Propofol Stimulates Immune Activity and Decreases Inflammatory Cytokines via NF-κB-Mediated JAK1-STAT3 Pathway in Gastric Cancer Patients Undergoing Radical Surgery

Haili Zhang (haili_zhang@yeah.net)
Qiqihar Medical University
Shi Qian
Qiqihar Medical Unversity
Wang Jing
Qiqihar Medical University

Research article

Keywords: Gastric cancer, propofol, CD4, CD8, NF-κB, JAK1, STAT3

Posted Date: August 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-53225/v1

Abstract

Background

Propofol is the most commonly used general anesthesia for patients with gastric cancer undergoing radical surgery. Studies have suggested that propofol exerts beneficial effects on the immune function of patients with cancer. However, the potential mechanism underlying propofol-mediated immune regulation remains to be elucidated. The present study investigated the regulatory effects of propofol on immune function in patients with gastric cancer undergoing radical surgery.

Methods

ELISA, reverse transcription-quantitative polymerase chain reaction, western blotting, gene transfection and immunohistochemistry were used to analyze the effects of propofol on gastric cancer cells.

Results

Results demonstrated that propofol general anesthesia resulted in an increased percentage of cluster of differentiation (CD)4^+ and $CD8^+$ cells, increased serum concentrations of interleukin (IL)-2 and tumor necrosis factor (TNF)-α, decreased serum concentrations of IL-1β and IL-8 following propofol general anesthesia in gastric cancer patients undergoing radical surgery compared with midazolam. In addition, propofol general anesthesia induced an imbalance in T helper (Th)1/Th2 cells, increased the number of natural killer cells and B cells, decreased the expression of prognostic factors, and improved tumor metastasis, recurrence and survival in patients with gastric cancer compared with midazolam. Furthermore, immunohistochemistry demonstrated that propofol downregulated nuclear factor (NF)-κBp65, and upregulated Janus kinase 1 (JAK1) and signal transducer and activator of transcription 3 (STAT3) expression level in gastric cancer tissues, downregulated the protein expression levels of NF-κBp65, JAK1, STAT3, TNF-α, IL-1β and IL-8 protein expression in gastric cancer cells isolated from gastric cancer tissues. NF-κBp65 overexpression inhibited propofol-mediated upregulation of JAK1, STAT3, TNF-α, IL-1β and IL-8 expression in gastric cancer cells.

Conclusions

These data indicate that that propofol may increase the number of T cells, stimulate T-cell proliferation, upregulate IL-2 and TNF-α expression, and enhance immune function via the NF-κB-mediated JAK1-STAT3 signaling pathway in patients with gastric cancer undergoing radical surgery.

Background

Gastric cancer is the one of the most common causes of cancer-associated mortality (1, 2). Gastric carcinogenesis is thought to be associated with genetic factors, as well as numerous environmental factors (3, 4). Gastric cancer is also associated with higher morbidity and mortality rates compared with other types of digestive system-derived carcinoma (5, 6). Gastric signet ring cell carcinoma frequently

presented with diffuse and infiltrating myositis, which increases the difficulty of clinical diagnosis and treatment for patients with suspected gastric cancer (7). At present, apoptosis resistance of gastric cancer was inevitable in the development of cancer progression, thus increasing the risk of metastasis in patients with gastric cancer. Previous studies indicated that surgery combined with immunotherapy was more efficient for the treatment of gastric cancer (8, 9); therefore, stimulating the immune function of patients with gastric cancer may be beneficial for tumor eradication.

Currently, propofol is one of the most widely used general anesthetics. Propofol attenuated the surgical stress-induced adverse immune response, which has short-term consequences in clinical patients receiving cancer or cardiac surgery (10, 11). A previous study revealed that propofol attenuated the surgical stress-induced adverse immune response better than isoflurane anesthesia, and can regulate the T helper cell (Th)1/Th2 ratio following surgery in patients undergoing craniotomy (12). A retrospective analysis demonstrated that general anesthesia with propofol was associated with a higher overall 1-year survival rate than sevoflurane in patients following radical colon and breast cancer surgery (13). A randomized trial also demonstrated that patients receiving propofol exhibited increased interleukin (IL)-2/IL-4 and cluster of differentiation (CD)4^+/$CD8^+$ T cell ratio compared with in desflurane-treated patients undergoing breast cancer surgery (14). Interleukin-1 alpha (IL-1α) plays an important role in tumorigenesis and angiogenesis of gastric cancer and the interleukin-1 receptor antagonist (IL-1RA) significantly inhibited the proliferation and migration of human gastric cancer cells (15). Data found that suppression of IL-8-Src signaling inhibited adhesion, migration and invasion of gastric cancer cells (16). Nevertheless, to the best of our knowledge, no study has evaluated the effects of propofol on perioperative immune function in patients undergoing radical surgery for gastric cancer.

The present study aimed to investigate the effects of propofol anesthesia on immune function, including the expression of cytokines [IL-1β, IL-8, IL-2 and tumor necrosis factor (TNF)-α], the number of immune cells ($CD4^+$ and $CD8^+$ cells), and the balance between $CD4^+$/$CD8^+$ and Th1/Th2 in patients undergoing radical surgery for gastric cancer. In addition, the immunoregulatory effects of propofol were compared pre- and post-operation in patients with gastric cancer. The findings provide evidence to suggest that propofol may increase the production of immune cells for patients undergoing radical surgery for gastric cancer.

Methods

Ethics statement.

The present study (registration number: TPHRZ20150412) was approved by the Ethics Committee of the Qiqihar Medical University. All patients agreed to the use of their samples in scientific research and provided written informed consent.

Patients. Between May 2015 and July 2016, patients (n = 122) with gastric cancer requiring radical surgery provided written informed consent prior to the trial. The mean age of clinical patients was 48.5 ±

10.5 years, and the mean body weight was 70.4 ± 10.6 kg. The number of male and female patients (n = 61/61) was almost equal. The operation time for each patient was ~ 3 h. All of the patients were scheduled for radical surgery under propofol general anesthesia (3 mg/kg/h, n = 61) or midazolam anesthesia (3 mg/kg/h, n = 61). For all patients, there was no history of endocrine, immune or circulatory system diseases. Patients with gastric cancer with major surgical complications were excluded from the present study. The standardized protocols for the surgical procedure and postoperative patient care were used to minimize any difference in surgical impact among patients.

Patient survival, tumor metastasis and recurrence. A total of 22 patients with gastric cancer received propofol general anesthesia (3 mg/kg/h) prior to surgery, and were followed-up for 60 months. In addition, another 22 patients with gastric cancer (men/women, 10/12; mean age, 36.5 years; mean body weight, 57.5 kg) received midazolam general anesthesia. Patient survival, and tumor metastasis and recurrence were recorded within the 60-month follow-up period.

ELISA. A sample (10 ml) of central venous blood was collected immediately prior to surgery and 4 days after surgery. Following centrifugation at 3,000 x g for 15 min at 4˚C, the serum was separated to analyze serum levels of IL-1β (cat. no. 201-LB; R&D Systems, Inc., Minneapolis, MN, USA), IL-2 (cat. no. 202-IL; R&D Systems, Inc.), IL-6 (cat. no. D6050; R&D Systems, Inc.), IL-8 (cat. no. D8000C; R&D Systems, Inc.), TNF-α (cat. no. DTA00D; R&D Systems, Inc.), IL-10 (cat. no. D1000B; R&D Systems, Inc.) caveolin (Cav)-1 (cat. no. MAB5736; R&D Systems, Inc.), programmed death-ligand (PDL)-1 (cat. no. DB7H10; R&D Systems, Inc.), methyl-CpG binding protein 2 (MECP2; cat. no. M7443-200UL; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and Y-box-binding protein (YB)-1 (cat. no. LS-B11709-50; LifeSpan BioSciences, Inc., Seattle, WA, USA) by ELISA according to the manufacturers' protocols. The results were detected using an ELISA microplate reader (Spectra Max 190; Molecular Devices, LLC, Sunnyvale, CA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from total peripheral blood samples using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and RNA purity and quantity were detected by ultraviolet spectrometry. PCR specific primers (Table I) were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. cDNA was synthesized by RT using RNeasy Mini kit (Qiagen Sciences, Inc., Gaithersburg, MD, USA), according to the manufacturer's protocol, after which qPCR was conducted using a FastStart Universal SYBR Green Master (cat. no. 4913850001; Roche Diagnostics, Basel, Switzerland) and a LightCycler 480 Real-Time PCR system (Roche Diagnostics) under the following conditions: 95˚C for 30 sec, followed by 28 cycles at 95˚C for 30 sec, 56˚C for 45 sec and 72˚C for 35 sec, and a final extension step at 75˚C for 10 min. Quantification cycle (Cq) values of standard samples were calculated based on the internal reference gene β-actin. PCR results were quantitatively analyzed using the $2^{-\Delta\Delta Cq}$ method, as described previously (17). The Th1/Th2 balance was analyzed using the IL-2^{+}TNF/IL-4^{+}IL-10 ratio.

Blood cell counts. A complete blood count was performed on all blood samples using an automated hemoanalyzer (cat. no. WD-5000; Weier Medical Equipment Co., Ltd., Changchun, China), according to the

manufacturer's protocol. Total and differential leukocyte, lymphocyte, monocyte and neutrophil counts were determined as described previously (18).

Flow cytometry. Blood samples (5 ml) were obtained from individuals and lymphocyte subsets were determined by flow cytometry (Cytomics FC500; Beckman Coulter, Inc., Brea, CA, USA), as previously described (19). CD3-fluorescein isothiocyanate (FITC) (cat. no. ab34275, Abcam, Cambridge, UK)/CD4-phycoerythrin (PE) (cat. no. ab18282, Abcam) (helper T cells, $CD4^+$ T cells) and CD3-FITC (cat. no. ab34275, Abcam)/CD8-PE (cat. no. ab210327, Abcam) (suppressor/cytotoxic T cells, $CD8^+$ T cells) antibodies were used to analyzing percentage of CD4 + T and $CD8^+$ T cells by conducting flow cytometry.

Nuclear factor (NF)-κBp65 overexpression. Gastric cancer cells were isolated from gastric cancer tissues obtained from patients that received propofol general anesthesia, as previously described (20). Cells (1×10^5) were cultured in a 6-well plate in Dulbecco's modified Eagle's medium (Sigma-Aldrich; Merck KGaA) supplemented with 10% fetal bovine serum (Sigma-Aldrich; Merck KGaA), 100 U/ml penicillin and 100 µg/ml streptomycin at 37˚C in an atmosphere containing 5% CO_2 until they reached 85% confluence. Subsequently, the medium was removed and the cells were washed three times with PBS. Gastric cancer cells (1×10^6) were then infected with plentivirus-NF-κBp65 (pNF-κB; 100 mM; 5'-TTCCCTGAAGTGGAGCTAGGA-3'; Invitrogen; Thermo Fisher Scientific, Inc.) or plentivirus-Vector (control; 100 mM; 5'-GCTTCGGCAGCATATACTCTAAAT-3'; Invitrogen; Thermo Fisher Scientific, Inc.) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Expression level of NF-κB was analyzed after 48 h transfection using RT-qPCR (supplementary materials Figure S1). NF-κB-overexpressed gastric cells were treated with propofol (2 mg/ml) for 12 h at 37˚C for further analysis.

Western blotting. Gastric cells and NF-κB-overexpressed cells were lysed in radioimmunoprecipitation assay buffer (M-PER reagent for cells; Thermo Fisher Scientific, Inc.) and were homogenized at 4˚C for 10 min. Protein concentration was measured using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). Subsequently, protein extracts (20 µg) were separated by 12.5% SDS-PAGE and were then transferred to polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were incubated in blocking buffer (5% milk) for 2 h at 37˚C prior to incubation with primary antibodies at 4˚C overnight. The primary rabbit anti-human antibodies used in the immunoblotting assays were: NF-κBp65 (1:1,200, cat. no. ab16502; Abcam), IL-1β (1:1,000, cat. no. ab2105; Abcam), IL-8 (1:1,200, cat. no. ab7747; Abcam), TNF-α (1:1,000, cat. no. ab6671; Abcam), Janus kinase 1 (JAK1; 1:1,200, cat. no. ab47435; Abcam), signal transducer and activator of transcription 3 (STAT3; 1:500, cat. no. ab68153; Abcam) and β-actin (1:2,000, cat. no. ab8226; Abcam). After incubation, the membranes were washed three times in Tris-buffered saline containing 1.0% Tween (TBST) and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) monoclonal antibody (1:5,000, cat. no. PV-6001; OriGene Technologies, Inc., Beijing, China) for 1 h at 37˚C. After three washes with TBST, the membranes were developed using a chemiluminescence assay system (Roche Diagnostics) and exposed

to Kodak films (Kodak, Rochester, NY, USA). Densitometric semi-quantification of the immunoblots was performed using Quantity-One software (version 1.2; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Immunohistochemistry. Immunohistochemical analysis was performed as described previously (21). Gastric cancer tissues were obtained from patients and were fixed with 10% formalin for 2 h at 25˚C. Subsequently, the tissues were washed with PBS for 10 min, incubated with xylene for 5 min and incubated with an ethanol gradient (80, 90, 95 and 100%) for 3 min at 25˚C. Tissues were then embedded in paraffin at 56˚C and paraffin-embedded gastric cancer tissues sections (4 µm) were prepared for further analysis. The paraffin-embedded sections were treated with hydrogen peroxide (3%) for 10–15 min at 37˚C and were hydrated in a decreasing series of ethanol. Antigen retrieval was performed using an antigen retrieval kit (cat. no. ab93684; Abcam) at 65˚C for 10 min. Subsequently, 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) was used to block nonspecific binding at 37˚C for 2 h and tissue sections were incubated with the following primary antibodies at 4˚C for 12 h: NF-κBp65 (1:1,200, cat. no. ab16502; Abcam), JAK1 (1:1,200, cat. no. ab47435; Abcam) and STAT3 (1:500, cat. no. ab68153; Abcam). The tissue sections were then washed three times and incubated with a HRP-conjugated IgG monoclonal antibody (1:2,000, cat. no. PV-6001; OriGene Technologies, Inc.) for 12 h at 4˚C. Sections were incubated with diaminobenzidine (Sigma-Aldrich; Merck KGaA) at room temperature for 10 sec, in order to detect positive signals, and six random views were observed under a microscope. Images were recorded using an inverted light microscope (Olympus Corporation, Tokyo, Japan). Protein density was analyzed using ImageJ software 4.6 (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. All data are presented as the means ± standard deviation of triplicate experiments, and were analyzed by SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA). The comparisons of means between two matched groups were conducted using a paired Student's t-test. Unpaired t-test or ANOVA were used to compare statistical difference between two groups. One-way ANOVA analysis followed by Tukey's test was used to compare statistical difference among multiple groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinicopathological features of patients with gastric cancer. In the present study, the propofol group comprised 61 patients with gastric cancer, and midazolam comprised 61 patients with gastric cancer. There were no significant differences in heart rate, respiration rate and body temperature between propofol and midazolam group (Table II). Data revealed that post-operation of propofol had less pain that midazolam for patients with gastric cancer.

Effects of propofol on immune cells. The number of leukocytes in patients with gastric cancer was increased 4 days after propofol general anesthesia compared with pre-operation and midazolam (Fig. 1A). In addition, the number of lymphocytes was decreased 4 days after propofol general anesthesia compared with midazolam (Fig. 1B). The number of monocytes was increased 4 days after general anesthesia compared with midazolam (Fig. 1C). Neutrophil levels were also increased 4 days after

propofol general anesthesia compared with midazolam (Fig. 1D). These findings indicated that propofol general anesthesia regulated immune cells in patients with gastric cancer undergoing radical surgery.

Effects of propofol on cytokine expression. The effects of propofol on the expression levels of cytokines were analyzed 4 days after propofol general anesthesia in patients with gastric cancer undergoing radical surgery. Serum levels of IL-2 and TNF-α were increased following propofol general anesthesia compared with midazolam ($P < 0.01$; Fig. 2A and B). Compared with pre-operation, the serum concentrations of IL-1β and IL-8 were decreased in patients following propofol treatment compared with midazolam ($P < 0.01$; Fig. 2C and D). These findings suggested that propofol general anesthesia regulated cytokine expression in patients with gastric cancer undergoing radical surgery.

Effects of propofol on $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ cells. As shown in Fig. 3A and B, there was a statistically significant increase in $CD4^+$ and $CD8^+$ T cells following propofol anesthesia compared with midazolam ($P < 0.05$). In addition, the $CD4^+/CD8^+$ T cell ratio was increased 4 days after propofol anesthesia compared with midazolam ($P < 0.05$; Fig. 3C). These findings suggested that propofol general anesthesia increased the percentage of $CD4^+$ and $CD8^+$ cells in patients with gastric cancer undergoing radical surgery.

Effects of propofol on the Th1/Th2 balance. ELISA was used to determine the effects of propofol anesthesia on the serum levels of Th1 and Th2 cytokines. The results indicated that the mRNA expression levels of Th1 cytokines, IL-2 and TNF-α, were increased 4 days after propofol anesthesia ($P < 0.01$; Fig. 4A). In addition, the expression levels of Th2 cytokines, IL-4 and IL-10, were increased 4 days after propofol anesthesia in patients with gastric cancer undergoing radical surgery ($P < 0.05$; Fig. 4B). However, the Th1/Th2 ratio was decreased 4 days after propofol anesthesia ($P < 0.05$; Fig. 4C). These findings suggested that propofol general anesthesia regulated cytokine expression in patients with gastric cancer following radical operation.

Effects of propofol on prognostic factors. The prognostic factors Cav-1, PDL-1, MECP2 and YB-1 were evaluated in patients with gastric cancer undergoing radical surgery. The serum levels of Cav-1, PDL-1, MECP2 and YB-1 were decreased in patients with gastric cancer following propofol anesthesia compared with midazolam ($P < 0.01$; Fig. 5A-D). These findings indicated that propofol general anesthesia may decrease prognostic factors in patients with gastric cancer undergoing radical surgery.

Effects of propofol on tumor metastasis, recurrence and survival. The effects of propofol general anesthesia on tumor metastasis, recurrence and patient survival were analyzed in the present study. As shown in Fig. 6A, propofol general anesthesia inhibited gastric cancer metastasis following radical surgery compared with midazolam. In addition, the findings indicated that gastric cancer recurrence was decreased following propofol general anesthesia in patients undergoing radical surgery (Fig. 6B). Notably, survival was prolonged by propofol general anesthesia in patients with gastric cancer undergoing radical surgery compared with midazolam (Fig. 6C). These results suggested that propofol general anesthesia

improved tumor metastasis, recurrence and survival for patients with gastric cancer undergoing radical surgery during a 60-month follow-up period.

Effects of propofol on the NF-κB-mediated JAK1-STAT3 signaling pathway. A previous study demonstrated that NF-κB can regulate responses in the immune system (22). Therefore, the present study further analyzed the potential mechanism underlying the effects of propofol on gastric cancer cells. The results revealed that propofol downregulated NF-κBp65 expression, and upregulated JAK1 and STAT3 expression in gastric cancer tissues (Fig. 7A). Western blotting demonstrated that propofol treatment decreased the protein expression levels of TNF-α, IL-1β and IL-8 in gastric cancer cells (Fig. 7B). Furthermore, propofol downregulated NF-κBp65, and upregulated JAK1 and STAT3 protein expression in gastric cancer cells (Fig. 7C). The present study also demonstrated that NF-κBp65 overexpression canceled propofol-induced upregulation of JAK1 and STAT3 protein expression in gastric cancer cells compared to cells transfected empty vector (Fig. 7D). In addition, propofol-induced decreases in TNF-α, IL-1β and IL-8 expression were attenuated by NF-κBp65 overexpression in gastric cancer cells (Fig. 7E). These results indicated that propofol regulated immune responses via the NF-κBp65-mediated JAK1-STAT3 signaling pathway.

Discussion

Propofol has been reported to present fewer adverse and toxic side-effects, and exhibits reduced toxicity in the heart and central nervous system compared with midazolam (23). In addition, it has been demonstrated that propofol general anesthesia increased the percentage of $CD4^+$ and $CD8^+$ cells in patients undergoing laparoscopic radical hysterectomy for cervical cancer (24). Therefore, it was hypothesized that propofol increased the production of cytokines and provided protection for circulating lymphocytes during the perioperative period for patients undergoing radical surgery for gastric cancer. Although a previous report indicated that propofol anesthesia had less of an effect on immune function in patients with lung adenocarcinoma (25), the present study demonstrated that propofol general anesthesia was able to regulate immune cells and cytokine production in patients with gastric cancer undergoing radical surgery.

Gastric cancer is an important health problem, which is particularly intractable due to the existence of gastric tumor cells in the hydrochloric acid-containing gastric juice, which represents a relatively more extreme acidic environment compared with other human tissues (26, 27). In recent years, the inhibitory effects of propofol on human cancer cells have been widely studied. It has been revealed that propofol suppressed invasion of human lung cancer cells by downregulating aquaporin-3 and matrix metalloproteinase-9 expression (28). Notably, propofol anesthesia attenuated the surgical stress-induced adverse immune response, which may regulate the Th1/Th2 ratio (12). The present study indicated that propofol anesthesia not only had an anesthetic effect, but also stimulated immune regulation in patients undergoing radical surgery for gastric cancer. Zhou *et al* demonstrated that propofol was able to attenuate sevoflurane-induced cellular injury of human peripheral lymphocytes (29). The present findings indicated that propofol general anesthesia decreased the levels of lymphocytes, and increased

monocytes and neutrophils after general anesthesia compared with pre-operation for patients with gastric cancer undergoing radical surgery.

Several kinds of cancer surgeries with propofol-based total intravenous anesthesia may be associated with improved survival in gastric cancer patients who undergo resection (30). Propofol general anesthesia achieved a more stable hemodynamics and a shortened time to awakening (30). Data in the current study found that propofol general anesthesia shortened the hospital stays and time to awakening compared to previous study. Propofol sedation presented more benefits during endoscopic treatment for early gastric cancer compared to midazolam (31). Notably, our study found an association between propofol anesthesia and an increased anti-inflammatory cytokines. This may be due to the fact that propofol anesthesia induced activated immune functions. Propofol can promote the secretion of insulin during radical gastrectomy, and inhibited the excessive secretion of cortisol and hyperglycemia (32). In this study, propofol decreased serum levels of Cav-1, PDL-1, MECP2 and YB-1 for gastric cancer patients undergoing for gastric surgery. However, the influence of propofol on stress responses did not investigate in patients undergoing for gastric surgery.

IL-2 and IL-4 were associated with Th1 and Th2 cells, and the balance between Th1 and Th2 cells serves a significant role in patients with cancer (33). A previous study revealed that propofol anesthesia regulated the Th1/Th2 balance in spinal cord injury (34). The effects of propofol and dexmedetomidine anesthesia have also been detected on the Th1/Th2 balance in rat spinal cord injury (34). The present study demonstrated that the Th1/Th2 ratio was decreased after propofol anesthesia in patients with gastric cancer undergoing radical surgery, which may contribute to a reduction in inflammation. In addition, a previous study reported that propofol anesthesia for breast cancer surgery induced a favorable immune response in terms of preservation of IL-2/IL-4 and $CD4^+/CD8^+$ T cell ratio during the perioperative period (14). The present study revealed that IL-2 and TNF-α expression were significantly increased following propofol anesthesia. These findings suggested that propofol anesthesia may increase the percentage of $CD4^+$ and $CD8^+$ cells in patients undergoing radical surgery.

A previous study demonstrated that continuous infusion of midazolam affected immune function by decreasing the expression levels of IL-1β, IL-8 and TNF-α in pediatric patients post-surgery (35). Romano *et al* revealed that IL-2 immunotherapy enhanced tumor-infiltrating lymphocytes in patients with gastric cancer (36). The present study demonstrated that propofol anesthesia decreased the serum levels of IL -1β and IL-8 in patients undergoing radical surgery. Furthermore, A. Ní Eochagáin et al showed that neutrophil-lymphocyte ratio increased significantly in the propofol-paravertebral breast cancer patients (37). Results in this study observed the increasing neutrophil serum level in propofol group. However, neutrophils did not incarcerated on postoperative day 4 in the midazolam group. Definitely, neutrophil stimulated by propofol may contribute to the chemotactic attraction of suppressive gastric cancer cells. A previous study found that there could be a much defined axis where IL-8 plays an important role in the recruitment of certain lymphocyte populations and tumor development, including the way in which tumors are capable of developing metastasis (38). In this study, although we observed the increasing of neutrophil levels after propofol general anesthesia, the serum concentration of IL-8 was decreased in

patients following propofol treatment. These mechanisms mediated by IL-8 may be relevant in the establishment of immune system that helps the immune cells to monitor the tumor microenvironment. Although TNF-α and IL-2 were proinflammatory cytokines for patients with gastric cancer, IL-2 stimulated the host reaction against tumor tissues by lymphocyte/eosinophil infiltration (39), and TNF-αinduced apoptosis of human gastric cancer cells via upstream caspase-3 protease activation (40). The present study reported that propofol anesthesia increased the serum levels of IL-2 and TNF-α in patients undergoing radical surgery, which may enhance anticancer therapy for patients with gastric cancer. However, the *in vitro* assay demonstrated that propofol decreased TNF-α in gastric cancer cells. These differences may be due to the differences between intracellular inflammation and systematic inflammatory responses. These outcomes indicated that propofol anesthesia exerted beneficial effects for patients with gastric cancer undergoing radical surgery. However, the sample size of this clinical study was small and needed further clarify in our future work.

Cav-1 promoted bladder cancer metastasis, and is therefore considered a potential therapeutic target in invasive bladder cancer (41). In addition, it has been reported that tumor expression of PDL-1 was associated with poor prognosis in patients with gastric cancer (42). This study reported that the serum levels of Cav-1 and PDL-1 were decreased in patients with gastric cancer following propofol anesthesia. Furthermore, dysregulated expression of MECP2 was correlated with clinicopathological parameters in the development of gastric cancer (43). A previous study also indicated that silencing the YB-1 gene led to inhibition of cell migration in gastric cancer *in vitro* (44). In the present study, propofol anesthesia decreased MECP2 and YB-1 serum levels in patients with gastric cancer, thus suggesting that propofol may be a potential anesthetic with anti-metastatic activity for patients with gastric cancer. A previous study revealed that inhibition of NF-κB is required for properly balanced immune responses, and it appeared to be evolutionarily conserved (45). In addition, it has been reported that propofol inhibited invasion and growth of ovarian cancer cells by regulating the NF-κB signaling pathway (46). In the present study, propofol was shown to downregulate NF-κB expression in gastric cancer tissues and cells. Furthermore, Lu *et al* demonstrated that the JAK1/STAT3 pathway was involved in the protective effects of propofol against hypoxia-induced inflammation and apoptosis in BV2 microglia (47). Chen *et al* found that activation of JAK1/STAT3 signaling inhibited tumorigenesis and induced cell apoptosis repression in gastric cancer (48). Notably, inhibiting the activation of the JAK1/Stat3 pathway is a practical anti-tumor approach to restrain tumor progression in in gastric cancer (49). The present study also reported that propofol regulated immune function via the NF-κB-mediated JAK1/STAT3 signaling pathway in gastric cancer cells. These results provided a potential target for the regulation of immune function in patients with gastric cancer undergoing radical surgery.

Conclusion

In conclusion, treatment with propofol during radical surgery exerted favorable effects on the immune system, particularly with regards to the Th1/Th2 balance and $CD4^+/CD8^+$ T cell ratio, during the perioperative period. The present findings indicated that propofol anesthesia decreased the Th1/Th2

ratio, which attenuated adverse effects on the immune system during radical surgery for patients with gastric cancer. Further studies are required to determine the clinical implications of using propofol compared with other types of general anesthesia, such as etomidate, in a large population.

Declarations

The authors declare that they have no conflict of interests.

Acknowledgements

Not applicable.

Funding

This study was supported by Qiqihar Medical Research Fund (Self-exposure palliative care for alexithymia in patients with coronary heart disease, grant number: QMSI2019M-28). The funders had no role in the design of the study, data collection, and interpretation of data and in writing the manuscript.

Availability of data and materials

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

Authors' contributions

FJ and M SJ performed the experiments. ZQ and NL Z designed experiments and wrote manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Qiqihar Medical University. All patients agreed to the use of their samples in scientific research and provided written informed consent.

Patient consent for publication

All patients provided consent for publication.

References

1. Chen SY, Zhang RG, Duan GC. Pathogenic mechanisms of the oncoprotein CagA in H. pylori-induced gastric cancer (Review). Oncol Rep. 2016;36:3087–94.
2. Ding Y, Yang Q, Wang B, Ye G, Tong X. The Correlation of MGMT Promoter Methylation and Clinicopathological Features in Gastric Cancer: A Systematic Review and Meta-Analysis. PloS one. 2016;11:e0165509.

3. Zeng XQ, Wang J, Chen SY. Methylation modification in gastric cancer and approaches to targeted epigenetic therapy (Review). Int J Oncol. 2017;50:1921–33.
4. Emadi-Baygi M, Sedighi R, Nourbakhsh N, Nikpour P. Pseudogenes in gastric cancer pathogenesis: a review article. Briefings in functional genomics, 2017.
5. Pimenta-Melo AR, Monteiro-Soares M, Libanio D, Dinis-Ribeiro M: Missing rate for gastric cancer during upper gastrointestinal endoscopy: a systematic review and meta-analysis. European journal of gastroenterology & hepatology, 2016.
6. Veisani Y, Delpisheh A. Survival rate of gastric cancer in Iran; a systematic review and meta-analysis. Gastroenterology hepatology from bed to bench. 2016;9:78–86.
7. Futtrup TB, Hasselby JP, Baeksgaard L. Gastric signet ring cell carcinoma presenting as diffuse, infiltrating myositis–a case report and review of the literature. Journal of gastrointestinal cancer. 2014;45(Suppl 1):62–5.
8. Li K, Li J: Current Molecular Targeted Therapy in Advanced Gastric Cancer: A Comprehensive Review of Therapeutic Mechanism, Clinical Trials, and Practical Application. Gastroenterology research and practice 2016: 4105615, 2016.
9. Ruzzo A, Catalano V, Canestrari E, et al. Genetic modulation of the interleukin 6 (IL-6) system in patients with advanced gastric cancer: a background for an alternative target therapy. BMC Cancer. 2014;14:357.
10. Jia L, Dong R, Zhang F, et al. Propofol Provides More Effective Protection for Circulating Lymphocytes Than Sevoflurane in Patients Undergoing Off-Pump Coronary Artery Bypass Graft Surgery. J Cardiothorac Vasc Anesth. 2015;29:1172–9.
11. Zhang T, Fan Y, Liu K, Wang Y. Effects of different general anaesthetic techniques on immune responses in patients undergoing surgery for tongue cancer. Anaesthesia intensive care. 2014;42:220–7.
12. Inada T, Yamanouchi Y, Jomura S, et al. Effect of propofol and isoflurane anaesthesia on the immune response to surgery. Anaesthesia. 2004;59:954–9.
13. Enlund M, Berglund A, Andreasson K, Cicek C, Enlund A, Bergkvist L. The choice of anaesthetic–sevoflurane or propofol–and outcome from cancer surgery: a retrospective analysis. Ups J Med Sci. 2014;119:251–61.
14. Woo JH, Baik HJ, Kim CH, et al. Effect of Propofol and Desflurane on Immune Cell Populations in Breast Cancer Patients: A Randomized Trial. J Korean Med Sci. 2015;30:1503–8.
15. Gong Z, Ma J, Su H, et al. Interleukin-1 receptor antagonist inhibits angiogenesis in gastric cancer. Int J Clin Oncol. 2018;23:659–70.
16. Shi J, Wei PK. Xiaotan Sanjie decoction inhibits interleukin-8-induced metastatic potency in gastric cancer. World journal of gastroenterology. 2015;21:1479–87.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402–8.

18. Khan HA, Alhomida AS, Sobki SH, Moghairi AA, Koronki HE. Blood cell counts and their correlation with creatine kinase and C-reactive protein in patients with acute myocardial infarction. Int J Clin Exp Med. 2012;5:50–5.
19. Verschoor CP, Kohli V. Cryopreserved whole blood for the quantification of monocyte, T-cell and NK-cell subsets, and monocyte receptor expression by multi-color flow cytometry: A methodological study based on participants from the canadian longitudinal study on aging. Cytometry Part A: the journal of the International Society for Analytical Cytology. 2018;93:548–55.
20. Yuan D, Chen L, Li M, et al. Isolation and characterization of circulating tumor cells from human gastric cancer patients. J Cancer Res Clin Oncol. 2015;141:647–60.
21. Selinger CI, Li BT, Pavlakis N, et al. Screening for ROS1 gene rearrangements in non-small-cell lung cancers using immunohistochemistry with FISH confirmation is an effective method to identify this rare target. Histopathology. 2017;70:402–11.
22. Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol. 2002;2:725–34.
23. Dispersyn G, Pain L, Touitou Y. Propofol anesthesia significantly alters plasma blood levels of melatonin in rats. Anesthesiology. 2010;112:333–7.
24. Liu S, Gu X, Zhu L, et al. Effects of propofol and sevoflurane on perioperative immune response in patients undergoing laparoscopic radical hysterectomy for cervical cancer. Medicine. 2016;95:e5479.
25. Liu J, Dong W, Wang T, et al. Effects of etomidate and propofol on immune function in patients with lung adenocarcinoma. American journal of translational research. 2016;8:5748–55.
26. Choi HS, Ha SY, Kim HM, et al. The prognostic effects of tumor infiltrating regulatory T cells and myeloid derived suppressor cells assessed by multicolor flow cytometry in gastric cancer patients. Oncotarget. 2016;7:7940–51.
27. Mori F, Canu V, Lorenzon L, Garofalo A, Blandino G, Strano S. Cancer Gastric Chemoprevention: Isolation of Gastric Tumor-Initiating Cells. Methods Mol Biol. 2016;1379:129–37.
28. Ye HJ, Bai JJ, Guo PP, Wang W, Lin CS. [Propofol suppresses invasion of human lung cancer A549 cells by down-regulating aquaporin-3 and matrix metalloproteinase-9]. Nan fang yi ke da xue xue bao = Journal of Southern Medical University. 2016;36:1286–90.
29. Zhou Y, Li E, Li Y, Liu S. Attenuating sevoflurane-induced cellular injury of human peripheral lymphocytes by propofol in a concentration-dependent manner. Arch Pharm Res. 2011;34:1535–43.
30. Zheng X, Wang Y, Dong L, et al. Effects of propofol-based total intravenous anesthesia on gastric cancer: a retrospective study. OncoTargets therapy. 2018;11:1141–8.
31. Kiriyama S, Naitoh H, Kuwano H. Propofol sedation during endoscopic treatment for early gastric cancer compared to midazolam. World journal of gastroenterology. 2014;20:11985–90.
32. Wu Y, Zhang L, Yin G, Liu Y, Chen L. Stress Response to Propofol versus Isoflurane Anesthesia in Patients Undergoing Gastric Surgery. Journal of the College of Physicians Surgeons–Pakistan: JCPSP. 2019;29:201–4.

33. Payne AS, Cornelius LA. The role of chemokines in melanoma tumor growth and metastasis. J Invest Dermatol. 2002;118:915–22.
34. He FY, Feng WZ, Zhong J, Xu W, Shao HY, Zhang YR. Effects of propofol and dexmedetomidine anesthesia on Th1/Th2 of rat spinal cord injury. Eur Rev Med Pharmacol Sci. 2017;21:1355–61.
35. Lu HB, Jia YP, Liang ZH, Zhou R, Zheng JQ. Effect of continuous infusion of midazolam on immune function in pediatric patients after surgery. Genetics molecular research: GMR. 2015;14:10007–14.
36. Romano F, Cesana G, Caprotti R, et al. Preoperative IL-2 immunotherapy enhances tumor infiltrating lymphocytes (TILs) in gastric cancer patients. Hepato-gastroenterology. 2006;53:634–8.
37. Ni Eochagain A, Burns D, Riedel B, Sessler DI, Buggy DJ. The effect of anaesthetic technique during primary breast cancer surgery on neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and return to intended oncological therapy. Anaesthesia. 2018;73:603–11.
38. Gonzalez-Aparicio M, Alfaro C: Influence of Interleukin-8 and Neutrophil Extracellular Trap (NET) Formation in the Tumor Microenvironment: Is There a Pathogenic Role? Journal of immunology research 2019: 6252138, 2019.
39. Romano F, Piacentini MG, Franciosi C, et al. Phase-II randomized study of preoperative IL-2 administration in radically operable gastric cancer patients. Hepato-gastroenterology. 2004;51:1872–6.
40. Park IC, Park MJ, Choe TB, Jang JJ, Hong SI, Lee SH. TNF-alpha induces apoptosis mediated by AEBSF-sensitive serine protease(s) that may involve upstream caspase-3/CPP32 protease activation in a human gastric cancer cell line. Int J Oncol. 2000;16:1243–8.
41. Liang W, Hao Z, Han JL, Zhu DJ, Jin ZF, Xie WL. CAV-1 contributes to bladder cancer progression by inducing epithelial-to-mesenchymal transition. Urol Oncol. 2014;32:855–63.
42. Tamura T, Ohira M, Tanaka H, et al. Programmed Death-1 Ligand-1 (PDL1) Expression Is Associated with the Prognosis of Patients with Stage II/III Gastric Cancer. Anticancer research. 2015;35:5369–76.
43. Zhang J, Zhao J, Gao N, Wang Y, Chen Y, Han J. MECP2 expression in gastric cancer and its correlation with clinical pathological parameters. Medicine. 2017;96:e7691.
44. Guo TT, Yu YN, Yip GW, Matsumoto K, Bay BH. Silencing the YB-1 gene inhibits cell migration in gastric cancer in vitro. Anat Rec (Hoboken). 2013;296:891–8.
45. Kim LK, Choi UY, Cho HS, et al. Down-regulation of NF-kappaB target genes by the AP-1 and STAT complex during the innate immune response in Drosophila. PLoS Biol. 2007;5:e238.
46. Huang X, Teng Y, Yang H, Ma J. Propofol inhibits invasion and growth of ovarian cancer cells via regulating miR-9/NF-kappaB signal. Brazilian journal of medical biological research = Revista brasileira de pesquisas medicas e biologicas. 2016;49:e5717.
47. Lu Y, Gu Y, Ding X, Wang J, Chen J, Miao C. Intracellular Ca2 + homeostasis and JAK1/STAT3 pathway are involved in the protective effect of propofol on BV2 microglia against hypoxia-induced inflammation and apoptosis. PloS one. 2017;12:e0178098.

48. Chen W, Wu G, Zhu Y, et al. HOXA10 deteriorates gastric cancer through activating JAK1/STAT3 signaling pathway. Cancer management research. 2019;11:6625–35.
49. Wang C, Wang M, Xing B, et al. C-terminal of E1A binding protein 1 enhances the migration of gastric epithelial cells and has a clinicopathologic significance in human gastric carcinoma. OncoTargets therapy. 2019;12:5189–200.

Figures

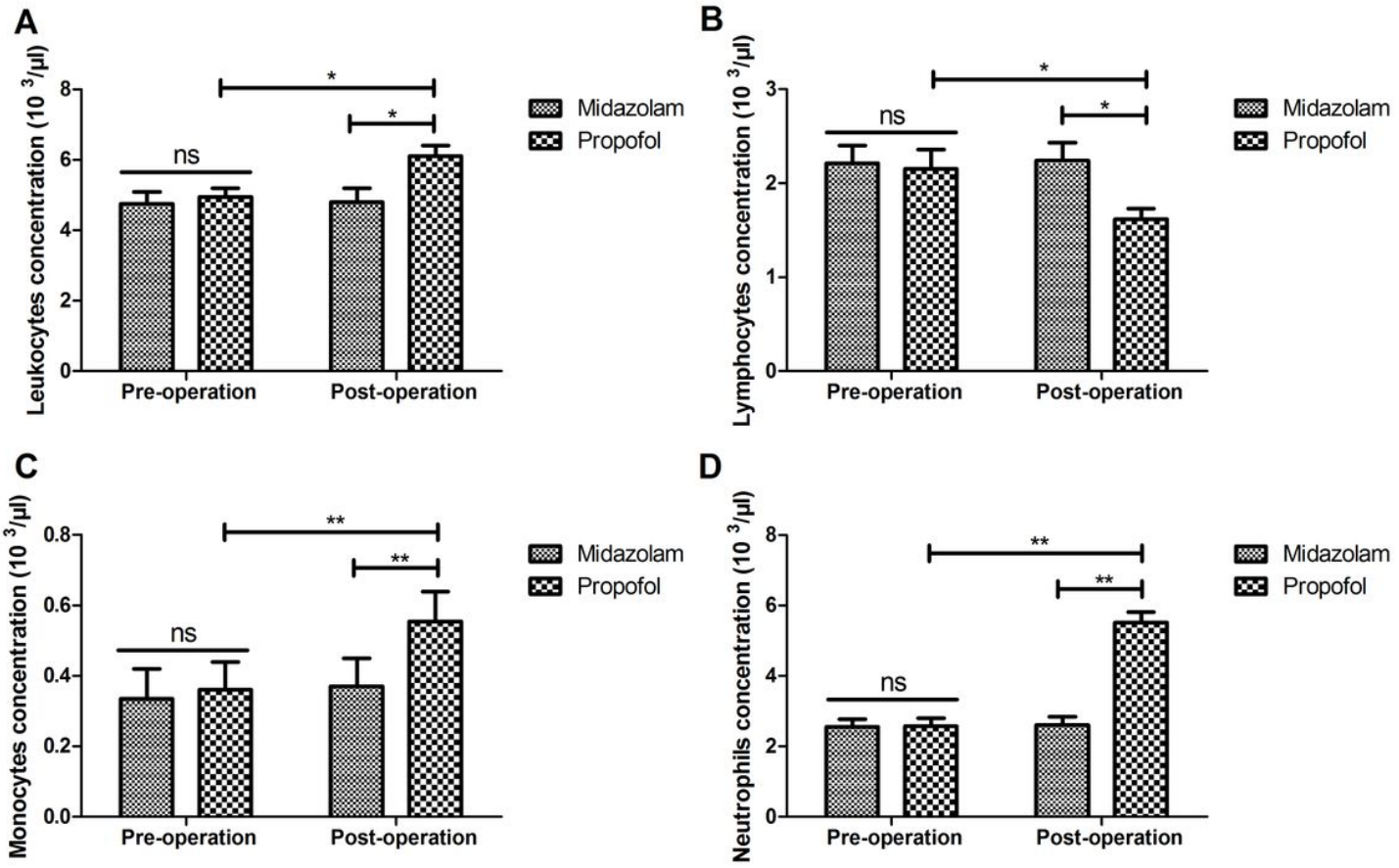

Figure 1

Effects of propofol on immune cells in patients with gastric cancer undergoing radical surgery. Propofol general anesthesia (A) increased leukocytes, (B) decreased lymphocytes, and increased (C) monocytes and (D) neutrophils in patients with gastric cancer following radical operation compared with midazolam. *P<0.05 and **P<0.01 vs. pre-operation.

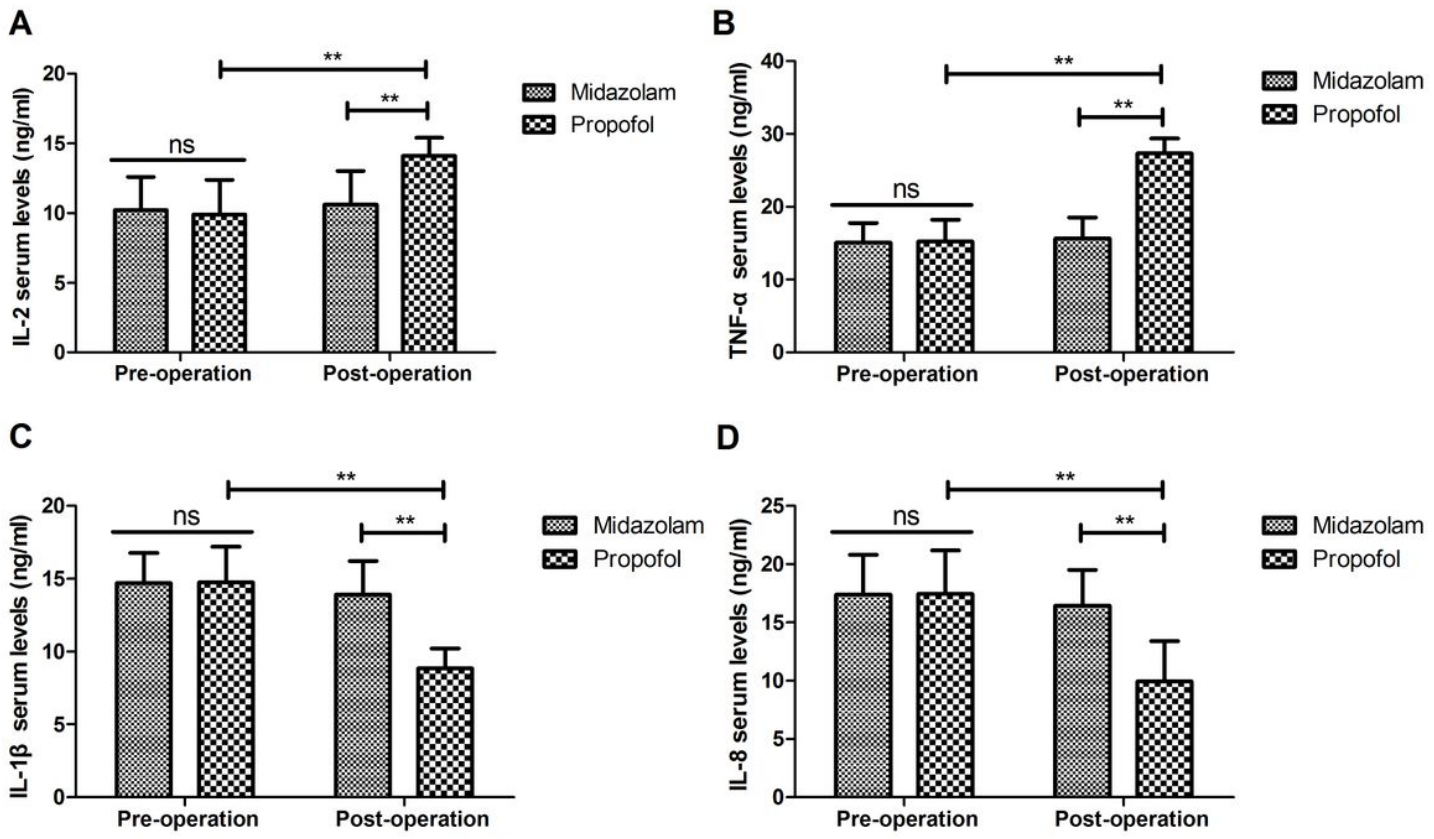

Figure 2

Effects of propofol on cytokine expression in patients with gastric cancer undergoing radical surgery. Propofol general anesthesia increased the serum levels of (A) IL-2 and (B) TNF-α in patients undergoing radical surgery compared with midazolam. Propofol general anesthesia decreased the serum levels of (C) IL-1β and (D) IL-8 in patients undergoing radical surgery compared with midazolam. **P<0.01 vs. pre-operation. IL, interleukin; TNF-α, tumor necrosis factor-α.

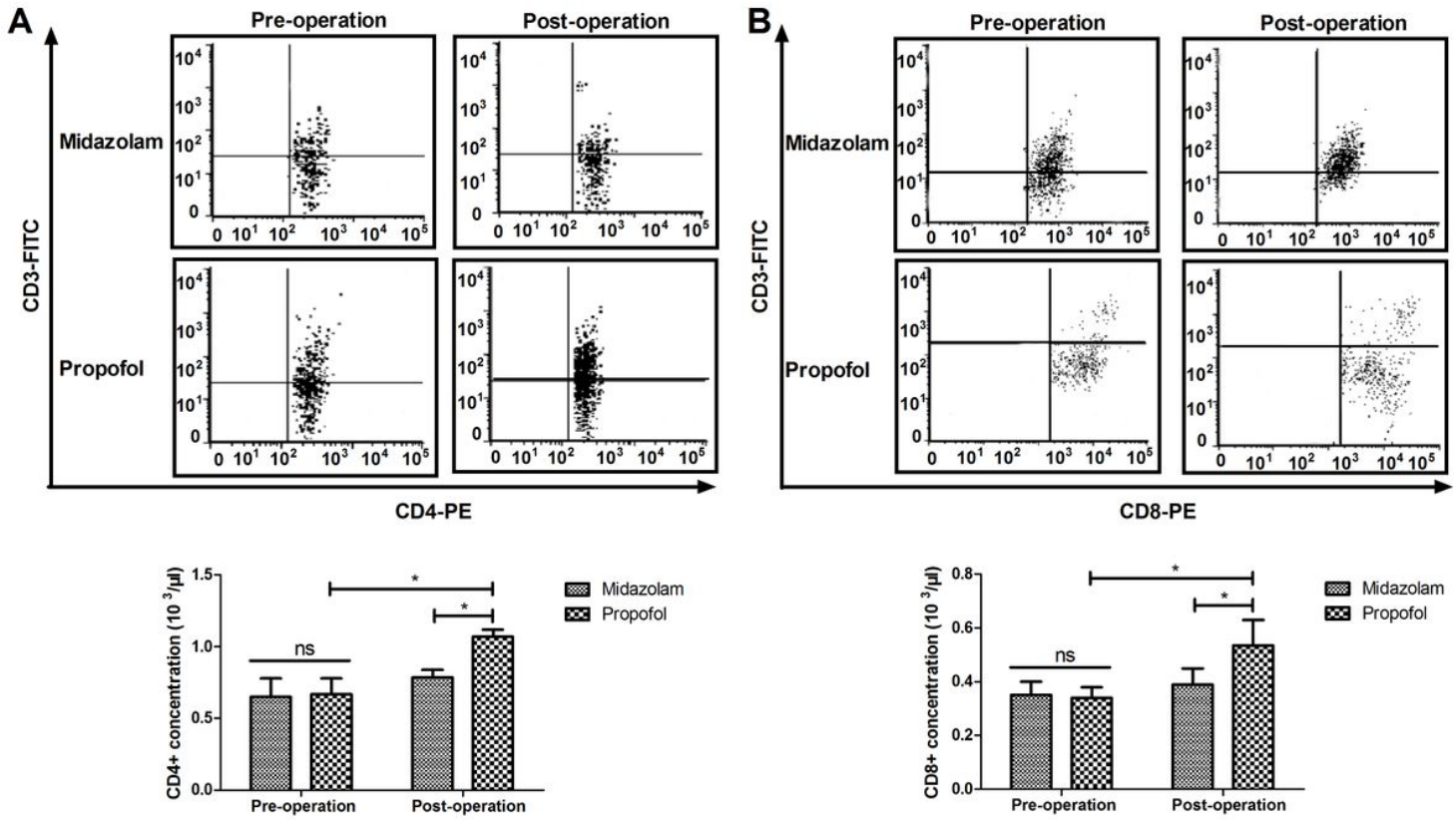

Figure 3

Effects of propofol on CD4+, CD8+ and CD4+/CD8+ cells. Propofol general anesthesia increased the percentage of (A) CD4+ and (B) CD8+ T cells compared with midazolam.

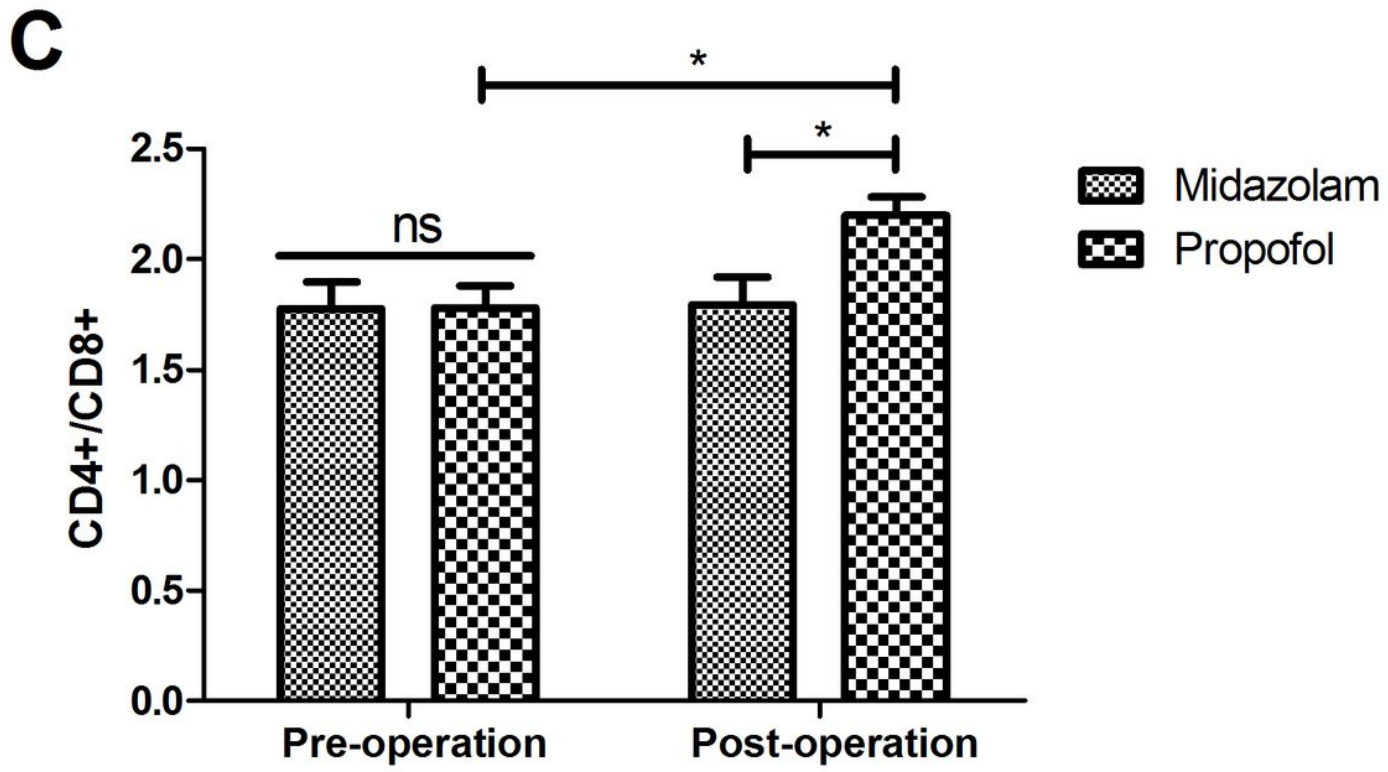

Figure 4

Propofol general anesthesia increased the CD4+/CD8+ T cell ratio compared with midazolam. *P<0.05 and **P<0.01 vs. pre-operation. CD, cluster of differentiation.

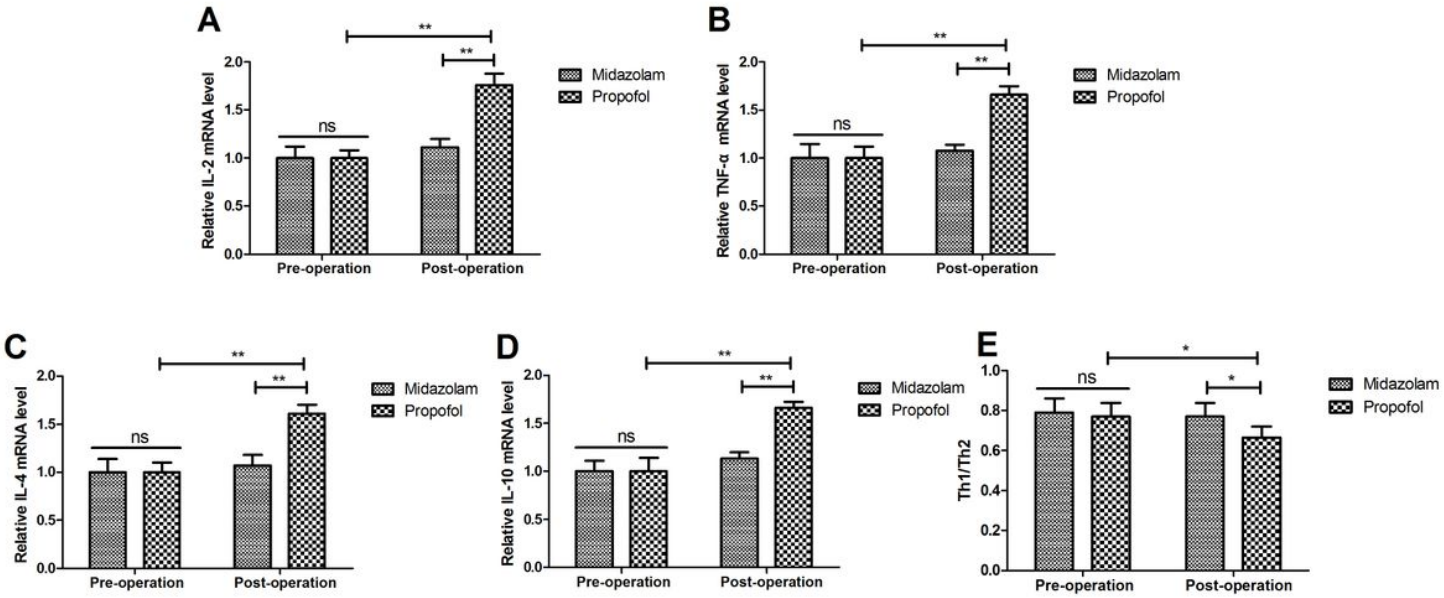

Figure 5

Effects of propofol on inflammatory cytokines expression and the Th1/Th2 balance. (A-B) Propofol general anesthesia increased the mRNA expression level of IL-2 and TNF-α (A), IL-4 and IL-10 (B) compared with midazolam. (C) Propofol general anesthesia decreased the Th1/Th2 ratio in patients with gastric cancer undergoing radical surgery compared with midazolam. *P<0.05 and **P<0.01 vs. pre-operation. IL, interleukin; Th, T helper; TNF-α, tumor necrosis factor-α.

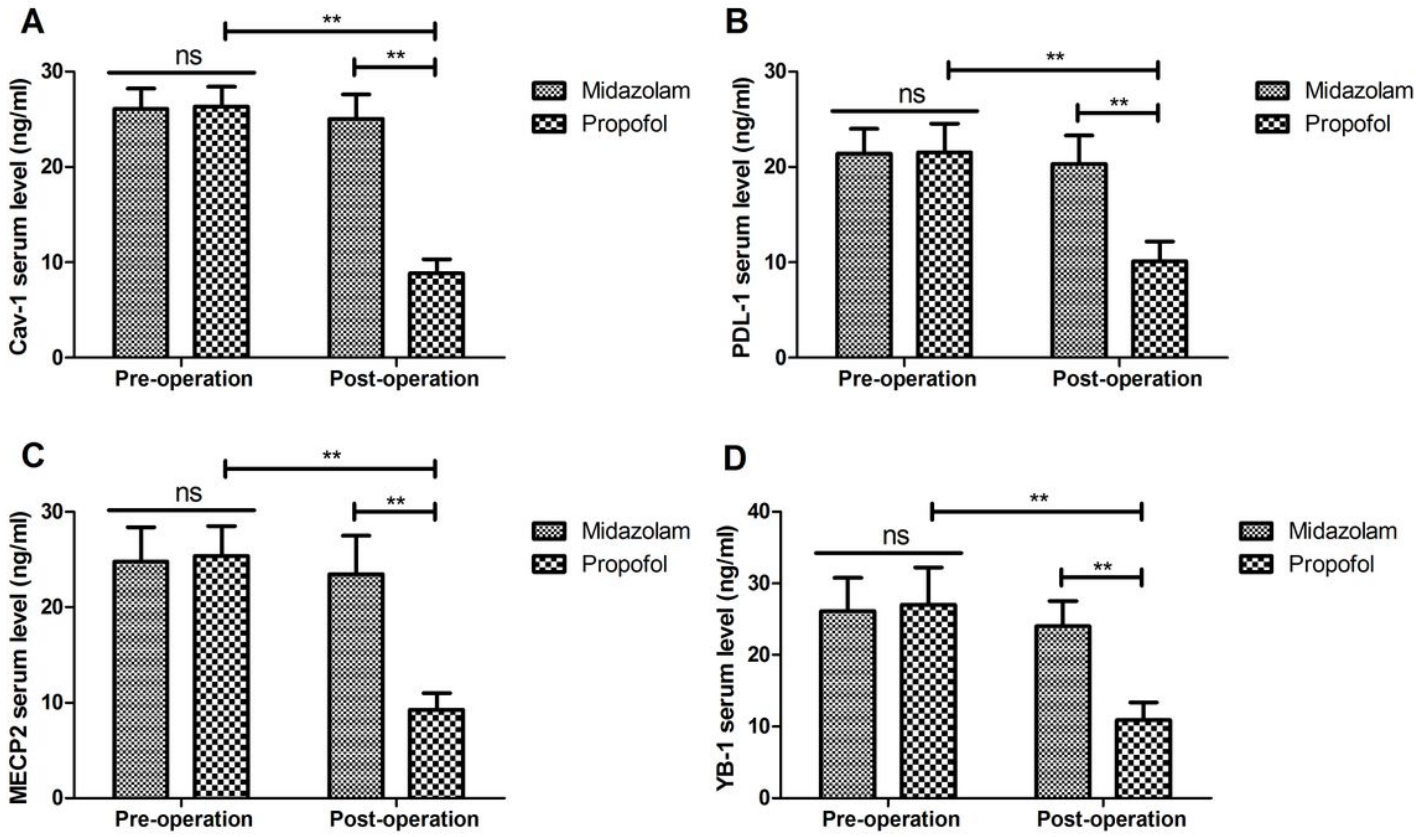

Figure 6

Effects of propofol on prognostic factors. Propofol general anesthesia decreased the expression levels of the following prognostic factors: (A) Cav-1, (B) PDL-1, (C) MECP2 and (D) YB-1 in patients with gastric cancer undergoing radical surgery compared with midazolam. **P<0.01 vs. pre-operation. Cav-1, caveolin-1; MECP2, methyl-CpG binding protein 2 ; PDL-1, programmed death ligand-1; YB-1, Y-box-binding protein-1.

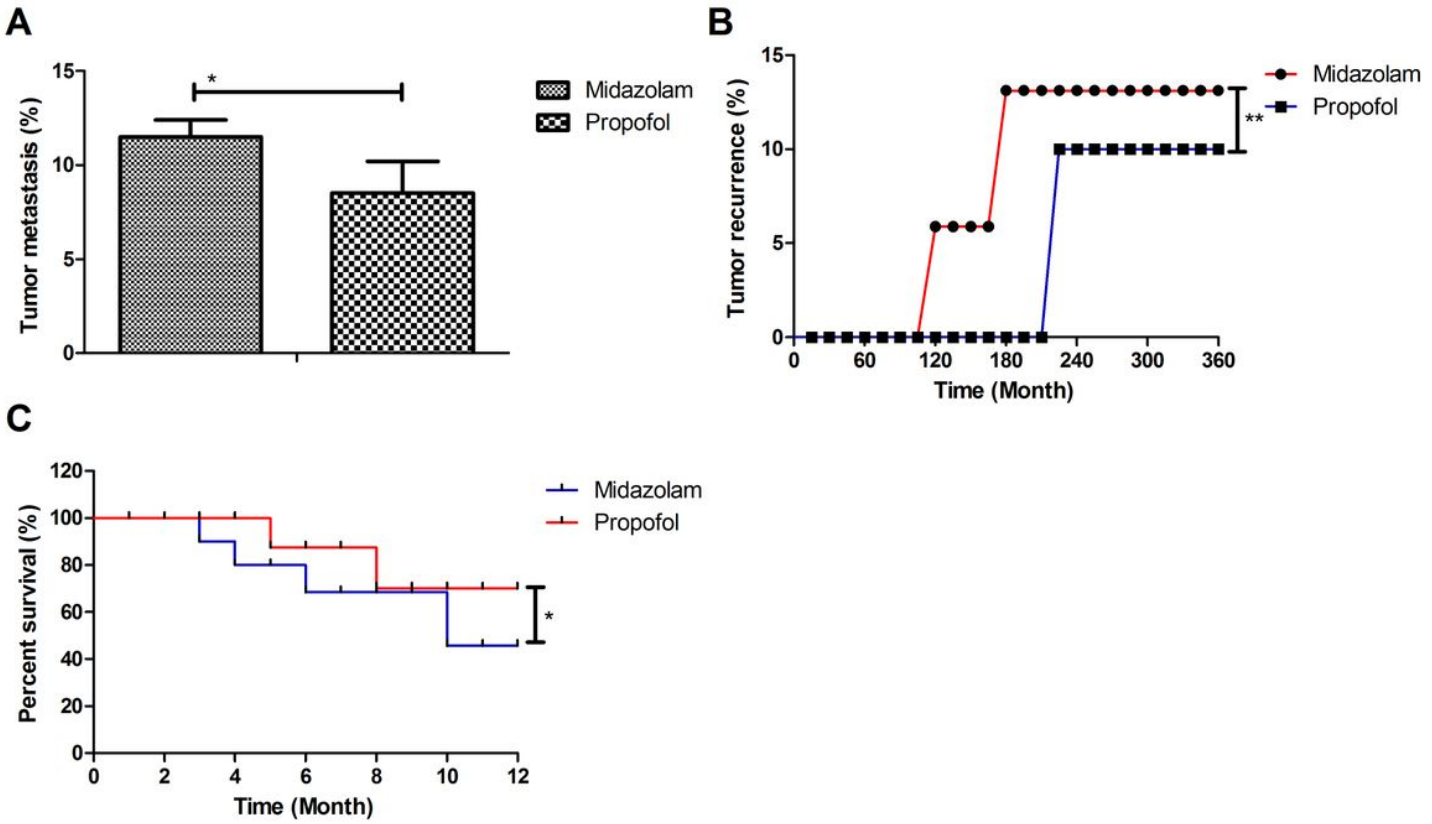

Figure 7

Effects of propofol on tumor metastasis, recurrence and survival. (A) Propofol general anesthesia inhibited gastric cancer metastasis following radical surgery compared with midazolam. (B) Gastric cancer recurrence was decreased in patients with gastric cancer by propofol general anesthesia compared with midazolam. (C) Propofol general anesthesia prolonged survival for patients with gastric cancer following radical surgery compared with midazolam. *P<0.05 and **P<0.01 vs. midazolam.

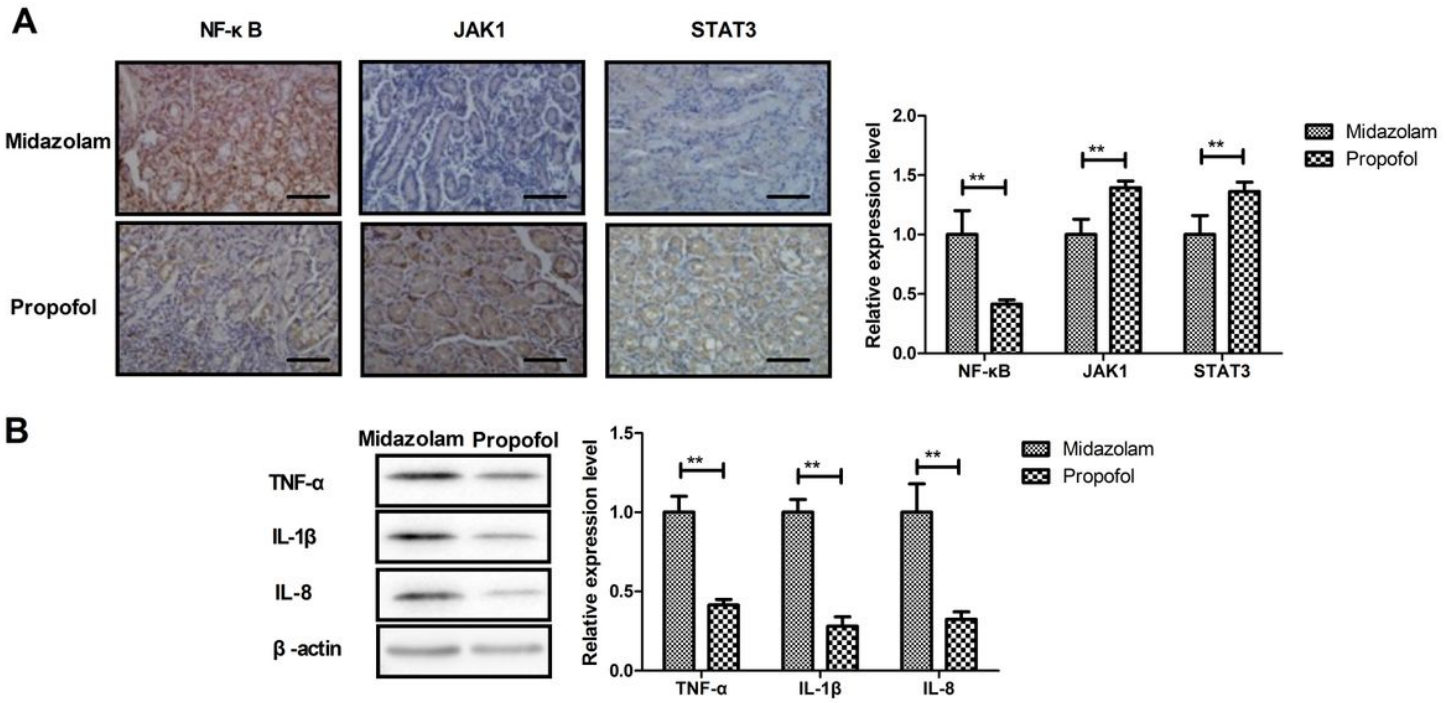

Figure 8

Effects of propofol on the NF-κB-mediated JAK1-STAT3 signaling pathway. (A) Effects of propofol on NF-κBp65, JAK1 and STAT3 expression in gastric cancer tissues, as determined by immunohistochemistry. Magnification, x40. Effects of propofol on (B) TNF-α, IL-1β and IL-8 protein expression, and

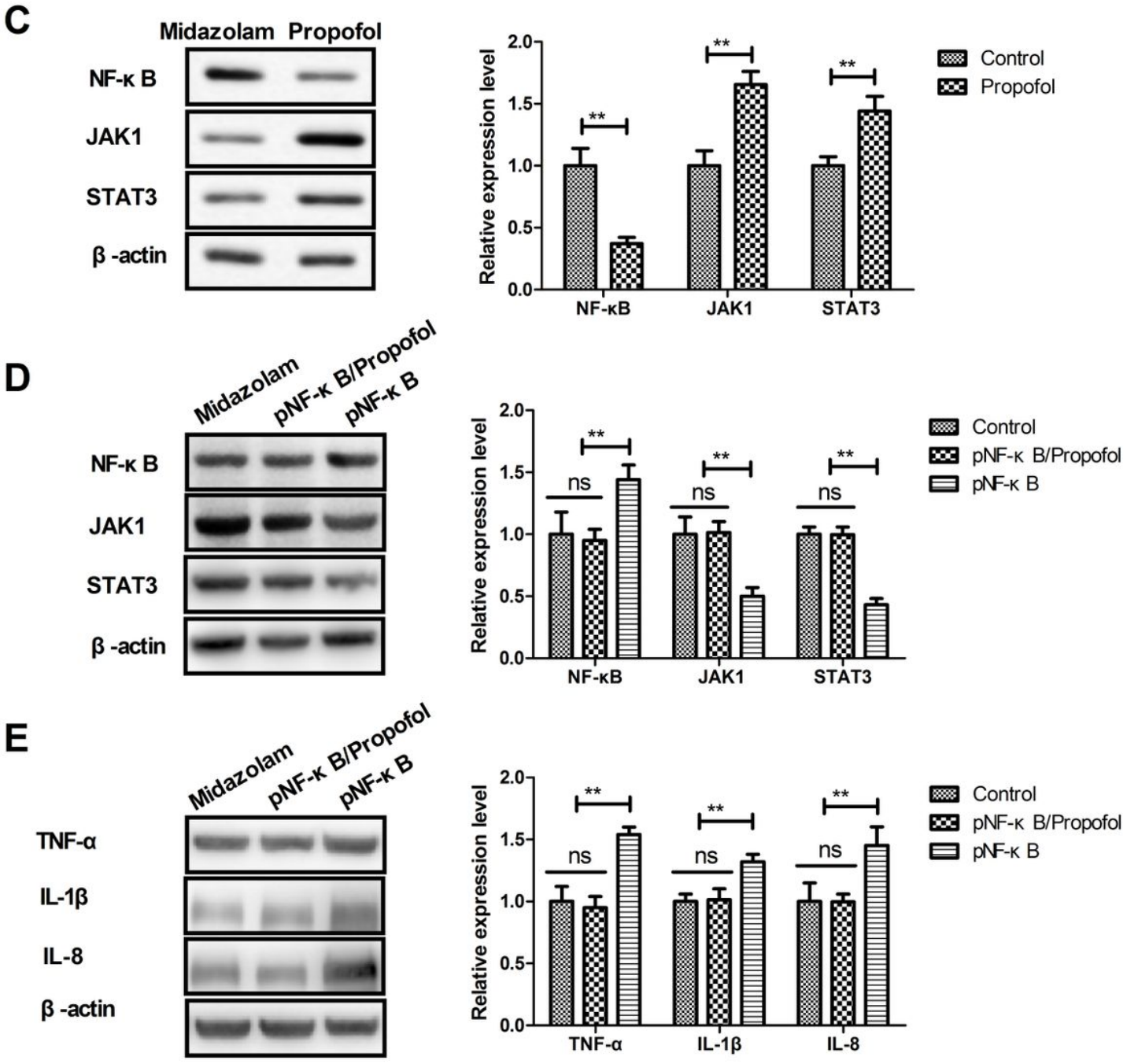

Figure 9

Effects of propofol on the NF-κB-mediated JAK1-STAT3 signaling pathway. (C) NF-κBp65, JAK1 and STAT3 protein expression in gastric cancer cells. (D) NF-κBp65 overexpression canceled propofol-induced upregulation of JAK1 and STAT3 protein expression in gastric cancer cells. (E) NF-κBp65 overexpression attenuated propofol-induced decreases in TNF-α, IL-1β and IL-8 expression in gastric cancer cells. **P<0.01. IL, interleukin; JAK1, Janus kinase 1; NF-κB, nuclear factor-κB; pNF-κB, plentivirus-NF-κBp65; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Uncroppedimagesoftheoriginalwesternblots.docx
- supplementarymaterials.docx