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Propofol suppresses tumor necrosis factor-α biosynthesis in lipopolysaccharide-stimulated macrophages possibly through downregulation of nuclear factor-kappa B-mediated *toll-like receptor 4* gene expression

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ABSTRACT

Lipopolysaccharide (LPS), a gram-negative bacterial outer membrane component, can activate macrophages via a toll-like receptor 4-dependent pathway. Our previous study has shown that propofol, an intravenous anesthetic reagent, has anti-inflammatory effects. This study was further aimed to evaluate the roles of toll-like receptor 4 in propofol-caused suppression of tumor necrosis factor-α (TNF-α) biosynthesis in LPS-stimulated macrophages and its possible molecular mechanisms. Exposure of macrophages to propofol and LPS did not affect cell viability. Meanwhile, the LPS-caused augmentations in the productions of TNF-α protein and mRNA were significantly decreased following incubation with a therapeutic concentration of propofol (50 μM). Analysis of toll-like receptor 4 small interference (si)RNA revealed that this membrane receptor might participate in the propofol-caused suppression of TNF-α biosynthesis. Treatment of macrophages with LPS-induced toll-like receptor 4 protein and mRNA productions. Propofol at a clinically relevant concentration could inhibit such induction. In parallel, the LPS-induced translocation and transactivation of transcription factor nuclear factor-kappa B (NFκB) were significantly alleviated following propofol incubation. There are several NFκB DNA-binding motifs found in the promoter region of *toll-like receptor 4*. Therefore, this study shows that propofol at a therapeutic concentration can downregulate TNF-α biosynthesis possibly via inhibition of NFκB-mediated *toll-like receptor 4* gene expression.

1. Introduction

Propofol (2,6-diisopropylphenol) is an intravenous anesthetic agent widely used to induce and maintain anesthesia during surgical procedures, or for sedation in the intensive care unit [1,2]. Previous studies showed that propofol may have immunosuppressive effects on the activities of leukocytes, neutrophils, and macrophages [3–5]. Studies in our lab further demonstrated that propofol at a clinically relevant concentration can suppress macrophage functions through reducing the mitochondrial membrane potential and adenosine triphosphate synthesis [5,6]. In the innate immunity system, macrophages play pivotal roles in cellular host defense against infection and tissue injury [7]. During inflammation, macrophages can produce and release a variety of inflammatory cytokines, which induce serial inflammatory reactions [8,9]. Tumor necrosis factor-α (TNF-α), a typical and critical inflammatory cytokine predominantly produced by macrophages, is reported to have pleiotropic effects in regulating the immune response, acute-phase reactions, hematopoiesis, and macrophage-mediated tumor cytotoxicity [10,11]. Levels of TNF-α in macrophages can be altered by endogenous or exogenous factors, which then lead to immunomodulation [12].

Toll-like receptors (TLRs) are type I transmembrane proteins with extracellular domains comprised largely of leucine-rich repeats and intracellular signaling domains [13]. In mammalian cells, there are at least 12 TLR members, which trigger host resistance to infection [13,14]. Lipopolysaccharide (LPS), a gram-negative bacterial outer membrane component, has been identified as one of the critical factors involved in the pathogenesis of sepsis [15]. In macrophages, LPS can specifically activate TLR4, which

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then stimulates translocation of the transcription factors, nuclear factor-kappa B (NFκB) and activator protein-1 (AP-1), from the cytoplasm to nuclei, ultimately inducing the expression of inflammatory cytokine genes [16,17]. Thus, levels of TLR4 in macrophages play crucial roles in immunoresponses. The expression of TLR4 in macrophages can be modulated [18,19]. In monocytes, LPS has been reported to induce TLR4 and TLR8 expressions [20]. NFκB DNA-binding motifs can be found in the promoter region of TLR4 [21]. Our previous studies showed that propofol can downregulate TNF-α production in LPS-stimulated macrophages [5,22]. Meanwhile, the roles of TLR4 in propofol-caused modulation of TNF-α biosynthesis are still unknown. Therefore, in this study, we attempted to evaluate the molecular mechanisms of propofol-involved regulation of *TNF-α* gene expression in LPS-activated macrophages and its possible mechanisms, especially in the roles of TLR4.

2. Materials and methods

2.1. Cell culture and drug treatment

Macrophage-like Raw 264.7 cells were purchased from American Type Culture Collection (Rockville, MD, USA). The cells were cultured in RPMI 1640 medium (Gibco-BRL, Grand Island, NY, USA), which was supplemented with 10% fetal calf serum, L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml). Macrophages were seeded in 75-cm^2 flasks at 37 °C in a humidified atmosphere of 5% CO_2.

Propofol, purchased from Aldrich (Milwaukee, WI, USA), was freshly prepared by dissolving it in dimethyl sulfoxide (DMSO) and protected from light for each independent experiment. DMSO in the medium was <0.1% to avoid the toxicity of this solvent to macrophages. According to the clinical application, propofol at ≤50 μM, which corresponds to clinical plasma concentrations [23,24], was chosen to be the administered dosages in this study. Control macrophages were treated with DMSO only.

2.2. Assay of cell viability

Cell viability was determined by a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [25]. Briefly, macrophages (5×10^3 cells per well) were seeded in 96-well tissue culture plates overnight. After drug treatment, macrophages were cultured with new medium containing 0.5 mg/ml MTT for a further 3 h. The blue formazan products in macrophages were dissolved in DMSO and spectrophotometrically measured at a wavelength of 550 nm.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Levels of TNF-α in the culture medium of macrophages were quantified following a previously described method [26]. Briefly, macrophages (1×10^4 cells/well) were seeded in 96-well tissue culture plates overnight. After drug treatment, the medium was collected and centrifuged. The amounts of TNF-α were quantified following the standard protocols of the ELISA kits purchased from Endogen (Woburn, MA, USA).

2.4. Reverse-transcription (RT) and quantitative polymerase chain reaction (qPCR) assays

Messenger (m)RNA from macrophages exposed to LPS, propofol, or a combination of propofol and LPS were prepared for RT or qPCR analyses of TNF-α, TLR4, or β-actin mRNA. Oligonucleotides for the PCR analyses of TNF-α, TLR4, and β-actin mRNA were designed and synthesized by Clontech Laboratories (Palo Alto, CA, USA). The oligonucleotide sequences of the respective upstream and downstream primers for these mRNA analyses were 5′-ATGAGCACAGAAAGCAT-GATCCGC-3′ and 3′-CTCAGGCCCGTCCAGATGAAACC-5′ for TNF-α [27], 5′-CAAGG-GATAAGAACGCTGAGA-3′ and 5′-GCAATGTCTCTGGCAGGTGTA-3′ for TLR4 [28], and 5′-GTGGGCCGCTCTAGGCACCAA-3′ and 5′-CTCTTTGATGTCACGCACGATTTC-3′ for β-actin [29]. The PCR reaction was carried out using 35 cycles of 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 UVI-DOCMW vers. 99.03 digital imaging system (UVtec, Cambridge, UK). A qPCR analysis was carried out using iQSYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad).

2.5. Immunoblotting analyses of TLR4 and β-actin

Protein analyses were carried out according to a previously described method [30]. After drug treatment, cell lysates were prepared in ice-cold radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris–HCl (pH 7.2), 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 0.15 M NaCl, and 1 mM EDTA). To avoid the degradation of cytosolic proteins by proteinases, a mixture of 1 mM phenyl methyl sulfonyl fluoride, 1 mM sodium orthovanadate, and 5 μg/ml leupeptin was added to the RIPA buffer. Protein concentrations were quantified using a bicinchonic acid protein assay kit (Pierce, Rockford, IL, USA). Proteins (50 μg/well) were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to nitrocellulose membranes. Immunodetection of TLR4 was carried out using a goat polyclonal antibody against mouse TLR4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Cellular β-actin protein was immunodetected using a mouse monoclonal antibody against mouse β-actin (Sigma, St. Louis, MO, USA) as the internal standard. These protein bands were quantified using a digital imaging system (UVtec).

2.6. TLR4 knock-down

Translation of TLR4 mRNA in macrophages was knocked-down using an RNA interference (RNAi) method following a small interfering (si)RNA transfection protocol provided by Santa Cruz Biotechnology as described previously [31]. TLR4 siRNA was purchased from Santa Cruz Biotechnology, and is a pool of 3 target-specific 20–25-nt siRNAs designed to knock-down TLR4's expression. Briefly, after culturing macrophages in antibiotic-free RPMI medium at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h, the siRNA duplex solution, which was diluted in the siRNA transfection medium (Santa Cruz Biotechnology), was added to the macrophages. After transfecting for 24 h, the medium was replaced with normal RPMI medium, and macrophages were treated with propofol, LPS, or a combination of propofol and LPS.

2.7. Extraction of nuclear proteins and immunodetection

Nuclear components were extracted and immunodetected following the method of Wu et al. [31]. After drug treatment, nuclear extracts of macrophages were prepared. Protein concentrations were quantified with a bicinchonic acid protein assay kit (Pierce). Nuclear proteins (50 μg/well) were subjected to SDS-PAGE, and transferred to nitrocellulose membranes. After blocking, nuclear NFκB was immunodetected using a rabbit polyclonal antibody against mouse NFκB (Santa Cruz Biotechnology). Proliferating cell

nuclear antigen (PCNA) was detected using a mouse monoclonal antibody against rat PCNA protein (Santa Cruz Biotechnology) as the internal standard. Intensities of the immunoreactive bands were determined using a digital imaging system (UVtec).

2.8. NFκB reporter assay

NFκB luciferase reporter plasmids (Stratagene, La Jolla, CA, USA) and pUC18 control plasmids were transfected into macrophages using a FuGene 6 transfection reagent (Roche Diagnostics, Mannheim, Germany) as described previously [32]. After transfection, macrophages were exposed to propofol and LPS. Then, cells were harvested. The luciferase activity in cell lysates was measured using a dual luciferase assay system (Promega, Madison, WI, USA).

2.9. Statistical analysis

Statistical differences were considered significant when the p value of Duncan's multiple-range test was <0.05. Statistical analysis between groups over time was carried out by two-way analysis of variance (ANOVA).

3. Results

3.1. Toxicity of propofol

Cell viability was assayed to evaluate the toxicity of propofol and LPS to macrophages (data not shown). Treatment of macrophages with 1, 10, 25, and 50 μM propofol for 24 h did not affect cell viability. However, when the concentration reached 100 μM, propofol caused a significant 33% decrease in cell viability (data not shown). Exposure of macrophages to 100 ng/ml LPS did not influence cell survival. Treatment of macrophages with a combination of LPS and 1, 10, 25, and 50 μM propofol for 24 h was not cytotoxic to the cells. However, when the combination of 100 μM propofol and 100 ng/ml LPS was used, the viability of macrophages was significantly reduced by 42% (data not shown).

3.2. Suppression of TNF-α expression by propofol

To determine the effects of propofol on TNF-α biosynthesis in LPS-stimulated macrophages, levels of this inflammatory cytokine in the culture medium were detected (Fig. 1). Exposure of macrophages to 100 ng/ml LPS for 24 h significantly increased cellular TNF-α levels by 11-fold (Fig. 1A). Exposure to 1 μM propofol did not change LPS-caused augmentation in the levels of TNF-α (Fig. 1A). Meanwhile, after treating macrophages with 10, 25, and 50 μM propofol, the LPS-caused enhancement in TNF-α synthesis was significantly decreased by 27%, 51%, and 60%, respectively (Fig. 1A). Treatment of macrophages with 1, 10, 25, and 50 μM propofol did not affect TNF-α synthesis (data not shown). Exposure to 100 ng/ml LPS for 1, 3, 6, 12, and 24 h augmented levels of TNF-α by 90% and 2.8-, 4.5-, 6-, and 11-fold, respectively (Fig. 1B). Treatment of macrophages alone with a therapeutic concentration of propofol (50 μM) for 1, 3, 6, 12, and 24 h did not affect TNF-α synthesis. However, exposure to 50 μM propofol for 1 and 3 h completely suppressed LPS-induced TNF-α production. Following co-treatment with propofol and LPS for 6, 12, and 24 h, the LPS-caused increases in cellular TNF-α amounts were significantly alleviated by 49%, 42%, and 56%, respectively (Fig. 1B).

TNF-α mRNA was analyzed using a qPCR to determine the mechanism of propofol-caused suppression of TNF-α syntheses in LPS-treated macrophages (Fig. 2). In untreated macrophages, low levels of TNF-α mRNA were detected (Fig. 2A). After exposure to 100 ng/ml LPS for 6 h, a 14-fold increase in TNF-α mRNA was induced. Propofol at 1 μM did not change the LPS-induced

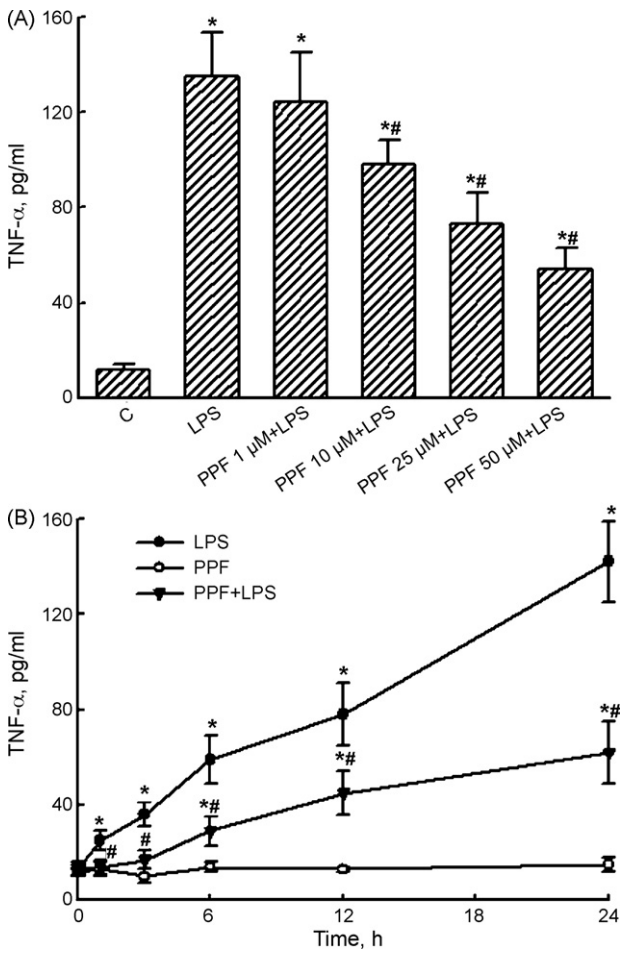

Fig. 1. Concentration- and time-dependent effects of propofol (PPF) on lipopolysaccharide (LPS)-induced TNF-α synthesis. Macrophages were exposed to 1, 10, and 50 μM PPF, 100 ng/ml LPS and a combination of LPS and PPF at 1, 10, 25, and 50 μM (A) for 24 h, or to 50 μM PPF, 100 ng/ml LPS, and a combination of PPF and LPS for 1, 3, 6, 12, and 24 h (B). Levels of TNF-α in the culture medium were determined using an enzyme-linked immunosorbent assay. Each value represents the mean ± SD for $n = 6$. The symbols, * and #, indicate that a value significantly ($p < 0.05$) differed from the control or LPS-treated groups, respectively. C, control.

TNF-α mRNA production. When the concentrations reached 10, 25, and 50 μM, LPS-induced TNF-α mRNA was significantly ameliorated by 29%, 45%, and 59%, respectively (Fig. 2A). Treatment with macrophages alone with 1, 10, 25, and 50 μM propofol did not influence TNF-α mRNA synthesis (data not shown). Exposure of macrophages to 100 ng/ml LPS for 3, 6, 12, and 24 h caused significant 2.8-, 3.6-, 5.5-, and 12-fold increases in TNF-α mRNA production (Fig. 2B). Treatment with 50 μM propofol for 1, 3, 6, 12, and 24 h did not affect the levels of TNF-α mRNA in macrophages. After treatment for 3, 6, 12, and 24 h, propofol significantly inhibited LPS-caused induction of TNF-α mRNA synthesis by 100%, 53%, 48%, and 56%, respectively (Fig. 2B).

3.3. Roles of TLR4 in propofol-involved suppression of TNF-α expression

To determine the roles of TLR4 in propofol-caused suppression of TNF-α production in LPS-activated macrophages, TLR4 siRNA was applied to macrophages to knock-down the translation of this membrane receptor (Fig. 3). After application of TLR4 siRNA for 24 and 48 h, levels of TLR4 protein in macrophages were obviously decreased (Fig. 3A, top panel, lanes 2 and 3). The amount of β-actin in macrophages was detected as the internal standard

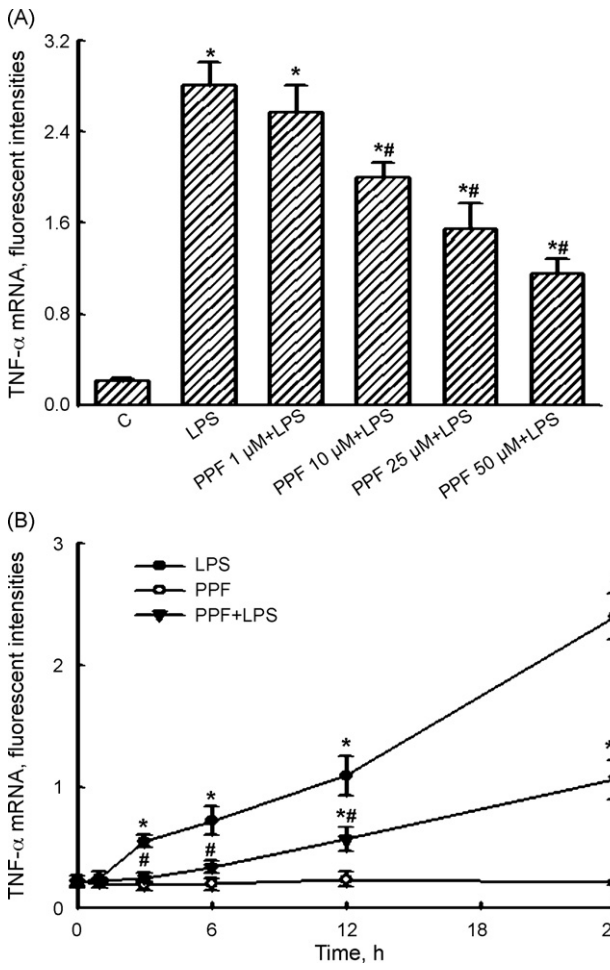

Fig. 2. Concentration- and time-dependent effects of propofol (PPF) on lipopolysaccharide (LPS)-induced TNF-α mRNA production. Macrophages were exposed to 1, 10, and 50 μM PPF, 100 ng/ml LPS and a combination of LPS and PPF at 1, 10, 25, and 50 μM (A) for 6 h, or to 50 μM PPF, 100 ng/ml LPS, and a combination of PPF and LPS for 1, 3, 6, 12, and 24 h (B). Quantitative PCR analysis of TNF-α mRNA was conducted according. Each value represents the mean ± SD for $n = 6$. The symbols, * and #, indicate that a value significantly ($p < 0.05$) differed from the control or LPS-treated groups, respectively. C, control.

(Fig. 3A, bottom panel). These protein bands were quantified and analyzed (Fig. 3B). Application of TLR4 siRNA to macrophages for 24 and 48 h significantly decreased cellular TLR4 levels by 59% and 81%, respectively. Incubation of macrophages solely with a therapeutic concentration of propofol (50 μM) or TLR4 siRNA did not change the basal level of TNF-α mRNA in macrophages (Fig. 3C). Meanwhile, propofol at a therapeutic concentration (50 μM) significantly inhibited LPS-induced TNF-α mRNA synthesis by 54%. After application of TLR4 siRNA to macrophages for 48 h, the LPS-induced increase in TNF-α mRNA production was significantly alleviated by 85%. Co-treatment of macrophages with propofol and TLR4 siRNA completely suppressed LPS-caused induction of TNF-α mRNA (Fig. 3C).

3.4. Inhibition of TLR4 expression by propofol

Analyses of TLR4 protein and mRNA were conducted to further evaluate the molecular mechanisms of propofol-involved suppression of TNF-α biosynthesis (Figs. 4 and 5). TLR4 was detected in untreated macrophages (Fig. 4A, top panel, lane 1). After exposure to LPS, the amounts of TLR4 were obviously augmented (lane 2). Treatment of macrophages with 50 μM propofol did not change TLR4 production (lane 3). Meanwhile, propofol at a clinically relevant concentration (50 μM) reduced TLR4 synthesis in LPS-activated macrophages (lane 4). Levels of β-actin were immunodetected as the internal standard (Fig. 4A, bottom panel). These immunoreactive protein bands were quantified and analyzed (Fig. 4B). Exposure of macrophages to LPS caused a significant 2.2-fold augmentation in the cellular TLR4 protein levels. The LPS-caused enhancement of TLR4 production was completely reduced following propofol treatment (Fig. 4B).

TLR4 mRNA was detected in untreated macrophages (Fig. 5A, top panel, lane 1). Incubation of macrophages with a therapeutic concentration of propofol (50 μM) did not affect TLR4 mRNA synthesis (lane 2). After treatment with LPS, the expression of TLR4 mRNA was obviously induced (lane 3). Treatment with propofol inhibited TLR4 mRNA production in LPS-stimulated macrophages (lane 4). Amounts of β-actin mRNA were quantified as the internal standard (Fig. 5A, bottom panel). These DNA bands were quantified and analyzed (Fig. 5B). Treatment with propofol to macrophages completely

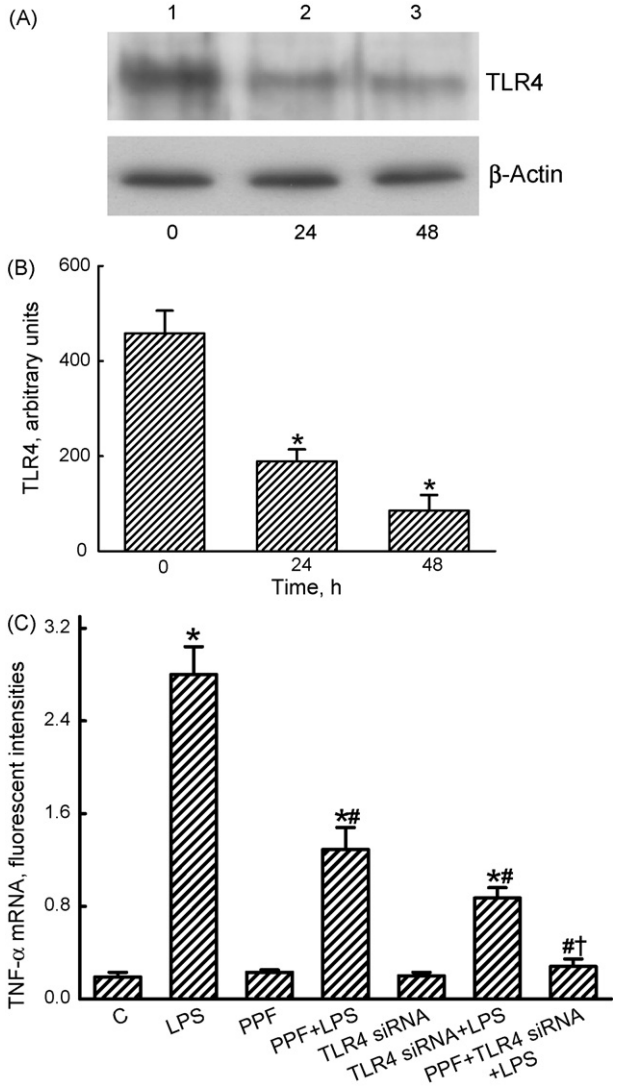

Fig. 3. Roles of TLR4 in propofol (PPF)-caused suppression of TNF-α synthesis in lipopolysaccharide (LPS)-stimulated macrophages. Application of TLR4 small interference (si)RNA into macrophages. Amounts of TLR4 were immunodetected (A, top panel). Levels of β-actin were quantified as the internal standard (bottom panel). These protein bands were quantified and analyzed (B). Amounts of TNF-α in control (C), PPF (50 μM)-, LPS (100 ng/ml)-, and TLR4 siRNA-treated macrophages were determined by an enzyme-linked immunosorbent assay (C). Each value represents the mean ± SD for $n = 4$. The symbols, *, #, and †, indicate that a value significantly ($p < 0.05$) differed from the control, LPS-, or PPF + LPS-treated groups, respectively.

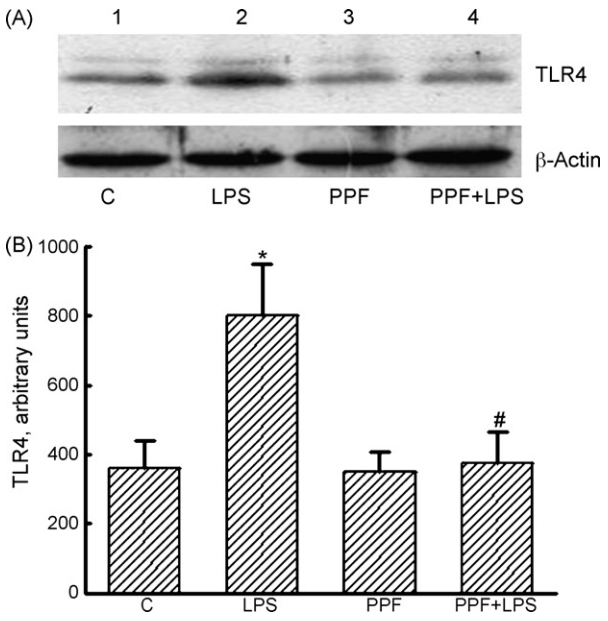

Fig. 4. Effects of propofol (PPF) on lipopolysaccharide (LPS)-caused increases in TLR4 synthesis. Macrophages were exposed to 50 μM PPF, 100 ng/ml LPS, and a combination of PPF and LPS for 24 h. Amounts of TLR4 were immunodetected (A, top panel). Levels of β-actin were determined as the internal standard (bottom panel). These immunorelated protein bands were quantified and analyzed (B). Each value represents the mean ± SD for $n = 7$. The symbols, * and #, indicate that a value significantly ($p < 0.05$) differed from the control or LPS-treated groups, respectively. C, control.

inhibited LPS-induced TLR4 mRNA. The qPCR further revealed that LPS increased TLR4 mRNA synthesis by 2.5-fold (Fig. 5C). Propofol at a clinically relevant concentration (50 μM) completely inhibited TLR4 mRNA production in LPS-activated macrophages.

3.5. Reduction of NFκB activation by propofol

To further evaluate the mechanism of propofol-caused inhibition of TLR4 mRNA and protein syntheses, the translocation of NFκB from the cytoplasm to nuclei and its transactivation activity was analyzed (Fig. 6). Exposure of macrophages to LPS obviously increased amounts of nuclear NFκB (Fig. 6A, top panel, lane 2). Propofol alone did not affect the translocation of NFκB (lane 3). The LPS-enhanced NFκB levels in the nuclei of macrophages were reduced following propofol treatment (lane 4). The amounts of PCNA were immunodetected as the internal standard (Fig. 6A, bottom panel). These protein bands were quantified and analyzed (Fig. 6B). Incubation of macrophages with LPS caused a significant 2.4-fold increase in the levels of nuclear NFκB. After propofol treatment, the LPS-induced augmentation in nuclear NFκB levels was alleviated by 41% (Fig. 6B). A reporter gene assay further showed that LPS increased NFκB-stimulated luciferase activity by 4.8-fold (Fig. 6C). Propofol alone did not affect the luciferase activity. Meanwhile, the LPS-enhanced transactivation activity of NFκB was significantly decreased by 55% following propofol incubation (Fig. 6C).

4. Discussion

Propofol at a therapeutic concentration (50 μM) can suppress TNF-α biosynthesis. Data by protein and RNA analyses revealed that the LPS-caused enhancements of TNF-α protein and mRNA productions were significantly inhibited by propofol. The concentrations of propofol used in this study were ≤50 μM, which are within clinically relevant concentrations [23]. The viability assay showed that propofol under such concentrations as administered herein was not cytotoxic to macrophages. Thus, propofol at a therapeutic concentration can downregulate TNF-α biosynthesis, and the suppressive mechanism occurs at least at a pretranslational level rather than due to a deadly effect on macrophages. TNF-α is a typical and critical inflammatory cytokine predominantly produced by macrophages [8]. During inflammation, macrophages can produce and release this inflammatory cytokine to the circulatory system to regulate the immune response, acute-phase reaction, and hematopoiesis [9,11]. Previous studies reported that propofol may cause immunosuppression of the activities of neutrophils, leukocytes, and macrophages [3–5]. Therefore, the propofol-caused suppression of TNF-α synthesis can partially explain the immunomodulatory effects of this intravenous anesthetic when it is clinically applied. Clinically, intralipid is a common solvent of propofol. Previous studies have reported that intralipid could activate NFκB in endothelial cells or induced immune responses during low dose endotoximia in human [33,34]. Thus, this study dissolved propofol in DMSO to rule out the possible effects of intralipid on LPS-stimulated macrophages.

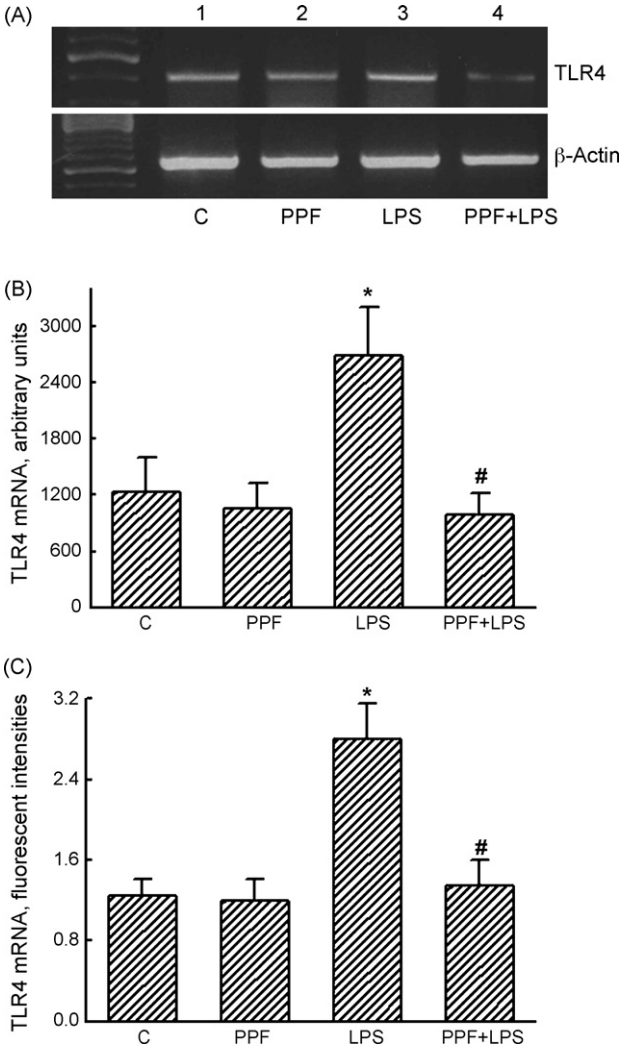

Fig. 5. Effects of propofol (PPF) on lipopolysaccharide (LPS)-caused increases in TLR4 mRNA production. Macrophages were exposed to 50 μM PPF, 100 ng/ml LPS, and a combination of PPF and LPS for 6 h. RT-PCR analyses of TLR4 mRNA were carried out (A, top panel). Levels of β-actin mRNA were detected as the internal standard (bottom panel). These DNA bands were quantified and analyzed (B). Quntitative PCR analysis of TLR4 mRNA were further conducted (C). Each value represents the mean ± SD for $n = 6$. The symbols, * and #, indicate that a value significantly ($p < 0.05$) differed from the control or LPS-treated groups, respectively. C, control.

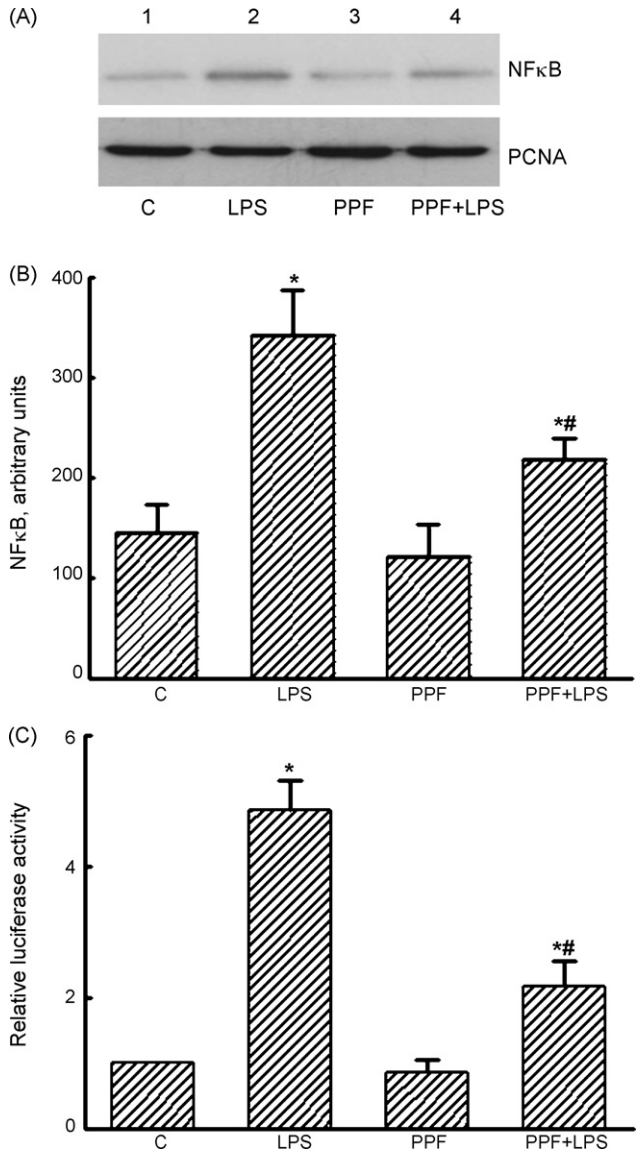

Fig. 6. Effects of propofol (PPF) on lipopolysaccharide (LPS)-induced translocation and transactivation of NFκB. Macrophages were exposed to 50 μM PPF, 100 ng/ml LPS, and a combination of PPF and LPS for 1 h. Amounts of nuclear NFκB were immunodetected (A, top panel). Levels of PCNA were analyzed as the internal standard (bottom panel). These protein bands were quantified and analyzed (B). A reporter gene assay was carried out to determine the transactivation activities of NFκB (C). Each value represents the mean ± SD for $n = 6$. The symbols, * and #, indicate that a value significantly ($p < 0.05$) differed from the control or LPS-treated groups, respectively. C, control.

TLR4 may participate in the propofol-involved reduction of TNF-α production in LPS-stimulated macrophages. In response to LPS stimuli, TLR4 plays critical roles in regulating macrophage-mediated inflammatory reactions [18]. Our present data reveal that application of TLR4 siRNA to macrophages simultaneously decreased cellular TLR4 levels and LPS-caused enhancement of TNF-α mRNA synthesis. Furthermore, co-treatment of macrophages with propofol and TLR4 siRNA completely inhibited TNF-α mRNA expression in LPS-activated macrophages. Thus, TLR4 may play a key role in propofol-caused suppression of TNF-α biosynthesis. In response to LPS stimuli, NFκB and AP-1 are two typical transcription factors which are triggered following TLR4 activation in macrophages [16,17]. In endothelial cells, propofol can inhibit NFκB-transduced signals to protect cells against LPS-induced barrier dysfunction [35]. NFκB DNA-binding elements are found in the promoter region of the *TNF-α* gene [36]. Therefore, the roles of TLR4 in the propofol-caused reduction of TNF-α synthesis may act via downregulating NFκB activation.

Propofol reduces TNF-α synthesis via inhibition of *TLR4* gene expression. Previous studies showed that TLR4 plays a critical role in regulating *TNF-α* gene expression in LPS-stimulated macrophages [26,37]. In parallel with the downregulation of TNF-α synthesis, levels of TLR4 protein in LPS-activated macrophages were significantly alleviated by a therapeutic concentration of propofol (50 μM). Thus, one possible reason to explain how propofol can suppress LPS-caused increases in biosynthesis of TNF-α is the inhibition of TLR4 synthesis by this intravenous anesthetic agent. Downregulation of TLR4 expression can lead to a weakness of immune responses [18]. Ciprofloxacin, a quinolone antibiotic, has immunomodulatory characteristics because it can preserve the LPS-induced expressions of *TLR4* and *TLR8* as well as TNF-α production in both peripheral blood mononuclear cells and blood mononuclear cells [20]. Thus, the propofol-involved inhibition of TLR4 production can partially explain the modulatory properties of this intravenous anesthetic agent. LPS-induced TLR4 mRNA expression was inhibited by propofol incubation. Our analysis of the NFκB transcriptional factor further revealed that propofol-influenced inhibition of *TLR4* gene expression occurs through transcriptional events.

Regulation of propofol-caused inhibition of *TLR4* gene expression occurs via downregulation of NFκB activation. NFκB is a common transcription factor, which participates in regulating expressions of diverse inflammatory genes [38]. NFκB DNA-binding motifs can be found in the promoter region of the *TLR4* gene [21]. In the process of NFκB activation, the translocation of this transcription factor from the cytoplasm to nuclei is important for regulating gene expression [39]. Our present results indicate that propofol at a clinically relevant concentration (50 μM) alleviated LPS-caused increases in the levels of nuclear NFκB. After translocation into nuclei, NFκB binds to its specific DNA element in the promoter region to induce *TLR4* gene expression [40]. Our present data from a reporter gene assay showed that LPS-induced NFκB transactivation was significantly reduced following propofol treatment. After lowering the activation of NFκB, propofol at a therapeutic concentration consequently inhibited TLR4 mRNA and protein productions. Thus, propofol can downregulate the translocation and transactivation of NFκB to suppress *TLR4* gene expression. In addition, NFκB is a downstream target of TLR4-transducing signals [16,17]. Therefore, propofol possibly attenuates TLR4-mediated signals in LPS-activated macrophages, then suppresses NFκB activation, and finally inhibits *TLR4* gene expression.

In conclusion, a clinically relevant concentration of propofol (50 μM) can ameliorate the biosyntheses of TNF-α mRNA and protein in LPS-stimulated macrophages. Analysis by RNA interference further showed that the mechanism of propofol-caused reduction in TNF-α synthesis may be TLR4-dependent. The LPS-induced enhancement of TLR4 mRNA and protein production significantly decreased following propofol treatment. Sequentially, propofol at a therapeutic concentration decreased the translocation of NFκB from the cytoplasm to nuclei and its transactivation activity to the luciferase reporter gene. NFκB activation enhanced TLR4 protein and mRNA productions, and suppression of NFκB led to inhibition of TLR4 expression which alleviated the LPS-induced TNF-α biosynthesis. Therefore, our present data suggest that propofol at a clinically relevant concentration can inhibit TNF-α biosyntheses in LPS-stimulated macrophages possibly through inhibiting NFκB-mediated *TLR4* gene expression. The propofol-induced suppression of TNF-α synthesis can partially explain the anti-inflammatory and antioxidative effects of this intravenous anesthetic agent in the clinic.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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