Basic Research Paper

Antitumor effects of raddeanin A on S180, H22 and U14 cell xenografts in mice

Ming-Kui Wang,* Li-Sheng Ding and Feng-E Wu

Natural Product Research Center; Chengdu Institute of Biology; Chinese Academy of Sciences; Chengdu, Sichuan, P.R. China

Key words: anemone raddeana Regel, raddeanin A, antitumor effect, mouse, xenograft

Background and Objective: Raddeanin A, a triterpenoid saponin from Anemone raddeana Regel, has good antitumor activity in vitro. This study was to investigate its antitumor effects on tumor cell xenografts in mice. Methods: The inhibitory effects of raddeanin A on the proliferation of human nasopharyngeal carcinoma KB cells and ovarian cancer SKOV3 cells were measured by MTT assay. The inhibitory effects of raddeanin A injection on the growth of sarcoma S180, liver cancer H22 and cervical carcinoma U14 cell xenografts in mice and the effect of raddeanin A lavage on the growth of S180 cell xenografts were measured. The acute toxicity of raddeanin A was also measured. Results: The 50% inhibition concentration (IC50) of raddeanin A was 4.64 µg/mL for KB cells and 1.40 µg/mL for SKOV3 cells. When injected with raddeanin A at a dose of 4.5 mg/kg, the growth inhibition rates of S180, H22 and U14 cell xenografts were 60.5%, 36.2% and 61.8%, respectively. When lavaged with raddeanin A at a dose of 200 mg/kg, the growth inhibition rate of S180 cell xenografts was 64.7%. The median lethal dose (LD50) of raddeanin A lavage was 16.1 mg/kg. Conclusion: Raddeanin A has good antitumor activity both in vitro and in vivo, and would be a potential antitumor medicine.

"Liang Tou Jian," also known as "Zu Jie Xiang Fu" and Anemone raddeana Regel in China, belongs to the Ranunculaceae Family, Anemone Tribe and raddeana Regel Plantae, and is used to treat cancer, rheumatism, paralysis, and pain sensation around waist and legs.1 Vast amount of researches has already conducted on the component analysis of "Liang Tou Jian," in which the primary components are triterpenoid saponins, and varieties of them have already isolated and identified.2-7 Our research discovered that one of the triterpenoid saponins, raddeanin A, exhibited cytotoxicity in vitro.8 Ren et al.9 also confirmed the anti-tumor activity of "Liang Tou Jian." Because the active component is triterpenoid saponin with big molecular weight and water-solubility, it would be possible to lose the antitumor activity due to the solubility and saponin hydrolysis in vivo although it has cytotoxicity in vitro. Therefore, in order to assess the application perspective of this compound, it is necessary to confirm its anti-tumor activity in vivo.

Material and Methods

Materials. Main drugs and reagents. Cyclophosphamide injection was produced by the 12th Pharmaceutical Manufacturer of Shanghai. Other reagents were all analytical purities. "Liang Tou Jian" herb was purchased from Lanzhou Herbal Medicine Wholesales Station, and identified by Professor Xiao Shun-chang, Chengdu Institute of Biology, Chinese Academy of Sciences.

Animals. ICR mice, with weight range of 18–22 g and without limitation on sex specification, were provided by the Experimental Animal Room of the Antibiotic Industrial Research Facility in Sichuan. The qualification certificate was Chuan Animal Quality Control No. 68. For the same batch of mice, they were of the same sex. Every group contained at least 10 mice.

Cell line. Human nasopharyngeal carcinoma cell line KB was provided by Beijing Pharmaceutical Laboratory of China Medical Science Institute. Human ovarian cancer cell line SKOV3 was from the Huaxi Medical Center of Sichuan University. Mouse granuloma cell line S180, hepatic carcinoma cell line H22, and cervical cancer cell line U14 were all provided by the Pharmacology Department of Antibiotic Industrial Research Facility in Sichuan.

Methods. Preparation of raddeanin A. "Liang Tou Jian" herb (3 kg) was powdered and extracted with 95% ethanol (15 L) for 3 times at room temperature. The ethanol was evaporated to obtain 800 g extracts, which was then dispersed in 2500 mL of water and extracted with ethyl acetate (1500 mL) for 3 times. The lower layer was then placed in a round bottom flask, and solid KOH (75 g) added. The solution was heated at 80°C for hydrolysis for 2 h, adjusted pH to 7 with 5% hydrochloric acid, then extracted with n-butanol (500 mL) for 4 times. The extracted solutions were combined and n-butanol recovered in reduced pressure to obtain crude saponin (540 g). The crude saponin (180 g) was dissolved in methanol (1000 mL) and added with silica gel (100–200 mesh, 600 g). Silica gel with absorption of saponin was obtained by evaporating methanol in...
Antitumor effects of raddeanin A on S180, H22 and U14 cell xenografts in mice

### Results

**Inhibitory effects of raddeanin A on proliferation of KB and SKOV3 cells.** Raddeanin A had certain antitumor effect on the proliferation of KB and SKOV3 cells. The IC_{50} values of raddeanin A were 4.64 µg/mL for KB cells and 1.40 µg/mL for SKOV3 cells.180

**Inhibitory effect of raddeanin A on tumor growth.** The effects of raddeanin A injection on the growth of S180, H22, and U14 tumor cell xenografts and the effect of raddeanin A lavage on the growth of S180 tumor cell xenografts in mice were shown in Table 1 and Table 2.

When injected with raddeanin A at a dosage of 4.5 mg/kg for 10 days, the growth inhibition rates were 60.5% for S180 cell xenografts, 61.8% for H22 cell xenografts, and 61.8% for U14 cell xenografts; when the dosage was 1.5 mg/kg, the growth inhibition rates were 43.3%, 34.6%, and 38.2%, respectively. Raddeanin A injection showed significant inhibitory effect on tumor growth (p < 0.01, p < 0.05, p < 0.01), with obvious dosage-effect relation. When lavaged with raddeanin A at dosages of 200, 100, and 50 mg/kg for 10 days, the growth inhibition rates of S180 cell...
Each group contained 10 mice.

### Table 3 The acute toxicity of raddeana A lavage in mice

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>Logarithmic dose</th>
<th>Dead mice [number (%)]</th>
<th>Probability unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.00</td>
<td>0.477</td>
<td>10 (100)</td>
<td>7.12</td>
</tr>
<tr>
<td>2.10</td>
<td>0.322</td>
<td>9 (90)</td>
<td>6.38</td>
</tr>
<tr>
<td>1.47</td>
<td>0.167</td>
<td>7 (70)</td>
<td>5.64</td>
</tr>
<tr>
<td>1.03</td>
<td>0.013</td>
<td>5 (50)</td>
<td>4.90</td>
</tr>
<tr>
<td>0.72</td>
<td>-0.143</td>
<td>2 (20)</td>
<td>4.17</td>
</tr>
</tbody>
</table>

### Table 4 The acute toxicity of mice by raddeana A injection

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Logarithmic dose</th>
<th>Dead mice dose [number (%)]</th>
<th>Probability unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.0</td>
<td>1.556</td>
<td>10 (100)</td>
<td>6.61</td>
</tr>
<tr>
<td>25.2</td>
<td>1.401</td>
<td>8 (80)</td>
<td>5.89</td>
</tr>
<tr>
<td>17.6</td>
<td>1.246</td>
<td>5 (50)</td>
<td>5.18</td>
</tr>
<tr>
<td>12.6</td>
<td>1.100</td>
<td>2 (20)</td>
<td>4.46</td>
</tr>
<tr>
<td>8.6</td>
<td>0.934</td>
<td>2 (20)</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Each group contained 10 mice.

xenografts were 64.7%, 42.3%, and 37.8%, respectively. Raddeanin A lavage also showed significant inhibitory effect on tumor growth (p < 0.01), with obvious dosage-effect relation.

**Acute toxicity of raddeanin A.** The death of mice within one week after the completion of raddeanin A lavage is shown in Table 3. The LD₅₀ of raddeanin A was 1.08 g/kg and the 95% CI was 0.80 to 1.33 g/kg. At 5 min after raddeanin A lavage, spontaneous activities of these mice were reduced, with ataxia and polypnea; Death occurred 30 min afterward. Autopsies of these mice showed no abnormality in organs. Acute toxicity primarily resulted in dysfunction of the central nervous system and respiratory system.

The death of mice within two weeks after the completion of raddeanin A injection is shown in Table 4. The LD₅₀ of raddeanin A was 16.1 mg/kg and the 95% CI was 12.7 to 20.1 mg/kg. At 2 min after raddeanin A injection, the mice manifested contracted abdomen, body twisting, and other symptoms similar to those observed after raddeanin A lavage.

**Discussion**

Both injection and lavage of raddeanin A have significant inhibitory effect on tumor growth, with obvious dosage-effect relation. During the experiment, raddeanin A caused less death of mice as compared with cyclophosphamide. During the acute toxicity experiment, no death occurred in alive mice after the termination of raddeanin A administration, suggesting that raddeanin A has no toxicity accumulation. Because of hemolytic property, the application of saponin injection is limited, therefore, it is important that saponins could be administered orally.

Oleanolic acid type saponins exist widely in plants, even in foods, but few reports are available on their antitumor activity. We had previously isolated many oleanolic acid type saponins, and discovered that only a few of saponins have antitumor effect. However, no matter used in vivo or in vitro, its anti-tumor activity is weaker than that of raddeanin A although their differences in structure are only the type and sequence of 3-sugar chain. In addition, because of large polarity of raddeanin A, it is difficult to be absorbed in the digestive tract. However, raddeanin A lavage still shows good antitumor activity. Therefore, the mechanism of raddeanin functioning in vivo is worthy of further investigation.

In our study, the therapeutic index of raddeanin A injection was 3.6, while that of raddeanin A oral administration reached 5.4. In comparison with other anti-tumor drugs with a therapeutic index of 1, raddeanin A showed much less toxicity. With its unique advantage, raddeanin A may be a potential antitumor drug.

**References**