts of TNF-α, IL-1β, and IL-6 in the culture medium of macrophages exposed to LPS, propofol, and a combination of propofol and LPS were determined following the standard protocols of the ELISA kits purchased from Endogen (Woburn, MA).

### *Immunoblotting Analysis*

After pretreatment with the drugs for 24 h, macrophages were washed with phosphate-buffered saline (PBS) (0.14 M NaCl, 2.6 mM KCl, 8 mM $Na_2HPO_4$, and 1.5 mM $KH_2PO_4$), and cell lysates were collected after dissolving the cells in 50 μL of ice-cold radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris-HCl, pH 7.2; 0.1% sodium dodecylsulfate; 1% Triton X-100; 1% sodium deoxycholate; 0.15 M NaCl; and 1 mM EDTA). Protein concentrations were quantified by a bicinchonic acid (BCA) protein assay kit (Pierce, Rockford, IL). Cytosolic proteins (100 μg) were resolved on a 12% polyacrylamide gel and electrophoretically blotted onto a piece of nitrocellulose membrane. Cellular iNOS protein was immunodetected using a mouse monoclonal antibody against the mouse iNOS protein (Transduction Laboratories, Lexington, KY). Cellular β-actin was immunodetected using a mouse monoclonal antibody against mouse β-actin (Sigma, St. Louis, MO) as an internal standard. Intensities of immunoreactive bands were determined using a UVIDOCMW version 99.03 digital-imaging system (Uvtec, Cambridge, UK).

### *RT-PCR Assay*

Messenger RNA from macrophages exposed to LPS, propofol, and a combination of LPS and propofol were prepared for the RT-PCR analyses of TNF-α, IL-1β, IL-6, iNOS, and β-actin following manufacturer's instructions of the ExpressDirect™ mRNA Capture and RT System for the RT-PCR kit (Pierce, Rockford, IL). Oligonucleotides for PCR analyses of TNF-α, IL-1β, IL-6, iNOS, and β-actin were designed and synthesized by Clontech Laboratories (Palo Alto, CA). The oligonucleotide sequences of these primers were as follows:

TNF-α: 5′-ATGAGCACAGAAAGCATGATCCGC-3′ and
3′-CTCAGGCCCGTCCAGATGAAACC-5′

IL-1β: 5′-ATGGCAACTGTTCCTGAACTCAACT-3′ and
3′-TTTCCTTTCTTAGATATGGACAGGAC-5′

IL-6: 5′-ATGAAGTTCCTCTCTGCAAGAGACT-3′ and
3′-CACTAGGTTTGCCGAGTAGATCTC-5′

iNOS: 5′-CCCTTCCGAAGTTTCTGGCAGCAGC-3′ and
5′-CGACTCCTTTTCCGCTTCCTGAG-3′

β-actin: 5′-GTGGGCCGCTCTAGGCACCAA-3′ and
3′-CTTTAGCACGCACTGTAGTTTCTC-5′.

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 were 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 digital-imaging system.

### *Statistical Analysis*

The statistical significance of differences between the control and drug-treated groups was evaluated by Student's *t*-test; the difference was considered statistically significant at $P$ values of $<0.05$. The statistical difference between LPS and a combination of the propofol- and LPS-treated groups was considered significant when the $P$ value of the Duncan multiple range test was $<0.05$.

## RESULTS

Exposure to 50 μM propofol for 1, 6, and 24 h was not cytotoxic to macrophages (TABLE 1). Survival of macrophages was not influenced by LPS administration. Co-treatment with propofol and LPS for 1, 6, and 24 h did not affect macrophage viability.

ELISA analyses revealed that levels of TNF-α, IL-1β, and IL-6 proteins were low but detectable in untreated macrophages (FIG. 1). In macrophages, administration of LPS for 1 h caused significant 12-, 7-, and 6-fold increases of TNF-α, IL-1β, and IL-6 levels, respectively (FIG. 1A). Propofol did not change the amounts of TNF-α, IL-1β, and IL-6 in macrophages. However, co-treatment with propofol and LPS significantly reduced levels of LPS-enhanced TNF-α, IL-1β, and IL-6 by 45, 77, and 83%, respectively. In macrophages treated for 6 h, LPS increased the levels of TNF-α, IL-1β, and IL-6 by 10-, 12-, and 5-fold, respectively (FIG. 1B). Propofol did not change the basal levels of these inflammatory cytokines. Meanwhile, propofol significantly

**TABLE 1. Effects of propofol and LPS on macrophage viability[a]**

| Treatment | Cell viability, OD values at 570 nm | | |
|---|---|---|---|
| | 1 h | 6 h | 24 h |
| Control | 0.623 ± 0.063 | 0.668 ± 0.072 | 0.769 ± 0.082 |
| Propofol | 0.661 ± 0.059 | 0.683 ± 0.051 | 0.743 ± 0.081 |
| LPS | 0.642 ± 0.076 | 0.660 ± 0.089 | 0.785 ± 0.075 |
| Propofol plus LPS | 0.617 ± 0.068 | 0.652 ± 0.074 | 0.767 ± 0.075 |

[a]Macrophages were exposed to 50 mM propofol, 1 ng/mL LPS and a combination of propofol and LPS for 1, 6, and 24 h. Cell viability was determined by the MTT assay as described in MATERIALS AND METHODS. Each value represents the mean ± SEM for $n = 6$.

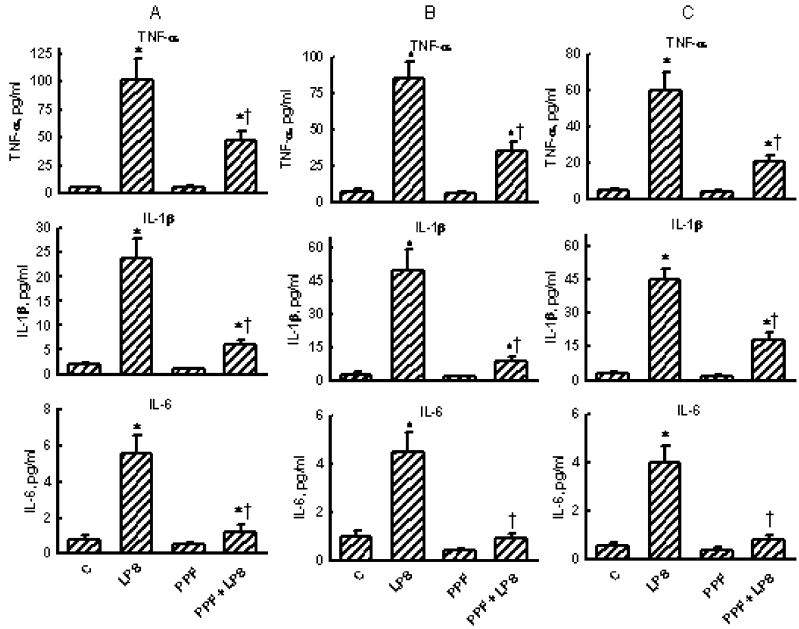


**FIGURE 1.** Effects of propofol on LPS-enhanced TNF-α, IL-1β, and IL-6 protein levels. Macrophages were exposed to 50 μM propofol (PPF), 1 ng/mL lLPS, and a combination of PPF and LPS for 1 h (**A**), 6 h (**B**), and 24 h (**C**). The amounts of TNF-α, IL-1β, and IL-6 in the culture medium were analyzed by the ELISA method. Each value represents the mean ± SEM for $n = 9$. *Values are significantly different from the respective control, $P < 0.05$.†Values are significantly different between the combined PPF and LPS and LPS treatment groups.

**TABLE 2. Effects of propofol and LPS on TNF-α, IL-1β, and IL-6 mRNA production by macrophages**

| | 1-h-treated macrophages | | |
|---|---|---|---|
| Treatment | TNF-α | IL-1β | IL-6 |
| Control | n.d. | n.d. | n.d. |
| LPS | 5673 ± 872 | 1310 ± 672 | 682 ± 79 |
| Propofol | n.d. | n.d. | n.d. |
| Propofol + LPS | 1522 ± 256† | 533 ± 87† | 299 ± 55† |
| | 6-h-treated macrophages | | |
| Treatment | TNF-α | IL-1β | IL-6 |
| Control | 876 ± 491 | n.d. | n.d. |
| LPS | 3712 ± 546 | 7124 ± 917 | 1565 ± 226 |
| Propofol | 665 ± 569 | n.d. | n.d. |
| Propofol + LPS | 1279 ± 330† | 723 ± 177† | 257 ± 59† |
| | 24-h-treated macrophages | | |
| Treatment | TNF-α | IL-1β | IL-6 |
| Control | n.d. | n.d. | n.d. |
| LPS | 4316 ± 774 | 5819 ± 748 | 827 ± 116 |
| Propofol | 513 ± 491 | n.d. | n.d. |
| Propofol + LPS | 1198 ± 311† | 477 ± 88† | n.d. |

[a]Macrophages were exposed to 50 μM propofol, 1 ng/mL lipopolysaccharide (LPS), and a combination of propofol and LPS for 1, 6, and 24 h. RT-PCR analyses of TNF-α, IL-1β, and IL-6 mRNA were carried out, and DNA bands were quantified as described in MATERIALS AND METHODS. Each value represents the mean ± SEM for $n = 6$. †Values significantly differ between the combined propofol and LPS treatment and the LPS treatment groups, $P < 0.05$. n.d. not detected.

reduced LPS-enhanced levels of TNF-α, IL-1β, and IL-6 proteins by 62, 84, and 76%, respectively. In macrophages treated for 24 h, administration of LPS resulted in 3-, 3-, and 4-fold increases in TNF-α, IL-1β, and IL-6, respectively (FIG. 1C). Propofol did not influence macrophage TNF-α, IL-1β, and IL-6 levels. Exposure to propofol significantly reduced LPS-increased levels of TNF-α, IL-1β, and IL-6 proteins by 67, 67, and 80%, respectively.

RT-PCR analyses showed that administration of LPS in macrophages for 1, 6, and 24 h induced TNF-α, IL-1β, and IL-6 mRNA (FIG. 2). Co-treatment of macrophages with propofol and LPS for 1, 6, and 24 h inhibited LPS-induced TNF-α, IL-1β, and IL-6 mRNA. The DNA bands of RT-PCR products were quantified using a digital imaging system (TABLE 2). In macrophages treated for 1 h, propofol significantly reduced LPS-induced levels of TNF-α, IL-1β, and IL-6 mRNA by 83, 61, and 56%, respectively (TABLE 2). In groups treated for 6 h, the levels of LPS-induced TNF-α, IL-1β, and IL-6 mRNA were significantly decreased by 66, 90, and 84%, respectively, following propofol administration. In macrophages treated for 24 h, administration of propofol suppressed 72% and 92% of the LPS-induced TNF-α and IL-1β mRNA, respectively. Propofol completely inhibited LPS-induced IL-6 mRNA (TABLE 2).

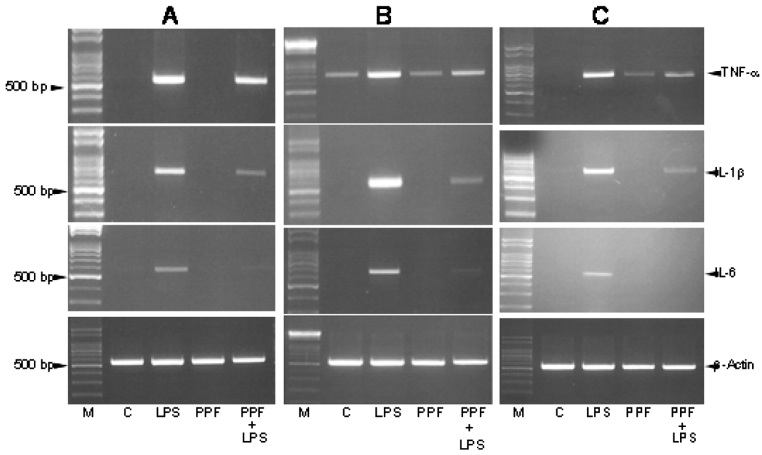


**FIGURE 2.** Effects of propofol on LPS-induced TNF-α, IL-1β, and IL-6 mRNA levels. Messenger RNA from macrophages exposed to 50 μM propofol (PPF), 1 ng/mL lipopolysaccharide (LPS), and a combination of PPF and LPS for 1 h (**A**), 6 h (**B**), and 24 h (**C**) was prepared for RT-PCR analyses of TNF-α, IL-1β, and IL-6 mRNA. 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. RT-PCR analysis of β-actin was used as the internal control. M, DNA marker; C, control.

**TABLE 3. Effects of propofol and LPS on nitrite production and iNOS expression by macrophages[a]**

| Treatment | Nitrite (μM) | iNOS protein (arbitrary units) | iNOS mRNA (arbitrary units) |
|---|---|---|---|
| Control | 5 ± 1 | n.d. | n.d. |
| Propofol | 4 ± 1 | n.d. | n.d. |
| LPS | 32 ± 7* | 679 ± 137 | 3217 ± 624 |
| Propofol plus LPS | 11 ± 3*,† | 198 ± 58*,† | 882 ± 177*,† |

[a]Macrophages were exposed to 50 μM propofol, 1 ng/mL LPS, and a combination of propofol and LPS for 24 h. The levels of nitrite produced by macrophages were determined by the Griess reaction method. Analyses by immunoblotting and RT-PCR were carried out to determine the levels of iNOS protein and mRNA. Each value represents the mean ± SEM for $n = 3$. *Asterisk* (*), values are significantly different from the respective control ($P < 0.05$); *dagger* (†), values are significantly different between the combined propofol and LPS treatment and the LPS treatment groups ($P < 0.05$); *n.d.,* not detected.

Nitrite was detectable in untreated macrophages, and propofol did not affect nitrite production. Administration of LPS caused a 6-fold increase of nitrite in the medium with macrophages (TABLE 3). Propofol significantly reduced LPS-augmented nitrite levels in macrophages by 66%. In untreated macrophages, iNOS protein and mRNA were undetectable (TABLE 3). Propofol did not influence iNOS protein or

mRNA levels, but LPS increased the protein and mRNA levels of iNOS in macrophages (TABLE 3). Co-treatment with propofol and LPS significantly inhibited LPS-induced levels of iNOS protein and mRNA by 71% and 73%, respectively.

## DISCUSSION

Macrophages play critical roles in cellular host defense against infection or tissue injury.[4,5] This study showed that in response to LPS stimulation, macrophages produced large amounts of TNF-α, IL-1β, IL-6, and NO. Induction of TNF-α, IL-1β, and IL-6 plays a major role in mediating endotoxin-induced pathogenesis of septic shock, such as fever, metabolic acidosis, diarrhea, hypotension, and disseminated intravascular coagulation.[7,12,17] Overproduction of NO can induce cell apoptosis.[14,18] Slowing the release of these inflammatory factors from macrophages may retard the inflammatory responses to LPS stimulation. One of our previous studies showed that propofol has immunosuppressive effects on macrophage functions.[13] The present study further shows that propofol significantly suppressed LPS-induced TNF-α, IL-1β, IL-6, and NO production by macrophages. The concentration of propofol used in this study was 50 μM, which corresponds to a clinical plasma concentration.[15] Therefore, a therapeutic concentration of propofol has anti-inflammatory and antioxidative effects on the biosyntheses of TNF-α, IL-1β, IL-6, and NO in LPS-activated macrophages.

LPS increased the levels of TNF-α, IL-1β, and IL-6 proteins in macrophages. Following LPS administration, TNF-α, IL-1β, and IL-6 mRNA were induced. Thus LPS pretranslationally induces the expression of TNF-α, IL-1β, and IL-6. Propofol reduced LPS-enhanced levels of TNF-α, IL-1β, and IL-6 proteins. RT-PCR analyses revealed that propofol could inhibit LPS-induced TNF-α, IL-1β, and IL-6 mRNA production. Data from the viability test showed that administration of macrophages with propofol, LPS, or a combination of these two drugs was not cytotoxic to macrophages. Therefore, propofol-induced suppression of TNF-α, IL-1β, and IL-6 production in macrophages occurred because of a pretranslational mechanism but not because of the cytotoxic effect of this sedative agent. Induction of inflammatory cytokines by LPS in macrophages has been reported to be via the toll-like receptor 4–mediated signal transduction pathway.[19] Toll-like receptor 4 is a type I transmembrane protein.[20] In response to LPS stimulation, propofol is so lipophilic that it might disrupt the plasma membrane and modulate the toll-like receptor 4–mediated induction of inflammatory cytokines in macrophages.

Exposure of macrophages to LPS significantly increased the levels of nitrite in the culture medium containing macrophages. Nitrite is an oxidative product of NO. The elevation of nitrite corresponds to the increase in NO by macrophages. LPS induced iNOS at the protein and mRNA levels. Co-treatment with propofol and LPS significantly decreased nitrite levels and iNOS expression. Thus the suppressive effects of propofol on LPS-induced NO biosynthesis are exerted by a pretranslational event. Our previous study showed that propofol could protect macrophages from exogenous NO-induced cell apoptosis.[14] The present study has further demonstrated that propofol inhibits LPS-induced endogenous NO biosynthesis in macrophages. LPS can increase endogenous NO through CD14-dependent induction of iNOS.[21] CD14 is a membrane protein in macrophages. Propofol could modulate the interac-

tion of CD14 and LPS, which leads to the inhibition of iNOS expression and a decrease in NO production by macrophages.

LPS is recognized as one of the best-characterized signal effectors for triggering macrophages.[3] Concentrations of LPS used in *in vitro* and *in vivo* studies are about 1 μg/mL and 1 mg/kg body weight, respectively.[5,22] Bysani *et al.*[23] reported that the plasma concentration of LPS in a patient with fatal *Klebsiella pneumoniae* sepsis was 25 ng/mL In this study, we used ELISA and RT-PCR analyses to demonstrate that LPS at a low concentration (1 ng/mL) could apparently induce TNF-α, IL-1β, IL-6, and iNOS at both the protein and RNA levels. Our previous study showed that the inhibition of LPS-induced NO production by *N*-monomethyl argentine, one of the L-arginine analogues, can transcriptionally reduce TNF-α and IL-1β at the protein and mRNA levels.[16] The propofol-induced inhibition of NO synthesis might contribute to the suppression of LPS-induced TNF-α and IL-1β production.

In conclusion, this study has shown that LPS induces production of TNF-α, IL-1β, IL-6, and NO. In LPS-activated macrophages, propofol suppresses the biosyntheses of TNF-α, IL-1β, IL-6, and iNOS at the protein and mRNA levels. Thus, the modulating mechanism of propofol on LPS-induced production of TNF-α, IL-1β, IL-6, and NO occurs at a pretranslational level. Taken together, the results of this study show that at the therapeutic concentration, propofol has anti-inflammatory and antioxidative effects on the biosyntheses of TNF-α, IL-1β, IL-6, and NO in LPS-activated macrophages.

## ACKNOWLEDGMENTS

The authors thank Ms.Wan-Lu Lee for technical support and data collection for the experiment. This study was supported by Grant NSC92-2314-B-038-010 from the National Science Council, Taiwan, ROC.

## REFERENCES

1. LYNN, W.A. & J. COHEN. 1995. Management of septic shock. J. Infect. **30:** 207–212.
2. TABBUTT, S. 2001. Heart failure in pediatric septic shock: utilizing inotropic support. Crit. Care Med. **29:** S231–S236.
3. RAETZ, C.R., R.J. ULEVITCH & S.D. WRIGHT. 1991. Gram-negative endotoxin: an extraordinary lipid with profound effects on eukaryotic signal transduction. FASEB J. **5:** 2652–2660.
4. MOLLOY, R.G., J.A. MANNICK & M.L. RODRICK. 1993. Cytokines, sepsis and immunomodulation. Br. J. Surg. **80:** 289–297.
5. WEST, M.A., M.H. LI, S.C. SEATTER, *et al.* 1994. Pre-exposure to hypoxia or septic stimuli differentially regulates endotoxin release of tumor necrosis factor, interleukin-6, interleukin-1, prostaglandin E2, nitric oxide, and superoxide by macrophages. J. Trauma **37:** 82–89.
6. STOUT, R.D. 1993. Macrophage activation by T cells: cognate and non-cognate signals. Curr. Opin. Immunol. **5 :** 398–403.
7. WEST, M.A., S.C. SEATTER, J. BELLINGHAM, *et al.* 1995. Mechanisms of reprogrammed macrophage endotoxin signal transduction after lipopolysaccharide pretreatment. Surgery **118:** 220–228.
8. SEBEL, P.S. & J.D. LOWDON. 1989. Propofol: a new intravenous anesthetic. Anesthesiology **71:** 260–277.

9. BRYSON, H.M., B.R. FULTON & D. FAULDS. 1995. Propofol. An update of its use in anaesthesia and conscious sedation. Drugs **50:** 513–559.
10. DAVIDSON, J.A., S.J. BOOM, F.J. PEARSALL, *et al.* 1995. Comparison of the effects of four i.v. anaesthetic agents on polymorphonuclear leucocyte function. Br. J. Anaesth. **74:** 315–318.
11. HOFBAUER, R., M. FRASS, H. SALFINGER, *et al.* 1999. Propofol reduces the migration of human leukocytes through endothelial cell monolayers. Crit. Care Med. **27:** 1843–1847.
12. CARSWELL, E.A., L.J. OLD & R.L. KASSEL. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA **72:** 3666–3670.
13. CHEN, R.M., C.H. WU, H.C. CHANG, *et al.* 2003. Propofol suppresses macrophage functions and modulates mitochondrial membrane potential and cellular adenosine triphosphate levels. Anesthesiology **98:** 1178–1185.
14. CHANG, H., S.Y. TSAI, T.L. CHEN, *et al.* 2002. Therapeutic concentrations of propofol protect mouse macrophages from nitric oxide-induced cell death and apoptosis. Can. J. Anesth. **49:** 477–480.
15. GEPTS, E., F. CAMU & I.D. COCKSHOTT. 1987. Disposition of propofol administered as constant rate intravenous infusions in humans. Anesth. Analg. **66:** 1256–1263.
16. WU, C.H., T.L. CHEN, T.G. CHEN, *et al.* 2002. Nitric oxide modulates pro- and anti-inflammatory cytokines in lipopolysaccharide-activated macrophages. J. Trauma **55:** 540–545.
17. BEUTLER, B. & A. CERAMI. 1986. Cachectin and tumor necrosis factor as two sides of the same biological coin. Nature **320:** 584–588.
18. CHEN, R.M., H.C. LIU, Y.L. LIN, *et al.* 2002. Nitric oxide induces osteoblast apoptosis through the de novo synthesis of Bax protein. J. Orthop. Res. **20:** 295–302.
19. HOSHINO, K., O. TAKEUCHI, T. KAWAI, *et al.* 1999. Toll-like receptor 4 (TLR 4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR 4 as the *lps* gene product. J. Immunol. **162:** 3749–3752.
20. MEDZHITOV, R., P. PRESTON-HURLBURT, E. KOPP, *et al.* 1993. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol. Cell **2:** 253–258.
21. KIRKLAND, T.N., F. FINLEY, D. LETURCO, *et al.* 1993. Analysis of lipopolysaccharide binding by CD14. J. Biol. Chem. **268:** 24818–24824.
22. MILLAR, C.G. & C. THIEMERMANN. 1997. Intrarenal haemodynamics and renal dysfunction in endotoxaemia: effects of nitric oxide synthase inhibition. Br. J. Pharmacol. **121:** 1824–1830.
23. BYSANI, G.K., J.L. SHENEP, W.K. HILDNER, *et al.* 1990. Detoxification of plasma containing lipopolysaccharide by adsorption. Crit. Care Med. **18:** 67–71.