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Sedative Drug Modulates T-Cell and Lymphocyte Function-Associated Antigen-1 Function

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Abstract

Background—Sedative drugs modify immune cell functions via several mechanisms. However, the effects of sedatives on immune function have been primarily investigated in neutrophils and macrophages, and to the lesser extent lymphocytes. Lymphocyte function-associated antigen-1 (LFA-1) is an adhesion molecule that plays a central role in regulating immune function of lymphocytes including interleukin-2 (IL-2) production and lymphocyte proliferation. Previous clinical studies reported that propofol and isoflurane reduced IL-2 level in patients, but midazolam did not. We previously demonstrated that isoflurane inhibited LFA-1 binding to its counter ligand, intercellular adhesion molecule-1 (ICAM-1), which might contribute to the reduction of IL-2 levels. Here, we examined the effect of propofol, midazolam and dexmedetomidine on LFA-1/ICAM-1 binding, and the subsequent biological effects.

Methods—The effect of sedative drugs on T-cell proliferation and IL-2 production was measured by calorimetric assays on human peripheral blood mononuclear cells. Because LFA-1/ICAM-1 binding plays an important role in T-cell proliferation and IL-2 production, we measured the effect

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DISCLOSURES

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Contribution: Study design, conduct of study, data analysis, and manuscript preparation

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of sedative drugs on ICAM-1 binding to LFA-1 protein (cell-free assay). This analysis was followed by flow cytometric analysis of LFA-1 expressing T-cell binding to ICAM-1 (cell-based assay). To determine if the drug/LFA-1 interaction is due to competitive or allosteric inhibition, we analyzed the sedative drug effect on wild-type and high affinity LFA-1 and a panel of monoclonal antibodies that bind to different regions of LFA-1.

Results—Propofol at 10–100 μ M inhibited ICAM-1 binding to LFA-1 in cell-free assays and cell-based assays ($p < 0.05$). However, dexmedetomidine and midazolam did not affect LFA-1/ICAM-1 binding. Propofol directly inhibits LFA-1 binding to ICAM-1 by binding near the ICAM-1-contact area in a competitive manner. At clinically relevant concentrations, propofol, but not dexmedetomidine or midazolam, inhibited IL-2 production ($p < 0.05$). Additionally, propofol inhibited lymphocyte proliferation ($p < 0.05$).

Conclusions—Our study suggests that propofol competitively inhibits LFA-1 binding to ICAM-1 on T-cells and suppresses T-cell proliferation and IL-2 production, while dexmedetomidine and midazolam do not significantly influence these immunological assays.

Introduction

The effects of sedative drugs on inflammation have been studied in laboratory and clinical settings.¹ Propofol and midazolam have shown antiinflammatory properties in a variety of experimental models.¹ Perturbation of leukocyte function by sedatives may impair the ability of surgical and critically ill patients to combat infections and sepsis. Likewise, suppression of inflammatory responses to tissue injury and noxious peripheral stimulation may have some benefit.² The effects of sedatives on immune function have been primarily investigated in neutrophils and macrophages and to a lesser extent lymphocytes. Since propofol, midazolam and dexmedetomidine are commonly used in the perioperative and the intensive care settings, the immunomodulatory effects of these drugs need to be examined more rigorously.

Integrin lymphocyte function-associated antigen-1 (LFA-1) is a heterodimeric cell adhesion molecule consisting of non-covalently associated α - and β - subunits, ubiquitously expressed on leukocytes.³ It is required for various intercellular functions that include T-cell interactions with antigen presenting cells, B-cells, and co-stimulation of T-cell responses.³ The binding of T-cell receptor with Class II major histocompatibility complex (MHC) is relatively weak and less stringent,^{4, 5} so the engagement of LFA-1 seems indispensable in the formation of stable immunological synapse and activation for CD4⁺ T-cells.⁶ The production of interleukin (IL)-2 is predominantly made by activated CD4⁺ T-cells,⁷ and is inhibited by anti LFA-1 blocking antibodies. T-cell proliferation was impaired in LFA-1 knockout mouse, suggesting that LFA-1 is also important in this process.⁸ Clinical reports suggested that isoflurane and propofol may reduce IL-2 levels,^{9–11} but midazolam has no effect on IL-2 levels.⁹ However, the mechanism of anesthetic (sedative) – related change in IL-2 levels is unclear.

The LFA-1 α subunit (α L) contains the inserted (I) domain, which is located at the most distal part of its extracellular structure and functions as the ligand binding domain.^{12, 13} The binding of LFA-1 to its major ligand intercellular adhesion molecule-1 (ICAM-1) is dynamically regulated by the conformational changes of the I domain from the low-affinity to the high-affinity form, and only the latter can tightly bind to ICAM-1.^{12–14} The conformational changes involve the structural rearrangement of the allosteric (distinct from the ligand binding site) cavity at the bottom of the I domain, to which small-molecule LFA-1 antagonists bind.¹⁵ We previously showed that isoflurane and sevoflurane inhibited the activation-dependent conversion of LFA-1 to the high-affinity conformation by binding to the allosteric cavity, suggesting one of the underlying mechanisms of anesthetic-mediated

immunomodulation.^{16–18} The inhibition of LFA-1/ICAM-1 engagement may be one of mechanisms of IL-2 reduction under isoflurane exposure. We hypothesize that propofol inhibits LFA-1 function, causing the reduction of IL-2 production by T-cells, while midazolam does not.

Methods

Cells and Reagents

Jurkat (human T lymphoma), and CHO (Chinese hamster ovarian) cell lines were from ATCC (Manassas, VA) and cultured in RPMI 1640 (Sigma, St. Louis, MO), 10% fetal bovine serum (FBS) at 37°C in 5% CO₂. Whole blood from healthy human individuals was purchased from Research Blood Components, LLC (Brighton, MA). Peripheral blood mononuclear cells (PBMCs) were purified using Ficoll/paque gradient sedimentation (Amersham-Pharmacia Biotech; Piscataway, NJ) from whole blood. Monoclonal antibodies for different epitopes of LFA-1 (TS1/22, TS2/14, CBR LFA-1/9 and MEM83) were used to identify specific binding sites on I domain (Immune Disease Institute, Boston, MA).¹⁹ 20 (R)-5-(4-bromobenzyl)-3-(3, 5-dichlorophenyl)-1,5-dimethylimidazolidine-2,4-dione (BIRT377) is a LFA-1 small allosteric antagonist (Immune Disease Institute).²¹ Lipid-free propofol, midazolam, staphylococcal enterotoxin B (SEB), phorbol 12-myristate 13-acetate (PMA), and ionomycin were from Sigma. Dexmedetomidine was from TOCRIS (Ellisville, MO). Propofol, midazolam and dexmedetomidine were dissolved in dimethyl sulfoxide (DMSO).

IL-2 production assays

To compare the effect of propofol, midazolam and dexmedetomidine on IL-2 production, PBMC assays with either SEB (2 µg/ml) or PMA (10 ng/ml)/ ionomycin (1 µg/ml) were performed as previously described.²¹ SEB induces LFA-1- dependent IL-2 production, while PMA/ionomycin does not. Purified human PBMCs suspended in RPMI 1640 and 10% FBS were plated in a 96-well plate at 2×10^5 cells/ well. Several concentrations of propofol (10 to 100 µM), midazolam (1 to 50 µM) or dexmedetomidine (1 to 50 µM) were tested. All experiments included mock-treated samples containing equal volumes of DMSO (0.5%). The cells were incubated at 37°C for 16 hours. IL-2 levels were measured with a human IL-2 ELISA kit (R&D Systems, Minneapolis, MN). The LFA-1 blocking antibody TS1/22 was used as a control to demonstrate LFA-1 dependent process.

The Effect of Sedative Drugs on Binding of soluble ICAM-1 to the immobilized extracellular part of LFA-1

To examine the effect of sedative drugs on LFA-1/ ICAM-1 binding, recombinant extracellular portion of wild-type (WT) and high-affinity (HA) LFA-1 protein were expressed in CHO cells and purified to homogeneity as previously described.²² The α LI domain of HA LFA-1 protein is locked in the constitutively high-affinity conformation by an engineered disulfide bond.²³ Soluble LFA-1 WT or HA (5 µg/ml) was immobilized indirectly on anti- LFA-1 capturing antibody CBR LFA1/2 (Immune Disease Institute) on ELISA plates. Nonspecific binding was blocked with HEPES-buffered saline (HBS) and 2% bovine serum albumin (Sigma). Human ICAM-1-Fc α fusion protein (5 µg/ml) ^{22, 23} was then added to wells with HBS containing 1mM MnCl₂ and propofol, midazolam or dexmedetomidine at various concentrations. All experiments included mock-treated samples containing equal volumes of DMSO (0.5%). After incubation for 1 hour at room temperature, unbound ICAM-1 was washed off. Bound ICAM-1 was detected by peroxidase-labeled goat antihuman immunoglobulin A (IgA) (KPL, Inc., Gaithersburg, MD) and substrate (BD, Franklin Lakes, NJ). After 15 minutes, absorbance was measured at

405nm. ICAM-1 binding % was defined as [(optical density (OD) at various concentrations of sedatives) / (OD of mock treated)] $\times$ 100 (%).

Binding of soluble ICAM-1 to Jurkat cells

ICAM-1 binding to LFA-1 on Jurkat cells was measured by flow cytometry. Briefly, Jurkat cells expressing LFA-1 were harvested and washed once with HBS containing 10 mM EDTA and 3 times with HBS, and then resuspended in HBS. Cells (5×10^5) in 300 μ l HBS were aliquoted to tubes and centrifuged. Cell pellets were given a 150- μ l aliquot of HBS, 2 mM $MnCl_2$ containing propofol at $2 \times$ final concentration; and another 150- μ l aliquot of HBS containing 25 μ g/ml fluorescein isothiocyanate (FITC)-conjugated goat antihuman IgA antibody (Pierce, Rockford, IL), and either 10 μ g/ml ICAM-1-Fc α fusion protein or control human IgA. Each experiment included mock-treated samples containing equal volumes of DMSO (0.5%). Cells were incubated for 30 minutes at room temperature. Bound ICAM-1 was detected by flow cytometry (FACScan, BD Bioscience, San Jose, CA). ICAM-1 binding % was defined as [mean fluorescence intensity at various concentrations of propofol / mean fluorescence intensity of mock treated] $\times$ 100 (%).

Inhibition of antibody binding to LFA-1

Soluble LFA-1 (10 μ g/ml) was immobilized directly on ELISA plates overnight at 4°C. Wells were blocked with HBS, 2% bovine serum albumin for 1 hour at room temperature. LFA-1 antibodies (final concentration, 10 μ g/ml) and propofol (final concentration 100 μ M) were added and allowed to bind for 1 hour at room temperature. Both propofol-treated and mock-treated samples contained 0.5% DMSO. Unbound antibodies were washed off and bound antibodies were detected using peroxidase-labeled goat antimouse IgG (Invitrogen, Carlsbad, CA) and substrate. After 15 minutes, absorbance was measured at 405 nm. Data were presented as [OD of propofol-treated sample / OD of mock-treated sample] $\times$ 100(%) for each antibody.

Mixed lymphocyte reaction (MLR) Proliferation assay

To determine the effect of sedatives on lymphocyte proliferation, human PBMCs were purified in each experiment. PBMCs 1×10^5 cells were used as stimulators (nondividing cells) and responders (cells with proliferative response), respectively. Stimulator cells were incubated with mitomycin C (Sigma) 25 μ g/ml for 30 minutes at 37°C, and washed three times before suspending in RPMI1640, 10% FBS. The cells were incubated for 5 days with various concentrations of sedatives or BIRT377 (25 μ M), a LFA-1 small molecule antagonist.²¹ Sedatives up to supraclinical concentrations were tested. BIRT377 served as an internal control for the inhibition of both LFA-1/ICAM-1 binding and LFA-1 mediated cell adhesion. All experiments included mock-treated samples containing equal volumes of DMSO (0.1 %). Four days after incubation, bromodeoxyuridine (BrdU) (Roche, Nutley, NJ) was added to each well. Cells were incubated for another 24 hours. The effect of sedatives on PBMC proliferation was assessed by using BrdU incorporation-based cell proliferation ELISA (Roche Applied Science, Indianapolis, IN). OD was measured at 370 nm. Stimulation index % was defined as [OD of sample at given dose of sedative] / [OD of mock-treated sample] $\times$ 100 (%).

Statistical analysis

Data are presented as mean $\pm$ standard error and analyzed using a Student's t test (two-tailed) or analysis of variance with Tukey post hoc pairwise comparisons. Significance was set at P values less than 0.05. All statistical calculations were performed using Prism 5 (Graph Pad Software; La Jolla, CA).

Results

Propofol at a clinically relevant concentration inhibits IL-2 production by LFA-1 in a dose-dependent manner

We measured the effect of sedative drugs on LFA-1- dependent and independent IL-2 production from T-cells. SEB cross-links class II MHC with the T-cell receptor. PBMCs containing T-cells (LFA-1) and antigen-presenting cells (ICAM-1) produce IL-2 in LFA-1- dependent manner with SEB.²¹ However, PBMCs produce IL-2 in a LFA-1-independent fashion with PMA/ionomycin.²¹ The dependency of LFA-1 in each assay was confirmed with the anti-LFA-1 blocking antibody TS1/22 (Figure 1A and B). Propofol at 10 and 50 μ M (clinically relevant concentrations 24–26) attenuated SEB-mediated IL-2 production but not PMA/ionomycin-elicited IL-2 production (Figure 1A and B). Propofol at 100 μ M (supraclinical concentration) showed some LFA-1-independent IL-2 inhibition, while LFA-1-dependent IL-2 production was almost completely inhibited. Dexmedetomidine (clinically relevant concentration $< 0.01 \mu$ M 27–28) and midazolam (clinically relevant concentration $< 5 \mu$ M 29–30) at up to 50 μ M did not significantly affect IL-2 production (Figure 1 A and B).

Propofol inhibits the binding of LFA-1 to ICAM-1 in the cell-free system

To examine the effect of the three sedatives on extracellular LFA-1/ICAM-1 binding, we performed LFA-1 binding assays in the cell-free system. In a control experiment, BIRT377 and TS1/22 inhibited WT LFA-1/ ICAM-1 binding (data not shown). Propofol inhibited ICAM-1 binding to Mn^{2+} -stimulated WT LFA-1 (Figure 2). Dexmedetomidine and midazolam (up to 100 μ M) did not inhibit LFA-1/ ICAM-1 binding (Figure 2). The lack of effect by dexmedetomidine and midazolam on LFA-1/ICAM-1 binding may explain their lack of effect on IL-2 production.

Propofol inhibits the binding of LFA-1 to ICAM-1 in cells

Since propofol was the only drug to decrease LFA-1/ICAM-1 binding, we examined its effect using human T-cell line Jurkat cells that express LFA-1 at high levels. Propofol inhibited ICAM-1 binding to LFA-1 (Figure 3A). The suppression of ICAM-1 binding by propofol is not due to its effects on LFA-1 expression, because LFA-1 surface expression detected by TS1/18 was not affected (Figure 3B).

Propofol inhibits ICAM-1 binding to HA LFA-1

LFA-1/ICAM-1 binding can be blocked sterically and/or allosterically.²² To understand the mechanism of inhibition, we examined the binding of ICAM-1 using HA LFA-1. While WT LFA-1 was blocked by both direct and allosteric inhibitors (TS1/22, and BIRT377, respectively), HA LFA-1 mutant was blocked only by direct inhibitors (data not shown). We previously showed that isoflurane and sevoflurane inhibited ICAM-1 binding to the allosteric cavity at the bottom of the I domain.^{16–18} While volatile anesthetics inhibited ICAM-1 binding only to WT-LFA-1, propofol inhibited ICAM-1 binding to both WT and HA LFA-1 (Figure 4).

The effect of propofol on the binding of anti α L I domain antibodies to LFA-1

Since propofol inhibited ICAM-1's interaction with WT-LFA-1 and HA-LFA-1 similarly, we cannot exclude the possibility that propofol binds to ICAM-1, not LFA-1. Therefore, we examined the competitive binding of anti LFA-1 antibodies to LFA-1 against propofol. We mapped potential propofol binding site(s) within the I domain, using a panel of 4 LFA-1 antibodies that bind to different regions of the I domain: CBR LFA1/9, TS1/22, and TS2/14 map to different regions on the top of the I domain, just outside the ICAM-1-contacting area,

at which those monoclonal antibodies inhibit ICAM-1 binding sterically.³¹ In contrast, MEM83 maps to the side of the I domain, distant from the ICAM-1-contact area.³¹ Propofol reduced only TS1/22 binding (Figure 5). This suggests that propofol binds to or near the TS1/22 epitopes. Since TS1/22 blocks LFA-1 sterically, propofol may block it in a similar fashion.

Propofol inhibits lymphocyte proliferation

MLR is an assay to study allorecognition and cellular immunity.³² LFA-1/ ICAM-1 interaction is involved in lymphocyte proliferation in MLR, because the inhibition of LFA-1 inhibits MLR.^{33, 34} We investigated whether sedatives will block lymphocyte proliferation using one-way MLR with a BrdU incorporation assay. BIRT377-mediated antagonism of LFA-1 resulted in a 60% reduction in lymphocyte proliferation. Propofol, but not midazolam or dexmedetomidine, attenuated lymphocyte proliferation in a dose-dependent manner to a lesser degree (Figure 6).

Discussion

We demonstrated that propofol inhibited LFA-1/ICAM-1 interface sterically, which may be one of the mechanisms for the observed dose-dependent suppression of T-cell proliferation and IL-2 production by propofol. Midazolam and dexmedetomidine did not alter LFA-1/ ICAM-1 binding and IL-2 production.

We previously demonstrated that isoflurane and sevoflurane bind to the binding site for LFA-1 allosteric antagonists and inhibited ligand binding allosterically.^{16–18} The ability of sedatives to bind and inhibit LFA-1 has not previously been reported. Since propofol only inhibited LFA-1- dependent IL-2 production, we hypothesized that propofol would bind and inhibit LFA-1. LFA-1 enhances the binding to ICAM-1 by 1) strengthening affinity through the conformational changes of an individual LFA-1 molecule, and/or 2) lateral clustering of multiple LFA-1 molecules.³⁵ To study the direct action of propofol on LFA-1, we measured the effect on LFA-1/ICAM-1 binding using a cell-free assay. Propofol significantly inhibited LFA-1/ICAM-1 binding and affinity. We also examined LFA-1/ ICAM-1 binding in T-cells. Inhibition of LFA-1 by propofol was more potent in a cell assay than in a cell-free system. The actin regulates LFA-1 clustering.³⁶ Since propofol affects actin function,^{37–39} the higher inhibition observed in the cell-based assay suggests that clustering through intracellular processes may be inhibited. However, we also observed that the decreased LFA1/ICAM-1 binding was not due to changes in LFA-1 surface expression, which is in line with the report in a rodent abdominal sepsis model.⁴⁰ Therefore, propofol-induced inhibition of LFA-1/ICAM-1 binding is likely due to decreased affinity and clustering of LFA-1.

Interestingly, the mode of action of propofol on LFA-1 appeared to be different from that of isoflurane. Isoflurane inhibits WT, not HA-LFA-1, implying an allosteric inhibition. Propofol inhibited both WT- and HA- LFA-1, thereby suggesting the direct inhibition. Using a panel of 4 monoclonal antibodies mapping to different regions of the I domain, we have shown that propofol specifically inhibited the TS1/22 binding. TS1/22 maps to Gln²⁶⁶ and Ser²⁷⁰ that are located in β 5- α 6 loop and α 6 helix of I domain, which are the region near ICAM-1 binding site (Figure 7).³¹ Given that other antibodies did not affect propofol binding, it is unlikely that propofol globally perturbs structural integrin of the I domain by inducing protein unfolding. Our findings support a direct mode of inhibition by propofol on/ near the TS1/22 epitope(s), while not excluding the possibility that propofol binds to other sites including the allosteric cavity because our monoclonal antibodies mapping does not cover the entire I domain surface.

LFA-1 binding to ICAM-1 on antigen-presenting cells upregulates IL-2 gene expression,^{41–43} through protein kinase C δ phosphorylation, and c-Jun N-terminal kinase/mitogen-activated protein kinase (MAPK) activation.⁴⁴ PMA/inomocin directly activates protein kinase C δ , leading to the activation of MAPK.⁴⁴ Since propofol suppresses MAPK activity,⁴⁵ it may penetrate cell membrane and interact with intracellular signaling molecules at 100 μ M. The proposed mechanism of IL-2 reduction by propofol is illustrated in Figure 8. IL-2 supports proliferation and survival of T-cells, differentiation of naïve T-cells into effector and memory cells, secondary expansion of memory T-cells when they re-encounter an antigen.^{7, 46} IL-2 has been used as an immunotherapy to restore T-cell functions in patients with acquired immune deficiency syndrome or cancer.^{47–49} Anesthesia induction and maintenance with propofol were associated with a decrease in plasma IL-2 level in patients undergoing cholecystectomies.¹⁰ Forty-eight hours of continuous propofol infusion to surgical patients in the intensive care unit reduced serum IL-2 levels by 70%.⁹ Our results suggest that propofol directly acts on T-cells to inhibit IL-2 production. The suppression of IL-2 production potentially induces iatrogenic immune defects. Propofol also inhibited lymphocyte proliferation. These effects of propofol on lymphocytes could potentially impair innate immunity, since an adaptive immune system could affect innate immunity.^{50, 51}

We examined the effect of three sedatives at a concentration range of 0 to 100 μ M. A clinically relevant plasma concentration of propofol is 3–11 μ g/ml (17–62 μ M) for the maintenance of anesthesia,^{24, 25} and approximately 2 μ g/ml (11.3 μ M) for adequate sedation in the intensive care unit.²⁶ The concentration of propofol at 10–50 μ M is in the clinically relevant range, supporting the relevance of our *in vitro* findings to clinical practice. The clinically relevant plasma concentrations for midazolam and dexmedetomidine were within the lower end of the range used in our assays, where no changes in IL-2 production and LFA-1/ICAM-1 binding were detected. Because sedatives are often administered to immuno-compromised, and critically ill patients, our understanding of immunomodulation by sedation will be critical. For example, secondary analysis of data from the MENDS trial revealed a mortality benefit in septic patients sedated with dexmedetomidine relative to lorazepam.⁵² Based on our results, propofol infusion may lead to iatrogenic T-cell dysfunction. Because lipid emulsion itself can decrease lymphocyte proliferation,⁵³ propofol emulsion infusion may be harmful to patients with impaired T-cell function. Its clinical significance remains to be determined. On the other hand, midazolam and dexmedetomidine may be preferable for this patient population.

This study only examines the effect of sedatives on LFA-1/ICAM-1 binding. We did not examine the effects on downstream intracellular signaling. Direct interaction of sedatives on intracellular proteins could also attenuate LFA-1 activation. Further investigations into the effect of these sedative drugs on intracellular signaling and protein interactions are needed.

We demonstrated that propofol interacts with LFA-1 by inhibiting LFA-1/ICAM-1 binding, lymphocyte proliferation and IL-2 production. Midazolam and dexmedetomidine do not significantly affect these *in vitro* assays. The competitive mode of propofol-induced inhibition of LFA-1 represents one of the underlying mechanisms of sedation-mediated immunomodulation.

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Figure 1. The effect of sedative drugs on IL-2 production in PBMCs

Interleukin-2 (IL-2) production by T-cells in peripheral blood mononuclear cells (PBMCs) elicited by the stimulation of (A) staphylococcal enterotoxin B (SEB) or (B) phorbol 12-myristate 13-acetate (PMA)/ionomycin was studied using a calorimetric ELISA assay in the presence of propofol, midazolam and dexmedetomidine at various concentrations. Each experiment included a mock-treated sample that contained an equal amount of dimethyl sulfoxide (DMSO) (0.5%) during incubation. Lymphocyte function-associated antigen (LFA) blocking antibody TS1/22 (10 μg/ml) was used as a reference LFA-1 antagonist. The data represent mean $\pm$ standard error of triplicate samples. Similar results were obtained in two other independent experiments. Data were analyzed using one-way ANOVA with Tukey post hoc comparison to compare the data at different concentrations. (* $p < 0.05$ versus mock treated samples.)

Figure 2. The effect of sedative drugs on the binding of ICAM-1 to purified recombinant LFA-1 on plates

Binding of intercellular adhesion molecule-1 (ICAM-1) to the extracellular portion of wild-type lymphocyte function-associated antigen (WT LFA-1) immobilized on plates was studied using a calorimetric ELISA-type assay in the presence of various concentrations of propofol, midazolam and dexmedetomidine. Each experiment included a mock-treated sample that contained an equal amount of dimethyl sulfoxide (DMSO) (0.5%) during incubation. Bound ICAM-1 was detected at an optical density (OD) of 405 nm using peroxidase-labeled goat antihuman immunoglobulin A (IgA) and substrate. The data represent mean $\pm$ standard error of three independent experiments and are expressed as percentage of mock-treated samples. The data were analyzed using one-way ANOVA followed by a Tukey post hoc comparison to compare the data at different concentrations. (* represents $p < 0.05$ versus mock treated samples)

(A)

(B)

Figure 3. The effect of propofol on the binding of soluble ICAM-1 to LFA-1 in a human T-cell line Jurkat cells

(A) Binding of intercellular adhesion molecule-1 (ICAM-1) to lymphocyte function-associated antigen-1 (LFA-1) on Jurkat cells was studied using flow cytometry in the presence of propofol at various concentrations. Each experiment included a mock-treated sample that contained an equal amount of dimethyl sulfoxide (DMSO) (0.5%) during incubation with soluble ICAM-1. Each experiment included a mock-treated sample that only contained 0.5% DMSO during incubation with soluble ICAM-1. The data represent mean $\pm$ standard error of triplicate samples. Similar results were obtained in two other independent experiments. Data were analyzed using one-way ANOVA with Tukey post

hoc comparison to compare the data at different concentrations. (* $p < 0.05$ versus mock treated samples.) (B) The effect of propofol on LFA-1 surface expression in Jurkat cells was studied using flow cytometry in the presence of propofol at various concentrations. The surface expression was probed with TS1/18. Each experiment included a mock-treated sample that contained an equal amount of DMSO (0.5%). The data represent mean $\pm$ standard error of triplicate samples. Similar results were obtained in two other independent experiments. Data were analyzed using one-way ANOVA with Tukey post hoc comparison to compare the data at different concentrations. (* $p < 0.05$ versus mock treated samples.)

Figure 4. The effect of propofol on the binding of ICAM-1 to HA LFA-1

Binding of intercellular adhesion molecule-1 (ICAM-1) to the extracellular part of high-affinity (HA) lymphocyte function-associated antigen-1 (LFA-1) immobilized on plates was studied using a calorimetric ELISA-type assay in the presence of propofol at various concentrations. Each experiment included a mock-treated sample that contained an equal amount of dimethyl sulfoxide (DMSO) (0.5%) during incubation with soluble ICAM-1. Bound ICAM-1 was detected at optical density (OD) of 405 nm using peroxidase-labeled goat antihuman immunoglobulin A (IgA) and substrate. Each experiment included a mock-treated sample that only contained 0.5% DMSO during incubation with soluble ICAM-1. The data represent mean $\pm$ standard error of triplicate samples. Similar results were obtained in two other independent experiments. Data were analyzed using one-way ANOVA with Tukey post hoc comparison to compare the data at different concentrations. (* $p < 0.05$ versus mock-treated samples.)

Figure 5. The effect of propofol on the binding of LFA-1 antibodies

Binding of anti α L I domain monoclonal antibodies to lymphocyte function-associated antigen-1 (LFA-1) was studied using a calorimetric ELISA-type assay with or without 100 μ M propofol. Each sample contained 0.5% dimethyl sulfoxide (DMSO). Bound anti- α L I domain antibodies was detected calorimetrically at an optical density (OD) of 405nm using peroxidase-labeled goat antimouse immunoglobulin (Ig)G and substrate. The data represent mean $\pm$ standard error of triplicate samples. Similar results were obtained in two other independent experiments. A two-tailed student t test was used to compare mock- and propofol- treated samples within each antibody. (* $p < 0.05$ versus mock-treated samples with each antibody) Statistical significance was only observed with TS1/22.

Figure 6. The effect of propofol on lymphocyte proliferation

Lymphocyte proliferation was evaluated with one-way mixed lymphocyte reaction in the presence of sedatives at various concentrations using bromodeoxyuridine (BrdU) incorporation based cell proliferation ELISA assay. BIRT377 (25 μM) was used as a reference lymphocyte function-associated antigen-1 (LFA-1) antagonist. Each including mock-treated sample contained an equal amount of dimethyl sulfoxide (DMSO) (0.1%) during incubation. The data represent mean $\pm$ standard error of three independent experiments and were analyzed using one-way ANOVA with Tukey post hoc comparison. (* $p < 0.05$ versus mock-treated samples)

Figure 7. LFA-1 I domain structure with predicted allosteric binding sites

The site to which the ligand intercellular adhesion molecule-1 (ICAM-1) binds is shown as a purple asterisk. The hydrophobic cavity that contains the allosteric inhibitor- and isoflurane/sevoflurane-binding sites is marked with a red asterisk. Proposed propofol binding site(s) are circled in red.

Figure 8. The proposed mechanism of propofol-mediated interleukin-2 reduction

The action sites of staphylococcal enterotoxin B (SEB) and phorbol 12-myristate 13-acetate (PMA)/ionomycin, and major signaling pathway for interleukin-2 production are shown. At 10–100 μ M of propofol, interleukin-2 production was inhibited with SEB stimulation in peripheral blood mononuclear cells (PBMCs), whereas only at 100 μ M, its production was inhibited with PMA/ionomycin stimulation (Figure 1). Since propofol inhibits mitogen-activated protein kinase (MAPK) activity,²⁸ we proposed the mechanism of propofol-mediated interleukin-2 reduction in our experiment.