Multiple Biotherapy Effects of Salidroside on Gastric Carcinoma

Xiaoping Wang, Qiaoxia Wang, Huanping Lin, Bing Xu, Na Chang, Yanfang Pan and Yan Fang

ABSTRACT

To verify the anti-tumor effect of salidroside on gastric cancer, BALB/C mice grafted with a mouse gastric adenocarcinoma cell line MFC was used as the experimental model. The mice were divided into four groups, one control and the other three different drug administration experimental groups. Animals in the three experimental groups received Shenfoweikang herb over a 60-day period starting at the first day after grafting. Animals received saline as controls. All the mice were sacrificed at 61 days after being grafted. The anti-tumor effect was assessed by three ways: (1) tumor size was periodically measured during the life of the animals and tumor weight was determined by an electron balance immediately after the animals killed. (2) Apoptotic indices (AI) were examined by the TUNEL method and flow cytometric analysis. (3) The expression of VEGF, STAT3 and DEC1 in tumor tissues was examined by immunostaining and analyzed by Image J analysis system. Compared with controls, tumor growth (size and weight) was significantly inhibited with salidroside treatment ($P<0.05$). AI in gastric cancer grafted mice was significantly increased in the salidroside treatment group compared with the controls. The expressions of VEGF, STAT3 and DEC1 in tumor tissues were down-regulated after treated with salidroside. The anti-tumor effect of gastric cancer cell growth in vivo by salidroside is related with the induction of the cell apoptosis and down-regulation of VEGF, STAT3 and DEC1 signal transduction system.

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INTRODUCTION

In clinic studies, Chinese herb salidroside had been found to have effect on premalignant lesion, especially on gastric diseases [1,2]. Salidroside might inhibit gastric carcinoma cell proliferation and cause tumor cell death. Apoptosis plays a crucial role in the proliferation and turnover of cells in various tumors. It has been clear that its extent is often enhanced in tumor by many anticancer drugs, such as cytotoxic drugs, hormone, or some Chinese herbal medicine [3-5]. Researches indicated that Chinese herbs could enhance apoptosis of human gastric cancer grafted in mice [5-7]. Differentiated embryo chondrocyte gene 1 (DEC1), a nuclear transcription factor, can translocate into nucleus, bind DNA and regulate related gene expression. Recent results showed that the expression of DEC1 was significantly higher in the gastric cancer cells than the adjacent normal tissues [8]. JAK/STATs (signal transducers and activators of transcription) signaling pathway is closely related to cell proliferation, differentiation and apoptosis, which can lead to abnormal proliferation and malignant transformation. Research confirmed that there was a high expression of STAT3 in gastric cancer, which was closely related to TNM stage, invasion depth, lymph node metastasis and tumor grade [9]. Studies showed that the overexpression of STAT3 and VEGF in tumor cells can increase the microvessel density and promote the progression of gastric cancer [10].

Based on the previous studies, we presumed whether salidroside could affect the apoptotic indices of gastric cancer grafted onto mice and the expression of VEGF-STAT3-signal transduction in gastric cancer, further confirming the anti-tumor mechanism of the Chinese Shenfoweikang herbs.

MATERIAL AND METHODS

Mice and Chemical Reagents

Forty 6-8 weeks old BALB/C mice (weight 18-22 g) and a mice gastric carcinoma cell line MFC were obtained from the Fourth Military Medical University. The mice were subcutaneously grafted with the MFC cells. The tumor transplantation procedure was described previously [6]. Rabbit anti-mouse DEC1, STAT3 and VEGF polyclonal antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). EnVisionTM kits were purchased from Dako Corp (Carpinteria, CA, USA). Salidroside was purchased from Sigma (MO, USA). Salidroside was dissolved in normal saline (NS) at the stock concentration of 10mmol/L, then stored at -20°C. This is the basic solution, which can be diluted in NS as needed.
Administration

After grafting the mice were randomly divided into 4 groups, one control and the other three experimental groups assigned to receive salidroside solution. Each animal in the three experimental groups was given 2.0mL, 1.0 mL and 0.5mL of salidrosidesolution by gastric perfusion every day over a 60-day period beginning at 1st day after grafting. The control animals received normal saline according to the same schedule. Animals were killed 61 days after being grafted.

Assessment of Tumor Growth

The effect of therapy was assessed by two ways: (1) tumor size was measured twice a week by multiplying two perpendicular diameters. (2) tumor weight was determined immediately by electron balance after the animals were killed. The tumor tissues were fixed in 10% formalin, embedded in paraffin and cut into 5μm sections coated on the slides for staining.

Detection of Apoptosis

For detection of apoptotic cells, apoptotic indices were examined by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate fluorescence nick end labeling (TUNEL) method and flow cytometry analysis. (1)TUNEL: In situ cell death detection Kit POD (Roche Applied Science, Indianapolis, USA) was used to detect the apoptotic cell. The procedures were referred to the kit protocol.

Immunostaining Methods

All sections were deparaffinized and rehydrated with graded alcohols. Endogenous peroxidase was then blocked with 3 mL/L H2O2 diluted in methanol for 30 min at room temperature. Antigen retrieval was performed by treating the slides in citrate buffer in a microwave for 10 min. The slides were incubated in a moist chamber with DEC1, STAT3 or VEGF rabbit polyclonal antibody (1:100) at 4°C overnight. After a complete wash in phosphate buffered saline (PBS), the slides were incubated with horseradish peroxidase labeled goat anti-mouse antibody (1:100) for 45 min at 37°C. After a complete wash in PBS, the slides were developed in 0.5g/L freshly prepared 3,3’-diaminobenzidine solution (DAB, Sigma Co, St. Louis, Mo, USA) for 8 min, and then counterstained with hematoxylin, dehydrated, air dried, and mounted. All data expressed as means ± S.D. The Student’s t test was performed to analyze the significance of differences in different groups of mice. P<0.05 was considered statistically significant.
RESULTS

Inhibition of Tumor Growth by Salidroside

Compared with the control group, tumor growth (size and weight) was significantly inhibited by treatment with salidroside solution (P<0.05, Table 1). The results showed that the higher the concentration of salidroside treatment, the less the tumor weight and size.

Induction of Tumor Cell Apoptosis by Salidroside

Apoptotic index (AI) in mice loaded with gastric cancer cells was significantly elevated to 19.83±2.36 % by TUNEL method and 17.75±5.52 % FACScan in salidroside treatment group, compared with the controls (TUNEL: 2.79±1.53 %, P<0.05; FACScan: 5.78±2.43 %, P<0.05, Table 2).

TABLE I. SALIDROSIDE INHIBITED THE GROWTH OF GASTRIC CANCER (X±S).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor weight (g)</th>
<th>Tumor size (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-dose salidroside solution</td>
<td>0.49±0.22</td>
<td>255.42±33.54</td>
</tr>
<tr>
<td>Middle-dose salidroside solution</td>
<td>0.63±0.27</td>
<td>332.25±31.32</td>
</tr>
<tr>
<td>Low-dose salidroside solution</td>
<td>0.81±0.33</td>
<td>451.46±26.56</td>
</tr>
<tr>
<td>Saline</td>
<td>2.03±0.26</td>
<td>578.32±35.43</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.05, ‡P>0.05 vs control group.

TABLE II. SALIDROSIDE INDUCED APOPTOSIS ON GASTRIC CANCER CELLS (X±S).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Apoptotic index (AI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TUNEL</td>
</tr>
<tr>
<td>High-dose salidroside solution</td>
<td>19.83±2.36</td>
</tr>
<tr>
<td>Middle-dose salidroside solution</td>
<td>11.65±5.46</td>
</tr>
<tr>
<td>Low-dose salidroside solution</td>
<td>3.12±1.45</td>
</tr>
<tr>
<td>Saline</td>
<td>2.96±1.82</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.05, ‡P>0.05 vs control group.

TABLE III. EXPRESSIONS OF VEGF, STAT3 AND DEC1 IN GASTRIC CANCERS (X±S).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Optical density (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEGF</td>
</tr>
<tr>
<td>High-dose salidroside solution</td>
<td>0.021±0.012</td>
</tr>
<tr>
<td>Middle-dose salidroside solution</td>
<td>0.026±0.015</td>
</tr>
<tr>
<td>Low-dose salidroside solution</td>
<td>0.048±0.014</td>
</tr>
<tr>
<td>Saline</td>
<td>0.049±0.017</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.05, ‡P>0.05 vs control group.
Expressions of VEGF, STAT3 and DEC1 in Gastric Cancers

DEC1, STAT3 and VEGF immunoreactivities were detected in 40 mice gastric tumors. The optical density (OD) values of DEC1, STAT3 and VEGF in salidroside solution treated groups were significantly lower than that in control (P < 0.05, Table 3).

DISCUSSION

Gastric carcinoma is one of the most common malignant gastrointestinal carcinoma in the world. At present gastric carcinoma is still detected later in most patients throughout the world, and even with curative resection, they remain at a high risk of relapse and mortality. Thus, there is a great need for effective adjuvant therapy for patients with gastric carcinoma. Some studies suggested that Chinese herb salidroside have therapeutic effects on gastric pre-malignant lesion, with increasing the reversal of the atrophic gastritis, decreasing the recurrence and improving the life quality [6-8]. Because of its lower toxic side-effect compared with chemical therapy, it is worth to make a further research on its anti-cancer mechanism.

Similar to the other malignant tumors, gastric carcinoma is always accompanying with abnormal cell proliferation and differentiation. In the present study, we found that after treated with salidroside, the growth of tumor was inhibited compared with the control group. Apoptosis is a complex, tightly regulated, and active cellular process by which individual cells are triggered to undergo programmed cell death, and simultaneously will not injury neighboring cells or elicit any inflammatory reactions [11,12]. Various triggering factor initiate corresponding proteolysis cascade reaction depending on mitochondrion or APO-1/FAS/CD95 receptor mediate apoptotic pathways [13,14]. There are many oncogenes and tumor suppressor gene products in the regulation and execution of apoptosis. The results suggest that the mechanism of the inhibition of gastric cancer cells in vivo by Shenheweikang herbs is related with activating immune cells and further inducing apoptosis. The VEGF-STAT3-DEC1 signal transduction pathways play an important role in the development and progression of gastrointestinal cancers. Our results suggested that salidroside could down-regulate the expression of VEGF-STAT3-DEC1 signal transduction proteins in gastric cancer, thus inhibiting the growth of tumor cells.

In conclusion, salidroside inhibited gastric cancer cell growth. The anti-tumor effect of salidroside lies in inducing gastric cancer cells apoptosis and down-regulating the expression of VEGF-STAT3-DEC1 signal transduction protein in tumor cells. The detailed molecular mechanism of salidroside inhibiting gastric cancer cells still needs further investigation.
ACKNOWLEDGEMENTS

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REFERENCES