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ORIGINAL ARTICLE

Prescription consisting of Vitamin C and Baicalin inhibits tumor growth by enhancing the antioxidant capacity *in vivo*

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Summary

Purpose: *To explore the antitumor effect of prescription consisting of Vitamin C (Vc) and Baicalin (PVB).*

Methods: *To explore the antitumor effect of PVB, using U14 cervical tumor-bearing mice model was used and the drugs were administrated through the gavages. Spectrophotometry was used to determine the content of superoxide dismutase (SOD), malondialdehyde (MDA) and cytokines IL-2, Il-4 and IFN-γ.*

Results: *PVB had a better antitumor effect than baicalin and Vc used alone with an inhibition rate of 58.18% ($p<0.05$); PVB significantly improved the spleen index ($p<0.01$), and significantly reduced MDA content ($p<0.01$) but increased SOD activity in liver tissue and serum ($p<0.01$).*

Conclusions: *PVB shows better antitumor effect than Vc and baicalin used alone, and it can significantly enhance the immunity and antioxidant capacity of the mice.*

Key words: *antitumor effect, baicalin, large doses, PVB, vitamin C*

Introduction

Cervical cancer, a common gynecological tumor, is a serious threat to women's health in developing countries. There are about 371,000 new cases of invasive cervical cancer around the world, accounting for about 10% of the different kinds of cancer in females. China adds about 135,000 cases per year, accounting for 1/3 of the total incidence. About 50,000 patients die from cervical cancer each year, and in recent years a trend for increasing in younger ages has been noticed [1].

Vitamin C (Vc), also known as ascorbic acid, is a water-soluble hexose derivative with a variety of biological functions, plays a role in protecting against many disease including heart and cancer with no significant side effects [2]. In recent years, studies have shown that large doses of vitamin C significantly inhibited the growth of liver cancer, bladder cancer, prostate cancer, leukemia and other malignancies with no obvious effects on normal cells [3].

Baicalin is a traditional Chinese medicine derived from *Scutellaria* baicalensis with a wide range of physiological activities. Its active ingredients are flavonoids [4]. Currently, flavonoids with different structures extracted from *Scutellaria* have been identified, of which baicalin and its aglycone baicalein, Wogonin and its aglycone wogonoside contents are rich. Pharmacological studies have demonstrated that baicalin has antibacterial, antihypertensive, sedative, diuretic, choleretic, antiinflammatory, antiallergic and other active effects [5]. Baicalin (Baicalein, BAI), the main active ingredient of *Scutellaria* (skullcap) is the primary metabolite of baicalein [6]. The difference between baicalin and baicalein is the molecular structure of the 7-position substituent that in the former is a phenolic hydroxyl and in the latter a glycoside. Studies have shown that Baicalein has

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a variety of pharmacological effects, such as antiviral, antioxidative, antiallergic, antiinflammatory and anticoagulant, blocking cacium channels, regulating immunity and so on [7]. Recent studies have shown that baicalein plays an important role in colorectal cancer, liver cancer, prostate cancer and other malignancies, suggesting that it has broad-spectrum antitumor effects [8-15].

Methods

Establishment of U14 cervical tumor-bearing mice model

Three mice were intraperitoneally injected 0.3 ml of 1×10^7/ml U14 cervical cancer cells. The ascites was drained when the mouse abdomen enlarged significantly, and diluted at a concentration of 1×10^6 cells/ml with saline, and then inoculated with 0.3 ml of cervical cancer cells in the healthy mice' forelimb.

Dosing regimen

The successfully inoculated mice were divided into 10 groups, 8 for each group, named as follows:

Blank control group: no treatment;

Negative control group: intragastric administration of distilled water, 0.2 ml;

Positive control group: intraperitoneal injection of 25 mg/kg cyclophosphamide, 0.2 ml;

Baicalin group: intragastric administration of 80 mg/kg Baicalin, 0.2 ml;

Vc group: intragastric administration of 40 mg/kg Vc, 0.2 ml

Vc-baicalin high-dose group: 80 mg/kg Vc +80 mg / kg baicalin, gavage 0.2 ml;

Vc-baicalin low-dose group: 40 mg/kg Vc +80 mg / kg baicalin, gavage 0.2 ml;

The mice were administered the above-mentioned agents for 15 days, daily weighed, and growth conditions were recorded every day. On the 16 th day, the mice were eyeball-blooded and sacrificed, and then liver, kidneys, thymus, spleen and tumor tissues taken from the mice were used to detect relevant indicators.

Determination of inhibition rate, thymus index, spleen index

The tumor tissues, thymus and spleen were taken from each group of mice and weighed. Inhibitory rate, thymus and spleen indices (the ratio of thymus or spleen mass divided by the body weight of the animal) were calculated according to the formulae 1, 2 and 3:

Inhibition rate = (negative group tumor mass - experimental group tumor mass) / negative group tumor mass × 100% (Formula 1);

Thymus index (mg/10g weight) = thymus mass (mg) / weight (g) × 10 (Formula 2);

Spleen index (mg/10g weight) = spleen mass (mg) / weight (g) × 10 (Formula 3).

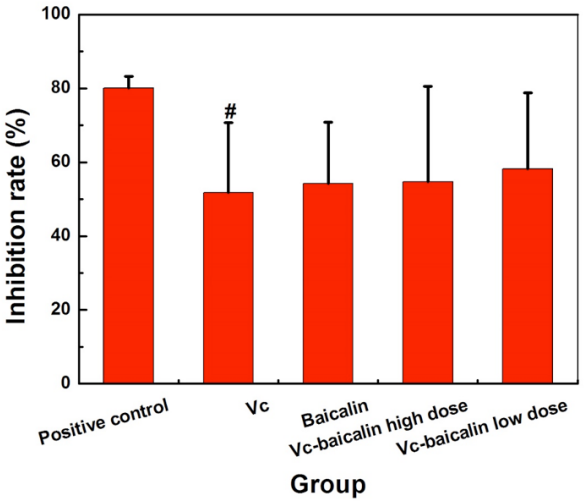

Figure 1. Histogram of inhibition rate of mice in each group. Compared with positive group, #p<0.05.

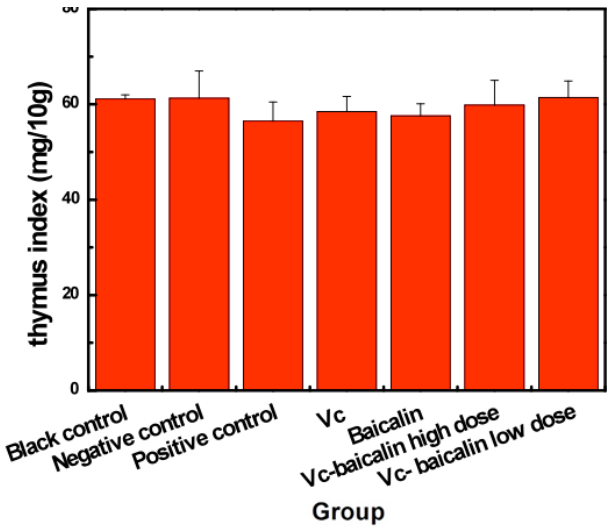

Figure 2. Histogram of thymus index of mice in each group. No significant differences were noticed.

Determination of protein, SOD and MDA in serum and liver tissue

1. Determination of protein, SOD and MDA in serum:

The obtained whole blood 1.5 ml was stayed for 30 min, and then centrifuged for 10 min and the supernatant was separated. According to the instructions of kits [total superoxide dismutase (T-SOD) assay kit (hydroxylamine method) and malondialdehyde (MDA) assay kit (TBA method), Nanjing Jiancheng Bioengineering Institute, Nanjing, China], the protein content, SOD and MDA of each group were measured in serum.

2. Determination of protein, SOD and MDA in liver tissue:

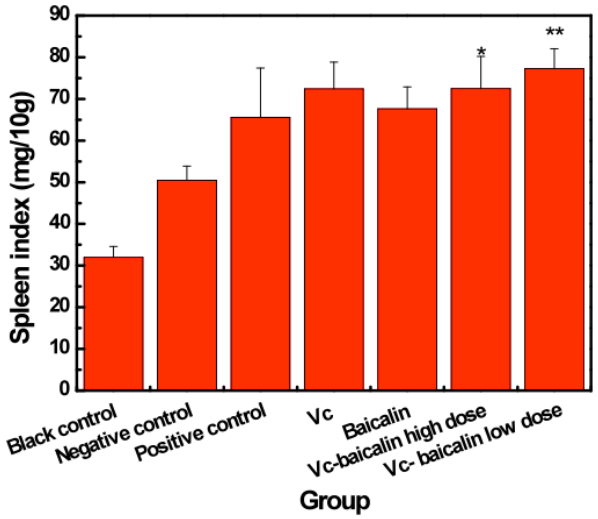

Figure 3. Histogram of spleen index of mice in each group. Compared with the negative group, *p<0.05, **p<0.01.

The liver tissue of mice was washed with cold saline, weighted and then made into 10% homogenate by adding normal saline to a ratio of 1:9. The homogenate was then centrifuged at 2500 rpm for 10 min at 4^{0}C to prepare the supernatant. SOD activity and MDA content were measured directly with commercial kits [Total superoxide dismutase (T-SOD) assay kit (hydroxylamine method) and malondialdehyde (MDA) assay kit (TBA method), Nanjing Jiancheng Bioengineering Institute, Nanjing, China].

Determination of IL-2, IL-4 and IFN-γ content in serum

The standard curves were drawn and the levels of IL-2, IL-4 and IFN-γ were determined in serum according to the manufacturer's instructions (Interleukin-2, Interleukin-4 and Interefon-γ assay kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistics

Data analyses were performed using SPSS 18 software (SPSS Inc., Chicago, Ill, USA) and the results are shown as mean ± standard deviation. Student's t-test was used to evaluate the differences between inhibition rates, thymus index, spleen index, cytokines' levels, SOD activity and MDA content vs blank control, negative control, positive control, Vc, Baicalin and Vc-Baicalin low-dose group. p<0.05 was considered as statistically significant.

Results

Mouse tumor model

On the third day after inoculation of U14 cervical cancer cells, tumors grew well at mice armpits, indicating that the vaccination was successful, and the success rate was above 99%.

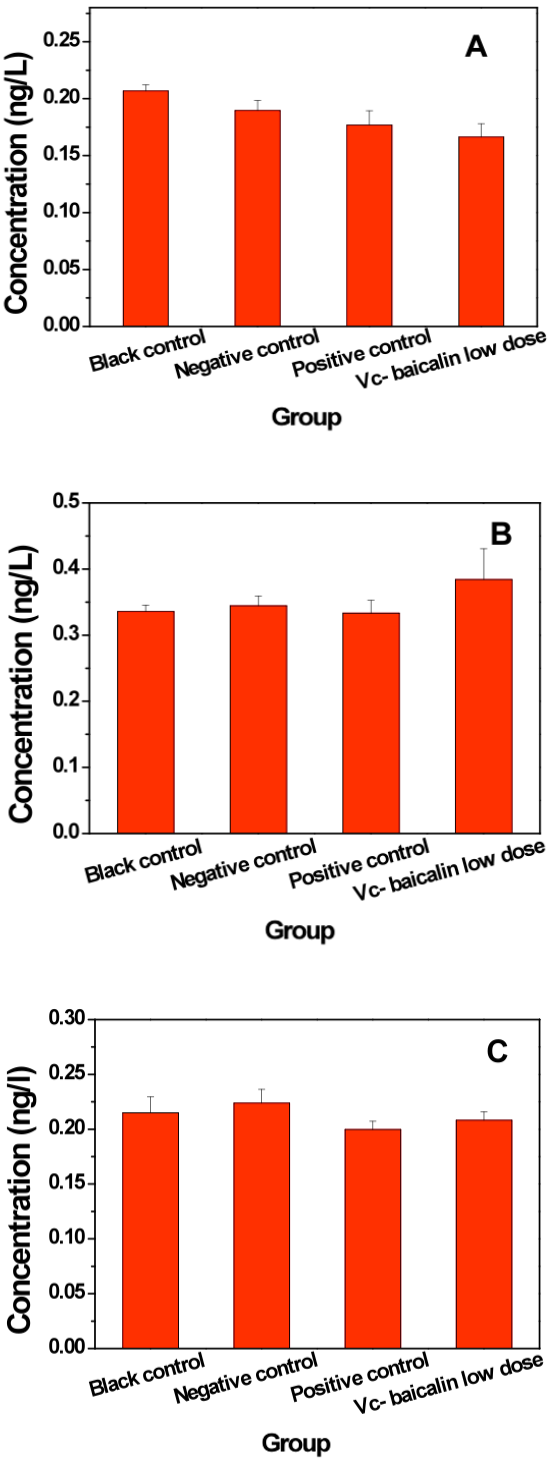

Figure 4. The serum IL-2, IL-4 and IFN-γ levels. **A**: IL-2 (p: 0.0126), **B**: IL-4 (p: 0.303), **C**: IFN-γ (p: 0.281).

Effect of PVB on tumor growth

The tumors of each mouse group were weighed and the inhibition rate was calculated

according to formula 1. The results are shown in Figure 1. We found that the low-dose Vc-baicalin group had the best inhibitory effect on tumor growth among the entire experimental groups. PVB improved the inhibition rate compared with Vc- and baicalin-alone groups.

Effect of PVB on thymus index and spleen index

The thymus index and spleen index in each group were calculated. Figures 2 and 3 show that the thymus index of PVB group was higher than that of Vc and baicalin groups. Both the low and high-dose PVB improved the spleen index, but the improvement was stronger in the low-dose PVB group.

Effects of PVB on antioxidant ability

The effects of PVB on antioxidant ability are shown in Figure 4 and 5. Low-doses Vc- baikalin significantly reduced the MDA content ($p<0.01$) and increased the SOD activity both in liver tissue and in the serum ($p<0.01$). Thus, PVB could significantly improve the antioxidant ability of mice.

Effect of PVB on cytokines

The results of the content of IL-2, IL-4 and IFN-γ are shown in Figure 6. Vc-baicalin did not alter significantly the immune system of mice. Thus, Vc-baicalin may directly induce apoptosis of tumor cells and inhibit tumor growth instead of improving the immune system of mice.

Discussion

In this study we found that adding a proper amount of baicalin in Vc can enhance the antitumor effect of the latter ($p<0.05$). The tumor inhibition rate of PVB was higher than Vc and baicalin used alone. This may be related to PVB ability to improve the body's antioxidant mechanisms significantly ($p<0.01$). PVB could significantly increase the spleen index but had little effect on IL-2, IL-4 and IFN-γ ($p<0.01$). This phenomenon may be related with side effects of baicalin. Therefore, PVB inhibits the growth of tumor by improving the antioxidant capacity and directly killing tumor cells. Zhang et al. studying the baicalin effects on osteosarcoma cells in 2012 [16], showed that baicalin can inhibit their growth significantly, creating cell cycle arrest in the G1 phase; caspases were also activated which induced apoptosis. But these authors did not do in-depth study of its mechanism. The study of Wang et al. [17]

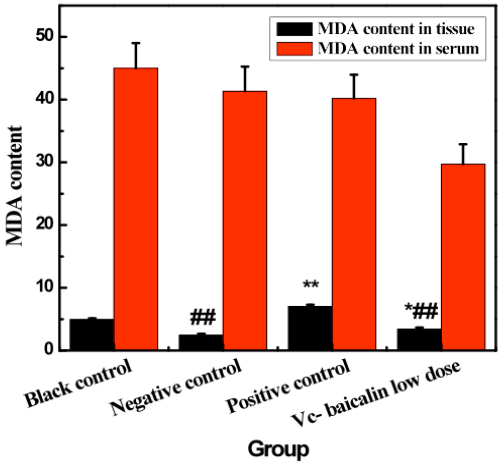

Figure 5. Histogram of MDA content of mice in each group. Compared with the negative group, *p<0.05, **p<0.01; compared with positive group, ##p<0.01.

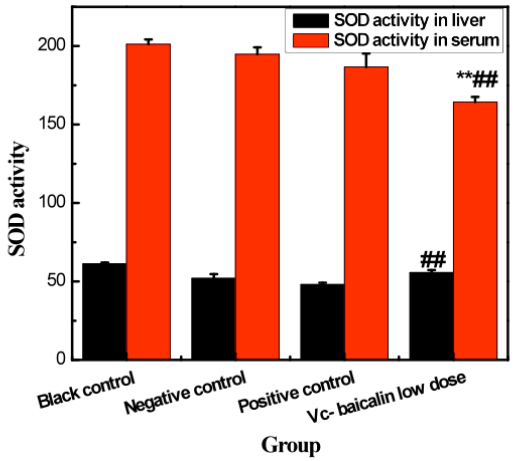

Figure 6. Histogram of SOD activity of mice in each group. Compared with the negative group, **p<0.01; compared with positive group, ##p<0.01;

showed that baicalin inhibited the growth of human oral squamous cell carcinoma Tca-8113 cells and induced apoptosis, and they concluded that its mechanism may be related to downregulation of Bcl-2 expression and upregulation of Bax expression. Li et al. [18] considered that baicalin could inhibit the proliferation of Tca-8113 cells by inhibiting the transition from G1 to S phase and ascribed its antitumor functions to enhanced expression of Fas. Chen's research [19] showed that the mechanism of the inhibitory effects of baicalin was related to inhibition of the expression of PCNA, p53, hMLHl, hMSH2, TGF-P 1 and IGF-IIR,

and to the apoptosis and cell cycle arrest in the G2/M phase. Li et al [20] found that baicalin has an obvious inhibitory effect on both the mouse timor and hepatocellular carcinoma cell line H22 via a mechanism of telomerase activity inhibition.

Wang et al. [21] prepared a recombinant protein (avian influenza virus NS1A) and combined this recombinant protein with baicalin for the treatment of lung cancer cell line SPC-A1; they found that the antitumor effect of this combination of recombinant protein with baicalin was significantly increased, which showed superiority of drug combination and proved the theoretical basis for cancer drug combination therapy.

The present study, using the U14 cervical cancer cell line in a tumor-bearing mice model studied the antitumor effect of baicalin combined with Vc (PVB). The results showed that PVB had better antitumor effect than Vc and baicalin used alone and that it can enhance considerably the antioxidant capacity of mice. Further research is needed to elucidate the mechanisms of the enhanced antitumor activities of PVB.

Acknowledgements

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