

Original Research Article

Effect of propofol on lung metastasis of circulating tumor cells after colorectal cancer surgery

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Abstract

Purpose: To investigate the effect of propofol on lung metastasis of circulating tumor cells (CTCs) after colorectal cancer (CRC) surgery, and the mechanism involved.

Methods: Specific-pathogen-free (SPF) male BALB/c mice ($n = 30$; mean age = 7 ± 1 weeks; mean weight = 19 ± 3 g) were used for this study. To establish mouse model of CRC, 100 μ L of CRC (CT26) cells was injected into the caudal vein of each mouse. Three groups of 10 mice were used: control, 5 μ L/mL propofol, and 10 μ L/mL propofol groups. Changes in pulmonary superficial nodules of mice lungs were determined. Colorectal cancer cell (CT26) proliferation and apoptosis were measured using Ki-67 immunohistochemical staining and in situ terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) assays, respectively.

Results: The number of pulmonary nodules and proportion of proliferating cells (Ki-67 expression level) were significantly higher in 5 μ L/mL propofol-treated mice, relative to control, but markedly lower in 10 μ L/mL propofol group than in 5 μ L/mL propofol group ($p < 0.05$). Although apoptotic index increased in 5 μ L/mL propofol group, cell apoptosis was comparable among the groups ($p > 0.05$).

Conclusion: These results suggest that propofol promotes pulmonary metastasis of CTCs after CRC surgery via stimulation of CTC proliferation in the lungs. Therefore, caution should be exercised in the use of propofol in colorectal cancer surgery.

Keywords: Apoptosis, Colorectal cancer, Circulating tumor cell, Lung metastasis, Propofol

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INTRODUCTION

The number of tumor patients has increased significantly over time, with malignant tumor becoming one of the most important causes of death globally. Colorectal cancer (CRC) is a type of cancer prevalent among the Chinese. It is characterized by high incidence and mortality which have continued to rise on annual basis [1]. Colorectal cancer (CRC) patients often miss

diagnosis and treatment because early symptoms of the disease are usually not obvious. Prognosis is also usually poor, since CRC patients are either in the middle or advanced stage of the disease at the time of diagnosis [2].

Metastasis seriously affects effectiveness of cancer treatment. At present, surgical resection is the main treatment strategy for CRC. However, studies have shown that even after complete

resection of primary tumor, undetected micro-metastases are capable of causing recurrence, thereby making postoperative metastasis and recurrence the most important factors that affect prognosis of CRC [3]. Colorectal cancer (CRC) cells directly or indirectly enter the systemic circulation through the lymphatic route, thereby colonizing distant tissues/organs, and eventually proliferating to form metastasis focus [4]. Circulating tumor cells (CTCs) are tumor cells that are passed into the blood from primary or metastasis focus [4]. They are responsible for distant metastasis.

Anesthesia, an indispensable part of perioperative period, has close relationship with patients' immune function and postoperative tumor recurrence and metastasis [5]. Propofol is a frequently used intravenous anesthetic with the advantages of quick induction time, minimal toxicity and low side effects. Moreover, propofol exerts immunomodulatory, antioxidant, neuroprotective, and anxiolytic effects. Studies have shown that propofol has little effect on immune function of patients undergoing CRC surgery, but it has regulatory effect on CRC formation [6]. This study investigated the effect of propofol on lung metastasis of CTCs after CRC surgery, as well as the mechanism involved.

EXPERIMENTAL

Materials

Colorectal cancer cell line (CT26) was bought from Shanghai Keyuandi Biotechnology Co. Ltd. Carbon dioxide incubator (HH.CP-T) was purchased from Beijing Hengtai Fengke Test Equipment Co. Ltd. Constant temperature water bath (HWT-6B) was product of Tianjin Hengao Technology Development Co. Ltd. Full-automatic tissue dehydrator (TSJ-1C) was obtained from Shanghai Kehuai Instrument Co. Ltd. Paraffin slicer (RM2245) was bought from Beijing Shengke Xinde Technology Co. Ltd. Fetal bovine serum (FBS) was purchased from Shanghai Jianglin Biotechnology Co. Ltd. Trypsin was product of Shanghai Ruji Biotechnology Co. Ltd., while propofol was bought from Sichuan Guorui Pharmaceutical Co. Ltd.

Cell culture

Colorectal cancer cells (CT26) were cultured in DMEM supplemented with 10 % FBS and 1 % penicillin/streptomycin solution at 37 °C for 24 h in a 5 % CO₂ incubator until the cells attained 85 % confluency. The resultant adherent cells were trypsinized with 2 mL of 0.25 % trypsin, and centrifuged at 1500 rpm for 10 min. The cell

mass was then reduced to single cell suspension which was inoculated into a 25 cm² culture dish at a density of 2 x 10⁴ cells/well (100 µL per well) and incubated. When the cells attained 60 - 70 % confluency, they were treated with serum-free medium and varied concentrations of propofol for 24 h. Normal cell culture without propofol served as control group. Cells in logarithmic growth phase were counted and seeded in 6-well plates at a concentration of 4 x 10⁴ cells/well. Each group had 4 replicates.

Mice

Specific-pathogen-free (SPF) male BALB/c mice (n = 30) aged 6 to 8 weeks, and weighing 16 - 22 g (mean age = 7 ± 1 weeks; mean weight = 19 ± 3 g) were obtained from School of Medicine, Shantou University, Guangdong, China. They were exposed to equal durations of light and darkness at 25 °C and humidity of 45 - 55 %. The study protocol was approved by the Institutional Animal Ethics Committee (approval no. 2019-03-015), and the study procedures used conformed with the directives of AAALAC [7].

Study design

A mouse model of CRC was established by injecting 100 µL of CT26 cells into the caudal vein of each mouse. Three groups of 10 mice were used: control, 5 µL/mL propofol, and 10 µL/mL propofol groups. Mice in propofol groups were intraperitoneally injected with 5 and 10 µL/mL of propofol. The mice were then fed adaptively for 1 week and euthanized. Fresh lung from each mouse was fixed in 10 % formaldehyde solution and pulmonary superficial nodules of mice in each group were counted. Pulmonary superficial nodules > 10, but not filled with lungs were counted as 15, while pulmonary superficial nodules > 10 and filled with lungs were counted as 20.

Immunohistochemical staining

Cell proliferation was determined using Ki-67 immunohistochemical staining method. The cells were plated on gelatin-coated coverslips, followed by fixation in 4 % paraformaldehyde in an oven at 60 °C for 20 min, and washing thrice in phosphate-buffered saline for 5 min. The slides were deparaffinized with xylene, dehydrated with ethanol, and exposed to briefly 3 % H₂O₂ to minimize background stain. Following treatment with citric acid buffer for 15 min in a microwave oven, the slides were exposed to Ultra V Block stain at laboratory temperature for 10 min. Thereafter, rabbit anti-Ki-67 monoclonal antibody was added, and the slides were

incubated for 2 h. Ultra-vision LP System was used for measurement of binding of antibody. The slides were stained with DAB, followed by counterstaining using hematoxylin. The proportion of positive cells was calculated. Results < 6 % were considered *low*, while counts of 6 – 10 % and > 10 % were considered *intermediate* and *high*, respectively. The Ki-67 expression level in each group of cells was determined using western blotting.

Apoptosis assay

Cell apoptosis was measured using TUNEL assay. The cells were plated on gelatin-coated coverslips, fixed with 4 % paraformaldehyde in an oven at 60 °C for 20 min, and washed thrice with PBS for 5 min. After blocking with 1 % bovine serum albumin for 60 min at laboratory temperature, 1° antibody was added and further incubated for 12 h at 4 °C. After washing thrice with PBS, FITC-labeled secondary antibody was added and incubated for 1 h at room temperature. The slides were thereafter stained with 4', 6-diamidino-2-phenylindole (DAPI) for 15 min and washed for 5 min. The slides were observed under a fluorescence microscope in the dark, and photographed after the addition of anti-fluorescent quenching agent. The apoptotic index of each group of cells was calculated.

Statistical analysis

Results are presented as mean ± SEM, and were statistically analyzed with SPSS (20.0). Group comparison was done using Duncan's multiple range test and Student's *t*-test. Values of $p < 0.05$ were taken as indicative of statistically significant differences.

RESULTS

Changes in the number of pulmonary nodules of mice

The number of pulmonary nodules was significantly higher in 5 µL/mL propofol-treated mice, relative to control, but was markedly lower in 10 µL/mL propofol group than in 5 µL/mL propofol group ($p < 0.05$; Table 1).

Effect of propofol on CT26 cell proliferation

As presented in Table 2, the proportion of proliferating cells (Ki-67 expression level) was significantly higher in 5 µL/mL propofol-treated mice, relative to control, but was markedly lower in 10 µL/mL propofol group than in 5 µL/mL propofol group ($p < 0.05$).

Table 1: Changes in the number of pulmonary nodules of mice (mean ± SD, n = 10)

Group	No. of pulmonary nodules
Control	4.26 ± 0.93
Propofol (5 µL/mL)	16.52 ± 5.87 ^a
Propofol (10 µL/mL)	12.23 ± 6.22 ^{a,b}
<i>f</i>	15.69
<i>P</i> -value	< 0.001

^a $P < 0.05$, vs control; ^b $p < 0.05$, vs 5 µL/mL propofol group

Table 2: CT26 cell proliferation (mean ± SD, n = 4)

Group	Proportion of proliferating cells (%)
Control	20.67 ± 10.36
Propofol (5 µL/mL)	51.52 ± 5.74 ^a
Propofol (10 µL/mL)	39.53 ± 13.47 ^{a, b}
<i>F</i>	9.02
<i>P</i> -value	0.007

^a $P < 0.05$, vs control; ^b $p < 0.05$, compared with 5 µL/mL propofol group

Effect of propofol on CT26 cell apoptosis

Although apoptotic index was increased in 5 µL/mL propofol group, percentage cell apoptosis was comparable among the groups ($p > 0.05$; Table 3).

Table 3: Comparison of cell apoptosis among the groups (mean ± SD, n = 4)

Group	Apoptotic index
Control	5.02 ± 3.46
Propofol (5 µL/mL)	7.23 ± 3.58
Propofol (10 µL/mL)	5.56 ± 2.17
<i>F</i>	0.54
<i>P</i> -value	0.601

DISCUSSION

Colorectal cancer (CRC) is a tumor with high incidence. In-depth understanding of the biological behavior of CRC has led to the development of individualized comprehensive treatment comprising surgery, chemoradiotherapy, endocrine therapy and molecular targeted therapy. However, postoperative metastasis and recurrence have limited the effectiveness of such treatment plans [8]. Statistics show that 15 % of CRC patients eventually develop lung metastasis. Metastasis involves the breaking away of tumor cells from the primary tumor into the systemic circulation. Most of the tumor cells get recognized and phagocytized by the body's immune system or apoptosis, while the few remaining tumor cells spread through the blood circulation in a dormant state known as CTCs [9].

The CTCs represent an intermediate stage of metastasis. They exist individually in the blood and cluster to form circulating tumor microemboli (CTM). Moreover, CTCs and CTM have become invaluable resources for research on various aspects of tumors. Most tumor cells that enter blood vessels get phagocytized by the body's autoimmune system; others undergo self-apoptosis after leaving the primary site, while the remaining tumor cells remain in a relatively static state, thereby playing a role in blood circulation, as well as becoming an important factor in tumor recurrence and metastasis [10]. Studies have shown that in an appropriate environment, CTCs become planted and transformed into tumor stem cells which may form the basis for local recurrence and distant metastasis [11]. Circulating tumor cells (CTCs) have prognostic significance in patients with early and metastatic cancers.

Anesthesiology is a perioperative discipline. The effect of anesthesia on immune function of patients may be an important factor that affects prognosis of tumor patients. Propofol is a commonly used phenolic intravenous anesthetic with rapid effect and short duration of action. It is widely used in the induction and maintenance of general anesthesia and auxiliary sedation of patients with regional block. Propofol has been reported to enhance the antioxidant capacity of cells via inhibition of cell apoptosis [12]. Studies have shown that propofol dose-dependently inhibited the invasion and migration of MCF7 cells [13]. This study investigated the effect of propofol on lung metastasis of CTCs after CRC surgery and the mechanism involved.

As a rapid mechanism of tissue growth, cell proliferation leads to an exponential increase in cell number. The compound Ki-67 is an intracellular protein that binds DNA in the nucleolar region. The protein has a short half-life and is not affected by other growth factors [14]. It has been reported that Ki-67 antibody has significant influence on a variety of tumors, and it is of great significance in the quantitative analysis of tumor growth fraction, tumor grading, degree of hyperplasia and prognosis [15]. The results of previous studies showed that the expression of Ki-67 was significantly upregulated in CTCs, but it was significantly downregulated at G0 phase of cell division cycle [16]. There is a link between Ki-67 level and tumor cell proliferative potential. Indeed, this link is used routinely used as an index of proliferation in pathological studies. It is one of the most reliable indices for determination of proliferative capacity of primary tumor cells. The results of this study indicate that propofol may promote the

proliferation of CTCs in the lungs and markedly increase the number of pulmonary metastatic nodules.

Apoptosis is a process of programmed cell death. At present, it is believed that induction of apoptosis is one way of treating cancer [17]. In this study, propofol had no significant effect on the apoptosis of colorectal CTCs.

CONCLUSION

The results obtained in this study indicate that propofol promotes pulmonary metastasis of CTCs after CRC surgery via stimulation of CTC proliferation in the lungs. Therefore, caution should be exercised in the use of propofol in colorectal cancer surgery.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shi Wu designed the study, supervised the data collection, and analyzed the data. Shiting Jia interpreted the data and prepared the manuscript for publication. Houwen Long supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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