Biotherapeutic mechanism of salidroside on gastric carcinoma cells

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Biotherapeutic mechanism of salidroside on gastric carcinoma cells

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Abstract. To study the biotherapy mechanisms of salidroside in treatment of gastric carcinoma, we inoculated the BALB/C mice with a gastric carcinoma cell line as the research model. The mice were divided into four groups. Mice in the experimental groups received different doses of salidroside for sixty days after grafting. Mice were fed with normal saline as control. All the mice were executed at the 61st day. Tumor volume was detected periodically and tumor weight was measured immediately after the mice sacrificed. Serum cytokines, perforin and granzyme B were detected by ELISA. The anti-tumor effect was examined by cytotoxic T lymphocyte (CTL) method. Our results demonstrated that salidroside could suppress the proliferation of gastric carcinoma cells by activating the immune cells and inducing the secretion of cytokines, perforin and granzyme B. Our study suggests that salidroside inhibited the growth of gastric cancers by activating the immune cells, which may lay a better basis for further study on gastric cancer biotherapy.

1. Introduction

Chinese herb salidroside had been verified to have the function on precancerous lesions, especially for chronic gastritis [1,2]. Salidroside is likely to suppress gastric cancer cell growth and induce the apoptosis of tumor cells. Apoptosis plays an important role in the growth and progression of various tumor cells. It was confirmed that apoptosis is always up-regulated in tumors by lots of drugs, such as hormone, anti-cancer cytotoxic drugs, or Chinese herbs [3-5]. Studies demonstrated that Chinese herbs are able to increase the apoptosis of gastric carcinoma cells grafted in mice [5-7].

In the present study, we investigated whether salidroside could induce the effects of the immune cells to suppress gastric carcinoma transplanted into mice, further verifying the anti-cancer mechanisms of salidroside.

2. Material and Methods

2.1 Tumor Cell Lines and Mice

The MFC mouse gastric carcinoma cell line was given from the Molecular Center of the first clinical hospital of Xi'an Jiaotong University. 6 to 8 weeks old female BALB/C mice were purchased from the Experimental Animal Center at Air Force Military Medical University. All the mice were raised under specific pathogen free conditions, and all operations were conducted in accordance with approved protocols for the care of experimental animals. The research was granted by the Ethics Committee of

animal Study at Shaanxi University of Chinese Medicine (2004-4B).

2.2 Drugs

Salidroside was provided from Sigma Biotechnology Company (MO, USA). Normal saline (NS) was used to dissolve salidroside, and stored the stock solution at -20°C. The stock solution could be further diluted in NS for use.

2.3 Administration

After transplanted with the gastric carcinoma cells, the mice were randomly separated into four groups, three experimental groups and one control group. The experimental groups were received salidroside solution. Each mouse of the experimental groups was fed with 2.0mL, 1.0mL and 0.5mL of salidroside solution every day for 60 days after grafting. The control group received NS according to the procedure. All the mice were sacrificed at the 61st day.

2.4 Perforin and Granzyme B ELISA Assay

The ELISA was used to measure the cytokine perforin and granzyme B in serum collected from above mice according to manufacturer's instruction. The OD values were obtained by an ELISA Reader System.

2.5 Cytotoxic T lymphocyte (CTL) Method

All the mice were administrated as above-mentioned. At the last day, 2.5×10^7 spleen cells were collected from the different mice groups and cultured with 10 units/ml of mouse interleukin-2 in RPMI 1640 in 5% CO_2 at 37 °C. Five days later, the spleen cells were collected as effector cells, and the MFC gastric cancer cells were used as target cells. The Non-Radioactive Cytotoxicity Lactate Dehydrogenase release assay Kit (Promega, USA) was applied to detect the spleen cells inhibiting the MFC cells in 10:1, 20:1 and 40:1 ratios. Specific lysis was calculated as the following formula: specific lysis % = [(experimental OD value − effector spontaneous OD value − target spontaneous OD value)/(target maximum OD value − target spontaneous OD value)]×100. Results are repeated three times.

2.6 In Vivo Tumor Therapeutic Test

To confirm whether salidroside suppressing the proliferation of cancers, we assigned three experimental groups to fed with salidroside solution. Each mouse in the experimental groups was given different concentrations of salidroside solution for 60 days after grafted. 5×10^6 MFC cancer cells were rinsed after digested with enzyme and resuspended in 0.2 ml of PBS. Each mouse was s.c. injected into the left flank. Tumor volume was examined with calipers every 3 days after grated. The volume was detected as the formula: $V = (a^2b)/2$.

2.7 Statistical Analysis

All data expressed as means ± S.D. The SPSS software was used to analyze the significance of differences. $P<0.05$ was regarded statistically significant.

3. Results

3.1 Suppressing the Proliferation of Cancers by Salidroside

Tumor volume and weight were significantly suppressed by salidroside solution treatment, compared with the control mice, ($P<0.05$, Table 1). The results demonstrated that the higher the dose of salidroside, the less the tumor volume and weight. The morphological changes of cancer were showed that the gastric carcinoma glands were smaller and less in salidroside solution treated group. There was a significant difference between the control and experimental group.

Table 1. Salidroside inhibited the tumor proliferation for gastric cancer (x±s)

Treatment	Tumor weight (g)	Tumor volume (mm^3)
High-dose salidroside solution	0.49±0.22[a]	255.42±33.54[a]
Middle-dose salidroside solution	0.63±0.27[b]	332.25±31.32[b]
Low-dose salidroside solution	0.81±0.33[c]	451.46±26.56[c]
Saline	2.03±0.26	578.32±35.43

[a]P<0.05, [b]P<0.05, [c]P<0.05 vs control group.

3.2 Salidroside Induced the Up-regulation of Granzyme B and Perforin in Vivo

The concentrations of granzyme B and perforin in the serum of the experimental mice were higher compared with the control group (P<0.05, Table 2). The results suggested that salidroside can elicit specific immune T cells response.

Table 2. The serum concentrations of granzyme B and perforin from salidroside treated mice (x±s)

Treatment	Perforin (μg/ml)	Granzyme B (μg/ml)
High-dose salidroside solution	368.23±15.26[a]	306.56±12.35[a]
Middle-dose salidroside solution	255.84±14.54[b]	250.34±10.82[b]
Low-dose salidroside solution	154. 33±12.68[c]	134.65±8.74[c]
Saline	7.85±1.26	6.68±2.54

[a]P<0.05, [b]P<0.05, [c]P<0.05 vs control group.

3.3 Salidroside Elicited CTLs Response

Cytotoxicity assay showed that spleen cells from mice fed with salidroside demonstrated higher cytolytic lysis on gastric cancer cells than that from mice treated with NS (P < 0.05, Table 3). In contrast, spleen cells from the control group exhibited a weaker ability on cancer cells lysis. The results indicated that salidroside were effective inducers for immune cell activation.

Table 3. Salidroside elicited effective immune cell cytotoxicity on gastric cancer cell (x±s)

Treatment	Inhibition Rate (%)	
	Splenocytes: MFC cells (10:1)	Splenocytes: MFC cells (40:1)
High-dose salidroside solution	38.72±7.65[a]	69.73±10.25[a]
Middle-dose salidroside solution	23.64±8.32[b]	44.32±8.64[b]
Low-dose salidroside solution	14.52±6.87[c]	25.53±7.46[c]
Saline	6.64±1.25	5.89±2.46

[a]P<0.05, [b]P<0.05, [c]P<0.05 vs control group.

4. Discussion

Gastric cancer is one of the malignant gastroenterological carcinoma around the world. Until now most patients with gastric cancer is still found at late stage when they are diagnosed. Even with surgical treatment, they are at a high risk of recurrence and mortality. Therefore, there is a big demand for effective therapeutic strategy on gastric cancer. Some research indicated that Chinese herb salidroside possess anti-tumor effects on gastric precancerous lesions, increasing the elimination of inflammation and repair of injury of the chronic gastritis [7,8]. Compared with chemotherapy, Chinese herbs have less toxic side effect, it deserves to carry out the further investigation on its anti-tumor mechanisms.

Same as other cancers, gastric cancers is always remaining atypical cell growth and differentiation. In this study, we showed that after treatment with salidroside, the proliferation of cancer cells was suppressed. We presumed that the herbs could elicit the immune cells to damage the cancer cells as well as bring about the apoptosis of the cancer cells [8-10]. The results showed that salidroside could induce the immune cells to synthesize cytokines, granzyme B and perforin, which are able to result in the apoptosis and necrosis of cancer cells. We also verified that spleen cells were activated and elicited the definite cytotoxic lysis on cancer cells, which were likely to secret cytokines, such as granzyme B and perforin to exert their immune effects. It has been verified that immune cells could induce the apoptosis of cancer cells [11]. Apoptosis is a strictly regulated and active complex process by which cells are initiated to undergo programmed cell death, and would not hurt adjacent cells or induce any inflammatory cells infiltration [11,12]. Various factors trigger the proteo-lysis cascade reaction depending upon mitochondrion or APO-1/FAS/CD95 signal transduction pathways [12,13]. There are a number of oncogene or suppressor gene translation products taking part in the regulation and implementation of apoptosis. The results indicate that the mechanism of suppressing gastric carcinoma cells by salidroside is correlated with activating immune cells and further inducing apoptosis.

5. Conclusions

Salidroside inhibited gastric carcinoma cell proliferation. The anti-cancer mechanism of salidroside lies in activating immune cells to secret certain cytokines, such as perforin and granzyme B to inhibit and kill tumor cells. The definitive mechanisms of salidroside inhibiting gastric cancer cells still needs further investigation.

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