A PRELIMINARY STUDY OF THE EFFECTS OF CHEMOTHERAPEUTIC AGENTS IN COMBINATION WITH CHINESE HERBAL COMPOUNDS AS IMMUNOMODULATORS IN MOUSE S180

Jin Xingquan* 金杏泉  Lu Wanqin 陆婉琴  Xu Jingfang 徐静芳
Sun Zengyi* 孙曾一

Department of Medical Oncology, Cancer Hospital, Shanghai Medical University
* Laboratory of Clinical Pharmacology, Cancer Institute, Shanghai Medical University

The effect of the Chinese herbal compound (CHC) on solid sarcoma 180 (S180) in Swiss mice was studied either alone or in combination with 5-flourouracil (5FU), cyclophosphamide (CYT) or mitomycin C (MMC). The preliminary results indicated that combination treatment seemed to possess better antitumor activity than chemotherapy alone. The treatment with CHC alone however had neither an obvious antitumor effect in tumor bearing mice nor toxicity in normal mice. These results show that CHC may stimulate organs of the immune system such as the spleen to be immunomodulators and enhance the antitumor activity of some chemotherapeutic agents.

Until now, the majority of clinical immunotherapy trials have focused on active non-specific immunotherapy; i.e., exploiting so called non-specific immunomodulatory substances or biological response modifiers. Traditional Chinese medicine, used in China for the treatment of cancer patients for hundreds of years, is still a useful tool in the multidisciplinary treatment of cancer. Some Chinese herbs, such as Radix candonopsis pilosulae (党参); Radix astragali (黄芪); Ligustrum lucidum (女贞子); Polyporus umbellatus (猪苓); Caulis millettiae (鸡血藤), have been used to restore the normal immunological function of the body and to consolidate the constitution. These potential immunomodulating functions have been evidenced by many clinical and experimental investigations. Some traditional Chinese herbs, such as Rhizoma curcumae zedoariae (莪术); Oldenlandia diffusa (蛇舌草) Dioscorea opposita (淮山药) appear to have an antitumor effect. We used a Chinese herbal compound (CHC) containing the herbs mentioned above as immunomodulators and observed their effects when combined with 5-flourouracil (5FU), cyclophosphamide (CYT) or mitomycin C (MMC) on solid S180 tumors in Swiss mice.

MATERIALS AND METHODS

Drugs and Treatment

Oral CHC was formed from the water extracted from the eight herbs and made into water soluble granules. Each 100 gm of CHC contained 9 gm Radix candonopsis pilosulae; 9 gm Radix astragali; 9 gm Ligustrum lucidum; 15 gm Polyporus umbellatus; 12 gm Caulis millettiae; 9 gm Dioscorea opposita; 9 gm Rhizoma curcumae zedoariae; and 15 gm Oldenlandia diffusa. The granules were dissolved in saline and administered orally from day 1-10 or day 1-14 after tumor cell inoculation (day 0). A solution of 5FU (841004, Shanghai 13th Pharmaceutical Company), CYT (851008, Shanghai 12th Pharmaceutical Company), or MMC (Lot No. 967, Kyowa Hakko Kogyo Company, Ltd., Japan) was prepared with saline just before use and injected intraperitoneally on day 1 at the dosage indicated for each experiment.

Tumors and Animals

Tumor S180 was supplied by the Shanghai Institute of Materia Medica, Academic Sinica. It was maintained in Swiss mice via intraperitoneal passage every week. For this study, an inoculant of $5 \times 10^6$ or $10^6$ viable tumor cells in 0.2 ml of saline was injected subcutaneously or intramuscularly respectively. Viable cells were counted.
by means of the trypan blue exclusion test. Swiss mice were supplied from the Shanghai Institute of Cancer. The mice were 6-8 weeks old and weighed 18-24 gm at the onset of the study. They were housed in groups in suspended wire cages and food and water were freely available.

**Evaluation of Antitumor Activity**

Tumor size was measured as two diameters at right angles using vernier calipers. The volume was calculated by the following formula: tumor volume (cm³) = d²D/2 (d=width of tumor; D=length of tumor). Tumor weight refers to the actual measurement of dissected tumor mass at autopsy. At least 9-10 mice per each experimental group were used throughout and the results were evaluated by the significance test (t test).

### RESULTS

**Effect of CHC in Normal Mice**

The effect of CHC in normal Swiss mice was investigated first. The effect of CHC on body weight, liver weight and spleen weight was observed. The mice given CHC were divided into two groups, a low dosage group (120 mg/kg) and a high dosage group (1200 mg/kg, equal to the dosage given to patients). As shown in table 1, both low and high dosages increased the spleen weight in normal mice. Although the high dosage group markedly increased the spleen weight (p<0.01), there was no statistically significant difference between the control group and the low or high dosage group in body weight gain as seen on day 4, 8, 14 and liver weight on day 15.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Treatment schedule (mg/kg, po)</th>
<th>Av. body weight (g±S.E.)</th>
<th>Liver* weight (g±S.E.)</th>
<th>Spleen* weight (mg±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>22.20 ± 1.18</td>
<td>23.83 ± 3.09</td>
<td>24.50 ± 4.50</td>
</tr>
<tr>
<td>Low dosage</td>
<td>120mg/kg/d × 14d</td>
<td>23.25 ± 2.86</td>
<td>24.30 ± 3.45</td>
<td>25.22 ± 3.88</td>
</tr>
<tr>
<td>High dosage</td>
<td>12000mg/kg/d × 14d</td>
<td>22.75 ± 2.4</td>
<td>24.40 ± 2.90</td>
<td>27.70 ± 4.28</td>
</tr>
</tbody>
</table>

*: Determined on day 15  **: p<0.01 compared to control group

### Effect of 5FU with CHC on the Growth of S180

The mice were inoculated subcutaneously with viable 5 × 10⁶ cells of S180, and 24 hours after the inoculation, they were randomly divided into control, 5FU alone, CHC alone and 5FU plus CHC groups. The dosage and administration are shown in table 2. Tumor sizes were measured on day 8 and 15 after implantation (day 0). The results showed that 5FU alone and 5FU plus CHC resulted in significant tumor growth inhibition on day 8 (p<0.05). Furthermore, in this group, four mice had no tumor growth on day 8. On day 15, the volume of the tumor in this combination group was smaller than in the 5FU alone group, while the CHC alone group has no significant tumor growth inhibition.

### Effect of CYT with CHC on Growth of S180

Mice were implanted intramuscularly with viable 10⁶ cells of S180. The treatment schedule of each group is shown in table 3. The mice were killed 15 days after tumor implantation and the tumor mass was removed and weighed. Results showed that there was not only a significant statistical difference in average tumor weight between the CYT plus CHC group and the control group (p<0.001), but also between the CYT plus CHC and the CHC alone groups (p<0.001).

### Effect of MMC with CHC on Growth of S180

The effect of MMC plus CHC against S180 was also studied. As shown in table 4, the average tumor weight on day 15 in the MMC plus CHC