Effects of anesthetic agents on inflammation in Caco-2, HK-2 and HepG2 cells

WEIJING LI[1*], XIAOGUANG HAO[2*], YAN LIU[1], TONG TONG[1], HONGMENG XU[1] and LI JIA[1]

Departments of [1]Anesthesiology and [2]Radiology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, P.R. China

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Abstract. Anesthetic agents are often used in surgical procedures to relieve pain in patients with traumatic injuries. Several anesthetic agents can cause immunosuppression by suppressing the secretion of immune factors such as cytokines. However, the effects of different anesthetic agents on inflammation are not completely understood. In the present study, three cell lines, Caco-2, HK-2 and HepG2, were treated with five anesthetic agents, including sodium barbiturate, midazolam, etomidate, ketamine and propofol, to investigate the effects of different anesthetic agents on inflammation in *in vitro* models. The expression levels of inflammatory genes, including NF-κB and its downstream cytokines, were detected via reverse transcription-quantitative PCR. The results indicated that anesthetic agents, including sodium barbiturate, ketamine and propofol, but not midazolam and etomidate, exerted significant inhibitory effects on NF-κB expression in the three different cell lines. Sodium barbiturate, ketamine and propofol also decreased the expression levels of the NF-κB downstream cytokines, including IL-1β and IL-18. Moreover, sodium barbiturate, ketamine and propofol reduced the effect of TNF-α on inflammatory activity in the three cell lines. The results of the present study may provide novel insight into the effects of anesthetic agents on inflammation and may aid with selecting the most appropriate anesthetic agent in surgical procedures.

Introduction

Anesthetic agents serve an important role in relieving pain in patients with traumatic injuries and in surgical procedures, but managing the utilization of anesthetic agents is a challenge for the anesthesiologist (1). A variety of anesthetic agents have been developed, including volatile anesthetics, nitrous oxide, xenon, propofol, ketamine, etomidate, dexmedetomidine, opioids, benzodiazepines, barbiturates, lidocaine and regional anesthesia (1). Different anesthetic agents exert distinct biological and physiological functions in the body, whereas determining the molecular and cellular functions of anesthetic agents is a challenge due to their pleiotropic effect (2). Besides their anesthetic effects, a number of anesthetic agents exert other functions, such as antimicrobial effects (3), synaptic inhibition (4), disruption of brain circuit formation (5) and left ventricular systolic function (ketamine and xylazine increasing the left ventricular ejection fraction and decreasing the left ventricular end diastolic diameter) (6). It has also been reported that certain anesthetic agents display cytotoxicity, neurotoxicity and genotoxicity (7). Inflammation is an innate physiological process in the body, which may have both favorable and unfavorable consequences for an individual's health. Inflammation protects against harmful pathogens and is activated during acute and chronic diseases (8). Inflammation is a complex biological network that involves a number of transcription factors, including NF-κB and STAT3, inflammatory enzymes and inflammatory cytokines (8). The NF-κB signaling pathway serves a central role in regulating inflammation, and functions in various organs and tissues. Emerging evidence indicates that inflammation is related to surgical processes, which should be considered during the management of surgery. For example, if trauma-induced inflammation is not appropriately regulated, neuro-inflammation may interfere with synaptic plasticity to affect learning and memory aspects of cognition (9). By contrast, surgical manipulation causes stress responses, inhibits immune cells and suppresses cell-mediated immunity (10). Increasing evidence indicates that anesthetic agents regulate immune reactions in the body (11). Therefore, the optimal choice of anesthetic agents serves an important role in health management during surgery. However, how anesthetic agents affect the immune system is not completely understood.

It has been reported that several anesthetic agents exert immunosuppressive functions by suppressing the viability of immune cells (11). Different anesthetic agents exert different effects on a variety of immune cells, for example, ketamine and thiopental, but not propofol, inhibit natural killer cells, whereas ketamine, but not midazolam, causes T-lymphocyte

Correspondence to: Dr Xiaoguang Hao, Department of Radiology, The Fourth Hospital of Hebei Medical University, 12 Ji'an'kang Road, Shijiazhuang, Hebei 050000, P.R. China
E-mail: haoxiaoguang566@163.com

*Contributed equally

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apoptosis (11). Investigating the effects of different anesthetic agents on inflammation is important due to the link between surgery and inflammation, and understanding this link is important for regulating the effects of anesthetic agents on inflammation (10).

The present study aimed to investigate the effects of five anesthetic agents, including sodium barbiturate, midazolam, etomidate, ketamine and propofol, on inflammation in three cell lines (Caco-2, HK-2 and HepG2). The results of the present study may aid with choosing the suitable anesthetic agent for surgical procedures.

Materials and methods

Study design. To investigate the effects of various commonly used anesthetic agents on different cell lines, five commonly used anesthetic agents including sodium barbiturate, midazolam, etomidate, ketamine and propofol at different concentrations were used to treat Caco-2 (intestine), HK-2 (kidney) and HepG2 (liver) cells. Caco-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells, which has been used for intestine studies (12,13). HK-2 cell line is an immortalized proximal tubular cell line derived from normal kidney, which has been widely used for kidney studies (14,15). HepG2 is a liver cancer cell line, which has been used for liver studies (16,17). Reverse transcription-quantitative PCR (RT-qPCR) and western blotting assays were used to determine the expression levels of NF-κB and its downstream cytokines in cells treated with different anesthetic agents.

Chemicals and drugs. The following anesthetic agents were used: Sodium barbiturate [$C_4H_3N_2NaO_3$; molecular weight, 150.07 g/mol; Chemical Abstracts Service (CAS) no. 4390-16-3], midazolam ($C_{18}H_{13}ClFN_3$; molecular weight, 325.77 g/mol; CAS no. 59467-70-8), etomidate ($C_{14}H_{16}N_2O_2$; molecular weight, 244.29; CAS no. 33125-97-2), ketamine ($C_{13}H_{17}Cl_2NO$; molecular weight, 274.19; CAS no. 1867-66-9) and propofol [$(CH_3)_2CH_2C_6H_3OH$; molecular weight, 178.27; CAS no. 2078-54-8]. All chemicals were supplied by Sigma-Aldrich; Merck KGaA, diluted according to manufacturer's instruction, aliquoted and frozen at -80˚C prior to use. Control groups were non-treated cells.

Cell culture. Caco-2, HK-2 and HepG2 cells were purchased from American Type Culture Collection and cryopreserved until use. After thawing, cells were sub-cultured at least twice prior to experimental use. All cells were cultured according to manufacturer's instructions. Briefly, cells were cultured in DMEM (cat. no. 10567014; Thermo Fisher Scientific, Inc.) supplemented with 10% (v/v) FBS (Invitrogen; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (cat. no. 15140163; Thermo Fisher Scientific, Inc.). The culture medium was refreshed every 3 days. Cells were maintained at 37˚C with 5% CO_2 in a humidified atmosphere and routinely sub-cultured twice a week at 70-80% confluence using 0.5% trypsin-EDTA (cat. no. 25200072; Thermo Fisher Scientific, Inc.).

Drug treatment. At >90% confluence in T75 flasks, cells were dissociated using TrpyLE (cat. no. 12604013; Thermo Fisher Scientific, Inc.) and seeded into 48-well plates ($2.5x10^4$ cell per well). At 30-40% confluence, cells were treated with 0, 0.1, 1.0, 2.0, 5.0 or 10.0 μM sodium barbiturate, midazolam, etomidate, ketamine or propofol for 48 h at 37˚C with 5% CO_2 in a humidified atmosphere. The doses of drugs were selected based on the clinical experience of the authors. A previous study indicated that the plasma concentration of barbiturate should be below 4.4 μg/ml (~24 μM; measured on the day when burst-suppression pattern had disappeared) (18). In addition, concentration of midazolam in patients was between 20 and 100 μM (blood was collected at 30-45 min before the patient came to the operating room) (19), the steady state plasma concentration of etomidate was 158 μg/l (~0.65 μM; measured for periods of up to 24 h after stopping the infusion) (20), the plasma concentration of ketamine at steady-state was 1,018.7 ng/ml (~4.29 μM; the average of the three plasma samples collected at 20, 42, and 54 half-lives during continuous infusion) (21) and the concentration of propofol was 4-6 μg/l (22.50-33.75 μM; the time point of the measurement was not mentioned) (22). According to the aforementioned clinical studies, the plasma concentration of anesthetic agents should be between 0.65 to 33.75 μM. In combination with the experience of the current authors, the maximum concentration of the anesthetic agents used was 10.0 μM

For the co-treatment of TNFα (100 nM) and anesthetic agents (10 μM) including sodium barbiturate, ketamine and propofol, cells were treated with a cocktail of TNFα and sodium barbiturate, TNFα and ketamine, and TNFα and propofol for 48 h at 37˚C with 5% CO_2 in a humidified atmosphere. Medium without any drugs was set as the control.

RNA isolation and RT-qPCR. Total RNA from cells including Caco-2, HK-2 and HepG2 cells was isolated using the RNAeasy™ kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Total RNA was eluted with nuclease-free water and treated with DNase I (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, total RNA (1 μg) was reverse transcribed into cDNA using the ReverTra Ace® RT system (Toyobo Life Science) according to the manufacturer's protocol. qPCR was performed using the iCycler iQ system (Bio-Rad Laboratories, Inc.) and IQ SYBR Green Supermix (Bio-Rad Laboratories, Inc.). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95˚C for 5 min; 40 cycles of 95˚C for 15 sec and 60˚C for 35 sec. The sequences of the primers used for qPCR are presented in Table I. The primers were designed using NCBI Pick Primers (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and manufactured by Sangon Biotech Co., Ltd. The mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method and normalized to the internal reference gene GAPDH according to a previous study (12). RT-qPCR was performed in triplicate.

Western blot analysis. Caco-2, HK-2 and HepG2 cells treated with 10 μM sodium barbiturate, ketamine and propofol were lysed using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology) for 30 min on ice. The supernatant was aspirated for the determination of protein concentration using a bicinchoninic acid assay kit, after centrifugation at 1,500 x g and 4˚C for 10 min. The samples were heated for 10 min at 95˚C. Afterwards, the samples (30 μg/lane)

Table I. Primers for reverse transcription-quantitative PCR.

Primer	Sequence (5'-3')	Product size, nt
NF-κB	F: ACAGCGGGGAAAGACACATC	221
	R: TCTGCCATTCTGAAGCTCTCTC	
IL-1B	F: AGCCATGGCAGAAGTACCTG	116
	R: CCTGGAAGGAGCACTTCATCT	
IL-18	F: TGCAGTCTACACAGCTTCGG	99
	R: GCAGCCATCTTTATTCCTGCG	
GAPDH	F: AATGGGCAGCCGTTAGGAAA	166
	R: GCCCAATACGACCAAATCAGAG	

F, forward; R, reverse; nt, nucleotides.

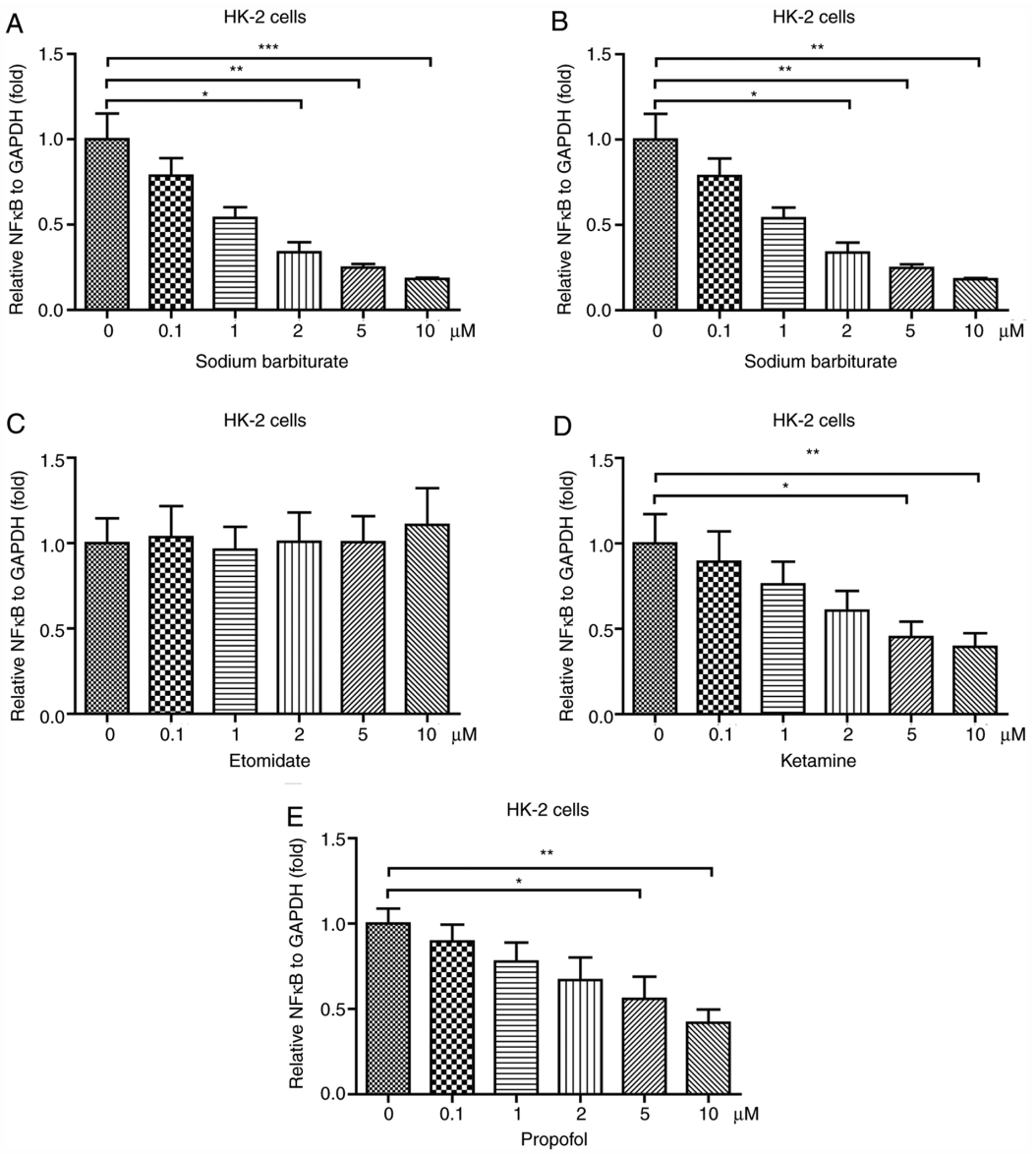

Figure 1. Sodium barbiturate, ketamine and propofol decrease NF-κB expression in HK-2 cells. Effects of (A) sodium barbiturate, (B) midazolam, (C) etomidate, (D) ketamine and (E) propofol on the mRNA expression levels of NF-κB in HK-2 cells (n=4). *P<0.05, **P<0.01 and ***P<0.001.

were subjected to SDS-PAGE (10% gel) at 110 V, followed by protein transfer to polyvinylidene difluoride membranes on ice at 100 V for 2 h. The membranes were blocked with 5% skimmed milk diluted in ddH_2O at room temperature

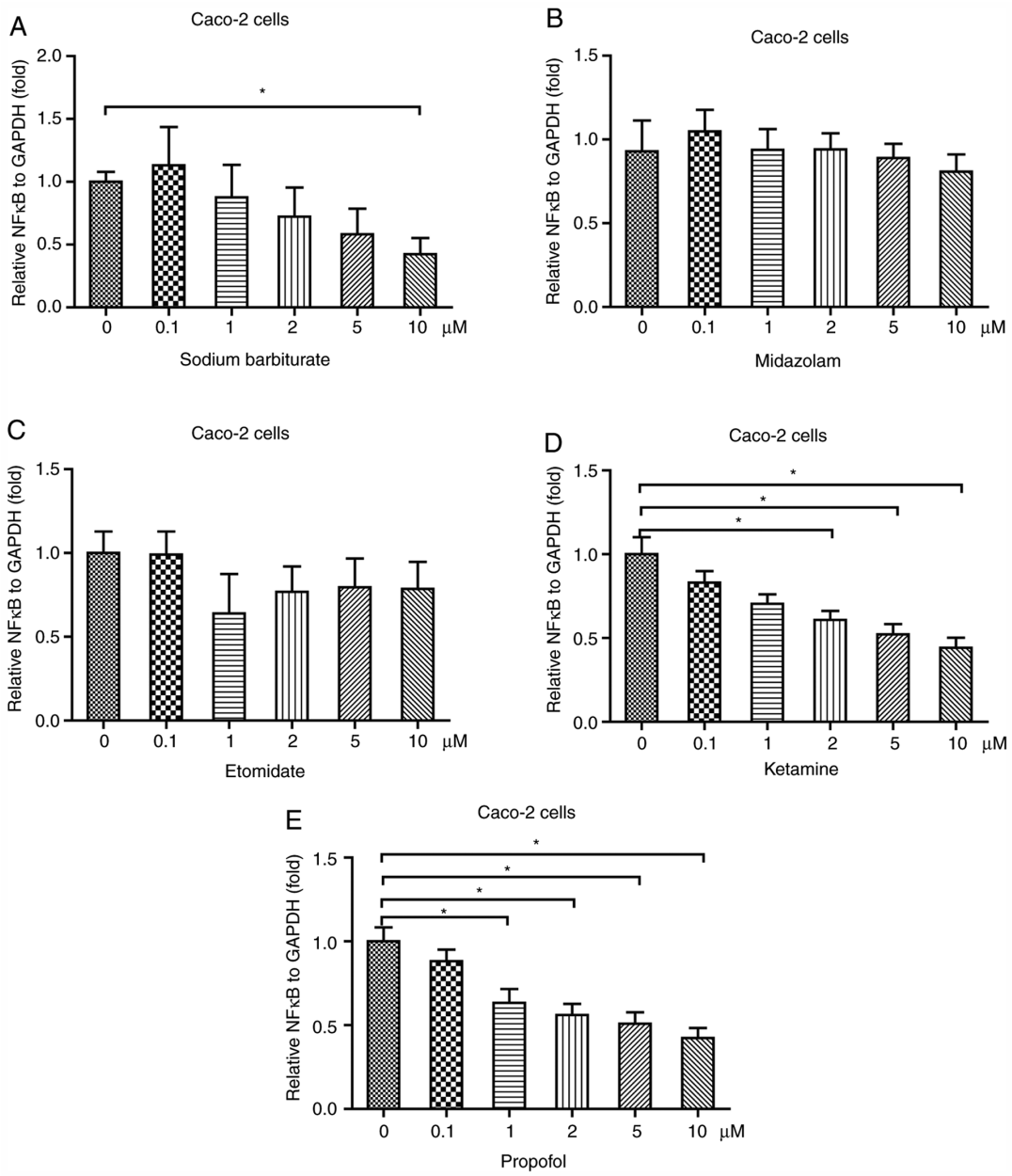

Figure 2. Sodium barbiturate, ketamine and propofol decrease NF-κB expression in Caco-2 cells. Effects of (A) sodium barbiturate, (B) midazolam, (C) etomidate, (D) ketamine and (E) propofol on the mRNA expression levels of NF-κB in Caco-2 cells (n=4). *P<0.05.

for 1 h and subsequently incubated with rabbit anti-IL-1β (D3U3E; cat. no. 12703), anti-IL-18 (D2F3B; cat. no. 54943) and anti-β-actin (13E5; cat. no. 4970) antibodies at 4˚C overnight (all at 1:1,000 dilution; Cell Signaling Technology, Inc.). Then, the membranes were washed three times with PBS supplemented with 0.1% Tween-20 for 15 min, followed by an incubation with HRP-conjugated goat anti-rabbit IgG H&L (1:5,000; cat. no. ab6721; Abcam) for 1 h at room temperature before washing with PBS with 0.1% Tween-20 three times for 15 min. β-actin was used as the endogenous control. The bands were visualized using Odyssey CLx Imager (LI-COR Biosciences) and quantified using ImageJ software (64-bit Java 1.8.0_172; National Institutes of Health).

Cell Counting Kit-8 (CCK-8). Caco-2, HK-2 and HepG2 cells at a density of $1x10^4$ cells/well were seeded into a 96-well-plate (Corning Inc.) and cultured as aforementioned for 24 h. Then, the cells were treated with the aforementioned concentrations of sodium barbiturate, ketamine and propofol for 48 h at 37˚C. Subsequently, the cells were treated with 10 μl CCK-8 reagent (cat. no. C0037; Beyotime Institute of Biotechnology) for an additional 1 h at 37˚C in the dark. The absorbance was measured using a microplate reader (Bio-Rad Laboratories, Inc.) at a wavelength of 450 nm.

Statistical analysis. Statistical analyses were performed using one-way ANOVA followed by Tukey's test using GraphPad Prism software (version 5.0; GraphPad Software, Inc.). Data are presented as the mean ± SEM of at least three independent experiments. P<0.05 was considered to indicate a statistically significant difference.

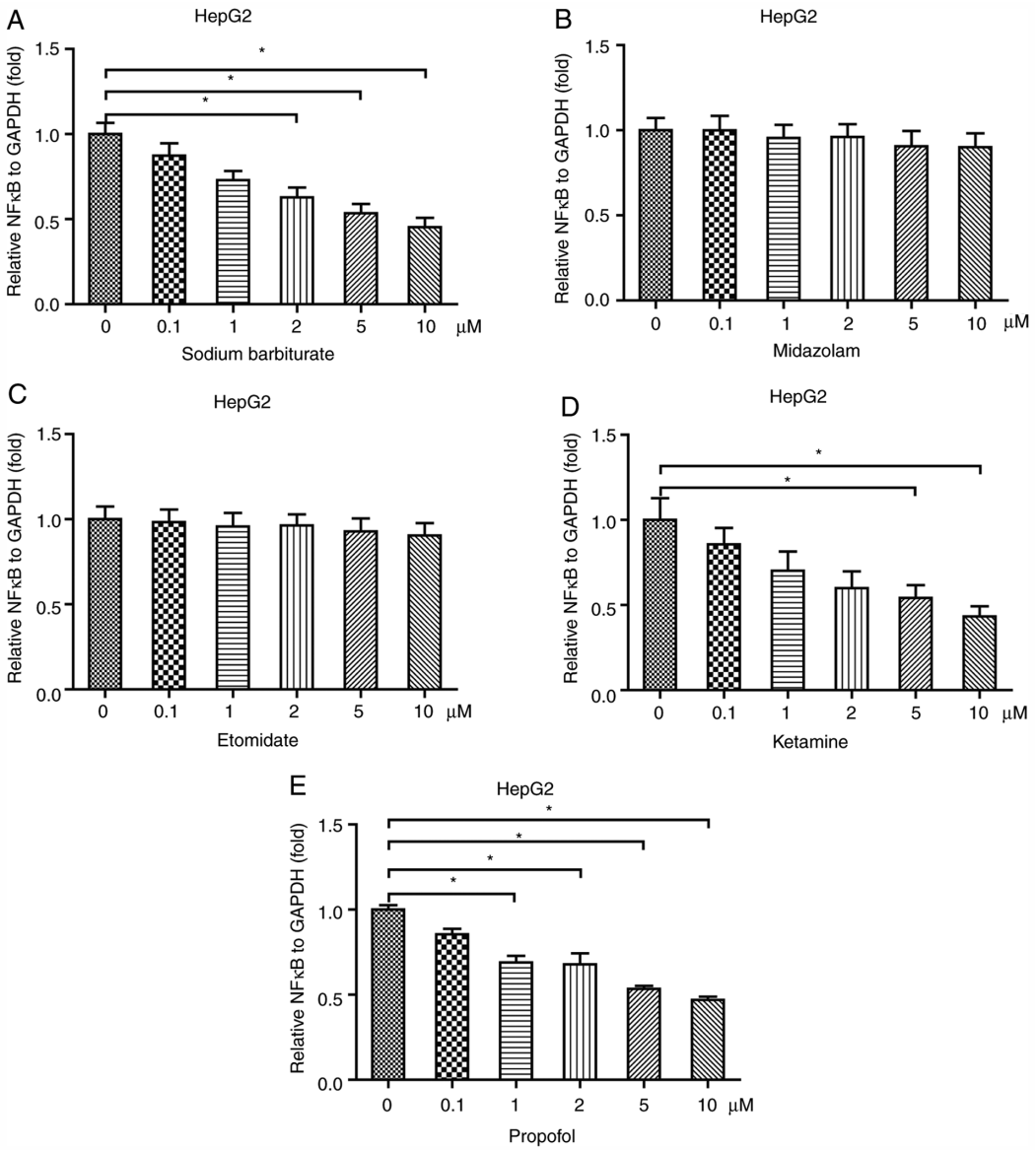

Figure 3. Sodium barbiturate, ketamine and propofol reduce NF-κB expression in HepG2 cells. Effects of (A) sodium barbiturate, (B) midazolam, (C) etomidate, (D) ketamine and (E) propofol on the mRNA expression levels of NF-κB in HepG2 cells (n=4). *P<0.05.

Results

Sodium barbiturate, ketamine and propofol decrease NF-κB expression in HK-2 cells. To investigate the effects of different anesthetic agents on inflammation in the kidney, an immortalized proximal tubule epithelial cell line HK-2 was used. HK-2 cells were treated with different concentrations of five different anesthetic agents, including sodium barbiturate, midazolam, etomidate, ketamine and propofol. The results indicated that sodium barbiturate (2, 5 and 10 μM) significantly decreased the mRNA expression levels of NF-κB in a dose-dependent manner (Fig. 1A). However, midazolam (Fig. 1B) and etomidate (Fig. 1C) did not significantly alter NF-κB mRNA expression levels in HK-2 cells compared with the control group. Ketamine and propofol (5 and 10 μM) significantly reduced mRNA expression levels of NF-κB in HK-2 cells (Fig. 1D and E). Collectively, the results suggested that anesthetic agents, including sodium barbiturate, ketamine and propofol, decreased NF-κB mRNA expression levels in HK-2 cells, whereas midazolam and etomidate displayed no effect on NF-κB mRNA expression levels compared with the control cells.

Sodium barbiturate, ketamine and propofol decrease NF-κB expression in Caco-2 cells. The intestine is a location where inflammation often occurs (23). To investigate the effects of anesthetic agents on inflammation in the intestine, a commonly used intestinal cell line (Caco-2) was used (12,24). The results indicated that 10 μM sodium barbiturate significantly decreased the mRNA expression levels of NF-κB in Caco-2 cells (Fig. 2A). Similar to in HK-2 cells, in Caco-2 cells, the results suggested that midazolam (Fig. 2B) and etomidate (Fig. 2C) did not significantly alter the mRNA expression levels of NF-κB. At 2, 5 and 10 μM, ketamine significantly decreased NF-κB mRNA expression levels in Caco-2 cells in a dose-dependent manner (Fig. 2D). Moreover, at 1, 2, 5 and 10 μM, propofol significantly reduced the mRNA expression levels of NF-κB in HK-2 cells in a dose-dependent manner

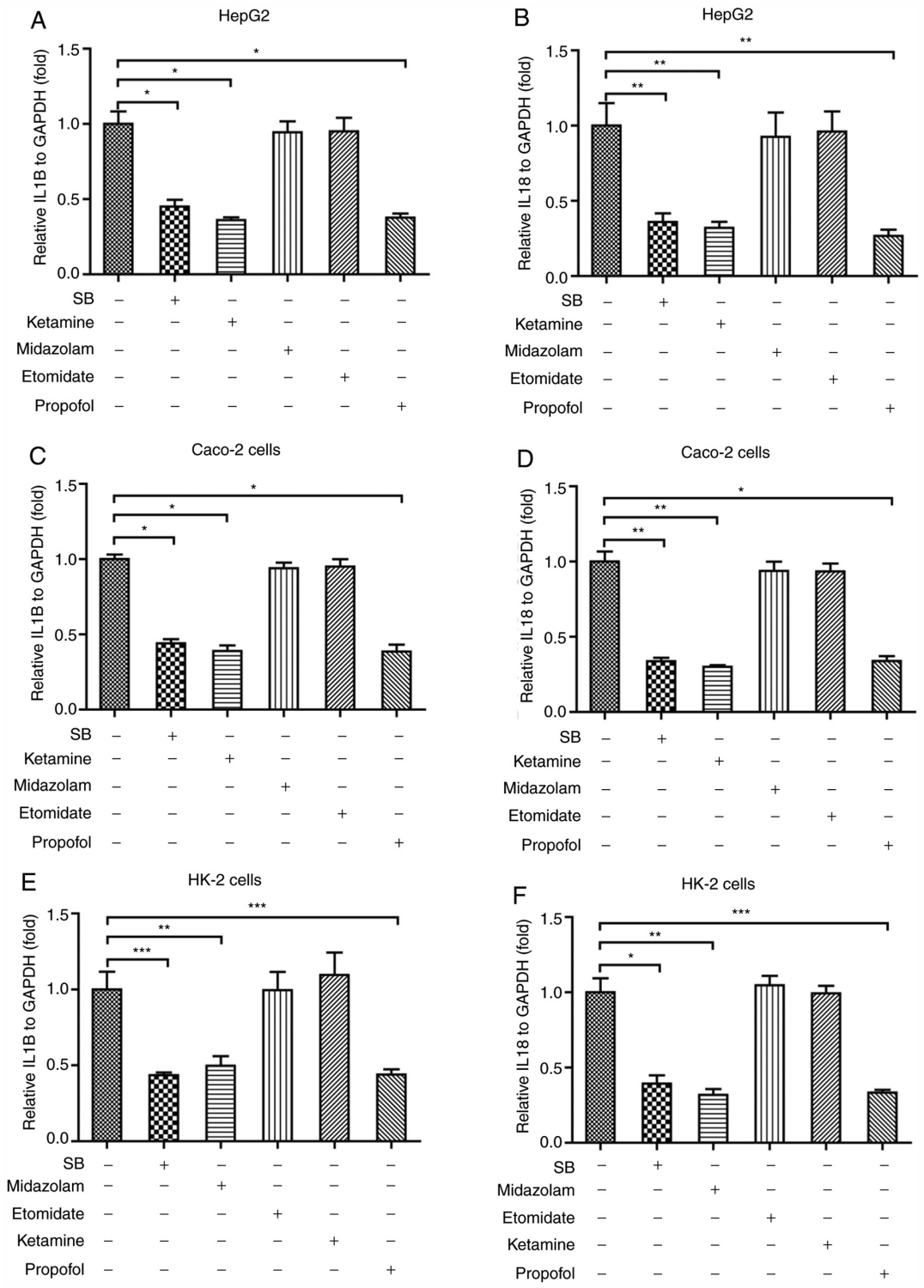

Figure 4. SB, ketamine and propofol inhibit mRNA expression of NF-κB downstream cytokines in the three cell lines. Effects of anesthetic agents on (A) IL-1β and (B) IL-18 mRNA levels in HepG2 cells, (C) IL-1β and (D) IL-18 mRNA levels in Caco-2 cells, and (E) IL-1β and (F) IL-18 mRNA levels in HK-2 cells. n=4. *P<0.05, **P<0.01 and ***P<0.001. SB, sodium barbiturate.

(Fig. 2E). Therefore, the results indicated that anesthetic agents, including sodium barbiturate, ketamine and propofol, rather than midazolam and etomidate, decreased the mRNA expression levels of NF-κB in Caco-2 cells.

Sodium barbiturate, ketamine and propofol reduce NF-κB expression in HepG2 cells. The liver is also an important organ where inflammation may occur (25). To examine the effects of anesthetic agents on inflammation in the liver, a liver cell line HepG2 was treated with different concentrations of sodium barbiturate, midazolam, etomidate, ketamine and propofol. Sodium barbiturate (2, 5 and 10 μM) significantly decreased the mRNA expression levels of NF-κB in HepG2 cells (Fig. 3A). Similar to in HK-2 and Caco-2 cells, the results indicated that midazolam (Fig. 3B) and etomidate (Fig. 3C) had no significant effect on the mRNA expression levels of

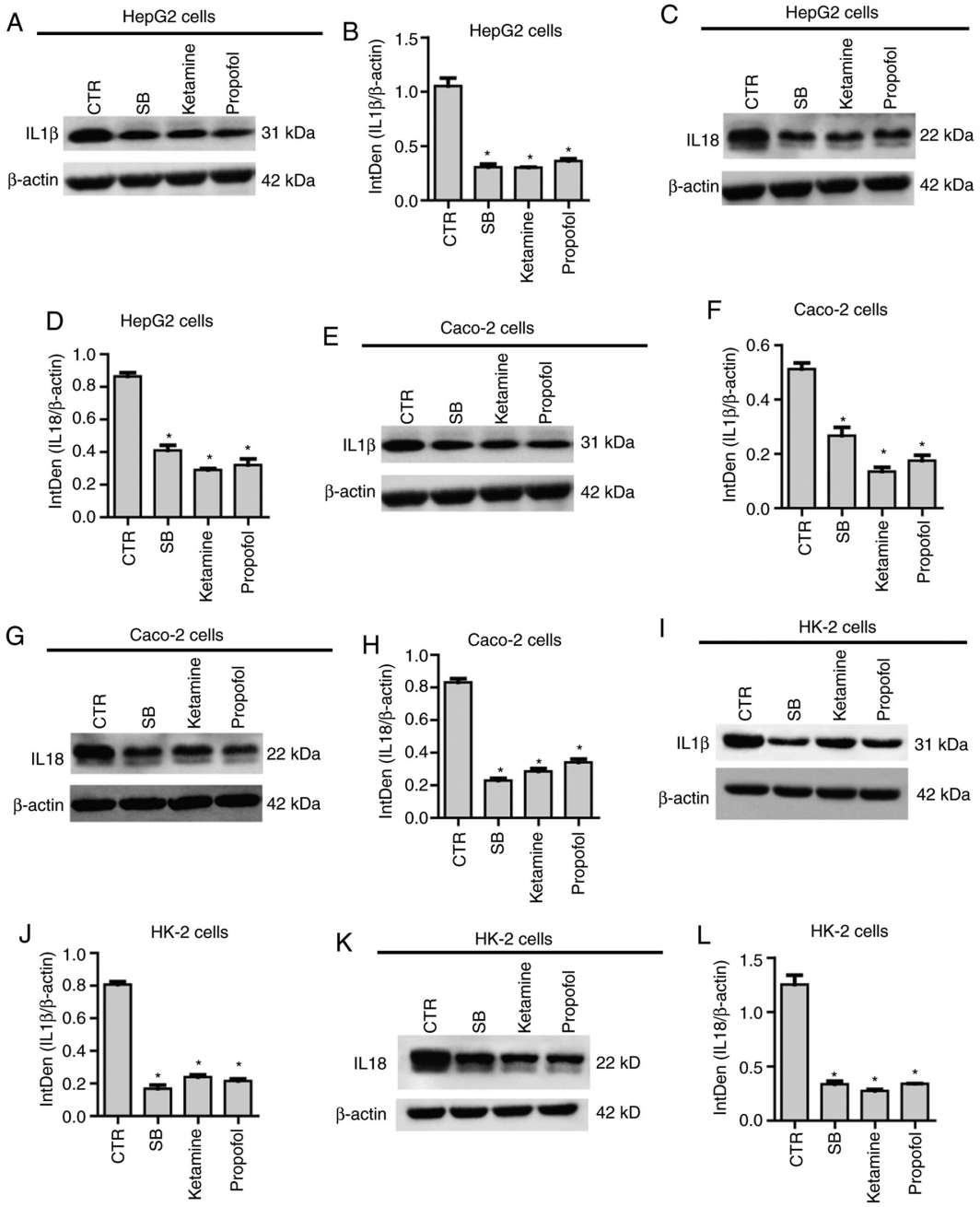

Figure 5. SB, ketamine and propofol inhibit protein levels of NF-κB downstream cytokines in the three cell lines. The western blotting results indicated that sodium barbiturate, midazolam and propofol decreased the protein expression levels of (A and B) IL-1β and (C and D) IL-18 in HepG2 cells, (E and F) IL-1β and (G and H) IL-18 in Caco-2 cells, and (I and J) IL-1β and (K and L) IL-18 in HK-2 cells. n=3. *P<0.05 vs. CTR. SB, sodium barbiturate; CTR, control; IntDen, Integrated Density.

NF-κB in HepG2 cells. Ketamine (5 and 10 μM) significantly decreased NF-κB mRNA expression levels in HepG2 cells in a dose-dependent manner (Fig. 3D). Propofol (1, 2, 5 and 10 μM) significantly reduced the mRNA expression levels of NF-κB in HepG2 cells in a dose-dependent manner (Fig. 3E). Collectively, the results indicated that anesthetic agents, including sodium barbiturate, ketamine and propofol, but not midazolam and etomidate, decreased the mRNA expression levels of NF-κB in HepG2 cells.

Sodium barbiturate, ketamine and propofol downregulate NF-κB downstream cytokines in the three cell lines. The NF-κB signaling pathway serves a central role in regulating inflammatory activities in the body, and can activate a variety of downstream cytokines, including IL1β and IL18, to result in inflammation (26). To further verify the effects of anesthetic agents on inflammation, the effects of sodium barbiturate, midazolam, etomidate, ketamine and propofol on IL-1β and IL-18 expression levels were assessed in the three different cell lines. Sodium barbiturate, ketamine and propofol significantly decreased the mRNA expression levels of IL-1β (Fig. 4A) and IL-18 (Fig. 4B) in HepG2 cells, which was consistent with the observations that the three anesthetic agents downregulated NF-κB expression. As in HepG2 cells, sodium barbiturate, ketamine and propofol, but not midazolam and etomidate, significantly reduced the mRNA

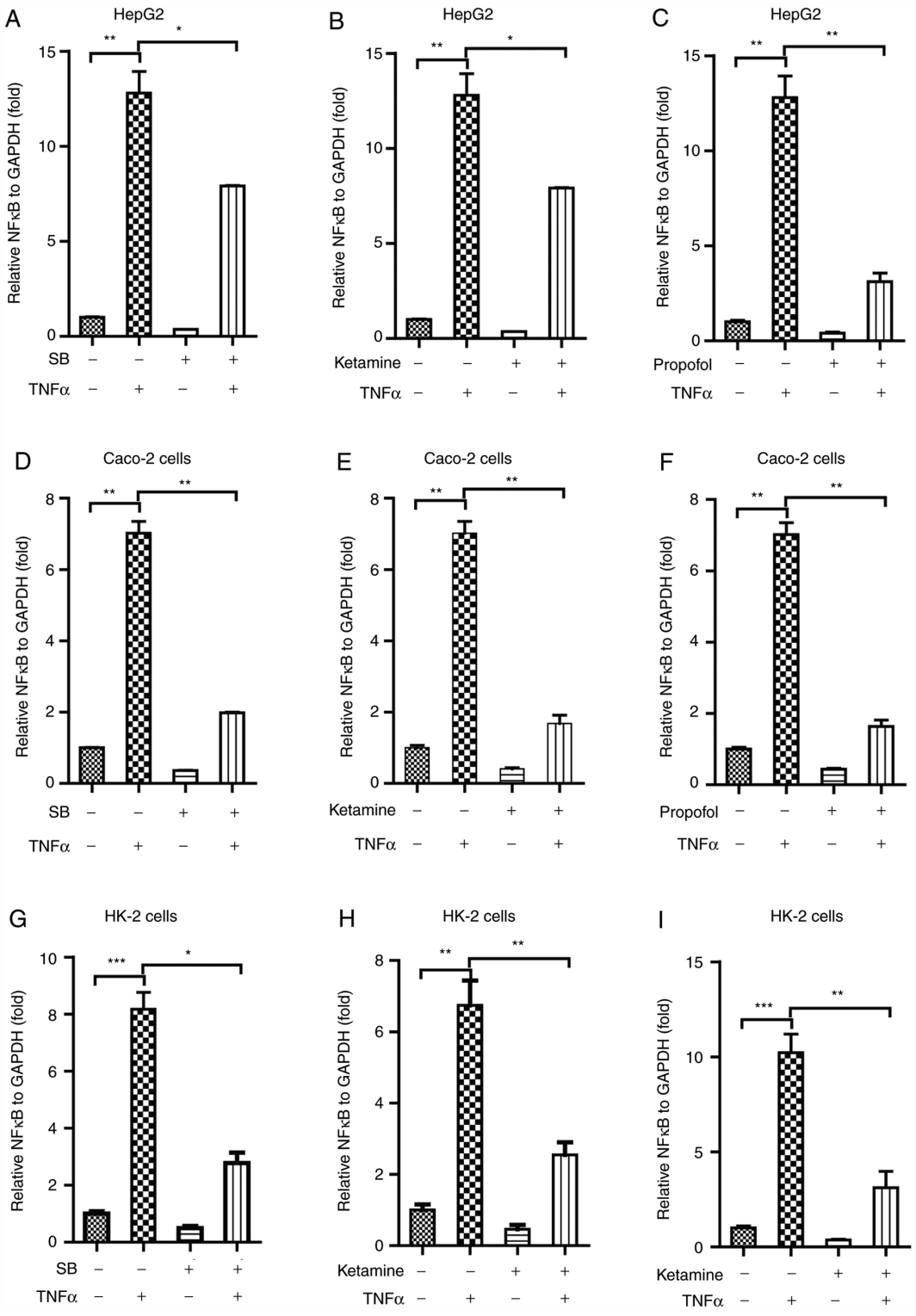

Figure 6. Sodium barbiturate, ketamine and propofol inhibit TNFα-mediated increase in NF-κB expression in the three cell lines. Effect of (A) SB, (B) ketamine and (C) propofol on TNFα-mediated NF-κB expression in HepG2 cells. Effect of (D) SB, (E) ketamine and (F) propofol on TNFα-mediated NF-κB expression in Caco-2 cells. Effect of (G) SB, (H) ketamine and (I) propofol on TNFα-mediated NF-κB expression in HK-2 cells (n=4). *P<0.05, **P<0.01 and ***P<0.001 SB, sodium barbiturate.

expression levels of IL-1β (Fig. 4C) and IL-18 (Fig. 4D) in Caco-2 cells. Finally, the results indicated that sodium barbiturate, ketamine and propofol, but not midazolam and etomidate, significantly decreased the mRNA expression levels of IL-1β (Fig. 4E) and IL-18 (Fig. 4F) in HK-2 cells. To further verify the effects of ketamine, midazolam and propofol on IL-1β and IL-18 expression levels, western blotting was performed. The results indicated that sodium barbiturate, midazolam and propofol decreased the protein expression levels of IL-1β (Fig. 5A and B) and IL-18 (Fig. 5C and D) in HepG2 cells. In parallel, sodium barbiturate, midazolam and propofol decreased the protein expression

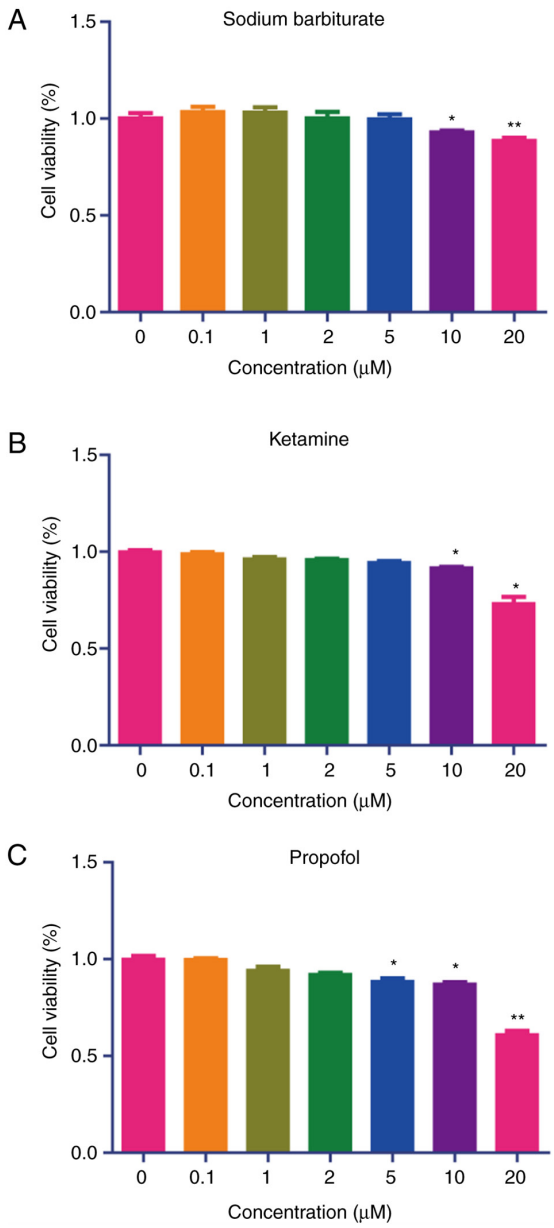

Figure 7. Cell viability following treatment with sodium barbiturate, ketamine and propofol in HepG2, Caco-2 and HK-2 cells. Viability of (A) HepG2 cells, (B) Caco2 cells and (C) HK-2 cells treated with sodium barbiturate. *P<0.05 and **P<0.01 vs. 0 μM.

levels of IL-1β (Fig. 5E and F) and IL18 (Fig. 5G and H) in Caco-2 cells. Similarly, sodium barbiturate, midazolam and propofol decreased the protein expression levels of IL-1β (Fig. 5I and J) and IL-18 (Fig. 5K and L) in HK-2 cells. Therefore, the results indicated that sodium barbiturate, ketamine and propofol suppressed inflammation in the three different cell lines used in the present study.

Sodium barbiturate, ketamine and propofol inhibit TNFα-mediated activation of NF-κB signaling in the three different cell lines. TNF-α is an activator of inflammation (27). To further investigate the effects on anesthetic agents on inflammation, cells were co-treated with TNF-α and sodium barbiturate, ketamine or propofol. The three aforementioned anesthetic agents were used as the results indicated that these agents significantly reduced the expression levels of NF-κB and its downstream effectors. TNFα (100 nM) markedly increased the mRNA expression level of NF-κB in HepG2 cells, as determined using RT-qPCR (Fig. 6A-C). Of note, sodium barbiturate (Fig. 6A), ketamine (Fig. 6B) and propofol (Fig. 6C) significantly inhibited TNFα-mediated NF-κB expression in HepG2 cells. Similarly, TNFα markedly increased the mRNA expression levels of NF-κB in Caco-2 cells (Fig. 6D-F), and sodium barbiturate (Fig. 6D), ketamine (Fig. 6E) and propofol (Fig. 6F) significantly inhibited TNFα-mediated NF-κB expresion in Caco-2 cells, as determined using RT-qPCR. TNFα significantly increased the mRNA expression levels of NF-κB in HK-2 cells (Fig. 6G-I), and sodium barbiturate (Fig. 6G), ketamine (Fig. 6H) and propofol (Fig. 6I) significantly inhibited TNFα-mediated NF-κB expression in HK-2 cells, as determined using RT-qPCR. Collectively, the results indicated that sodium barbiturate, ketamine and propofol inhibited TNFα-mediated activation of NF-κB signaling in HepG2, Caco-2 and HK-2 cells.

Effects of sodium barbiturate, ketamine and propofol on HepG2, Caco-2 and HK-2 cell viability. To determine the effects of sodium barbiturate, ketamine and propofol on the viability of HepG2, Caco-2 and HK-2 cells, a CCK-8 assay was performed, and it was indicated that 10 and 20 μM sodium barbiturate had slight cytotoxicity on HepG2 and Caco-2 cells, respectively (Fig. 7A and B). Furthermore, 20 μM sodium barbiturate exhibited cytotoxicity on HK-2 cells (Fig. 7C). High concentrations (10 and 20 μM) of ketamine showed significant cytotoxicity on HepG2, Caco-2 and HK-2 cells, respectively (Fig. 7D, E and F). Similarly, 5, 10 and 20 μM of propofol showed significant cytotoxicity on HepG2 cells (Fig. 7G). Furthermore, 10 and 20 μM propofol produced cytotoxicity on Caco-2 (Fig. 7H), and 20 μM propofol produced cytotoxicity on HK-2 cells (Fig. 7I). Thus, low concentrations (<10 μM) of sodium barbiturate, ketamine and propofol had minor cytotoxic effects while high concentrations (≥10 μM) had significant effects on the viability of HepG2, Caco-2 and HK-2 cells.

Discussion

The association between surgical procedures and inflammation has received increasing attention (28). Since anesthetic agents are widely used during surgical procedures to manage the pain and comfort of patients, the effects of anesthetic agents on inflammation have received increasing attention. Therefore, the present study investigated the effects of five commonly used anesthetic agents, including sodium barbiturate, midazolam, etomidate, ketamine and propofol, on inflammation in Caco-2, HK-2 and HepG2 cells. The results indicated that three out of the five anesthetic agents, sodium barbiturate, ketamine and propofol, significantly decreased the expression levels of NF-κB and its downstream cytokines, including IL-1β and IL-18, in the three cell lines. Moreover, the results indicated that sodium barbiturate, ketamine and propofol inhibited TNFα-mediated activation of NF-κB signaling in HepG2, Caco-2 and HK-2 cells. The present study provided novel insight into the molecular mechanisms underlying anesthetic agent-mediated regulation of inflammation

and may aid in selecting an appropriate anesthetic agent for surgical procedures.

Surgery can cause inflammation, for example, it was reported that colorectal surgery often causes systemic inflammatory response syndrome, which may cause post-operative morbidity and mortality (28). The pathogenesis underlying inflammation induced by surgery is not completely understood. Numerous anesthetic agents are used in surgical procedures. Several studies have demonstrated that certain anesthetic agents are linked to inflammation. Getachew *et al* (29) reported that ketamine exerted antidepressant and anti-inflammatory effects via interacting with specific gut bacteria in rats. Ketamine was reported to control innate immunity in the body and regulate the functions of a number of cellular effectors in the inflammatory reaction (30). Chang *et al* (31) reported that ketamine could affect macrophages to suppress NF-κB-mediated responses to lipopolysaccharide (LPS). Chang *et al* (32) also reported that ketamine inhibited hypoxia-induced inflammatory responses in late-gestation ovine fetal kidney cortex. In the present study, ketamine markedly decreased NF-κB expression in kidney, intestine and liver cell lines, and further downregulated the expression levels of NF-κB downstream cytokines, which was consistent with previous reports. The present study also indicated that ketamine significantly suppressed TNFα-mediated activation of NF-κB signaling in kidney, intestine and liver cell lines. The results of the present study demonstrated the inhibitory effects of ketamine on inflammation. The kidney, intestine and liver contain rich immune cells, which often generate immune reactions in response physiological changes (33). The results of the present study may aid with the identification of a suitable anesthetic agent for surgical procedures on the kidney, intestine and liver.

Propofol has been used in surgery for a number of years (34). Accumulating evidence demonstrated that propofol exerted regulatory effects on inflammation. Jia *et al* (35) confirmed that propofol could suppress LPS-induced inflammation via the PI3K signaling pathway in microglia. Jia *et al* (35) reported that propofol suppressed the secretion of cytokines, including IL8, IL6 and TNFα, in LPS-treated RAW 264.7 cells. Consistently, the present study indicated that propofol downregulated the expression levels of TNFα, IL1β and IL18 in Caco-2, HK-2 and HepG2 cells. The results also indicated that propofol inhibited TNFα-mediated activation of NF-κB signaling in the three cell lines.

Furthermore, the present study suggested that sodium barbiturate exerted anti-inflammatory effects in different types of cells. A previous study reported that a barbituric acid derivative exerted an inhibitory effect on the NF-κB signaling pathway in hepatic stellate cells (36). In addition, O'Sullivan *et al* (37) reported that dinitrate-barbiturate served as an anti-inflammatory agent, which could be used for the treatment of inflammatory bowel disease.

It has been reported that several anesthetic agents exert immune regulatory effects in *in vitro* models. However, the present study had a number of limitations: i) There are other types of anesthetic agents in addition to sodium barbiturate, midazolam, etomidate, ketamine and propofol, thus, screening the effects of a large number of anesthetic agents on inflammation to obtain a profile of the effects of anesthetic agents on immunity is important; ii) cell line models are less reliable compared with more advanced *in vitro* models, such as organoid models (38), therefore, validating the results of the present study in more advanced organoids is required; and iii) although the preliminary mechanism underlying the effects of anesthetic agents on inflammation was identified in the present study, future studies should investigate the precise mechanism of action.

In conclusion, the inflammatory reaction serves a key role in maintaining body homeostasis and survival, especially in the context of surgical procedures (39). Moreover, three out of the five anesthetic agents, including sodium barbiturate, ketamine and propofol, displayed anti-inflammatory effects in kidney, intestine and liver cells. Midazolam and etomidate did not display significant effects on inflammation in kidney, intestine and liver cells. Sodium barbiturate, ketamine and propofol decreased TNFα-mediated effects on inflammatory gene expression levels. The results of the present study provide further understanding of the effects of anesthetic agents on inflammation and may aid in selecting a suitable anesthetic agent in surgical procedures.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WL and XH contributed to study conception, design and management. WL, YL, TT, HX and LJ performed the experiments and collected and analyzed the data. WL contributed to manuscript writing and draft preparation. XH contributed to manuscript revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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