

Original Article

Effect of celecoxib combined with chemotherapy drug on malignant biological behaviors of gastric cancer

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Abstract: Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, has been reported to have antitumor effects. In some tumor models, the combination of celecoxib with chemotherapy agents has shown synergistic antitumor effect; however, the effect of celecoxib combination with tegafur/gimeracil/oteracil potassium on the malignant biological behaviors of gastric cancer in nude mice is unclear. In this study, female nude mice were subcutaneously transplanted with SGC-7901 gastric cancer cells. When the tumor model formed, the mice were divided into control group, celecoxib group, tegafur/gimeracil/oteracil potassium group, and the combination of both drug regimens group. Mice were treated for 3 weeks. Following treatment, the proliferating index was calculated, apoptosis related proteins, COX-2, vascular endothelial growth factor-C (VEGF-C) and lymphatic vessel density were quantified in tumor tissues by immunohistochemistry. Apoptosis was evaluated by TUNEL staining. The results revealed that celecoxib and tegafur/gimeracil/oteracil potassium alone significantly inhibited tumor growth. The combination of these two drugs showed a synergistic antitumor effect. Both celecoxib and tegafur/gimeracil/oteracil potassium alone inhibited proliferation and promoted apoptosis. The combination of these two drugs further enhanced this anticancer effect. Both celecoxib and the combination treatment inhibited lymphangiogenesis and the expression of COX-2 and VEGF-C. However, tegafur/gimeracil/oteracil potassium treatment had no obvious effect on lymphangiogenesis. These results suggested that the combination of celecoxib and tegafur/gimeracil/oteracil potassium produced a synergistic antitumor effect, possibly by inhibiting the proliferation of tumor cells and promoting apoptosis. Celecoxib and celecoxib in combination with tegafur/gimeracil/oteracil potassium possibly by reducing the expression of COX-2, in turn down-regulating the expression of VEGF-C, resulted in the inhibition of lymphangiogenesis.

Keywords: Gastric cancer, COX-2, VEGF-C, lymphangiogenesis, celecoxib, tegafur/gimeracil/oteracil potassium

Introduction

Gastric cancer is the second leading cause of cancer-related death worldwide, with a 5-year survival rate of less than 25% [1, 2]. While the incidence of gastric cancer has decreased in the past 30 years, gastric cancer remains a serious threat to human health in China. Further study of the biological mechanism of gastric cancer occurrence and development and discovery of new targeted therapies will provide new strategies for the treatment of gastric cancer.

Cyclooxygenase-2 (COX-2) is a key enzyme that catalyzes arachidonic acid to prostaglandins.

COX-2 is generally not expressed in normal tissues in adults, but its expression is induced by mitogenic and inflammatory stimuli. COX-2 is overexpressed in many human cancers, including gastric cancer [3]. It has been reported that COX-2 contributes to carcinogenesis and cancer progression by promoting proliferation, inhibiting apoptosis, inhibiting immune surveillance and promoting angiogenesis [4-8]. In addition, COX-2 is a new factor of lymphangiogenesis. Vascular endothelial growth factor-C (VEGF-C) has been demonstrated to induce lymphangiogenesis by activating VEGFR-3, which is expressed on lymphatic endothelial cells. Timoshenko AV et al [9] studied breast cancer cell lines and specimens and found that

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COX-2 may up-regulate the expression of VEGF-C through the prostaglandin receptor EP1-/EP4-. COX-2 and VEGF-C are significantly positively correlated with the lymphangiogenesis of breast cancer. Celecoxib is a highly selective COX-2 inhibitor that has been shown to have antitumor effect in many human tumors, including head and neck cancer [10], gastric cancer [11], and colon cancer [12]. In addition, several preclinical and clinical studies indicated that celecoxib increased the sensitivity of tumor cells to chemotherapy drugs [13] or radiotherapy [14], enhancing their antitumor effect.

Tegafur/gimeracil/oteracil potassium is a new oral anticancer drug composed of three components: tegafur and two modulators, 5-chloro-2,4-dihydropyridine (CDHP) and potassium oxonate. Tegafur is the prodrug of 5-FU, and CDHP increases the plasma concentration of 5-FU by inhibiting dihydropyrimidine dehydrogenase (DPD). Oxonate reduces the gastrointestinal toxicity of 5-FU. Both CDHP and oxonate do not have antitumor effect [15]. The antitumor mechanism of tegafur/gimeracil/oteracil potassium is the same as 5-FU; however, it has fewer side effects.

The aim of this study was to investigate the effect of celecoxib and tegafur/gimeracil/oteracil potassium combination treatment on gastric cancer malignant biological behaviors in nude mice and to analyze the change of related molecular biological indicators.

Materials and methods

Reagents

Celecoxib was purchased from Pfizer Pharmaceuticals Ltd (NY, America). Tegafur/gimeracil/oteracil potassium was purchased from the Hengrui pharmaceutical company (Jiangsu, China). Both celecoxib and tegafur/gimeracil/oteracil potassium were suspended in 0.5% CMC.

Animals

Female BALB/c nude mice (4-6 weeks, 13.5-14.5 g) were purchased from the Institute of Experimental Animals of Peking Union Medical College. The mice were maintained under specific pathogen-free conditions at the Center for Animal Experimentation of the First Affiliated Hospital of Chongqing Medical University. The mice were fed sterilized diet and water *libitum*. The animal experiment strictly follow the relat-

ed laws and regulations about rational use of experimental animals issued by ministry of science and technology and the ministry of health of China.

Cell culture

The human gastric cancer cell line SGC-7901 was preserved by the central laboratory of the First Affiliated Hospital of Chongqing Medical University. SGC-7901 cells were cultured in RPMI1640 medium (Hyclone, UT, USA) supplemented with 10% fetal bovine serum (Hyclone, UT, USA) in an atmosphere of 5% CO₂ at 37°C.

Gastric cancer xenograft tumor model and treatment of nude mice

SGC-7901 cells (2×10^6 cells/200 μ l) were injected subcutaneously in the right side of the back near the axilla in nude mice to establish a xenograft tumor model of human gastric cancer. After the largest diameter of tumors reached approximately 5 mm, the mice were randomly divided into four treatment groups: the control group, the celecoxib group, the tegafur/gimeracil/oteracil potassium group, and the combination group. Mice (6 per group, n=6) were treated with vehicle (0.5% CMC), celecoxib (50 mg/kg) [16], tegafur/gimeracil/oteracil potassium (10 mg/kg) [17], or the combination of drug regimens for 5 consecutive days a week for 3 weeks by gavage. Every 3 days during treatment, we measured the tumor's long diameter (a) and short diameter (b) using vernier calipers. Tumor volume was calculated according to the formula $TV=1/2ab^2$, and these values were used to generate tumor-growth curves. The weight change of the nude mice before and after treatment was measured, and the side effects of drug therapy were assessed. After 3 weeks of treatment, the mice were sacrificed, and the tumor inhibition rate was calculated. Tumor tissues were fixed in 4% paraformaldehyde for analysis by immunohistochemistry and TUNEL assay.

Immunohistochemical analysis

Immunohistochemistry procedures were performed as described by the immunohistochemistry SP kit (Zhongshan company, Beijing, China). Consecutive 4- μ m-thick sections were cut from the paraffin samples. The sections were deparaffinized in xylene, rehydrated through a graded series of alcohols (100%, 95%, 85%, 70%), and then washed with PBS. For

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Table 1. The weight change of each group after treatment (g, $\bar{x} \pm s$, n=6)

Group	Before treatment	After treatment
Control	18.42±0.98	20.87±1.48 ^a
Celecoxib	17.78±0.74	19.18±0.77 ^a
Tegafur/gimeracil/Oteracil potassium	17.85±0.97	18.82 ± 1.32 ^b
Combination	17.75±0.62	19.03±1.37 ^b

^aP < 0.01, ^bP < 0.05 compared with before treatment.

antigen retrieval, the sections were incubated in citrate buffer in a microwave for 15 min. Endogenous peroxidase was blocked with 3% H₂O₂ for 30 min. Goat serum was then added to the sections for 30 min at 37°C to block non-specific antibody binding. The sections were incubated with primary antibodies for COX-2 (diluted 1:200, Bioworld, MO, USA), VEGF-C (diluted 1:100, Epitomics, CA, USA), PCNA, Bcl-2 (diluted 1:150, Epitomics, CA, USA), caspase-3 (diluted 1:300, Beyotime Institute of Biotechnology, Shanghai, China), and the Syrian hamster anti-mouse podoplanin antibody (diluted 1:200, Biolegend, CA, USA) overnight at 4°C. A biotinylated secondary antibody was then added for 30 min at 37°C. Streptavidin-horseradish peroxidase complex was added and colored with 3-3'-diaminobenzidine. For negative controls, no primary antibodies were used.

We analyzed the positive staining (brown) of tumor tissues. The positive staining of COX-2 and VEGF-C was located in the cytoplasm, but Bcl-2- and caspase-3-positive staining was located in both the cytoplasm and nucleus of the cancer cells. For the quantification of these proteins, five random fields from each section at × 200 magnifications were selected. We calculated the mean optical density of each protein expression in the tumor tissues of different groups using the Image Pro Plus 6.0 system.

The positive expression (brown) of PCNA was noted in the cell nucleus. The number of positive cells was quantified in five random fields at × 200 magnifications, and the proliferation index (PI) was calculated to be equal to the number of positive cells/total cells × 100%.

The positive expression of podoplanin was located in the cytoplasm of lymphatic endothelial cells. For the quantification of lymphatic vessel density (LVD), the three most highly vascularized areas detected by podoplanin immunostaining were examined carefully at low-power magnification (× 40), and LVD was expressed

as the average of three × 200 field counts of podoplanin-positive single endothelial cells, clusters of endothelial cells, and lymphatic vessels [18].

TUNEL staining

The TUNEL assay was performed following the procedure outlined by the apoptosis detec-

tion kit (Roche, Switzerland). Paraffin-embedded tissue sections were dewaxed and rehydrated according to standard protocols: heating at 60°C, followed by a xylene wash and rehydrated through a graded series of ethanol and double distilled water. Tissue sections were incubated for 15 min at 37°C with proteinase K. The sections were rinsed twice (5 min/each) with PBS, and the area around the sections was dried. TUNEL reaction mixture (50 µl) was added to the samples, which were then incubated for 60 min at 37°C in a humidified atmosphere. The sections were rinsed three times with PBS. Horseradish-peroxidase-labeled streptavidin was used to bind the biotinylated nucleotides. The sections were then colored with DAB and counterstained with hematoxylin.

To quantify TUNEL expression, five random fields from each section at × 200 magnification were selected, and the percentage of positively stained cells among the total number of cells was calculated: Apoptosis rate=the number of apoptotic cells/the total number of cells × 100%.

Statistical analysis

SPSS 17.0 software was used for the statistical analysis. Data are expressed as the mean ± SD. Differences among the groups were examined using one-way ANOVA. The differences between any two groups were analyzed using LSD-t. The differences of weight change after treatment in each group were analyzed using the paired Student's t-test. P < 0.05 was considered to be statistically significant.

Results

Establishment of a xenograft tumor model of gastric cancer in nude mice and the assessment of treatment side effects

The largest tumor diameter in twenty-six nude mice reached approximately 5 mm on the eigh-

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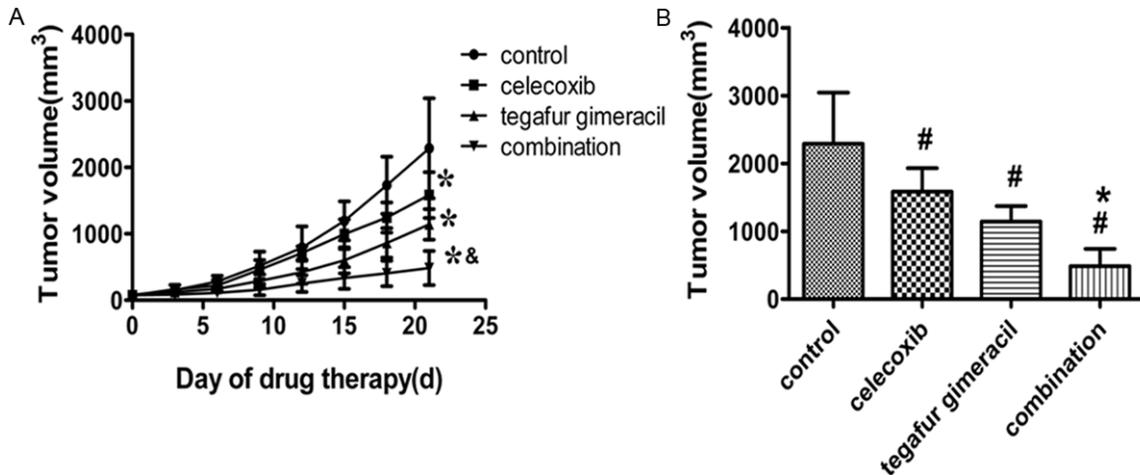


Figure 1. A. Growth curves of subcutaneous gastric cancer xenograft tumors in nude mice. * $P < 0.05$, compared with the control group; &#P < 0.05, compared with the celecoxib group and the tegafur/gimeracil/oteracil potassium group; B. Tumor volume of each group. # $P < 0.05$, compared with the control group; * $P < 0.05$, compared with the celecoxib group and the tegafur/gimeracil/oteracil potassium group.

teenth day of inoculation, while the other five nude mice did not form tumors. The tumor formation rate was 83.9%. The mean tumor volume of each group before treatment is as follows: control group $80.00 \pm 32.59 \text{ mm}^3$, celecoxib group $76.17 \pm 38.86 \text{ mm}^3$, tegafur/gimeracil/oteracil potassium group $79.75 \pm 27.74 \text{ mm}^3$, the combination group $75.08 \pm 23.22 \text{ mm}^3$. There is no difference between each group of tumor volume before treatment ($P > 0.05$). In the process of treatment, the mental state, activity and diet of the mice were normal and no obvious adverse effects were observed. There was no obvious malignant consumption in nude mice. The body weights were higher after the course of treatment ($P < 0.05$, **Table 1**).

The effect of celecoxib and tegafur/gimeracil/oteracil potassium on tumor growth

After treatment, tumors in the drug therapy groups grew slowly. Tumor volumes in the four groups were as follows: control group, $2288.67 \pm 753.87 \text{ mm}^3$; celecoxib group, $1583.75 \pm 345.25 \text{ mm}^3$; tegafur/gimeracil/oteracil potassium group, $1142.42 \pm 229.17 \text{ mm}^3$; and combination group, $485.00 \pm 255.25 \text{ mm}^3$. Compared to the control group, the tumor volumes in the drug therapy groups were smaller ($P < 0.05$). The tumors in the combination group were smaller than those in the single drug therapy group ($P < 0.05$), but there was no significant difference between the celecoxib group

and the tegafur/gimeracil/oteracil potassium group ($P > 0.05$). The tumor inhibition rates in these groups were as follows: celecoxib group, 30.8%; tegafur/gimeracil/oteracil potassium group, 50.1%; and combination group, 78.8%. According to the formula [19], $q = E_{A+B} / (E_A + E_B - E_A \cdot E_B)$, $q > 1.15$. Therefore, the combination of the two drugs had a synergistic antitumor effect (**Figure 1**).

The effect of celecoxib and tegafur/gimeracil/oteracil potassium on the proliferation and apoptosis of each treatment group

To analyze the change of proliferation and apoptosis in each group, we detected the proliferation index and performed a TUNEL assay. The proliferation index of each of the four groups was as follows: control group, $79.49\% \pm 3.47\%$; celecoxib group, $59.76\% \pm 3.86\%$; tegafur/gimeracil/oteracil potassium group, $52.70\% \pm 2.37\%$; and combination group, $32.24\% \pm 2.11\%$. The proliferation index in the drug therapy groups was lower than that in the control group ($P < 0.01$), was lower in the combination group than that in both the celecoxib group and the tegafur/gimeracil/oteracil potassium group ($P < 0.01$), and was lower in the tegafur/gimeracil/oteracil potassium group than that in the celecoxib group ($P < 0.01$).

The apoptosis rates in the four groups were as follows: control group, $11.19\% \pm 1.73\%$; celecoxib group, $30.98\% \pm 2.00\%$; tegafur/gimeracil/ot-

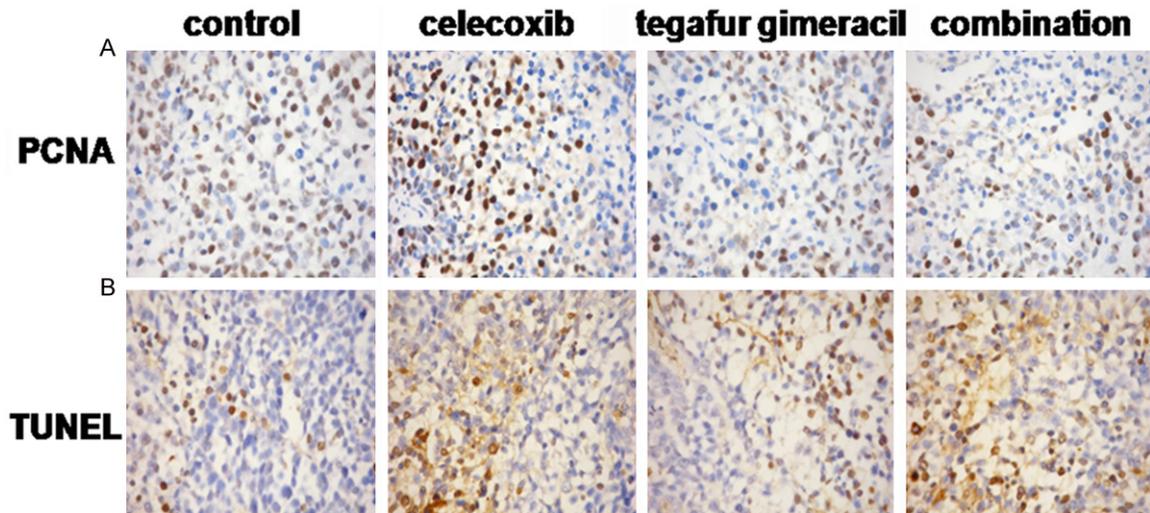


Figure 2. A. The expression of PCNA in subcutaneous gastric cancer xenograft tumors in nude mice by immunohistochemistry in the four groups. The positive expression (brown) of PCNA was located in the nucleus of tumor cells. The proliferation index in the combination group was lower than that in the celecoxib and tegafur/gimeracil/oteracil potassium group ($P < 0.01$); B. TUNEL staining of tumor tissue in each group. The nuclei of apoptotic cells are stained brown. The apoptosis rate in the combination group was higher than that in the celecoxib and tegafur/gimeracil/oteracil potassium groups ($P < 0.01$). Original magnification was $\times 200$.

eracil potassium group, $39.16\% \pm 2.74\%$; and combination group, $58.93\% \pm 3.70\%$. The apoptosis rate in the treatment groups was higher than that in the control group ($P < 0.01$). The apoptosis rate in the tegafur/gimeracil/oteracil potassium group was higher than that in the celecoxib group ($P < 0.01$), and the apoptosis rate in the combination group was higher than that in both the celecoxib and tegafur/gimeracil/oteracil potassium groups ($P < 0.01$) (Figure 2).

The effect of celecoxib and tegafur/gimeracil/oteracil potassium on apoptosis-related proteins

We detected the expression of apoptosis-related proteins by immunohistochemistry to better understand how the drug treatments affected apoptosis. The expression of Bcl-2 in the drug therapy groups was lower than that in the control group ($P < 0.01$) and was lower in the combination group than that in the celecoxib and tegafur/gimeracil/oteracil potassium groups ($P < 0.05$). However, there was no significant difference between the celecoxib group and the tegafur/gimeracil/oteracil potassium group ($P > 0.05$). The expression of caspase-3 in the drug-administered groups was higher than that of the control group ($P < 0.05$) and was higher in the combination group than that in the cele-

coxib group or in the tegafur/gimeracil/oteracil potassium group ($P < 0.01$). In addition, the expression of caspase-3 in the tegafur/gimeracil/oteracil potassium group was higher than that in celecoxib group ($P < 0.05$) (Figure 3).

The effect of celecoxib and tegafur/gimeracil/oteracil potassium on the protein expression of COX-2 and VEGF-C and on lymphangiogenesis

We detected COX-2 and VEGF-C expression and lymphatic vessel density by immunohistochemistry to understand the effect of celecoxib and tegafur/gimeracil/oteracil potassium on lymphangiogenesis. The expression of COX-2 and VEGF-C protein in the celecoxib group and in the combination group was lower than that in the control and tegafur/gimeracil/oteracil potassium groups ($P < 0.05$). There was no significant difference in COX-2 expression between the tegafur/gimeracil/oteracil potassium group and the control group. Additionally, there was no significant difference in COX-2 expression between the celecoxib group and the combination group ($P > 0.05$). The lymphatic vessel density of the groups was as follows: control group (8.60 ± 1.52)/HF; celecoxib group (4.60 ± 1.14)/HF; tegafur/gimeracil/oteracil potassium group (8.00 ± 1.58)/HF; and combination group (3.80 ± 1.30)/HF. The lymphatic vessel density of the celecoxib group and the combination

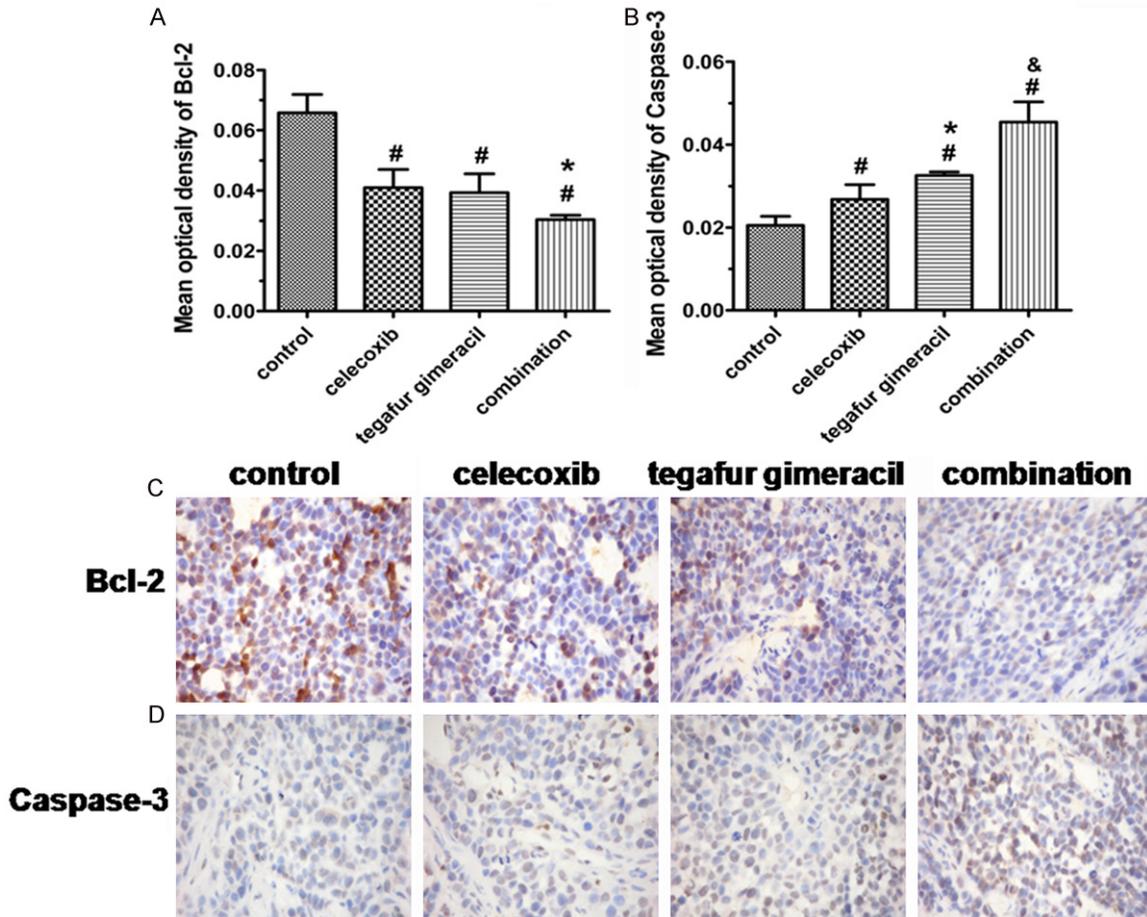


Figure 3. A. The average optical density value of Bcl-2 protein from tumor tissue in each group. Compared with the control group, [#] $P < 0.01$; Compared with the celecoxib group and the tegafur/gimeracil/oteracil potassium group, ^{*} $P < 0.05$; B. The average optical density value of caspase-3 protein from tumor tissue in each group. Compared with the control group, [#] $P < 0.01$; Compared with the celecoxib and tegafur/gimeracil/oteracil potassium groups, ^{*} $P < 0.01$; Compared with the celecoxib group, ^{*} $P < 0.05$; C. The expression of Bcl-2 protein in subcutaneous gastric cancer xenograft tumors in nude mice by immunohistochemistry in each group; D. The expression of caspase-3 protein in subcutaneous gastric cancer xenograft tumors in nude mice by immunohistochemistry in the four groups. Original magnification was $\times 200$.

group were lower than that in the control and tegafur/gimeracil/oteracil potassium groups ($P < 0.05$). There was no significant difference in lymphatic vessel density between the tegafur/gimeracil/oteracil potassium group and the control group. There was also no significant difference in lymphatic vessel density between the celecoxib group and the combination group ($P > 0.05$) (Figure 4).

Discussion

Many studies have indicated that the COX-2/PGE2 signaling pathway plays an important role in the development of malignant tumors [20]. Studies *in vitro* and *in vivo* found that the selective COX-2 inhibitor celecoxib could inhibit the

growth of a variety of tumors. In a spontaneous metastatic breast cancer mouse model, celecoxib effectively inhibited tumor growth. Celecoxib may have inhibited tumor growth through the inhibition of proliferation and angiogenesis, the up-regulation of Bax expression, and the down-regulation of Akt and Bcl-2 expression, thus promoting tumor tissue apoptosis [21]. Tegafur/gimeracil/oteracil potassium is a derivative of 5-FU. The mechanism of its antitumor effect is that tegafur is metabolized into 5-FU by cytochrome P450 in the liver. 5-FU inhibits DNA synthesis and alters gene expression. The antitumor effect of tegafur/gimeracil/oteracil potassium has been demonstrated in some solid tumors, including advanced gastric cancer [22], colorectal cancer [23], non-small

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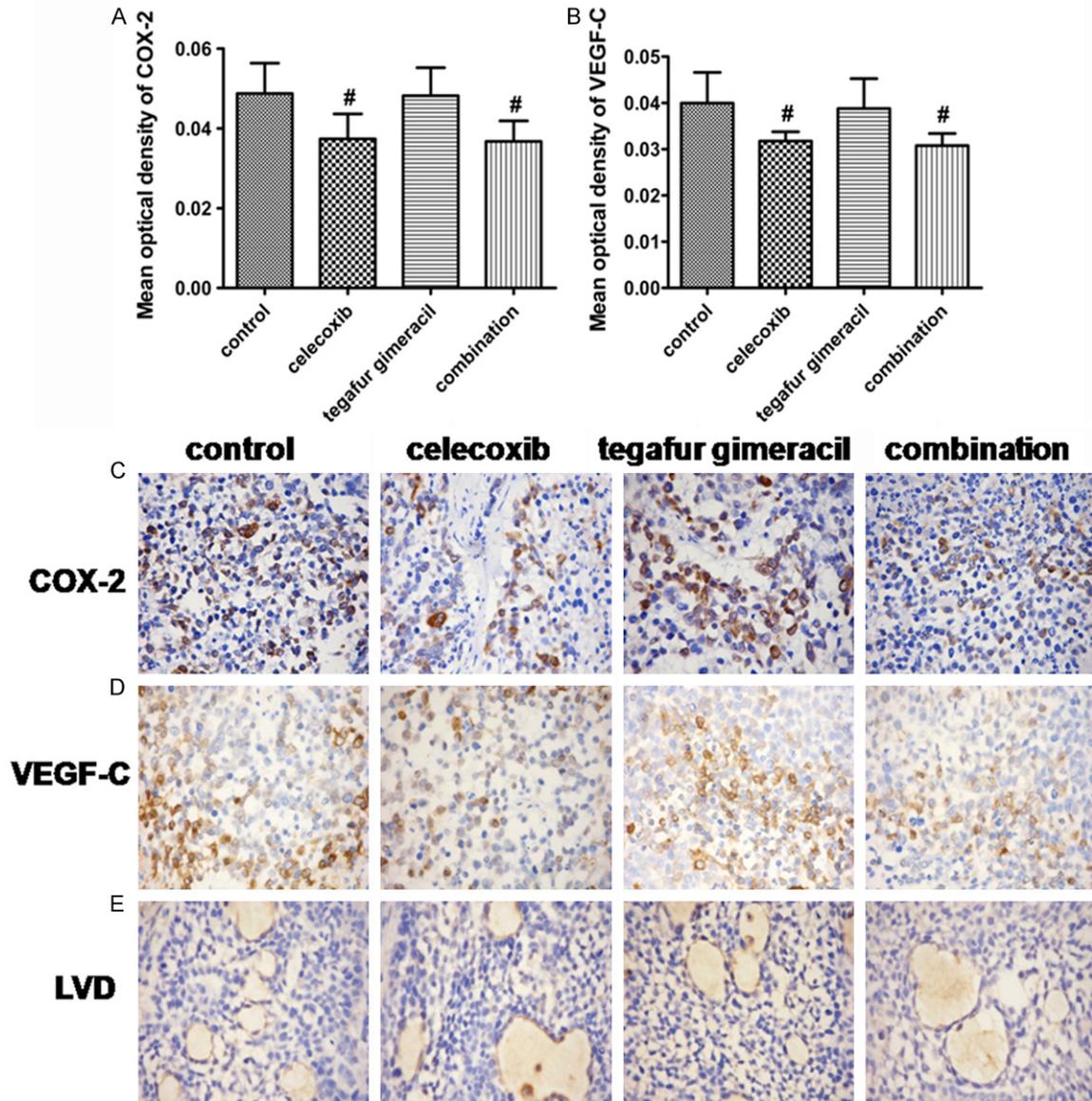


Figure 4. A. The average optical density value of COX-2 protein in tumor tissue from each group. Compared with the control group and the tegafur/gimeracil/oteracil potassium group, $^{\#}P < 0.05$; B. The average optical density value of VEGF-C protein in tumor tissue from each group. Compared with the control group and the tegafur/gimeracil/oteracil potassium group, $^{\#}P < 0.05$; C. The expression of COX-2 in subcutaneous gastric cancer xenograft tumors in nude mice by immunohistochemistry in each group; D. The expression of VEGF-C in subcutaneous gastric cancer xenograft tumors in nude mice by immunohistochemistry in each group; E. Lymphatic vessel density (LVD) in subcutaneous gastric cancer xenograft tumors in nude mice by immunohistochemistry. Original magnification was $\times 200$.

cell lung cancer [24], head and neck cancer [25], pancreatic cancer [26], and advanced biliary tract cancer [27].

In our study, celecoxib and tegafur/gimeracil/oteracil potassium significantly inhibited tumor growth. The combination of both drugs had a synergistic antitumor effect. There were no obvious side effects in each treatment group. In a randomized phase II clinical study, eleven

cachectic patients with head and neck or gastrointestinal cancer received celecoxib or placebo for 21 days. The results showed that there were no obvious side effects in the celecoxib group. Cachectic patients receiving celecoxib gained weight, their BMI increased, and their quality of life scores improved [28].

To our knowledge, this is the first report to show that celecoxib combined with tegafur/gimer-

acil/oteracil potassium had a better antitumor effect than either agent alone. To understand the change of related biological indicators, we first detected the expression of PCNA in each group. PCNA is an important indicator of tumor cell proliferation. We found that celecoxib and tegafur/gimeracil/oteracil potassium significantly inhibited the proliferation of tumor cells. The proliferation index in the combination group was lower than that in the celecoxib and tegafur/gimeracil/oteracil potassium groups ($P < 0.01$). Celecoxib and tegafur/gimeracil/oteracil potassium inhibited proliferation through different pathways. Tegafur/gimeracil/oteracil potassium may have inhibited proliferation by inhibiting the DNA synthesis of tumor cells. Celecoxib inhibited proliferation by inhibiting the activity of COX-2. In an ultraviolet B-induced skin tumor model, treatment with 5-FU and celecoxib displayed a synergistic antitumor effect. Celecoxib and 5-FU significantly inhibited the proliferation of tumor cells, and celecoxib enhanced the antitumor function of 5-FU [29].

We further illustrated the antitumor effect of the drugs by measuring apoptosis. Our study indicated that both celecoxib and tegafur/gimeracil/oteracil potassium promoted the apoptosis of gastric cancer xenograft tumors in nude mice. The apoptosis index in the combination group was higher than the celecoxib and tegafur/gimeracil/oteracil potassium groups ($P < 0.01$). The expression of Bcl-2 protein was down-regulated in the celecoxib group, the tegafur/gimeracil/oteracil potassium group and the combination group. The expression of Bcl-2 protein in the combination group was lower than that in the single drug group ($P < 0.05$). The expression of caspase-3 protein was up-regulated in the celecoxib, tegafur/gimeracil/oteracil potassium and combination groups. The expression of caspase-3 protein in the combination group was higher than that in the single-drug group ($P < 0.05$). Bcl-2, which inhibits apoptosis, is a member of the Bcl-2 family. Caspase-3 is a key enzyme in the apoptosis signaling pathway and mediates apoptosis. Flis et al [30] found that the combination of 5-FU or oxaliplatin with sulindac sulfide potently inhibited the growth of colorectal carcinoma cells *in vitro*. A possible mechanism was that the combination of these two drugs reinforced the S phase cell cycle block and increased cell apoptosis. In SGC-7901 gastric cancer cells [31] and Hela cervical cancer cells [32], celecoxib down-

regulated the expression of Bcl-2, promoted apoptosis and had a synergistic antitumor effect in combination with cisplatin. In a subcutaneous xenograft tumor model of colorectal cancer in nude mice, both celecoxib and 5-FU up-regulated the expression of caspase-9 and caspase-3, promoting apoptosis. The combination of the two drugs enhanced the antitumor effect [33]. Dandekar et al. [34] found that treatment with celecoxib and docetaxel displayed a synergistic antitumor effect. Both drugs significantly up-regulated the expression of caspase-9 and caspase-3 and promoted apoptosis. In conclusion, our study is consistent with previous findings.

Lymphatic metastasis is the main mechanism of metastasis in gastric cancer, thus determining the treatment selection and prognosis. The process of lymphangiogenesis promoting lymph node metastasis of malignant tumors has been confirmed in many experimental studies. Lymphangiogenesis is a process that generates new lymphatic vessels from pre-existing lymphatics [35] or lymphatic endothelial progenitors [36]. VEGF-C was the first lymphatic factor discovered, and COX-2 is a new lymphatic factor. Yonemura Y [37] studied 85 primary gastric cancers specimens. They found that there was a statistically significant positive correlation between the expression of VEGF-C and lymphangiogenesis, suggesting that VEGF-C may induce lymphangiogenesis in primary gastric cancers. Zhang J [38] studied 63 surgical resections from gastric cancer specimens. The study indicated that COX-2 expression was associated with lymphangiogenesis and lymph node metastasis in human gastric carcinoma. Immunohistochemical staining of 59 lung adenocarcinoma specimens showed that COX-2 levels were highly correlated with VEGF-C and lymphatic vessel density. These results provided evidence that COX-2 up-regulated VEGF-C and promoted lymphangiogenesis in human lung adenocarcinoma [39]. In our study, celecoxib and the combination of celecoxib and tegafur/gimeracil/oteracil potassium significantly inhibited the expression of COX-2 and VEGF-C and reduced the density of lymphatic vessels. However, tegafur/gimeracil/oteracil potassium alone did not have any obvious effects on these factors. Celecoxib may have down-regulated COX-2 levels, in turn inhibiting the expression of VEGF-C, and as a result reduced the density of lymphatic vessels. In xenograft tumor models of breast cancer in

nude mice, celecoxib inhibited the growth of transplanted tumors and the generation of new lymphatic vessels [40]. Celecoxib inhibited the expression of VEGF-C in Anip973 lung cancer cells, but PGE₂, the main metabolite of COX-2, elevated VEGF-C expression in AGZY83-a lung cancer cells. Furthermore, animal models have provided evidence that celecoxib decreased VEGF-C expression, lymphangiogenesis, and lymph node metastases. One mechanism may be that COX-2 regulated VEGF-C expression via the PGE₂ pathway and that EP1/EP4 receptors were involved in PGE₂-mediated VEGF-C production [41]. The conclusion of our study is in accordance with many studies, both domestic and foreign.

In conclusion, although both celecoxib and tegafur/gimeracil/oteracil potassium showed obvious antitumor effects when given alone, the combination of the two drugs produced synergistic antitumor efficacy in gastric cancer xenografts. The combination of celecoxib and tegafur/gimeracil/oteracil potassium inhibited tumor growth *in vivo* possibly through inhibiting the proliferation of gastric cancer cells and promoting apoptosis. Celecoxib and celecoxib in combination with tegafur/gimeracil/oteracil potassium may reduce COX-2 expression, in turn down-regulating VEGF-C expression, thereby inhibiting lymphangiogenesis.

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Disclosure of conflict of interest

None.

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