Propofol Inhibits Lung Cancer Invasion and Metastasis by Attenuating Icam-1/ Mmp-9 Axis

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Abstract Objective: To investigate the effect of propofol on invasion and metastasis of A549 cells and the expression of intercellular adhesion molecule 1 (ICAM-1) / matrix metalloproteinase-9 (MMP-9) axis.Methods: A549 cells were treated with gradient doses of propofol (25, 50, 100 μg/ml) and different times (12, 24 h). The influences of propofol on ICAM-1 mRNA of A549 cells were observed by qRT-PCR, and the roles of different concentrations of propofol on ICAM-1 and MMP-9 protein and invasion ability were detected by Western Blot and Transwell assay, respectively.Results: The maximum inhibitory influence of propofol was achieved in group 100 μg/ml for 24 h. ICAM-1 and MMP-9 protein in the treatment group with 25, 50 and 100 μg/ml propofol for 24 h were significantly lower than those in the control group. The number of migrated and invaded cells in the 25, 50 and 100 μg/ml propofol treatment group for 24 h was significantly lower than that in the blank group.Conclusion: ICAM-1 protein and MMP-9 protein in human lung cancer A549 cells could be down-regulated after treatment with 50 and 100 μg/ml propofol, and the invasion and metastasis ability of A549 cells could be attenuated through ICAM-1/ MMP-9 axis.

Keywords: Propofol; intercellular adhesion molecule 1; Matrix metalloproteinase 9; A549; Invasion and metastasis
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INTRODUCTION

Lung cancer is regarded as one of the most prevalent respiratory malignant tumors in the world. Its incidence and mortality rate are rising steadily [1]. Recurrence and metastasis are still the main account of death of lung cancer patients. In recent years, people have gradually realized that anesthetic drugs and anesthesia methods can also affect the postoperative outcome and long-term prognosis of tumor patients [2]. How to restrain the further development of tumor cells is a new challenge for anesthesiologists [3]. Anesthesiologists should choose anesthetics or anesthesia methods that may attenuate the growth and metastasis of tumor cells, so as to diminish the probability of tumor recurrence and metastasis.

The smooth and safe operation depends on the rational choices of anesthetic during the perioperative period. More and more basic and clinical studies have shown that perioperative anesthetics can affect the prognosis of breast cancer [4, 5], colon cancer [6], lung cancer [7], and other tumor surgery patients. Compared with general anesthesia, local anesthesia / general anesthesia can notably ameliorate the prognosis of patients with

tumor surgery [8, 9]. A retrospective clinical study showed mortality risk ratio of inhalation anesthesia compared with that of total intravenous anesthesia was 1.59 (1.30-1.95), suggesting that inhalation anesthesia has tumor promoting roles compared with intravenous anesthesia [10]. The effect of anesthetics on tumor cells includes indirect and direct function, which mainly refers to the influence of anesthetics on the immune system. For example, inhalation of isoflurane can dramatically reduce the activity and quantity of natural killer cells together with cytotoxic T cells in the circulation [11, 12], while local anesthetics have little effect on the immune system. However, there is still a lack of in vivo study of anesthetic drugs on tumor cells and the corresponding molecular mechanism is not clear.

Although propofol is a commonly used anesthetic, a large number of recent studies have reported that it plays an important role in the development of a variety of tumors, including lung adenocarcinoma [13]. Propofol can up-regulate the level of miR-486 in lung cancer cells, inhibit cell activity in a dose-dependent manner, and promote cell apoptosis, suggesting that propofol may become an ideal anesthetic for lung cancer surgery by effectively inhibiting the activity of lung cancer cells and inducing cell apoptosis [14].With the progress of research, more and more scholars have proved that the inflammatory mechanism may be closely related to tumor metastasis. For example, ICAM-1 is an essential cell surface receptor for leukocyte adhesion [15] and tumor invasion [16] in non-small cell lung cancer cells. ICAM-1 can also promote tumor cell extravasation by binding with neutrophils [17]. Matrix metalloproteinases (MMPs) are a kind of Zn2+ dependent endonuclease secreted in the non-active progenase form. MMP-9, or gelatinase B, is highly expressed in serum and lung tissue of NSCLC patients [18]. ICAM-1 can be involved in the development of early lung adenocarcinoma and regulate the biological function of lung cancer cells. The influence of propofol on ICAM-1 and MMP-9 in A549 cells remains unclear.

Local anesthetics (LAS) have antiarrhythmic and analgesic effects on voltage-gated sodium channels. Some studies have found that propofol and ropivacaine can inhibit TNF-α-induced invasion of lung adenocarcinoma cells by blocking the activation of Akt and focal adhesion kinase [19]. Other studies have found that propofol can inhibit the migration and invasion of cancer cells by down regulating TRPV6 [20]. Recent studies have shown that TRP and SOC channels, ORAI1 and matrix interaction molecule 1 (STIM1) are also closely associated with cancer cell metastasis [21]. TRP and SOC channels have become the focus of attention due to their potential roles in tumor diagnosis, prognosis and treatment targets.

In our study, the function of propofol on ICAM-1 and MMP-9 were observed to explore the function and mechanism of propofol on A549 cells. Propofol is widely used in clinical practice, and based on the in-depth study of propofol in tumor proliferation, apoptosis, invasion and metastasis, we believe that propofol will provide a new way for the prognosis of patients with cancer surgery and tumor prevention and treatment.

METHODS

Cell Lines and Cell Culture

Human cell lines A549 was purchased from the ATCC. The cell lines were routinely cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). Cells were cultured in a humidified incubator at 37 ℃ with 5% CO2.

Mtt Assay

After treatment, the cells were put back into a 37 ℃, 5% CO2 incubator. After continuous culture for 48 h, 20 uL MTT (Sigma-Aldrich) was added into each hole and incubated at 37 ℃ for 4 h. The culture medium was carefully suck out, added with 150 uL DMSO (Sigma-Aldrich) into each hole, and shook in the shaking table to make the crystal fully dissolved. The absorbance value (OD value) of each hole at 460 mm was measured by enzyme scale. The OD value was in direct proportion to the cell proliferation.

Detection of Apoptosis by Annexin V-Fitc / Pi

100 uL cell suspensions were taken and added 5 uL Annexin together with 2.5 uL PI. The mixture was shook gently, mixed well and kept away from light on ice for 10 min, then conducted flow cytometry within 30 min.

Transwell Assay

200 uL of cell suspension was taken and dropped evenly into chamber. The lower chamber was RIMP-1640 medium containing 10% fetal bovine serum. Propofol was used for treatment, and then put into the incubator for further culture, in which the migration experiment culture was 16 h, and the invasion experiment culture was 48 h. Subsequently, the cells were stained with 0.1% crystal violet for 20-30 min and observed and under the microscope with neutral resin seal.

Fluorescence Quantitative Pcr Was Used To Detect MRNA

Total RNA was extracted with trizol and 1g RNA was taken for reverse transcription at 42 ℃ for 2 min. An appropriate amount of cDNA products was taken for fluorescence quantitative PCR. The amplification conditions lasted for 5 s at 95 ℃ and 60 s at 60 ℃, with a total of 40 cycles. PCR primer sequences were retrieved from GeneBank database: ICAM-1 upstream primer 5'-TCAATGGCTTCTTTGACCAGTTCA-3',

downstream primer 5'-CTTCACATGGGCCAGCTTCACATT-3'; β-actin upstream primer 5' -CATGTACGTTGCTATCCAGGC-3 ', downstream primer
5'-CTCCTTAATGTCACGCACGAT-3'.
Internal beta actin serve as an internal reference, and mRNA expression of relative quantity was calculated, with 2-ΔΔCT to evaluate target mRNA expression level.

Western Blotting

The cells were cultured for 48 h after transfection. Total protein was extracted from the cells. About 25 μg of the sample was processed by 12% polyacrylamide gel electrophoresis and then transferred onto the PVDF membrane. The membrane was placed in the blocking solution and shaken slowly at 37 °C for 2 h. After blocking, the membrane was added with primary antibody ICAM-1 (Abcam, 1: 1000), MMP-9 (Abcam, 1:500) and GAPDH (Abcam, 1: 5000) then incubate at 4 °C overnight. The membrane was then added with secondary antibody IgG-HRP (Abcam, 1: 1000) and incubated at 37 °C for 2 h. ECL chemiluminescence was developed, and the gel imager was processed for analysis.

Statistical Analysis

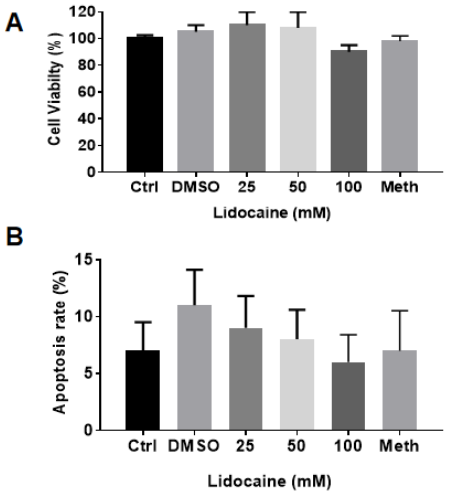
The experimental data were all expressed by mean ± SEM and analyzed by Graphpad Prism. According to the specific situation, independent sample t-test, one-way ANOVA and Bonferroni post-hoc were used to determine the difference. When $p < 0.05$, there was statistical difference.

RESULTS

Effects of Propofol on Proliferation and Apoptosis of A549 Cells

The experiment was divided into 6 groups: blank control group; DMSO Solvent Group, 25 μg/ml (L25 group), 50 μg/ml (L50 group), 100 μg/ml (L100 group) and ICAM-1 inhibitor Methimazole at a concentration of 500 μg/ml. The clinically effective concentration of propofol under intravenous anesthesia (0-100 μg/ml) had no evident influence on the activity of A549 cells (Figure 1A). In addition, there was no significant change in the proliferation level of A549 treated with ICAM-1 inhibitor Methimazole (500 μg/ml). We further investigated the roles of propofol on apoptosis of A549. 24 h treatment with propofol (0-100 μg/ml) could not dramatically increase or decrease the apoptosis rate, and Methimazole (500 μg/ml) had no function on the apoptosis rate of A549 cells. In conclusion, propofol does not affect the course of lung cancer patients by affecting its proliferation and apoptosis.

Figure 1. Effects of propofol on proliferation and apoptosis of A549 cells

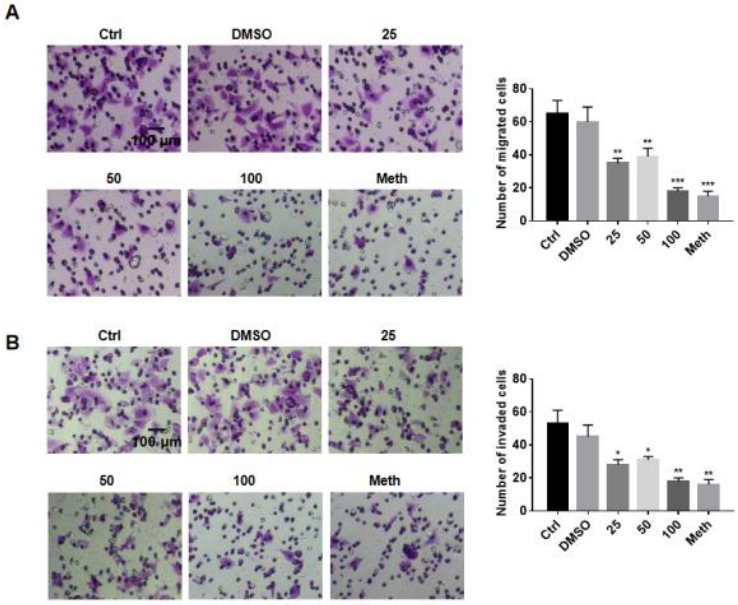


A.Effects of propofol on proliferation of A549. B. Effects of propofol on apoptosis of A549.

Effects of Propofol on Migration and Invasion of A549 Cells

Administration of different concentrations of propofol for 24 h attenuated the migration and invasion of A549 cells. The inhibitory influence of propofol increased with the concentration of propofol (0-100 μg/ml, as shown in Figure 2). As for the impact of Methimazole on the invasiveness, the results of the 500 μg/ml Methimazole treatment for 2 h showed that the amount of migrated and invaded cells in Group Me was dramatically restrained by Transwell experiment and matrix experiment (Figure 2A, B). It was suggested that inhibition of ICAM-1 in A549 cells may contribute to tumor cell migration and invasion.

Figure 2. Effects of propofol on migration and invasion of A549 cells



A. On the left, there are control group, DMSO Group, 25, 50 and 100 μg/ml propofol group, ICAM-1 inhibitor Methimazole group. On the right, the cell count of each field is shown, n = 5, (X200 times field). B. On the left, there are ccontrol group, DMSO Group, 25, 50 and 100 μg/ml propofol group, ICAM-1 inhibitor Methimazole group. On the right, the cell count of each field is shown, n = 5, (X200 times field). *p<0.05, **p<0.01, ***p<0.001.

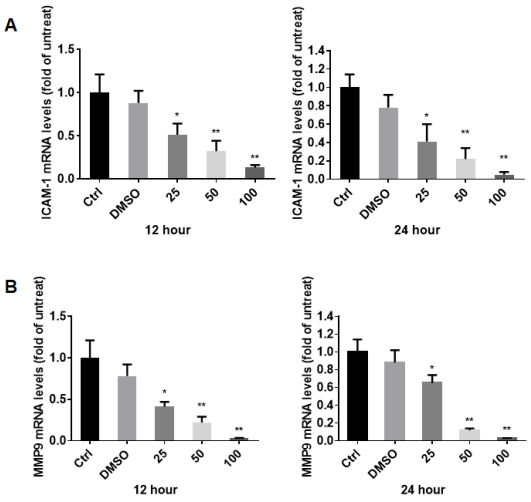
Effect of Propofol on Icam-1 Mrna and Protein Expression in A549 Cells

ICAM-1 mRNA level was significantly suppressed in the low, medium and high dose propofol groups at 12 h and 24 h, while ICAM-1 protein was reduced in the low, medium as well as high dose propofol groups at 24 h. Except for treatment for 12 h and 24 h in group D, the difference between the other groups and the blank group was statistically significant (Figure 3A).

Effect of Propofol on Mmp-9 Mrna and Protein Expression in A549 Cells

At 12 h and 24 h, MMP-9 in the middle and high dose propofol group (50 and 100 μg/ml) was dramatically decreased, indicating that propofol could inhibit MMP-9, while MMP-9 in the low dose propofol group (25 μg/ml) was enhanced at 24h, as shown in Figure 3B. It can be seen that the role of low doses of propofol on the invasion and metastasis remains to be further verified.

Figure 3. Effect of propofol on ICAM-1 and MMP-9 mRNA expression in A549 cells



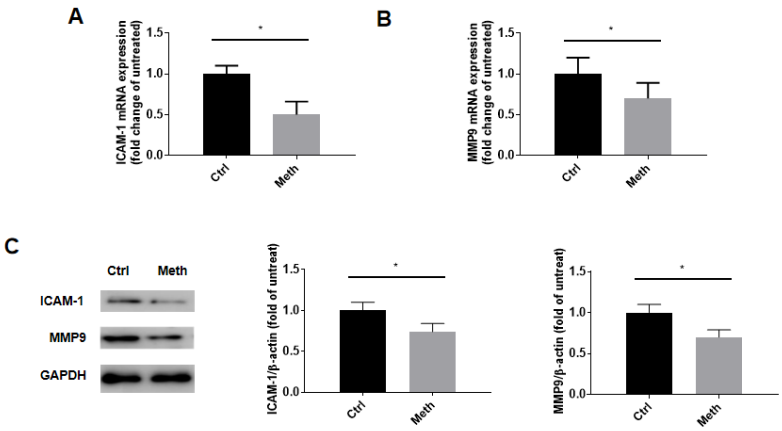
A.ICAM-1 mRNA in A549 cells for 12 h (left) and 24 h (right); B. MMP-9 mRNA in A549 cells for 12

h (left) and 24 h (right); There are ccontrol group, DMSO Group, 25, 50 and 100 μg/ml propofol group. *p<0.05, **p<0.01.

Effects of Methimazole on Icam-1 and Mmp-9 Protein Expression in A549 Cells

The expressions of ICAM-1 and MMP-9 mRNA in the Me group were markedly less than those in the blank group (Figure 4A, B). Similarly, ICAM-1 and MMP-9 protein in the Me group were 0.74±0.074 and 0.71±0.023, respectively (as shown in Figure 4C). These results suggested that methimazole can inhibit ICAM-1 and MMP9 to some extent. In addition, the decrease of ICAM-1 reduces MMP9, which indicates that ICAM-1 plays a role through MMP9.

Figure 4. Effects of Methimazole on ICAM-1 and MMP-9 expression in A549 cells

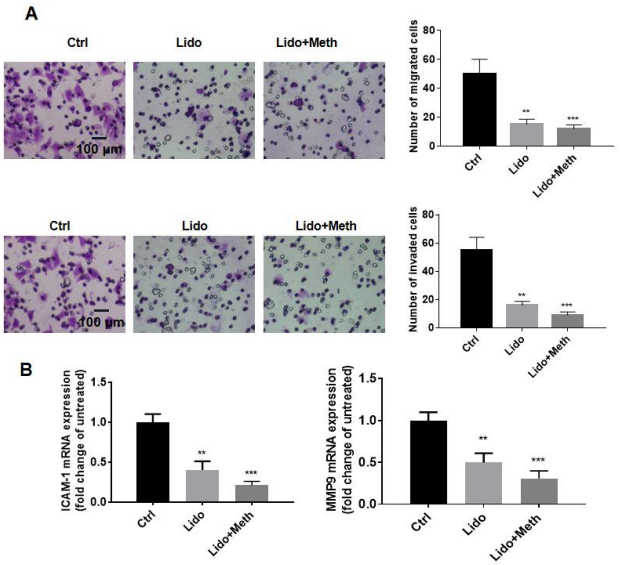


A. ICAM-1 mRNA expression in A549 cells. B. MMP-9 mRNA expressions in A549 cells. C. ICAM-1 and MMP-9 protein in A549 cells treated with propofol for 24 h. *p<0.05, **p<0.01, ***p<0.001.

Effects of Methimazole on Migration and Invasion of A549 Cells

Compared with propofol group, Methimazole combined with propofol can significantly inhibit the invasion and migration of A549 cells (Figure 5A), indicating that Methimazole plays a role in the effect of propofol on lung cancer cells. In addition, Methimazole combined with propofol significantly reduced the mRNA levels of ICAM-1 and MMP9 (Figure 5B). These results suggest that propofol inhibits the invasion and metastasis of lung cancer through ICAM-1 / MMP9 axis.

Figure 5. Effects of Methimazole on migration and invasion of A549 cells



A. There are control group, propofol Group, and propofol with Methimazole group, the cell count of

each field is shown, n = 5, (X200 times field). B. ICAM-1 and MMP-9 mRNA in A549 cells. *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

At present, the role of anesthetics in cancer is becoming more and more concerned. With the deepening of research, the role of propofol, a common intravenous anesthetic, in tumor has been gradually discovered and understood. Yang et al. [22]pointed out in their study on gastric cancer that propofol can inhibit the growth and survival of cancer cells in vitro by up-regulating ING3 expression. Du et al. [23]found that propofol could inhibit the proliferation, migration and invasion of endometrial cancer cells and promote cell apoptosis by down-regulating SOX4 expression. Liu et al. [24] pointed out that propofol may inhibit the survival, migration, invasion and EMT of lung cancer A549 cells by upregulation of miR-1284. Sun et al. [25] found that propofol could inhibit the growth, migration and invasion of lung cancer A549 cells by down-regulating miR-372. The above studies have confirmed that propofol can inhibit the malignant proliferation and metastasis of tumor cells through different mechanisms. To date, the effect of propofol on ICAM-1 in A549 tumor cells has not been clarified. In our study, 549 cell line was cultured in vitro to determine the effect of propofol at different concentrations and action time on ICAM-1 mRNA and protein in A549 cells. The results showed that the ICAM-1 mRNA was markedly decreased in the low, medium and high dose propofol groups at 12 h and 24 h, and the ICAM-1 was decreased in the low, medium as well as high dose propofol groups at 24 h, indicating that propofol has an inhibitory influence on the ICAM-1 level in time-dose dependence.

MMPs can degrade and destroy basement ECM, promote the release of vascular surface growth factors and participate in tumor angiogenesis and tumor growth [26]. MMP-9 is secreted out of cells in the form of proenzyme, mainly in mononuclear macrophages [27]. MMP-9 is considered to be a considerable molecule for the invasion and metastasis of lung cancer cells, and the level of MMP-9 in the serum and lung tissue of NSCLC patients is markedly increased. Diminishing the expression of MMP-9 can reduce the early metastasis of lung cancer and damage the vascular system of the tumor, thus achieving an anti-tumor effect [28]. Meta-analysis showed that positive MMP-9 immunohistochemical staining was lower than that of patients with negative results, suggesting the considerable role in the prognosis of NSCLC patients. In this study, MMP-9 was significantly decreased in the propofol group (50 and 100 μg/ml), while MMP-9 in the low dose propofol group (25 μg/ml) was increased. Propofol at 0~40 μg/ml promoted proliferation and invasion of GC-SD cells and inhibited apoptosis in dose-time dependent manner. Low dose (15, 20, 25 g/mL) propofol promoted MMP-9 in A549 cells for 24 h and inhibited it for 48 h [29]. It can be seen that medium and high doses of propofol have more precise inhibitory function of tumor cells, while the influence of low doses of propofol of tumor cells remains to be further verified. In addition to the dose factor, whether propofol has different function on metastasis of different tumor cells is worth exploring.

Studies on the mechanism found that knockout of ICAM-1 could reduce the invasion of lung cancer cells through the AKT/MMPs, the down-regulation of ICAM-1 could diminish glycerol uptake and mitochondrial ATP generation in tumor cells [30]. It can delay the growth of tumor cells, which may be related to the blocking of HIF-1α /VEGF pathways [31]. This study showed that treatment with 50 and 100 μg/ml propofol could down-regulat ICAM-1 mRNA, ICAM-1 protein and MMP-9 protein for 24 h[32]. 500 μg/ml Methimazole can markedly inhibit tumor cell invasion and ICAM-1 protein for 2 h [33].

This study showed that 50 and 100 μg/ml propofol could down-regulate the ICAM-1 and MMP-9, inhibit the invasion, and provide experimental basis for the selection of anesthesia methods and drugs for patients with tumor surgery. The specific mechanism and pathway of propofol's down-regulation of ICAM-1 and MMP-9, as well as animal experiments and clinical observation of propofol's effect on tumor metastasis, are worthy of further discussion.

CONCLUSION

ICAM-1 protein and MMP-9 protein in human lung cancer A549 cells could be down-regulated after treatment with 50 and 100 μg/ml propofol. The invasion and metastasis ability of A549 cells could be attenuated by propofol through ICAM-1/ MMP-9 axis.

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