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Propofol Inhibits Superoxide Production, Elastase Release, and Chemotaxis in Formyl Peptide–Activated Human Neutrophils by Blocking Formyl Peptide Receptor 1

Shun-Chin Yang,*† Pei-Jen Chung,‡ Chiu-Ming Ho,*§ Chan-Yen Kuo,*† Min-Fa Hung,‡ Yin-Ting Huang,‡ Wen-Yi Chang,‡ Ya-Wen Chang,‡ Kwok-Hon Chan,*§ and Tsong-Long Hwang*‡

Neutrophils play a critical role in acute and chronic inflammatory processes, including myocardial ischemia/reperfusion injury, sepsis, and adult respiratory distress syndrome. Binding of formyl peptide receptor 1 (FPR1) by N-formyl peptides can activate neutrophils and may represent a new therapeutic target in either sterile or septic inflammation. Propofol, a widely used i.v. anesthetic, has been shown to modulate immuno-inflammatory responses. However, the mechanism of propofol remains to be established. In this study, we showed that propofol significantly reduced superoxide generation, elastase release, and chemotaxis in human neutrophils activated by fMLF. Propofol did not alter superoxide generation or elastase release in a cell-free system. Neither inhibitors of γ-aminobutyric acid receptors nor an inhibitor of protein kinase A reversed the inhibitory effects of propofol. In addition, propofol showed less inhibitory effects in non-FPR1-induced cell responses. The signaling pathways downstream from FPR1, involving calcium, AKT, and ERK1/2, were also competitively inhibited by propofol. These results show that propofol selectively and competitively inhibits the FPR1-induced human neutrophil activation. Consistent with the hypothesis, propofol inhibited the binding of N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein, a fluorescent analog of fMLF, to FPR1 in human neutrophils, differentiated THP-1 cells, and FPR1-transfected human embryonic kidney-293 cells. To our knowledge, our results identify, for the first time, a novel anti-inflammatory mechanism of propofol by competitively blocking FPR1 in human neutrophils. Considering the importance of N-formyl peptides in inflammatory processes, our data indicate that propofol may have therapeutic potential to attenuate neutrophil-mediated inflammatory diseases by blocking FPR1.

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Propofol (2,6-diisopropylphenol) is a widely used i.v. non-opioid anesthetic, and it is mainly administered for the sedation of surgical or critically ill patients, usually those with an immunoinflammatory status. As well as its anesthetic effects, there is growing evidence in animal and human studies that propofol exerts protective effects during acute inflammatory processes (1). In animal studies, propofol has decreased cytokine release during sepsis (2, 3) and reduced neutrophil-mediated inflammation in acute pulmonary injury (4, 5). In human studies, propofol has attenuated myocardial reperfusion injury and pulmonary dysfunction following cardiopulmonary bypass by reducing free radical release and modulating the inflammatory process (6, 7).

It is well demonstrated that overwhelming activation of the immune cells may be a major contributor to tissue damage in inflammatory diseases. It is noteworthy that propofol suppresses chemotaxis, phagocytosis, the generation of reactive oxygen species (ROS), and/or the synthesis of cytokines by monocytes and macrophages, which are mediated by activation of the γ-aminobutyric acid (GABA)A receptor (8, 9); reduces the mitochondria potential (10); and inhibits the AKT/IKKβ/NF-κB pathways (11). Propofol impairs the chemotaxis and respiratory burst of neutrophils in response to fMLF (12, 13). However, other research revealed that propofol fails to alter respiratory burst in PMA-activated neutrophils (14). Indeed, the cellular mechanisms responsible for the pharmacological effects of propofol in human neutrophils are controversial and remain to be established.

Neutrophils are a major cell population in the human innate immune system, which is the first line of defense against bacterial invasion (15). However, neutrophils are regarded as destructive cells, releasing toxic ROS and proteolysis enzymes that destroy the surrounding tissue (16, 17). For example, accumulated neutrophils induce endothelial dysfunction and microcirculatory collapse in acute coronary syndrome and in a myocardial ischemia/reperfusion injury model (18). Similarly, in lung injury models, inappropriateantly activated neutrophils have resulted in acute respiratory
distress syndrome or transfusion-related acute lung injury (19, 20).
Interestingly, it has been shown in animal and human studies that propofol may diminish the oxidative or inflammatory injury induced by neutrophils (2, 4, 7). These observations are interpreted to mean that propofol has an antioxidant capacity. Clearly, additional research is required to define the mechanisms of the action of propofol on activated neutrophils.

In this study, we examined the pharmacological roles of propofol in the respiratory burst, degranulation, and chemotaxis of human neutrophils, and explored its potential anti-inflammatory mechanisms. Our results demonstrate that, at clinical concentrations, propofol inhibits superoxide production, ROS generation, elastase secretion, and chemotaxis by human neutrophils activated with fMLF. Many of the observations made in the study suggest that the anti-inflammatory effects of propofol are mediated by blocking the interaction between fMLF and its receptor, formyl peptide receptor 1 (FPR1), thus disrupting the receptor-mediated signaling pathways. FPR1 is a G-coupled G protein–coupled receptor and is known to be important in neutrophilic inflammatory disorders (21). Notably, our results indicate that propofol may have therapeutic potential to attenuate neutrophil-mediated inflammatory diseases by blocking FPR1.

Materials and Methods

Reagents
Propofol (2,6-diisopropylphenol) was purchased from Sigma-Aldrich (St. Louis, MO). Dihydrorhodamine 123 (DHR 123), fluo-3 acetomethoxyester (fluo-3/AM), and N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein (FNLFNKY) were obtained from Molecular Probes (Eugene, OR). The cAMP enzyme immunoassay kits were from GE Healthcare (Uppsala, Sweden). Leu-Glu-Ser-Ile-Phe-Arg-Ser-Leu-Phe-Arg-Val-Met (MKM-1), muscimol, baclofen, and CGP52432 were obtained from Tocris Bioscience (Ellisville, MO). X-[2-(p-Bromocinnamylamino)ethyl]-5-isooquinolinesulfonamide (HS9), methoxyxycinyl-α-Ala-α-Ala-Val-nitroamide, and rolipram were purchased from Calbiochem (La Jolla, CA). Abs directed against phosphor-(H89), methoxysuccinyl-Ala-Ala-Pro-Val-nitroanilide, and rolipram were purchased from Sigma-Aldrich.

Isolation of human neutrophils
This study was approved by the local institutional review board, and written informed consent was obtained from each healthy volunteer. Neutrophils were isolated from peripheral blood according to the standard method of dextran sedimentation, followed by centrifugation in a Ficoll-Hypaque gradient and the hypotonic lysis of the erythrocytes. The purified neutrophils contained ≥98% viable cells, as determined by Trypan blue exclusion, and were suspended in calcium-free HBSS at 4˚C before use.

Differentiation of human monocytic leukemia cells (THP-1)
THP-1 were cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 10% FBS, and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B). THP-1 were cultured in the presence of 300 µM dibutyryl cAMP for 48 h to induce cell differentiation (22).

Expression of FPR1 in human embryonic kidney cells (HEK-293)
HEK-293 were maintained in DMEM supplemented with 10% FBS, 2 mM glutamine, and antibiotics. HEK-293 were stably transfected with the P24V-AC vector containing the human FPR1 gene (NM_002029; OriGene, Rockville, MD) for 72 h using X-tremeGENE Hp DNA transfection reagent (Roche, Mannheim, Germany), according to the manufacturer’s instruction. After transfection, cells were cultured in the medium containing G418 (2 mg/ml). G418-resistant clones were used for further studies.

Measurement of superoxide generation
The measurement of the superoxide generated by the activated neutrophils was dependent on the reduction of ferricytochrome c. After neutrophils (6 × 10^6 cells/ml) were mixed with 0.5 mg/ml ferricytochrome c and 1 mM CaCl2 at 37˚C, they were treated with DMSO (as control) or propofol for 5 min, and were activated with fMLF, MMK-1 (300 nM), or sodium fluoride (NaF; 20 mM) in the pretreatment of cytochalasin B (1 µg/ml for fMLF and MMK-1; 2.5 µg/ml for NaF) or PMA (5 nM). The change in absorbance was monitored continuously at 550 nm with a spectrophotometer (U-3010; Hitachi, Tokyo, Japan). Calculation was dependent on the statement in the previous study (23).

Measurement of ROS production
The intracellular ROS production by the activated neutrophils was determined from the conversion of nonfluorescent DHR 123 to fluorescent rhodamine 123, detected with flow cytometry. After neutrophils (2.5 × 10^6 cells/ml) were incubated with DHR 123 (2 µM) for 15 min at 37˚C, they were treated with propofol (0–50 µM) for 5 min, and then fMLF (100 nM) was added for 15 min. The response was terminated by placing the cells on ice. The change in fluorescence was analyzed by flow cytometry.

Measurement of the elastase released
This study used elastase release as evidence of the degranulation of the activated neutrophils. Methoxyxycinyl-α-Ala-α-Ala-Pro-Val-nitroamide was used as the elastase substrate (24). After neutrophils (6 × 10^6 cells/ml) were mixed with 100 µM substrate and 1 mM CaCl2 at 37˚C, they were treated with DMSO (control) or propofol for 5 min, and were then activated by fMLF. MMK-1 (300 nM), NaF (20 mM), or leukotriene B4 (LTB4; 100 nM) in the pretreatment of cytochalasin B (0.5 µg/ml for fMLF, MMK-1, and LTB4; 2.5 µg/ml for NaF). The change in absorbance was monitored continuously at 405 nm with a spectrophotometer. The results are shown as percentages of the elastase released in the control group.

Analysis of superoxide scavenging
The superoxide-scavenging effect of propofol was determined in a cell-free xanthine/xanthine oxidase system. The assay buffer contained 50 mM/L Tris (pH 7.4), 0.3 mM/L WST-1, and 0.02 U/ml xanthine oxidase. After 0.1 mM/L xanthine was added to the assay buffer for 10 min at 30˚C, the change in absorbance reflecting the reduction of WST-1 induced by superoxide was measured at 450 nm.

Evaluation of lactate dehydrogenase release
Lactate dehydrogenase (LDH) was used as the symbol of cytotoxicity and determined by a commercially available method (Promega, Madison, WI). The calculation was based on LDH activity in the propofol group (0–100 µM/L) expressed as a percentage of the total LDH activity. The total LDH activity was determined with the lysis of neutrophils with 0.1% Triton X-100 for 30 min at 37˚C.

Chemotaxis assay
Cell migration was measured using a 24-well microchemotaxis chamber (pore size 3 µm; Millipore, Darmstadt, Germany). Neutrophils (5 × 10^6 cells/ml) were pretreated with propofol (0–100 µM/L) for 5 min at 37˚C in the top chamber. A total of 300 nM/L fMLF, 300 nM/L MMK-1, 100 nM/L LTB4, or 100 ng/ml IL-8 with propofol was placed in the bottom chamber. After incubation for 120 min, the numbers of migrated cells were determined by a Coulter counter (Beckman Coulter, Fullerton, CA) (25, 26).

Receptor-binding assay
Receptor binding was assayed by the FACScan analysis of the binding of FNLFNKY, a fluorescent analog of fMLF, as described previously (27, 28). Neutrophils (2 × 10^6 cells/ml) differentiated THP-1 (2 × 10^6 cells/ml), or FPR1-transfected HEK-293 (2.5 × 10^6 cells/ml) were incubated with propofol for 5 min at 4˚C and labeled with FNLFNKY. After 30 min, the cells were pelleted, resuspended in ice-cold HBSS, and immediately analyzed with flow cytometry.

Determination of cAMP concentration
Neutrophils were incubated with propofol (0–50 µM/L) or DMSO (control) for 5 min and stimulated with fMLF for another 1 min. The reaction was then terminated by the addition of 0.5% dodecylmethylammonium bromide. After centrifugation at 3000 × g for 5 min at 4˚C, the supernatant was assayed for cAMP with an enzyme immunoassay kit (Amersham Biosciences, Buckinghamshire, U.K.).
**Measurement of intracellular calcium concentration**

Neutrophils (3 x 10⁶ cells/ml) or differentiated THP-1 (7 x 10⁶ cells/ml) were labeled with fluo-3/AM (2 μmol/L) for 30 min at 37°C. The cytoplasmic calcium levels were measured in a quartz cuvette with a Hitachi F-4500 spectrofluorometer with a thermostat (37°C), and with continuous stirring. The excitation wavelength was 488 nm, and the emission wavelength was 520 nm. After they were treated with propofol for 5 min, stimulants were added in the presence of 1 mmol/L Ca²⁺ to increase intracellular calcium concentration [Ca²⁺]i. [Ca²⁺]i was calculated from the fluorescence intensity, as follows: [Ca²⁺]i = K_d × [(F - F_min)/(F_max - F)] ; where F is the observed fluorescence intensity, F_max and F_min were obtained by the addition to the neutrophils of 0.05% Triton X-100 and 20 mmol/L EGTA, respectively, and K_d was taken to be 400 nmol/L.

**Immunoblotting analysis**

Neutrophils were treated with propofol for 5 min and stimulated with fMLF (10–100 μmol/L) for 30 s. The reaction was terminated by placing the cells on ice. After centrifugation at 4°C and removal of the supernatant, the pellet was lysed in 150 μl buffer (50 mmol/L HEPES [pH 7.4], 100 mmol/L NaCl, 1 mmol/L EDTA, 2 mmol/L Na₃VO₄, 10 mmol/L p-nitrophenyl phosphate, 5% 2-ME, 1 mmol/L PMSF, 1% dilution of Sigma-Alrich protease inhibitor cocktails, and 1% Triton X-100). After brief sonication, the samples were centrifuged at 14,000 × g for 20 min at 4°C to yield whole-cell lysates. The lysates were used for Western blotting analysis. NaDodSO₄-PAGE with 12% polyacrylamide gels was used to separate the proteins. The samples were then blotted onto nitrocellulose membranes. Immunoblotting was performed with the indicated Abs and HRP-conjugated secondary anti-rabbit Abs (Cell Signaling Technology, Beverly, MA). The proteins were detected with the ECL system (Amersham Biosciences).

**Statistical analysis**

All experiments were performed at least three times, and the results are expressed as means ± SEM. The statistical analyses were based on Student t test or the Mann–Whitney U test, and all calculations were performed with SigmaPlot (Systat Software, San Jose, CA). A p value <0.05 was considered statistically significant.

**Results**

**Propofol inhibits superoxide generation and elastase release in fMLF-activated neutrophils, but fails in a cell-free system**

To determine whether propofol regulates neutrophil functions, superoxide generation and elastase release were measured in activated cells during the administration of propofol. Propofol (3–50 μmol/L) had a dose-dependent inhibitory effect on superoxide generation in the neutrophils activated by the FPR1 activator, fMLF (Fig. 1A). The IC₅₀ of propofol was 10.31 ± 3.02 μmol/L. Moreover, propofol (3–50 μmol/L) also reduced the elastase release from fMLF-activated neutrophils in a dose-dependent manner, with an IC₅₀ value of 25.94 ± 1.74 μmol/L (Fig. 1B). Propofol (50 μmol/L) did not alter basal superoxide generation and elastase release under resting conditions (Fig. 1A, 1B). In addition, the superoxide produced by NADPH oxidase in neutrophils can be converted to various ROS, which cause extensive tissue damage. The flow cytometric analysis showed that propofol reduced the intracellular ROS generated by the neutrophils treated with fMLF (Fig. 1C). In contrast, propofol did not induce the release of LDH, even at a high concentration (100 μmol/L), suggesting that inhibition of the neutrophils’ respiratory burst and degranulation by propofol were not attributable to its cytotoxicity (data not shown). The inhibitory effects of propofol were then tested in cell-free systems to determine whether propofol scavenges superoxide and inhibits elastase activity. We assayed the superoxide-scavenging effect of propofol at concentrations of up to 100 μmol/L, but superoxide generation was not affected in the cell-free xanthine/xanthine oxidase system. Superoxide dismutase was used as the positive control (Fig. 1D). We also found that propofol (10–100 μmol/L) had no direct inhibitory effect on the extracellular elastase activity (Fig. 1E). These data indicate that propofol impairs the respiratory burst and the degranulation of FPR1-activated neutrophils, but that these inhibitory effects do not occur in a cell-free system.

**Neither GABAA nor GABAB mediates the inhibitory effects of propofol**

It is well known that propofol exerts versatile pharmacologic functions through the activation of GABA receptors. Therefore, we next investigate whether propofol alters neutrophil functions through the activation of GABA receptors. Therefore, we next investigate whether propofol alters neutrophil functions through GABAA or GABAB receptor. To address this question, we used a pharmacological approach with GABAA or GABAB receptors in neutrophils. GABAA receptors agonists and antagonists. Neither muscimol (GABA receptors agonist) nor baclofen (GABA receptors agonist) altered the superoxide and elastase secretion by neutrophils under resting conditions or by fMLF-stimulated neutrophils (Supplementary Fig. 1A, 1B). Furthermore, neither SR95531 (GABAA receptor antagonist) nor CGP52432 (GABAB receptor antagonist) affected the propofol-mediated inhibition of these functions (Supplementary Fig. 1C, 1D). These data suggest that the activation of the GABAA and GABAB receptors does not play a role in the generation of...
Propofol selectively attenuates fMLF-induced human neutrophil chemotaxis

The migration of neutrophils into tissues is an essential event in the FPR1-induced inflammatory response. To investigate whether propofol reduces neutrophil migration, we examined the chemotaxis of neutrophils in response to different chemoattractants. Neutrophil migration induced by fMLF (30 nmol/L) was significantly reduced in the presence of propofol (50 and 100 μmol/L) (Fig. 3A). In contrast, propofol failed to inhibit chemotaxis induced by MMK-1 (300 nmol/L), LTB4 (100 nmol/L), and IL-8 (100 ng/ml) (Fig. 3B–D).

Propofol competitively binds FPR1 in human neutrophils

To examine whether propofol has a binding affinity for FPR1, the binding of FNLFNYK to the surface of neutrophils was monitored by flow cytometry. Fig. 4A showed that fMLF (10 μmol/L) completely inhibited the binding of FNLFNYK (4 nmol/L) to neutrophils. Compared with the control group, propofol (5, 50, and 100 μmol/L) significantly and dose dependently inhibited the binding of FNLFNYK to the fMLF receptor (Fig. 4A). Furthermore, the specific concentration-binding curve of FNLFNYK (2–12 nmol/L) was reduced by propofol (50 μmol/L) (Fig. 4B). These results indicate that propofol can competitively bind to FPR1.

Propofol exerts competitive inhibitory effects on fMLF-activated neutrophil responses

The concentration-response curves of fMLF for superoxide production and elastase release are shown in Fig. 5, and the EC50 values were 18.94 ± 3.35 and 39.94 ± 21.78 nmol/L, respectively. Significantly, propofol pretreatment produced right shifts in the concentration-response curves of fMLF for superoxide production and elastase release, with EC50 values of 176.17 ± 24.15 and 273.49 ± 114.69 nmol/L, respectively. In addition, propofol exerted higher degree of inhibitions in a low concentration of fMLF (10 nmol/L)-treated neutrophils, and the IC50 values for superoxide generation and elastase release were 0.38 ± 0.14 and 0.27 ± 0.11 μmol/L, respectively (Supplemental Fig. 2). These data support the proposition that propofol is a competitive inhibitor of FPR1.

cAMP/Protein kinase A pathway does not mediate the inhibitory effects of propofol

In the following experiments, we investigated whether the cAMP/protein kinase A (PKA) pathway is involved in the inhibitory effects of propofol. Rolipram (phosphodiesterase 4 inhibitor, 0.05 versus the control group. 

**Propofol less effectively inhibits non-FPR1 agonist-triggered responses**

We next asked whether propofol impairs the cellular responses of only those neutrophils challenged with fMLF. To answer this question, neutrophils were stimulated with other non-FPR1 activators, including MMK-1 (FPR2 agonist, 300 nmol/L), LTB4 (BLT1 receptor activator, 100 nmol/L), NaF (direct G protein activator, 20 mmol/L), and PMA (protein kinase C activator, 5 nmol/L). Fig. 2 shows that propofol even at high concentration of 100 μmol/L exerted only slight inhibitory effects on the MMK-1–, NaF–, LTB4–, or PMA-induced responses. Taken together, these results suggest that propofol has a selective inhibitory action on FPR1 agonist-activated neutrophils.
3 μmol/L, but not propofol (10 and 50 μmol/L), increased cAMP levels in fMLF-activated human neutrophils (Fig. 6A), suggesting that cAMP is not involved in the inhibitory effects of propofol. Consistent with this result, H89 (3 μmol/L), a PKA inhibitor, failed to reverse the inhibitory effects of propofol on superoxide generation and elastase release in activated cells (Fig. 6B, 6C).

Propofol attenuates fMLF-induced Ca2+ mobilization in human neutrophils

Ca2+ signals play an important role in many neutrophil functions. To determine whether treatment with propofol attenuates Ca2+ signals in activated neutrophils, their [Ca2+]i was assayed. The peak [Ca2+]i, induced by fMLF (10–1000 nmol/L) was significantly diminished by propofol (50 μmol/L) (Fig. 7). In contrast, the peak [Ca2+]i induced by MMK-1 (300 nmol/L), IL-8 (100 ng/ml), and LTB4 (100 nmol/L) was unaltered by propofol (50 μmol/L) (Supplemental Fig. 3). These results suggest that propofol specifically attenuates Ca2+ signals in FPR1 agonist–activated neutrophils.

Propofol inhibits AKT and ERK1/2 phosphorylation in fMLF-activated neutrophils

It is well known that the PI3K/AKT and MAPK pathways are involved in the downstream signaling of fMLF-stimulated neutrophils. Propofol (50 μmol/L) caused a significant reduction in the phosphor-AKT expression in neutrophils in response to different concentrations of fMLF (Fig. 8A). In contrast, the ERK1/2 activation induced by fMLF at low concentrations (10 and 30 μmol/L), but not that induced by a high concentration (100 μmol/L), was inhibited by propofol (Fig. 8B). However, the administration of propofol induced the expression of phosphor-p38 in resting cells, and it failed to suppress fMLF-triggered p38 activation (Fig. 8C).

Propofol binds FPR1 in differentiated THP-1 and FPR1-transfected HEK-293

The specificity of propofol for FPR1 was examined in dibutyryl cAMP-differentiated THP-1 and FPR1-transfected HEK-293. fMLF (10 μmol/L) completely inhibited the binding of FNLFNYK (4 nmol/L) to dibutyryl cAMP-differentiated THP-1. Also, propofol dose dependently inhibited the binding of FNLFNYK (4 nmol/L) to FPR1 in differentiated THP-1 (Fig. 9A). Furthermore, the peak [Ca2+]i, induced by fMLF (10–100 nmol/L), but not MMK-1 (300 nmol/L), was significantly diminished by propofol (50 μmol/L) (Fig. 9B). These data indicate that propofol inhibits fMLF-induced Ca2+ mobilization by blocking FPR1 in differentiated THP-1. Furthermore, the binding of FNLFNYK (4 nmol/L) to FPR1-transfected HEK-293 was inhibited by fMLF (10 μmol/L) and propofol (5, 50, and 100 μmol/L) (Fig. 10). These results indicate that propofol displays specificity for the FPR1.
Discussion

The immunomodulatory effects of propofol have been reported in a variety of experimental and functional research over the years (29). However, very little is known about the pharmacologic mechanisms of propofol, especially in impairing neutrophil functions. To our knowledge, our study shows, for the first time, that, at therapeutic concentrations, propofol inhibits the respiratory burst, degranulation, and chemotaxis of fMLF-activated neutrophils by competitively binding to FPR1 and thus attenuating downstream signaling, including in the Ca²⁺, AKT, and ERK1/2 pathways.

There is considerable evidence from clinical and experimental studies that propofol exerts significant protective effects against inflammatory and cardiovascular diseases, inhibiting the production of cytokines and the clearance of ROS (3, 30). Propofol has been shown to reduce oxidative injury to organ in endotoxemia animals (31) and in patients undergoing cardiac surgery (32). Human neutrophils play important roles in the pathogenesis of various diseases, including acute myocardial infarction, atherosclerosis, ischemic heart disease, and sepsis (15, 18). The oxidative stress produced by activated human neutrophils can directly or indirectly cause damage by destroying the surrounding tissue and is known to be involved in the pathogenesis of inflammatory diseases. Our data show that propofol significantly inhibits the superoxide production induced by fMLF in a concentration-dependent manner in intact human neutrophils, which is consistent with previous reports (33, 34). Propofol has been shown to display direct scavenging activity for free radical species (35). However, direct scavenging activity was ruled out because propofol failed to alter the superoxide generation in the cell-free xanthine/xanthine oxidase system. These controversial results may be explained by the different radical species examined in different studies. We suggest that propofol inhibits superoxide formation in activated neutrophils by modulating cellular signaling pathways. This hypothesis is supported by the evidence that propofol also markedly inhibited elastase release in fMLF-activated cells, but not in the cell-free system. Neutrophil granules contain many antimicrobial and potentially proinflammatory proteases. Elastase is a major serine protease secreted by stimulated human neutrophils and plays a critical role in inflammatory diseases (36). These data also support the proposition that propofol acts as an anti-inflammatory agent. Furthermore, the recruitment of neutrophils to sites of inflammation is one of the major biological...
functions for FPR1 (37). Our results show that propofol reduces neutrophil chemotaxis by fMLF in a concentration-dependent manner, which is consistent with previous reports (38, 39). GABA is an inhibitory neurotransmitter in the CNS and can modulate autoimmune inflammation (40). Through the activation of the GABA receptors, propofol exerts various pharmacological effects, including the inhibition of chemotaxis, phagocytosis, ROS generation, and/or cytokine synthesis in monocytes and macrophages (8, 9). Another research has demonstrated that human neutrophils express GABA\textsubscript{B}, but few GABA\textsubscript{A} receptors (41). However, the roles of the GABA\textsubscript{A} and GABA\textsubscript{B} receptors in the respiratory burst and degranulation of activated human neutrophils are still unknown. Data from the current study show that the GABA\textsubscript{A} and GABA\textsubscript{B} agonists, muscimol and baclofen, respectively, do not induce superoxide production or elastase release in either resting or activated human neutrophils. A role for the GABA\textsubscript{A} and GABA\textsubscript{B} receptor in the inhibitory action of propofol was excluded because either GABA\textsubscript{A} or GABA\textsubscript{B} antagonists failed to reverse the inhibitory effects of propofol.

The molecular and functional responses for human neutrophil recognition of formyl peptides are their binding to FPRs. Human neutrophils express two members of this family, FPR1 and FPR2.
FIGURE 10. Propofol binds the formyl peptide receptor in FPR1-transfected HEK-293. FPR1-transfected HEK-293 were incubated with propofol (0–100 μmol/L) or IMLF (10 μmol/L) for 5 min and labeled with FNLFNYK (4 nMol/L). Basal group (black line) was treated with DMSO alone in the absence of FNLFNYK, and the test groups (other colored lines) were treated with DMSO, propofol, and IMLF in the presence of FNLFNYK. The mean fluorescence intensity (MFI) is expressed as the mean ± SEM relative to the control group (100%). Data are representative of three experiments. *p < 0.05, ***p < 0.001 versus the control group.

(21, 42). In our study, the superoxide generation, elastase release, and chemotaxis induced by MMK-1, a FPR2 agonist, are less sensitive to inhibition by propofol than those induced by IMLF, a FPR1 agonist. In addition, the results obtained using different receptor activators, NaF, LTB4, PMA, and IL-8, clearly confirm that propofol selectively inhibits FPR1-mediated effects. It is noteworthy that propofol produced a parallel rightward shift in the IMLF concentration-response curves, whereas the maximum response remained unchanged. Besides, propofol exerted a higher degree of inhibition in a low concentration of IMLF-treated neutrophils. Based on these observations, we postulate that propofol has a selective and competitive blocking effect on FPR1. Our data confirm that propofol blocks the binding of FNLFNYK to FPR1 in human neutrophils in a concentration-dependent manner. Also, the specificity of propofol for FPR1 is obtained in the differentiated THP-1 and the FPR1-transfected HEK-293. To the best of our knowledge, this is the first study to show that propofol has a competitive binding affinity for FPR1.

The intracellular signaling mechanisms responsible for neutrophil activation are very complex and remain elusive. Several studies have established that the addition of chemotaxtractants to neutrophils leads to a small and temporary increase in the production of cAMP (43–45). We and others have reported that increased intracellular cAMP levels are associated with the inhibition of multiple intracellular activities, including the respiratory burst and the degranulation of neutrophils (24, 46). Rolipram, an inhibitor of phosphodiesterase 4, caused an increase in IMLF-induced cAMP levels in human neutrophils. In contrast, cAMP was ruled out because propofol failed to alter intracellular cAMP levels and the PKA inhibitor, H89, did not reverse the inhibitory effects of propofol on superoxide production or elastase release. The activation of FPR1 elicits multiple signaling pathways that trigger the human inflammatory responses. Phospholipase C catalyzes the conversion of phosphoinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate to cause the rapid release of Ca2+ (45, 47, 48). In addition to the increase in [Ca2+]i, the activation of PI3K/AKT signaling, as well as the ERK1/2 and p38 MAPK cascades, is mediated by the interactions between fMLF and FPR1 for multiple intracellular activities (49). In fact, propofol inhibits fMLF-induced neutrophil ROS production and chemotaxis by suppressing the [Ca2+]i, and phosphorylation of ERK (34, 39). The present study shows that propofol reduces the IMLF-induced peak [Ca2+]i, and phosphorylation of AKT and ERK1/2. The inhibitory potency of propofol is inversely related to the IMLF concentration, providing evidence that propofol inhibits fMLF-caused cell signals in a competitive manner. In contrast, we unexpectedly showed that propofol itself can induce the phosphorylation of p38 MAPK in human neutrophils. Nowadays, there are strong evidences supporting that propofol has anti-inflammatory effects in human and animal studies (1–7, 29, 30). However, studies showed that propofol increases neutrophil respiratory burst in the bronchoalveolar lavage fluid from patients undergoing tympanoplasty surgery (50), and it fails to affect neutrophil oxidative response in patients undergoing cataract surgery (51). Together, these studies demonstrate that the anti-inflammatory effects of propofol may differ by diseases. Obviously, further research is required to clarify the effects and action mechanisms of propofol in various in vivo models of inflammation.

The growing evidences have supported that FPR1 plays critical roles in sterile and septic inflammation. FPR1 is activated by N-formyl peptides, which are derived from either bacterial peptides or mitochondrial proteins (52, 53). The endogenous damage-associated molecular patterns from bone and liver mitochondria can activate neutrophils through FPR1 and induce severe inflammatory response syndrome (54–56). Therefore, concerns have been raised about the potential of functional FPR1 as a therapeutic target for the development of new drugs to treat inflammatory diseases (21, 57). In conclusion, to our knowledge, our results clearly demonstrate for the first time that propofol inhibits human neutrophil activations by selective and competitive blockade of FPR1. Given the importance of FPR1 in inflammatory diseases, these results also suggest that propofol may have potential benefits in protecting against FPR1-involved inflammatory diseases.

Disclosures
The authors have no financial conflicts of interest.

References


