Original Article

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Propofol protects against lipopolysaccharide-induced inflammatory response in human amnion-derived WISH cells

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Background: Nonobstetric surgery is sometimes required during pregnancy, and neck abscess or facial bone fracture surgery cannot be postponed in pregnant women. However, dental surgery can be stressful and can cause inflammation, and the inflammatory response is a well-known major cause of preterm labor. Propofol is an intravenous anesthetic commonly used for general anesthesia and sedation. Studies investigating the effect of propofol on human amnion are rare. The current study investigated the effects of propofol on lipopolysaccharide (LPS)-induced inflammatory responses in human amnion-derived WISH cells.
Methods: WISH cells were exposed to LPS for 24 h and co-treated with various concentrations of propofol (0.01–1 μg/ml). Cell viability was measured using the MTT assay. Nitric oxide (NO) production was analyzed using a microassay based on the Griess reaction. The protein expression of cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE 2), p38, and phospho-p38 was analyzed using western blotting.
Results: Propofol did not affect the viability and NO production of WISH cells. Co-treatment with LPS and propofol reduced COX-2 and PGE_2 protein expression and inhibited p38 phosphorylation in WISH cells.
Conclusion: Propofol does not affect the viability of WISH cells and inhibits LPS-induced expression of inflammatory factors. The inhibitory effect of propofol on inflammatory factor expression is likely mediated by the inhibition of p38 activation.

Keywords: Amnion; Inflammation; p38; Preterm Labor; Propofol.

INTRODUCTION

According to the World Health Organization, preterm birth (PTB) is when an infant is born before the 37th week of gestation. PTB has a worldwide prevalence of 5%–18% and is a major cause of morbidity and mortality at birth [1]. Therefore, research pertaining to PTB prevention and effects of drugs in pregnant women is important. The causes of PTB vary; however, spontaneous PTB is associated with intrauterine infection/inflammation [2,3].

Inflammation of fetal-maternal tissues during pregnancy is necessary to reform gestational tissues and accommodate fetal growth. During pregnancy, inflammation is a balanced process that maintains immune

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homeostasis. However, inflammatory overload in gestational tissues, caused by pregnancy-related risk factors, induces preterm labor [4]. Mothers with periodontitis have been reported to have an increased risk of preterm labor [5]. Therefore, prevention or inhibition of pathological responses to inflammation would have clinical value in terms of reducing the risk of preterm labor in pregnant women.

Anesthesia and surgery are sometimes required during pregnancy. Surgeries include those directly or indirectly related to pregnancy and those unrelated to pregnancy, such as dental surgery [6]. In dentistry, facial bone fractures or neck abscesses are emergency surgeries that cannot be delayed ill after the childbirth. During pregnancy, the risk of tooth decay and periodontitis increases due to physiological changes; however, treatment is often delayed due to pregnancy. In rare cases, serious conditions, such as Ludwig's angina, which obstructs the airway and can become life-threatening, have been known to occur [7]. Surgery can be stressful for the patient and can cause an inflammatory response, and anesthetics used in pregnant women during dental surgery can lead to PTB. Thus, it is necessary to identify the effects of anesthetics in pregnant women.

Propofol is an intravenous anesthetic commonly used for general anesthesia and sedation owing to its rapid onset of action and short recovery time [8]. In recent studies, propofol has been shown to have anti-inflammatory effects *in vitro* as well as *in vivo*.

During pregnancy, amniotic cells produce many factors, including cytokines and PGE_2. WISH cells, a human amnion-derived cell line, are most widely used in *in vitro* studies of amnion function [9]. However, few studies have investigated the effect of propofol on human amnion-derived WISH cells, which produce the factors necessary for pregnancy and delivery control. To clarify the anti-inflammatory effects of propofol during pregnancy, it is crucial to determine the inflammatory response pathways that are inhibited by propofol in WISH cells.

Lipopolysaccharide (LPS) is an outer membrane component of gram-negative bacteria and is a major stimulant of the acute inflammatory response. Toll-like receptor 4 (TLR4) is a pattern recognition receptor for LPS. Binding of LPS to TLR4 during the LPS-induced inflammatory response activates MAPK and nuclear factor-kappa B (NF-κB) signaling pathways [8,10,11]. LPS also enhances the production of cyclooxygenase-2 (COX-2), which is essential for prostaglandin E_2 (PGE_2) production [12].

This study was conducted to investigate the effect of propofol on the expression of inflammatory mediators in human amnion-derived WISH cells co-treated with LPS and propofol. The involvement of MAPK signaling pathway in the anti-inflammatory effects of propofol was also investigated.

METHODS

1. Cell culture

An established line of human amnion cells (WISH) was purchased from the American Type Culture Collection (ATCC® CCL25™, Manassas, VA, USA). The cells were cultured in Eagle's minimum essential medium (ATCC® 30-2003™) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) in a 5% CO_2 atmosphere at 37°C. After three days of culture, adherent cells were removed, and the culture was continued, with two replacements of the culture medium per week.

2. Propofol and LPS treatment

Commercially available propofol (Fresenius Kabi Austria GmbH, Hafnerstrabe, Austria) was used in all experiments. Propofol was diluted in the culture medium and added to cell cultures at various concentrations (0.01–1 μg/ml) along with LPS (1 μg/ml) for 24 h. The experimental groups were as follows: control group, LPS group, LPS + propofol 0.01 μg/ml group, LPS + propofol 0.1 μg/ml group, and LPS + propofol 1 μg/ml group.

3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

WISH cells (1×10^5 cells /well) were seeded in 24-well plates and cultured at 37°C in a 5% CO_2 incubator for 24 h. The cells were then exposed to LPS (1 μg/ml) and propofol (0.01–1 μg/ml) for 24 h. After the treatment, the MTT (Affymetrix, Cleveland, OH, USA) assay was performed. One hundred microliters of MTT solution (5 mg/ml in phosphate buffered saline at pH 7.4) was added to each well, and the plate was incubated at 37°C for 1 h. Then, the medium was removed, and 100 μl of dimethyl sulfoxide (Biosesang, Seongnam, Korea) was added into each well. The plates were gently rotated using an orbital shaker for 15 min to dissolve the precipitate completely. Absorbance at 540 nm was measured using a microplate reader (Model 680; Bio-Rad, Hercules, CA, USA). All experiments were repeated thrice.

4. Nitric oxide (NO) assay

WISH cells were seeded into 24-well plates at a density of 1×10^4 cells/well. NO concentration in the culture supernatants was determined as nitrite (NO_2^-) using Griess reagent (Cell Signaling Technology, Danvers, MA, USA). WISH cells were incubated with 0.01–1 μg/ml propofol and 1 μg/ml LPS for 24 h. Subsequently, the supernatants were collected and mixed with the same amount (1:1, v/v) of Griess reagent. The samples were incubated at room temperature for 10 min, after which the absorbance was measured at 540 nm using a microplate reader (Bio-Rad Model 680).

5. Western blotting

Total proteins were extracted from cells using chilled RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% NP40, 5 mM dithiothreitol, 0.2 mM sodium orthovanadate, 100 mM NaF, and 1 mM phenylmethylsulfonyl fluoride [PMSF]) containing a protease inhibitor/phosphatase inhibitor cocktail (Cell Signaling Technology). Proteins (25 μg protein/well) were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked in TBS-0.1% Tween-20 (TBST) containing 3% skim milk for 1 h. The membranes were then incubated overnight at 4°C with rabbit monoclonal antibodies against α-tubulin (1:1000; Santa Cruz, Santa Cruz, CA, USA), p38 MAPK (1:1000; Cell Signaling Technology), phospho-p38 MAPK (1:500; Cell Signaling Technology), PGE synthase 2 (A-2) (1:1000; Cell Signaling Technology), and COX-2 (D5H5) (1:1000; Santa Cruz) in TBST containing 3% skim milk. After three washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit (1:1000; Enzo Life Sciences, Farmingdale, NY, USA) and anti-mouse (1:1000; Santa Cruz) antibodies at room temperature for 1 h. After three washes with TBST, the protein bands were visualized using enhanced chemiluminescence detection reagents (Promega, Madison, WI, USA); α-Tubulin was used as the control. Target protein bands were normalized to the control band using ImageJ software (NIH, Bethesda, MD, USA).

6. Statistical analysis

Data are presented as mean ± standard deviation (SD). All experiments were repeated at least thrice. Statistical analyses were performed using SigmaPlot v.10. Statistical significance was set at $P < 0.05$.

RESULTS

1. Co-treatment of LPS and propofol was not toxic to WISH cells

We investigated the effects of propofol and LPS on WISH cells. After incubating WISH cells with propofol (0.01, 0.1, and 1 μg/ml) and LPS (1 μg/ml) for 24 h, cell viability was evaluated using MTT assay. The results showed that the viability of WISH cells was not affected by propofol at any of the tested concentrations (Fig. 1).

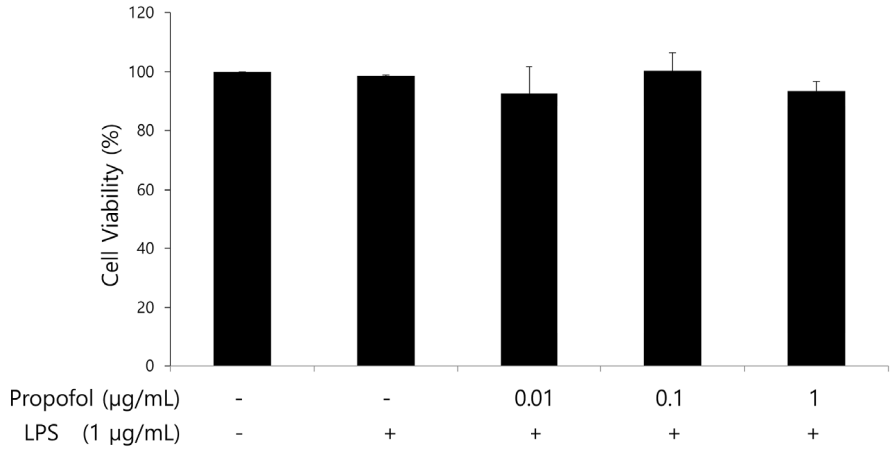

Fig. 1. Co-treatment of lipopolysaccharide (LPS) and propofol is not toxic to WISH cells. WISH cells were incubated with propofol (0.01–1 μg/ml) and LPS (1 μg/ml) for 24 h and were then subjected to MTT assay. Data are presented as the mean ± standard deviation. The experiment was repeated thrice.

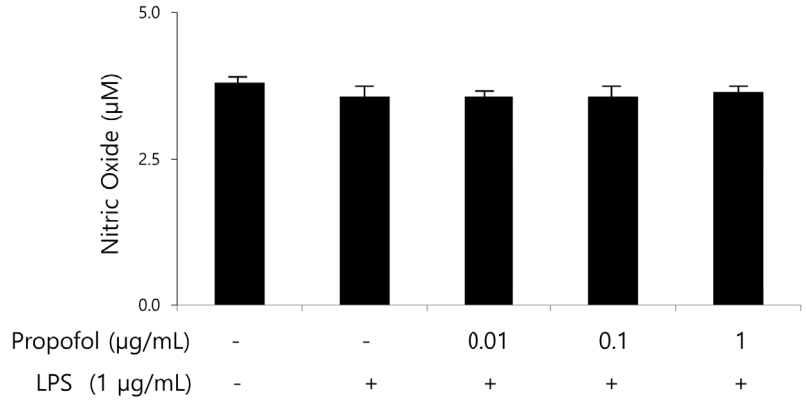

Fig. 2. Co-treatment of lipopolysaccharide (LPS) and propofol does not affect nitric oxide (NO) production. Stable nitrite end-products were measured using a microassay based on the Griess reaction. The results are presented as the mean ± standard deviation.. The experiment was repeated thrice.

2. Co-treatment of LPS and propofol did not affect NO production

NO production was measured using a microassay based on the Griess reaction. There was no difference in NO concentrations in the culture supernatants obtained from the propofol and LPS co-treated and control groups (Fig. 2). Thus, NO production in WISH cells was unaffected by propofol treatment.

3. Co-treatment of LPS and propofol suppressed COX-2 and PGE_2 protein expression in WISH cells

Western blot analysis revealed that LPS induced cellular inflammation and increased COX-2 and PGE_2 protein levels in WISH cells. Co-treatment with propofol suppressed LPS-induced upregulation of COX-2 and PGE_2 protein expression. Propofol significantly suppressed LPS-induced COX-2 and PGE_2 protein expression at 0.1 μg/ml concentration (Fig. 3).

4. Co-treatment of LPS and propofol inhibited p38 activation in WISH cells

To determine whether the anti-inflammatory effect of propofol was mediated via MAPK signaling pathway, p38 activation was investigated by measuring the expression of phospho-p38 using western blot analysis. LPS treatment significantly increased phospho-p38 levels. The levels of phospho-p38 were decreased upon co-treatment with propofol, with statistically significant decreases observed at 0.01 and 1 μg/ml propofol. These results

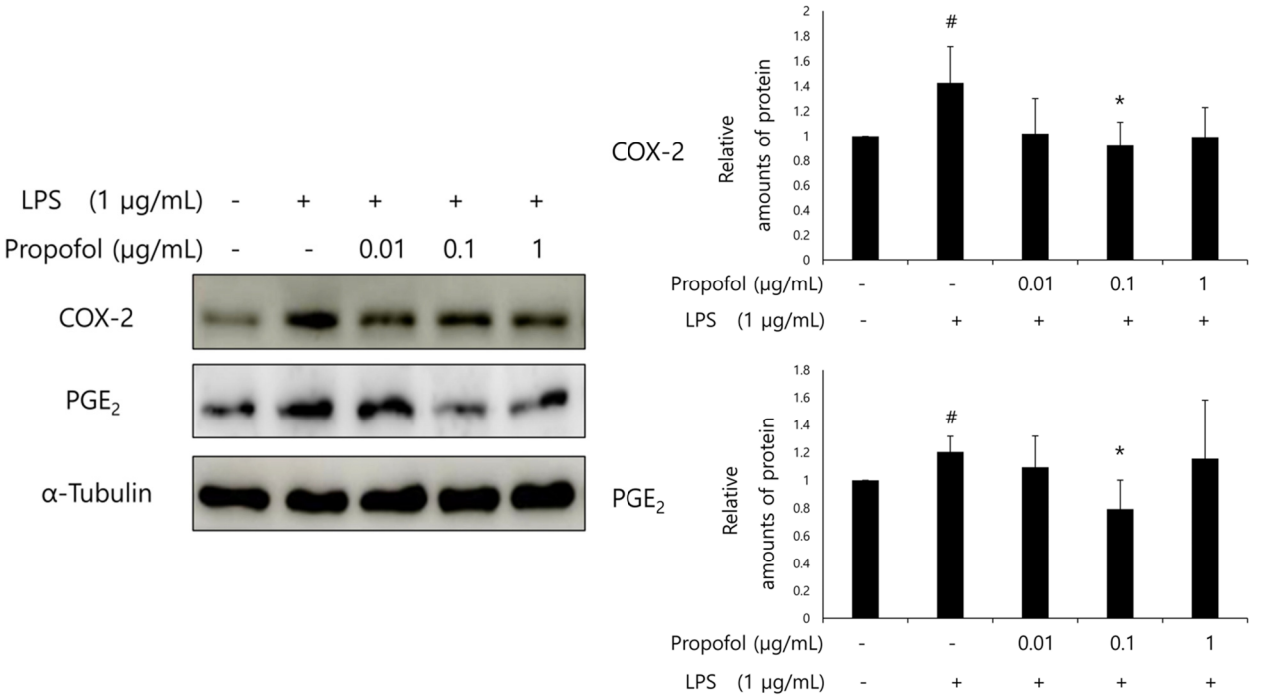

Fig. 3. Co-treatment of lipopolysaccharide (LPS) and propofol suppress cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) protein expression in WISH cells. COX-2 and PGE_2 protein expression was evaluated by western blotting. WISH cells were incubated with propofol (0.01–1 μg/ml) and LPS (1 μg/ml) for 24 h. Target protein levels were normalized to that of α-tubulin. Band intensities were measured using ImageJ software. Results are presented as the mean ± standard deviation. obtained from three independent experiments. #P < 0.05 versus control group; *P < 0.05 versus LPS group.

suggested that co-treatment of LPS and propofol inhibited p38 activation (Fig. 4).

DISCUSSION

We aimed to determine the effect of propofol treatment on LPS-induced inflammation in WISH cells and its underlying mechanism. Our findings suggest that propofol attenuates LPS-induced inflammatory response by inhibiting p38 phosphorylation in WISH cells.

Propofol did not affect the viability of WISH cells at any of the tested concentrations (0.01, 0.1, and 1 μg/ml). These results show that treatment with propofol in this concentration range is not toxic to WISH cells.

In addition, there was no difference in NO production between the propofol co-treated and control cells. NO, which increases during pregnancy, is an essential regulatory factor for uterine contractions. NO is a free radical produced from L-arginine, and maintains uterine relaxation during pregnancy [13,14]. In this study, the

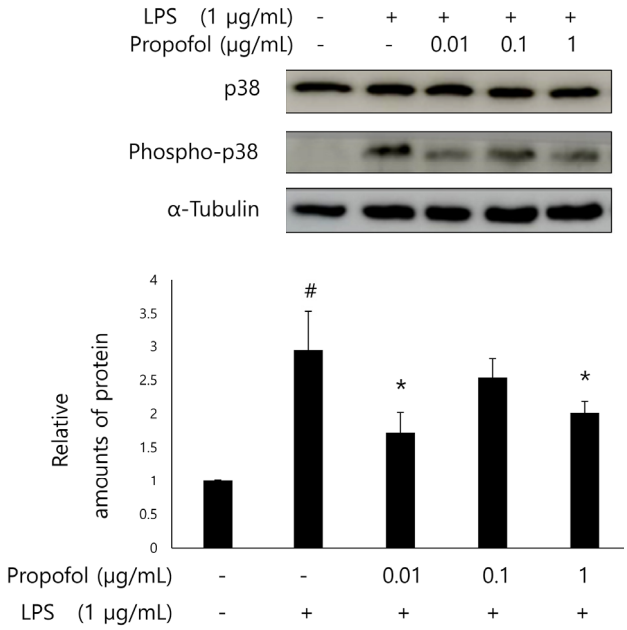

Fig. 4. Co-treatment of lipopolysaccharide (LPS) and propofol inhibits p38 activation in WISH cells. WISH cells were incubated with propofol (0.01–1 μg/ml) and LPS (1 μg/ml) for 24 h. p38 activation was subsequently evaluated by measuring phospho-p38 levels using western blotting. Phospho-p38 levels were normalized to that of p38. The assay was conducted in triplicate. #P < 0.05 versus control group; *P < 0.05 versus LPS group.

propofol concentration used for the co-treatment did not affect NO production. This is similar to a previous study that showed that propofol pretreatment did not affect NO production in LPS-stimulated WISH cells [15].

Propofol reduced COX-2 and PGE_2 protein expression in the LPS-induced inflammatory state. This finding is consistent with that of other studies, which have reported that propofol inhibits PGE_2 production by inhibiting COX-2 expression [16,17]. PGE_2 is an endogenous lipid mediator of inflammation and its production is regulated by COX-2 [12]. The amniotic membrane is an important source of PGE_2 during pregnancy and delivery [18]. PGE_2 plays vital roles, including those in cervical ripening and uterine contraction, during delivery. However, it can also cause excessive uterine contraction [19]. The finding that propofol reduced PGE_2 expression indicates that propofol can reduce uterine contractions.

MAPK is an important intracellular signal that mediates extracellular signaling responses in eukaryotic cells. MAPKs comprise three family members: extracellular signal-regulated protein kinase 1 and 2 (ERK 1/2), c-Jun N-terminal kinase (JNK), and p38 MAPK. In particular, p38 MAPK signaling pathway is the major MAPK pathway associated with inflammation. Pro-inflammatory factors, bacterial components, and UV irradiation can activate the p38 MAPK pathway. This pathway is activated by the phosphorylation of upstream MAPK kinases, such as MAPK kinase (MKK) 3/MKK6, and phosphorylates several transcription factors. At the cellular level, activated p38 regulates stress responses as well as cell growth, proliferation, differentiation, migration, and death. Activation of p38 MAPK signaling induces the expression of pro-inflammatory mediators, including COX-2 and tumor necrosis factor-α (TNF-α) [20-22]. Measurement of p38 phosphorylation levels in the blood monocytes of mice with endotoxemia revealed that LPS can induce p38 phosphorylation; propofol treatment could significantly inhibit p38 phosphorylation, indicating that propofol has an anti-inflammatory effect [8]. Furthermore, propofol was shown to inhibit p38 MAPK phosphorylation in alveolar epithelial cells and cardiomyocytes [20,23]. Therefore, we investigated whether the anti-inflammatory effect of propofol was mediated by phosphorylated p38 in WISH cells with LPS-induced inflammation. Propofol suppressed LPS-induced p38 phosphorylation; therefore, we suggest that propofol inhibits p38 signaling.

The major limitation of this study was that the positive effect of propofol was investigated only *in vitro*. Further *in vivo* and clinical studies are required. In addition, ample literature suggests that propofol may inhibit the release of inflammatory mediators, including IL-1β, IL-6, and TNF-α, which also requires further investigation [23,24]. Finally, the inhibition of COX-2 and PGE_2 expression may be influenced by the absence of amino acids and other substances involved in the production of COX-2 and PGE_2 in the medium.

In conclusion, this study demonstrated that propofol can reduce LPS-induced inflammation. Propofol inhibited p38 activation in LPS-induced WISH cells and suppressed the expression of COX-2 and PGE_2. Although further studies are needed to confirm the effect of propofol on preterm labor, the findings of the current study suggest that propofol may exert a protective effect against preterm labor induced by inflammation from nonobstetric surgeries.

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Eun-Jung Kim: Supervision
Jong Hoon Joo: Investigation, Software, Writing - original draft
Yeon Ha Kim: Investigation, Methodology, Software
Eun-Ji Choi: Supervision, Writing - review & editing

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