Study on the mechanism of oral administration of tetrandrine during neoadjuvant chemotherapy for colon cancer

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Abstract. Colon cancer is a digestive tract tumor with one of the highest frequencies worldwide, and with a high fatality rate. The present study aimed to investigate the expression and regulation of inflammatory factors in tumor tissues, monocytes and blood samples in patients with colon cancer (n=46) following treatment with neoadjuvant chemotherapy combined with tetrandrine. All patients underwent tumor resection after neoadjuvant chemotherapy. In the experimental group, 20 cases took tetrandrine during chemotherapy, while in the control group, 26 cases underwent chemotherapy without tetrandrine. Reverse transcription-quantitative PCR and western blotting were performed to detect the mRNA and protein expression levels of TNF-α. ELISA was used to detect the cytokine/chemokine expression levels [IL-15, IL-1β and IL-6, as well as chemokine ligand (CCL)2, CCL5, CCL20, chemokine (C-X-C motif) ligand CXCL1, CXCL2, CXCL3, CXCL5 and CXCL10 in the culture supernatant of colon cancer tissue]. Human blood mononuclear cells were cultured, and cytokine release was determined by ELISA. Cell proliferation ability was assessed using the MTT assay. Compared with the control group, the mRNA and protein expression levels of tumor necrosis factor-α (TNF-α) were downregulated in tumor tissues and serum and the serum levels of IL-15, IL-1β and IL-6 were relatively low in the experimental group. The expression levels of CCL5, CXCL2 and CXCL10 in the supernatant of cancer tissue culture were relatively low, compared with the conditioned medium prepared from tumor tissues of patients not receiving tetrandrine. When the cultured blood mononuclear cells were stimulated by the tissue culture supernatant from the experimental group, less IL-15, IL-1β and IL-6 were released, compared with the medium of tumor tissues of patients not taking tetrandrine. Following stimulation with the tissue culture supernatant from the experimental group, the proliferation ability of HCT116 colon cancer cells significantly declined. During chemotherapy of patients with colon cancer, tetrandrine may inhibit the expression of TNF-α in cancer tissues and blood, reduce the release of inflammatory factors and chemokines and decrease cancer cell proliferation. These findings provide a theoretical basis for the treatment of colon cancer in the clinic.

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Introduction

In recent years, the incidence of colon cancer has increased year by year in China, and currently ranks third (with an incidence rate of ~28/100,000), only after lung and gastric cancers (1). At present, the clinical treatment of patients with colon cancer is still based on the comprehensive treatment mode of surgical resection plus adjuvant chemoradiotherapy. The FOLFOX (oxaliplatin + calcium leucovorin + 5-fluorouracil, i.e., L-OHP+CF+5-Fu) regimen is currently a common chemotherapy regimen used to treat colon cancer. Colon cancer has a relatively low survival rate after chemotherapy (the five-year survival rate is ~31% in China) (2), which is detrimental to human health (2,3). In 2011, there were >12 million newly diagnosed cases of colon cancer worldwide, ranking it third for all malignant tumors in men, and the incidence in females was second only to breast cancer (4). At an early stage, colon cancer lacks typical signs and symptoms, and most patients are at the advanced stages (often with metastases) at the point of diagnosis, missing the opportunity for optimum treatment efficacy (5). The treatment of colon cancer is mainly based on surgical resection, combined with radiotherapy, chemotherapy and molecular-targeted therapy (6,7). Hence, it is of great importance to develop strategies for the prevention and treatment of colon cancer (8).

Tumor necrosis factor-α (TNF-α) is an important pleiotropic cellular signaling protein (cytokine), which has been associated with systemic inflammatory responses in the development of autoimmune diseases, including diabetes, and various cancers, such as colon, bladder, liver, stomach and breast cancer (9,10). TNF-α also exerts functions in angiogenesis

by promoting endothelial cell proliferation and increasing the expression of pro-angiogenic factors (11,12). In addition, TNF-molecules (such as E-cadherin and β-catenin) (13,14). In numerous malignant tumors (such as colon and kidney cancer), elevated TNF-α levels have been detected, which predicted poor prognosis of patients (15,16). In addition, TNF-α binds directly to its receptor, which leads to abnormal activation of the NF-κB, JNK and MAPK signaling pathways, further resulting in abnormal expression of numerous chronic inflammatory genes and the release of inflammatory factors and chemokines (such as semaphoring 3D and MMP3) (17-20).

Tetrandrine (Tet) is a bisbenzylisoquinoline alkaloid calcium antagonist, which can reduce the total peripheral vascular resistance and lower the blood pressure (21) (no reflex heart rate increases when blood pressure is reduced), increase cardiac output and muscle relaxation, is antipyretic, has analgesic and anti-inflammatory effects and exerts certain effects on various tumor cells (such as pituitary adenoma and nasopharyngeal carcinoma cells) (22,23). In addition, tetrandrine has been used for the treatment of lung cancer, in combination with low-dose radiation (24,25). Furthermore, tetrandrine can also be used in the treatment of patients with simple silicosis and coal sputum lung, early mild hypertension, rheumatic pain, joint pain and neuralgia (26-28). Studies have demonstrated that as a calcium antagonist, tetrandrine can directly and effectively inhibit intracellular calcium-dependent TNF-α production and can also indirectly inhibit the expression and production of TNF-α by cells (such as glial cells-neurons and monocytes) (29,30). However, whether the application of tetrandrine in the adjuvant chemotherapy of colon cancer is effective and efficient has not been fully elucidated.

In the present study, the efficacies and underlying mechanisms of tetrandrine combined with neoadjuvant chemotherapy in colon cancer were explored. Reverse transcription-quantitative (RT-q) PCR, western blotting, MTT assays and ELISA were performed to detect indicators in tumor tissue and blood samples from patients with colon cancer, subjected to tetrandrine combined with neoadjuvant chemotherapy.

Materials and methods

Patients and ethics. In total 46 patients with colon cancer with neuropathic pain who were admitted to the First Hospital of Zibo City (Shandong, China) between December 2015 and August 2018 were enrolled in the present study. All patients underwent tumor resection after neoadjuvant chemotherapy for colon cancer. Among these patients, 26 patients who did not take tetrandrine during chemotherapy were included in the control group, while 20 patients who had tetrandrine during chemotherapy were assigned to the experimental group. Tumors and blood samples (10 ml) were collected from all the patients and the paraneoplastic negative tissues were collected as control. In the control group, there were 16 males and 10 females, age range 35-68 years, median age of 52.2 years; while in the experimental group, there were 12 males and 8 females, age range 33-69 years and median age of 51.8 years. All of the patients suffered from first-time disease onset and were diagnosed and evaluated by pathologists and oncologists from the First Hospital of Zibo City. All the patients were subjected to 5-Fu-based adjuvant chemotherapy. Prior written and informed consent were obtained from every patient and the study was approved by the Ethics Review Board of the First Hospital of Zibo City.

Low-dose chemotherapy regimens and inclusion/exclusion criteria. Patient inclusion criteria were as follows: i) Patients who received a cycle of the L-OHP+CF+5-Fu regimen every 3 weeks [oxaliplatin (130 mg/m^2, day 1) + calcium leucovorin (200 mg/m^2, days 1-5) and 5-Fu (300 mg/m^2, days 1-5)]; ii) patients that had at least 1 measurable lesion before receiving L-OHP+CF+5-Fu adjuvant chemotherapy; iii) patients who did not receive chemotherapy within 6 months before receiving adjuvant chemotherapy or radiation therapy within 3 months of having received adjuvant chemotherapy; iv) patients who received 4 cycles of chemotherapy; and v) patients that after receiving adjuvant chemotherapy, based on a doctor's assessment could have the tumor removed. Subjects that did not meet the inclusion criteria were excluded from the present study. Patients in the experimental and treatment groups were subjected to the same basic treatments, mainly hydration diuretic treatment and routine liver protection, antiemetic, nutritional support and symptomatic treatment. General symptomatic treatment included a high-quality protein, low-salt and low-sodium diet; adequate energy and vitamins and appropriate exercise. During the chemotherapy period, the patients from the experimental group took tetrandrine tablets (specification: 20 mg/tablet; National Pharmaceutical Standard H20063338; Beihai Sunshine Pharmaceutical Co., Ltd.), according to the recommended dosage (60 mg 3 times per day, during chemotherapy), while the patients in the control group did not take the drug. The patients were followed-up after each chemotherapy.

Specimen collection. Following completion of chemotherapy, the patients underwent tumor resection and the specimens were collected. The freshly resected tumor tissues were kept at 4˚C. The tissue sample was cut into 1 cm x 1 cm pieces with surgical scissors in a sterile environment and stored in liquid nitrogen. The remaining tumor tissue was cut into 1 cm x 1 cm pieces (weight and dimensions were the same so that the tissue could fully release inflammatory factors), which were added into 1 ml complete DMEM medium containing 10% FBS (both Thermo Fisher Scientific, Inc.), supplemented with 100 U/ml penicillin and 0.1 mg/ml streptomycin, and incubated in a 37˚C (5% CO_2) incubator. After 24 h, the supernatant was collected and subjected to centrifugation at 1,000 x g at 4˚C for 15 min. The supernatant was collected in a 1.5 ml centrifugation tube and stored at -20˚C.

Peripheral blood (10 ml) was collected from all patients on the day of chemotherapy under fasting conditions. Monocytes were obtained with the Human Monocyte Separation kit (cat. no. P9260; Beijing Solarbio Science & Technology Co., Ltd.), according to the manufacturer's instructions. Some of the blood sample was centrifuged at 1,000 x g for 10 min, which led to separation of the sera and red blood cells and the serum was collected and stored at -20˚C.

Cell culture. The monocytes were cultured in a 37˚C, 5% CO_2 incubator for 1-2 h. Then adherent cells which represented the mononuclear cells were cultured with DMEM culture medium

containing 10% FBS (Thermo Fisher Scientific, Inc.) for 24 h before experiments. The HCT116 cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in a 37˚C, 5% CO_2 incubator with DMEM culture medium (Thermo Fisher Scientific Inc.) containing 10% FBS for 72 h prior to the MTT assay. HCT116 cells were challenged with the tissue culture supernatant a 37˚C for 24 h.

RT-q PCR. Total RNA was extracted from the serum and tissue samples with TRIzol® (cat. no. R0016; Beyotime Institute of Biotechnology) according to the manufacturer's instructions. cDNA was obtained by reverse transcription with the TIANScript II cDNA First Strand Synthesis Kit (Tiangen Biotech Co., Ltd.), according to the manufacturer's instructions. The RT-q PCR was performed with the SuperReal PreMix (SYBR Green) (cat. no. FP204; Tiangen Biotech Co., Ltd.) on the PCR-iQ5 RT-qPCR instrument (Bio-Rad Laboratories Inc.). The primer sequences were as follows: TNF-α forward, 5'-AGACCCTCACACTCAGATCATCTTC-3' and reverse 5'-CTCCGCTTGGTGGTTTGCTA-3'; and β-actin forward 5'-CACCAGGGCGTGATGGT-3' and reverse, 5'-CTCAAACATGATCTGGGTCAT-3'. The 20-μl PCR system consisted of 10 μl RT-qPCR-Mix, 0.5 μl primer each, 2 μl cDNA and 7 μl ddH_2O. The thermocycling conditions used were as follows: 95˚C for 3 min; 30 cycles of 94˚C for 15 sec, 58˚C for 30 sec and 72˚C for 1 min; followed by 72˚C for 5 min. The expression levels of the target gene were calculated using the $2^{-\Delta\Delta Cq}$ method (31). β-actin was used as the internal control.

Western blotting. Monocytes ($1x10^6$ cells; after being cultured for 4 h) and tissues were lysed with lysis buffer (Beyotime Institute of Biotechnology), according to the manufacturer's instructions. Protein concentration was determined using the bicinchoninic acid (BCA) method. Then, 20 μg protein per lane was separated with 10% SDS-PAGE and then electronically transferred onto PVDF membranes. Following blocking with 5% nonfat milk at room temperature for 1 h, the membrane was incubated with rabbit anti-human anti-TNF-α (1:1,000; cat. no. ab6671; Abcam,) or rabbit anti-human anti-β-actin (1:5,000; cat. no. ab129348; Abcam) primary antibody at 4˚C overnight. The membrane was then incubated with the goat anti-rabbit secondary antibody (1:3,000; cat. no. ab6721; Abcam) at room temperature for 1 h. Color development was performed with the ECL method (cat. no. ab65623; Abcam) and the protein bands were acquired and analyzed with the Image Lab v.3.0 software (Bio-Rad Laboratories, Inc.). β-actin was used as the loading control.

ELISA. Blood samples were centrifuged at 1,000 x g for 10 min for separating sera and red blood cells. The serum and tissue culture supernatants (after culturing with 1 ml complete medium supplemented with double antibodies, at 37˚C for 12 h) were used as specimens. ELISA was performed with the following kits, according to the manufacturer's instructions: Human TNF-α ELISA kit (cat. no. ab181421; Abcam), human IL-1β ELISA kit (cat. no. ab100562; Abcam), human IL-6 ELISA kit (cat. no. ab46027; Abcam), human IL-15 ELISA kit (cat. no. ab218266; Abcam), human chemokine ligand (CCL)2 ELISA kit (cat. no. ab179886; Abcam), human CCL20 ELISA kit (cat. no. ab178015; Abcam), human CCL5 ELISA kit (ab174446; Abcam), human chemokine (C-X-C) motif ligand (CXCL) 1 ELISA kit (cat. no. ab190805; Abcam), human CXCL2 ELISA kit (cat. no. ab184862; Abcam), human CXCL3 ELISA kit (cat. no. ab234574; Abcam), human CXCL5 ELISA kit (cat. no. ab212163; Abcam) and human CXCL10 ELISA kit (cat. no. ab83700; Abcam). Standard and sample wells were set separately. The standard wells were loaded with 50 μl standards at indicated concentrations. The sample wells were added with 10 μl sample, followed by the addition of 40 μl dilution. Nothing was added into the blank well. Except for the blank wells, 100 ml horseradish peroxidase (HRP)-labeled detection antibody was added to the standard and sample wells, which were then sealed with a sealing membrane and incubated for 1 h. After washing, 50 μl substrate A and B each was added into each well, followed by incubation at 37˚C for 15 min. Then 50 μl stop solution was added into each well. The optical density (OD) values at 450 nm were measured with the GloMax 20/20 luminometer (Promega Inc.) within 15 min.

MTT assay. HCT116 colon cancer cells (commonly used for their highly invasive and proliferative nature) in the logarithmic growth phase were collected and seeded onto the 96-well plates at a density of $2x10^3$ cells/well. Cell viability was assessed with the MTT assay (cat. no. JRDC000003; Kilton Biotechnology (Shanghai) Co., Ltd.). After 24, 48 and 72 h, respectively, all media were replaced with serum-free medium and 20 μl MTT (5 mg/ml) was added into each well and incubated in the dark at 37˚C for 4 h. After the medium was discarded, 150 μl DMSO was added into each well and the plate was shaken in the dark for 5 min. The absorbance (A) at 490 nm was measured to reflect the cell proliferation or number. The experiment was performed in triplicate.

Statistical analysis. Data were expressed as mean±SD. Statistical analysis was performed with the SPSS 18.0 (SPSS Inc.) software package. Unpaired *t*-tests were used for the pairwise group comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Tetrandrine decreases TNF-α expression in tumor tissues and blood samples. To detect the expression levels of TNF-α expression in tumor tissues and blood samples, RT-q PCR, western blotting and ELISA were performed. Compared with the control group, TNF-α mRNA and protein levels in the tumor tissues and blood samples significantly declined in patients with colon cancer treated with tetrandrine tablets during neoadjuvant chemotherapy ($P<0.05$; Figs. 1 and 2). These results suggested that the combination of tetrandrine tablets reduced TNF-α expression in neoadjuvant chemotherapy for colon cancer.

Tetrandrine reduces serum levels of IL-15, IL-1β and IL-6. To detect the expression levels of inflammation-related factors (IL-15, IL-1β and IL-6) in serum samples (32), ELISA was performed. Compared with the control group, the serum levels of IL-15, and IL-1β significantly declined (while IL-6 did not significantly decline) in the patients taking tetrandrine

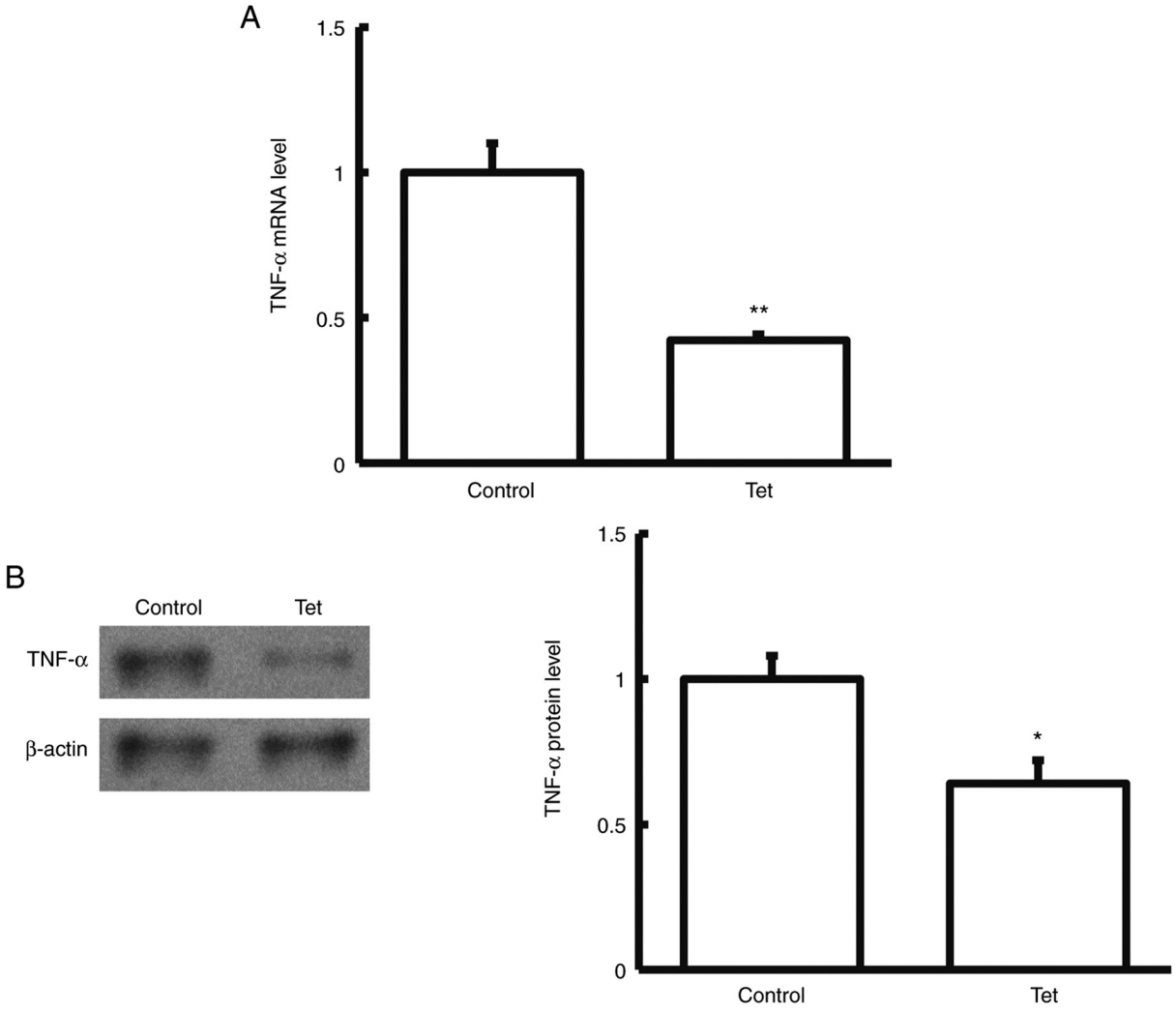

Figure 1. TNF-α expression levels in tumor tissues. (A) mRNA and (B) protein expression levels of TNF-α in the tumor tissues were detected with RT-qPCR and western blotting respectively. **P<0.01 compared with the control group. RT-q, reverse transcription-quantitative; control group, patients not receiving tetrandrine; tet group, patients recieving tetrandrine.

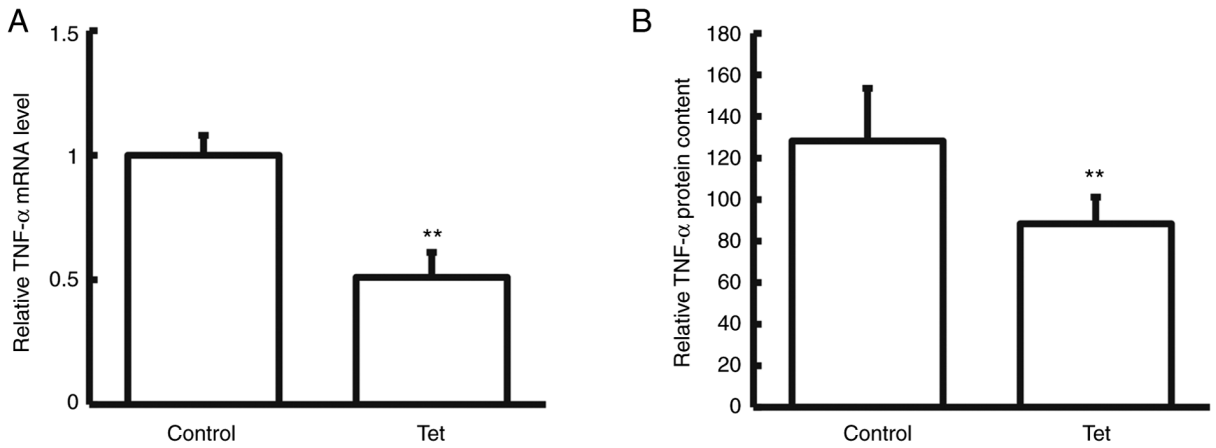

Figure 2. TNF-α in expression levels in blood samples. (A) mRNA and (B) protein expression levels of TNF-α in patient blood samples were detected with RT-qPCR and ELISA respectively. *P<0.05, **P<0.01 compared with the control group. RT-q, reverse transcription-quantitative; control group, patients not receiving tetrandrine; tet group, patients receiving tetrandrine.

tablets during the neoadjuvant chemotherapy (P<0.05; Fig. 3). These results indicated that tetrandrine can reduce the release of inflammatory factors in the blood of patients with colon cancer.

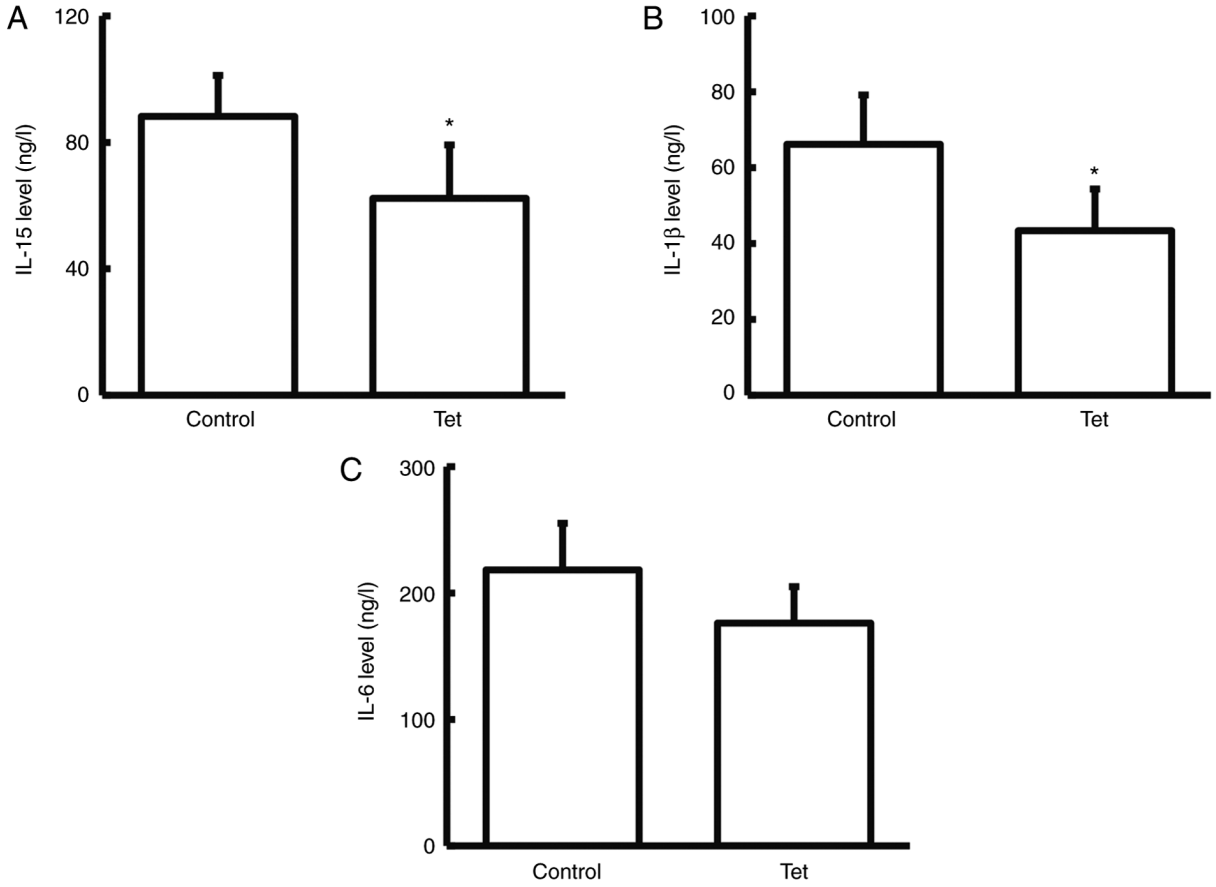

Figure 3. Expression levels of inflammatory cytokines in blood samples. The expression levels of (A) IL-15, (B) IL-1β and (C) IL-6 in blood samples from the patients of these groups were detected with ELISA. *P<0.05 compared with the control group. Control group, patients not receiving tetrandrine; tet group, patients receiving tetrandrine.

Tetrandrine reduces CCL5, CXCL2 and CXCL10 content in colon cancer tissue culture supernatant. Release of inflammatory cytokines and chemokines represents one of the common features of colon cancers (33). The chemotactic factors in the surgically resected fresh colon cancer tissue culture medium were detected with ELISA. It was revealed that compared with the control group, CCL5, CXCL2 and CXCL10 expression levels in the tissue culture supernatant significantly declined in the patients taking tetrandrine tablets during chemotherapy (P<0.05 for CXCL2; P<0.01 for CCL5 and CXCL10; Fig. 4). No significant differences were observed in the levels of the other chemokines investigated, such as CCL20, CCL2, CXCL1, CXCL3 and CXCL5 (data not shown). These results suggested that tetrandrine can reduce the release of chemokines from colon cancer cells.

Tetrandrine reduces release of IL-15, IL-1β and IL-6 in monocytes cultured with colon cancer tissue culture supernatant. Human blood mononuclear cells were cultured in the conditioned medium prepared from the surgically resected fresh colon cancer tissue and the release of IL-15, IL-1β and IL-6 in monocytes was detected. Compared with the control group, the release of IL-15, IL-1β and IL-6 in monocytes cultured with colon cancer tissue culture supernatant was significantly reduced (P<0.05; Fig. 5). Taken together, these results suggested that tetrandrine can reduce the release of colon cancer inflammatory cytokines in the colonic infiltrating monocytes.

Tetrandrine decreases colon cancer cell proliferation. HCT 116 cells (colon cancer cells) were cultured in a conditioned medium prepared from the surgically resected fresh colon cancer tissue and the cell proliferation was assessed with the MTT assay. The proliferation of the colon cancer cells cultured with the conditioned medium prepared from the surgically resected fresh colon cancer tissues from the patients taking the tetrandrine tablet during the chemotherapy was significant decreased compared with those taking no tetrandrine (P<0.05; Fig. 6).

Discussion

In the present study, the clinical efficacy of the combination application of tetrandrine in the neoadjuvant chemotherapy for patients with colon cancer was assessed. The changes of TNF-α expression in tumor tissues and blood samples, the changes of the expression levels of IL-15, IL-1β, and IL-6 in the blood samples and the release of chemokines in the tumor tissue culture supernatants were detected. In addition, after culturing with the tumor tissue supernatant, the release of human monocyte inflammatory factors from the monocytes of patients with colon cancer and the proliferation of colon cancer cell lines were investigated. The results of the present study preliminarily verified the anti-inflammatory effects of tetrandrine in the neoadjuvant chemotherapy for colon cancers.

The massive secretion of TNF-α cytokines in the tumor microenvironment can accelerate the growth and spread of

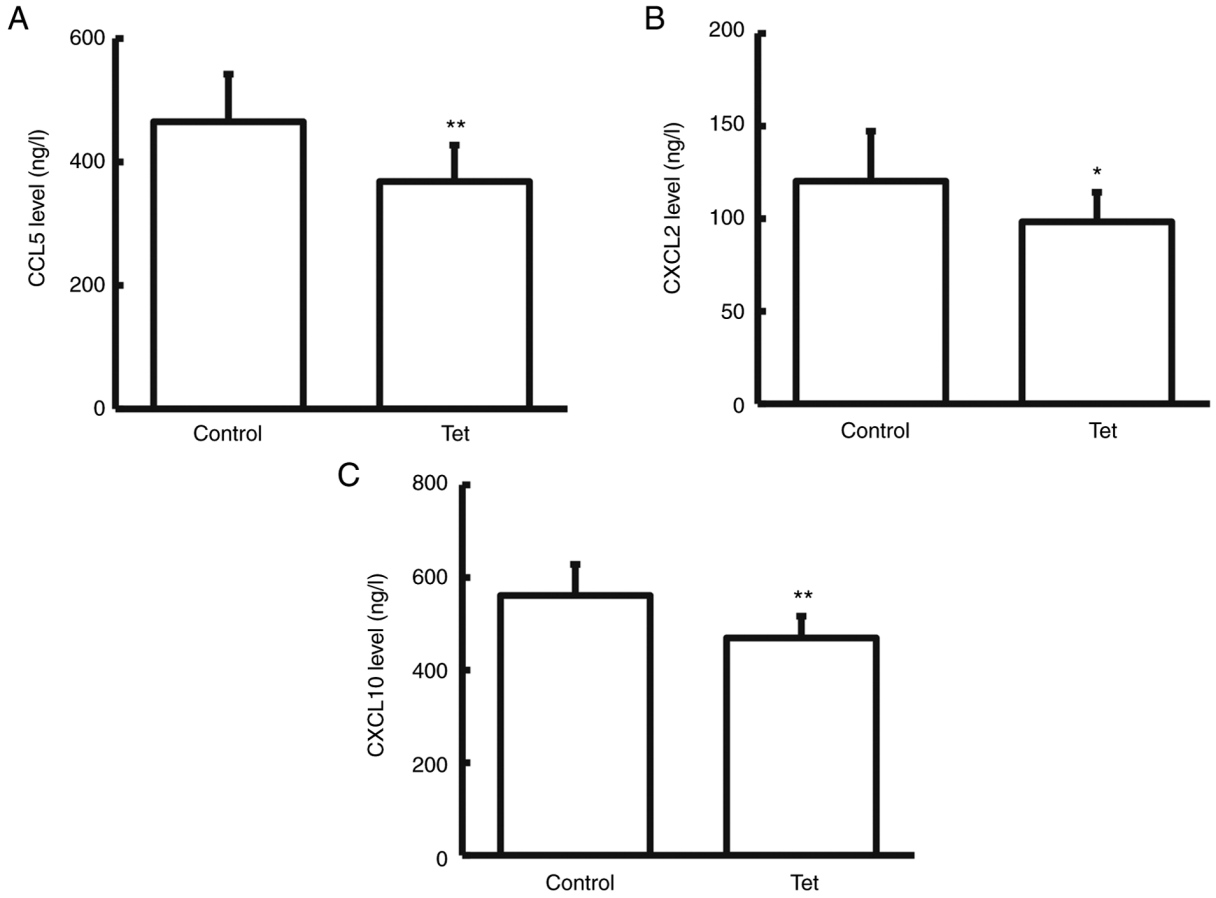

Figure 4. Expression levels of CCL5, CXCL2 and CXCL10 in colon cancer tissue culture supernatant. Expression levels of chemotactic factors, (A) CCL5, (B) CXCL2 and (C) CXCL10 in surgically resected fresh colon cancer tissue culture supernatant were detected with ELISA. *P<0.05, **P<0.01 compared with the control group. CCL, chemokine ligand; CXCL, chemokine (C-X-C) motif ligand; Control group, patients not receiving tetrandrine; tet group, patients receiving tetrandrine.

cancer cells (34). At the same time, cancer cells can bypass the immune system, promote the process of epithelial-mesenchymal transition and cause distant metastasis (35). Silencing of TNF-α expression in triple-negative breast cancer can inhibit the proliferation of TNBC cells and promote its apoptosis through the NF-κB pathway (36). In the TNBC mouse model, TNF-related apoptosis-inducing ligand receptor-2 can inhibit the proliferation and metastasis of cancer cells (37). A previous study has demonstrated the expression of TNF-α in the cytoplasm of solid tumors (such as such as lung cancer and colon cancer) (38). There is an important relationship between the expression of TNF-α in tumor tissues and tumor development (39). Tumor cells produce TNF-α and autocrine TNF-α promotes tumor cell growth and directly promotes tumor invasion (40,41).

The drug tetrandrine used in the present study is a natural non-selective Ca^{2+} channel blocker, which is very similar to the slow channel blocker verapamil (42). By inhibiting Ca^{2+}, tetrandrine can inhibit the effect and release of allergic media, reduce myocardial contractility, dilate peripheral blood vessels and relax muscles (42). Studies have reported that tetrandrine can eliminate oxygen free radicals, protect islet β-cell membranes, reduce Ca^{2+} overload, prevent excessive aggregation of superoxide in cells and finally reduce islet cell apoptosis (43,44). It is known that tetrandrine may serve a certain role in fighting against inflammation (45). At present, chronic inflammation has been recognized as one of the most common physiological causes for tumors and it is even believed that most tumors are caused by chronic inflammation (46). The control of inflammatory factors in tumors is also a new direction for preventing and treating cancers (47). The results of the present study demonstrated that administration of tetrandrine during neoadjuvant chemotherapy in patients with colon cancer reduced the expression of TNF-α in tumor tissues, suggesting that tetrandrine may inhibit colon cancer by reducing TNF-α expression to a certain extent. When colon cancer HCT116 cells were cultured in the present study with the supernatant of tumor tissue culture from patients treated with tetrandrine, cell proliferation dramatically declined, further confirming that the administration of tetrandrine during neoadjuvant chemotherapy can slow the growth of colon cancer.

It has been demonstrated that IL-15, IL-1β, and IL-6 are also very important factors in inflammatory processes (48). IL-15, IL-1β, and IL-6 in the tumor microenvironment can promote tumor proliferation and metastasis, strongly affecting disease prognosis (49-51). In addition, chemokines released by colon cancer cells can recruit immune cells to local infiltration within tumor tissues, amplifying the inflammatory response in tumor tissues, accelerating tumor progression and reducing

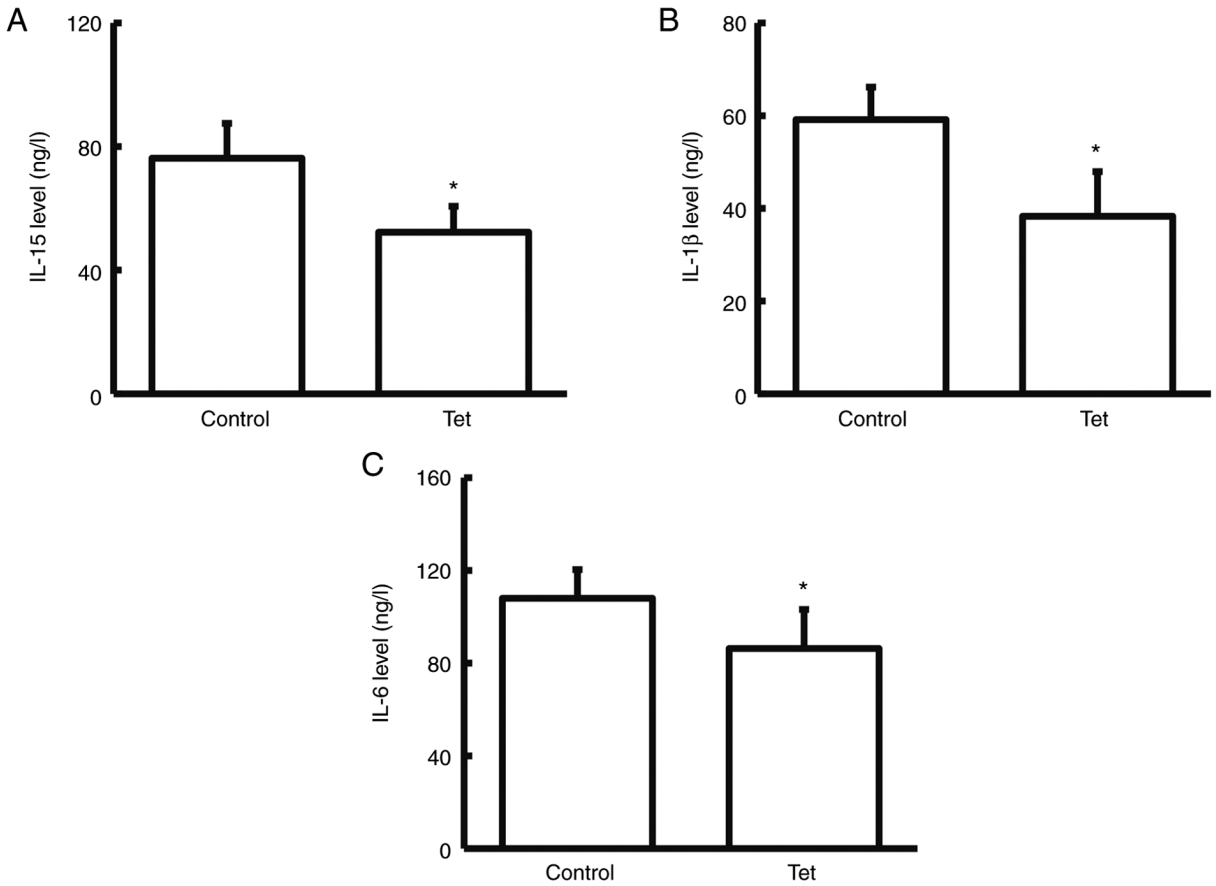

Figure 5. Release of IL-15, IL-1β and IL-6 in monocytes cultured with colon cancer tissue culture supernatant. Human blood mononuclear cells were cultured in the conditioned medium prepared from surgically resected fresh colon cancer tissues and the release of (A) IL-15, (B) IL-1β and (C) IL-6 in monocytes was detected with ELISA. *P<0.05 compared with the control group. Control group, patients not receiving tetrandrine; tet group, patients receiving tetrandrine.

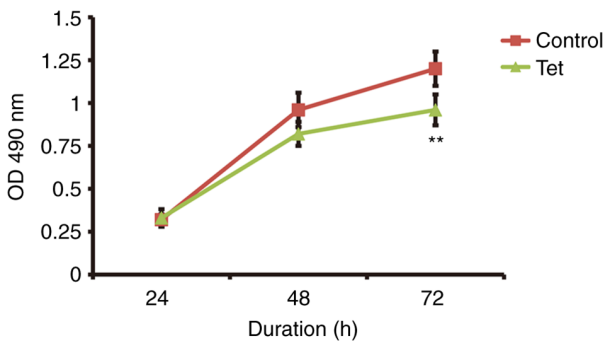

Figure 6. Detection of colon cancer cell proliferation. The colon cancer cell line (HCT-116) was cultured in a conditioned medium prepared from surgically resected fresh colon cancer tissues and the cells proliferation was assessed with the MTT assay. **P<0.01 compared with the control group. OD, optical density; Control group, patients not receiving tetrandrine; tet group, patients receiving tetrandrine.

the therapeutic effect of chemotherapy drugs (such as mitochondrial pyruvate carrier 1 and CCL7) (52,53). The relevant chemokines (CCL2, CCL5, CCL20, CXCL1, CXCL2, CXCL3, CXCL5 and CXCL10) in colon cancer tissue culture supernatants were assessed in the present study. The results of the present study demonstrated that the release of CCL5, CXCL2 and CXCL10 in colon cancer tissue culture supernatants from patients taking tetrandrine were relatively low, compared with those not taking tetrandrine, suggesting that administration of tetrandrine during the neoadjuvant chemotherapy process significantly reduced release of chemokines in colon cancer cells and decreased the recruitment of immune cells. In addition, the release of IL-15, IL-1β, and IL-6 in human monocytes cultured with colon cancer tissue culture supernatants was detected in the present study. The results demonstrated that the release amount of IL-15, IL-1β and IL-6 in these human monocytes cultured from colon cancer tissue supernatant from patients taking tetrandrine was significantly reduced compared with patients not taking tetrandrine, which further confirmed that the administration of tetrandrine during the adjuvant chemotherapy may reduce the activity and recruitment of human monocytes.

The present study had several limitations. For example, only some indicators in the blood samples and the effects on cells have been studied. A follow-up study on the patients' condition in the tested groups (i.e., overall survival, progression free survival and relapse rate) needs to be performed to determine whether the administration of tetrandrine upon neoadjuvant therapy in colon cancer is indeed beneficial.

In conclusion, the results of the present study demonstrated that the administration of tetrandrine during neoadjuvant chemotherapy for colon cancer reduced the inflammatory response and release of chemokines in cancer tissues, which

may delay tumor growth and the recruitment of immune cells. Hence, tetrandrine may have a positive effect on patients with colon cancers, reducing inflammatory indicators in the patient's blood.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JL, FY, BW, DL and BL contributed to the study design, experimental performance, data collection and analysis and manuscript preparation. All authors have read and approved the final manuscript. JL, FY, BW, DL and BL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Board of the First Hospital of Zibo City. Prior written and informed consent were obtained from every patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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