Anti-hepatoma activity and mechanism of corn silk polysaccharides in H22 tumor-bearing mice

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A B S T R A C T

Corn silk is a well known traditional Chinese herbal medicine and corn silk polysaccharides (CSP) possess multiple pharmacological activities. However, the antitumor effect of CSP on hepatocarcinoma has not been studied. This study aimed to investigate the effects of CSP on tumor growth and immune functions in H22 hepatocarcinoma tumor-bearing mice. The results demonstrated that CSP could not only inhibit the tumor growth, but also extended the survival time of H22 tumor-bearing mice. Besides, CSP administration could increase the body weight, peripheral white blood cells (WBC) count, thymus index and spleen index of H22 tumor-bearing mice. Furthermore, the production of serum cytokines in H22 tumor-bearing mice, such as IL-2, IL-6 and TNF-\(\alpha\), was enhanced by CSP treatment. In addition, no toxicological effects were observed on hepatic function and renal function in CSP-treated mice transplanted H22 tumor cells. In summary, this experimental finding indicated that CSP could elevate the immune functions in H22 tumor-bearing mice to enhance its antitumor activity and CSP seems to be a safe and effective agent for the treatment of hepatocellular carcinoma.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies throughout the world with a high incidence and mortality, which is often diagnosed at an advanced stage [1,2]. Although surgical interventions or non-surgical therapeutic modalities have been employed, HCC is rarely curative. Currently, chemotherapy is one of the important treatment methods, but the side effects are difficult to tolerate, and as a result, searching new compounds with better effectiveness and lower toxicity have received more and more attentions as a potential origin of new therapeutic anti-tumor drugs for HCC patients [3,4].

Previous reports have shown that immunosuppression can be clearly detected in both cancer patients and tumor-bearing animals [5–7], demonstrating that the immune system plays an important role in immunosurveillance against malignant cells. The immune system is able to identify and destroy nascent tumors and to thereby function as a primary defense against cancer [8]. Thus elevating immunity is much helpful to hepatocarcinoma immunotherapy and immunoprophylaxis [9,10]. Many attempts have been made during the past years to develop immunostimulating approaches to cancer treatment [11]. In these approaches, polysaccharides are being extensively explored for their potential for treatment and prevention of cancer. Some polysaccharides could scavenge free radical, induce differentiation of cancer cells, and enhance animal or human’s antitumor ability via activating different immune responses in the host [12–15].

Corn silk (\textit{Zea mays} L.) is a waste material from corn cultivation and available in abundance throughout the world [16]. Corn silk is another well-known traditional Chinese medicine that has been widely used for treatment of some diseases, such as cystitis, edema, kidney stones, diuretic, prostate disorder, and urinary infections as well as bedwetting and obesity [17–22]. Corn silk has been reported to contain various chemicals, including proteins, vitamins, alkaloids, tannins and mineral salts, steroids, flavonoids and polysaccharides [23–25]. Previous studies showed that polysaccharides from corn silk could lead to weight loss [26], regulate blood sugar [27] and improve gastrointestinal movement [28]. However, the data regarding its in vivo antitumor and immune enhancing properties are very limited. In this regard, the aim of this study was to prepare corn silk polysaccharides (CSP), and to investigate the antitumor and immunoregulatory activities of CSP in a H22 tumor-bearing mice model.

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2. Materials and methods

2.1. Materials and chemicals

Corn silk was gathered from corn field in September 2012 in Xi’an city of China.

2.2. Preparation of corn silk polysaccharides

Briefly, the dried powder (500 g) was defatted with anhydrous ethanol at 80 °C for three times, each for 1 h. After filtration, the residues were dried in air and then were extracted with water at 100 °C (1:15 (w/v), 1 h, 3 times). After cooling down to room temperature, the whole extract was filtered and centrifuged at 10,000 rpm for 30 min at 4 °C to obtain a clear solution. The supernatant was concentrated to 200 ml and three volumes of 95% alcohol were added slowly by stirring to precipitate the polysaccharide. After overnight precipitation at 4 °C, the sample was centrifuged as described above followed by repeated washing in sequence with anhydrous ethanol, acetone and diethyl ether. The refined polysaccharide pellets were completely dissolved in an appropriate volume of distilled water and intensively dialyzed through a cellulose bag for 24 h to remove the low molecular weight materials, and freeze dried, giving 10.3 g of crude corn silk polysaccharides (CSP).

2.3. Cells and animals

Mouse H22 hepatocarcinoma cells (purchased from Beijing Cowin Biotech Co. Ltd., Beijing, China) were cultured in DMEM (GIBCO, Carlsbad, CA, USA), supplemented with 10% fetal calf serum (FCS), 2 mmol/l glucose, 100 U/ml penicillin and 100 μg/ml streptomycin. Murine H22 cells were maintained in the ascitic form by sequential passages into the peritoneal cavities of Kunming mice, by weekly intraperitoneally (i.p.) transplanting 1 × 10⁷ tumor cells in 0.2 ml as previously described [29]. Male Kunming mice with body weight ranging from 18 to 22 g were purchased from the Experimental Animal Center of the Fourth Military Medical University (Xi’an, China). Animals were maintained in a pathogen-free environment (23 ± 2 °C, 55 ± 5% humidity) on a 12-h light/12-h dark cycle with food and water supplied ad libitum throughout the experimental period. All the animal experimental procedures are approved by the Animal Care and Use Committee of the Fourth Military Medical University.

2.4. Cell viability assay

The H22 cells were seeded at a concentration of 1 × 10⁵ cells/mL in a volume of 0.1 mL in 96-well plates in the presence of CSP (0–400 μg/ml) for 24, 48 and 72 h. The growth inhibitory effect was determined by measuring MTT [3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] assay [30, 31]. Briefly, each well was added 20 μL of 5 mg/mL of MTT and incubated for another 4 h. Then the culture media were removed, 150 μL of DMSO was added to each well. Absorbance at 490 nm was detected by microplate ELISA reader. The inhibition ratio of H22 cell proliferation was calculated as follows: inhibitory ratio (%) = 1 − average absorbance of treated group/average absorbance of control group × 100.

2.5. In vivo antitumor experiment

To establish murine solid tumors H22 transplanted model, corresponding ascites tumor cells (4 × 10⁵ cells in 0.2 mL PBS) were subcutaneously injected into the right axillary region of the Kunming mice in all groups. Twenty-four hours after inoculation, mice were divided randomly into five groups. CSP was administered orally (p.o.) to each group at different dosages (50, 100, 200 mg/kg), respectively. The positive control group received cisplatin at a dosage of 5 mg/kg by intraperitoneal injection and the vehicle control group was treated with 0.9% normal saline via p.o. The mice weights were recorded before and after each drug administration. 24 h after the last administration of tested drug on the 10th day of the experiment, blood samples were collected from the mice’s eyes and serum was harvested by centrifugation. Then, all the mice were sacrificed and the whole bodies, the segregated tumor, thymus and spleen of the mice were weighed immediately. The tumor inhibitory rate was calculated by the following formula: tumor inhibitory rate (%) = (W_control − W_treated)/W_control × 100. W_treated and W_control were the average tumor weight of the treated and vehicle control mice, respectively.

2.6. Survival assay

For the survival analysis, fifty mice were inoculated with tumor cells prepared by intraperitoneal inoculation (i.p.) and treatment was performed as mentioned above for 60 days. The survival time of animals was monitored and recorded daily. The test continued for 60 days and those that lived more than 60 days were defaulted as 60 days. The percent survival (%) was calculated using the following equations: percent survival (%) = [(10 – numbers of mice died in each group)/10] × 100.

2.7. Immune function analysis

Blood was collected from the mouse eye orbit, followed by being diluted with 2% acetate (acetic acid), and the white blood cell (WBC) count was determined by microscopy. The influence of different drugs on the immune organs was evaluated by the immune organ index [32]. The organ indexes of spleen and thymus were calculated as follows: organ index = average weight of organ (mg)/body weight (g).

2.8. Measurement of cytokines

The serum levels of cytokines IL-2, IL-6 and TNF-α were analyzed by a commercially available ELISA kit (Quantikine, R&D Systems, Minneapolis, MN, USA), following the manufacturer’s instructions.

2.9. Analysis of liver and kidney function

Biochemical indexes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), uric acid (UA) and creatinine (CRE) were recorded by automatic biochemical analyzer (Sysmex, Japan) using reagents purchased from Sysmex Incorporation.

2.10. Statistical analysis

Statistical analysis was performed using SPSS, version 11.0 (SPSS, Chicago, IL, USA). The data were expressed as the mean ± SD, and significant differences were assessed using Student’s t test. P < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Effect of CSP on H22 cell growth in vitro

To evaluate the effect of CSP on cell growth, proliferation assays were performed on H22 cells and cell viability was determined by the MTT assay. For these assays, cultured cells were exposed to CSP (0–200 μg/ml) for 24, 48 and 72 h. CSP did not inhibit H22 cell proliferation in all concentrations at any time point (data not shown). This result agreed with the reports in the literatures that
many polysaccharides usually have no direct toxicity to tumor cells in vitro [33–35].

3.2. Effect of CSP on H22 tumor growth in vivo

To further investigate whether CSP exerted an in vivo antitumor activity, H22 xenograft tumors were excised from each group and weighed following 10 day’s CSP pretreatment at dose of 50, 100 and 200 mg/kg. The antitumor effect of CSP on H22 tumor-bearing mice is summarized in Table 1. At the end of the study, the average tumor weight in the model control group was 2.34 ± 0.35 g. Compared to model control group, the tumors weight in the CSP groups was significantly decreased to 1.23 ± 0.33 g for 50 mg/kg (P < 0.01), 1.39 ± 0.41 g for 100 mg/kg (P < 0.01) and 1.41 ± 0.40 g (P < 0.01) for 200 mg/kg (P < 0.01), respectively. The tumor weights in the CSP groups were not significantly different to the cisplatin group (1.13 ± 0.31). According the tumor inhibitory rates of the CSP and cisplatin-treated groups were 47.44%, 40.60%, 39.74% and 51.71%, respectively.

3.3. Effect of CSP on life extension of mice

To determine the life prolonged effect of CSP, the ascites H22-bearing mice were treated with 200 mg/kg of CSP for 60 days. The average survival time of ascites H22-bearing mice treated with 200 mg/kg of CSP was extended more than three times compared with that in the model control group (Fig. 1). The effect was almost comparable to that of cisplatin, the standard drug. These data demonstrated that CSP treatment greatly prolonged the survival period of H22 tumor-bearing mice.

3.4. Effect of CSP on WBC count, spleen/thymus indexes and body weight

As shown in Table 2, the WBC count, spleen index, and thymus index of mice in cisplatin-treated group were significantly lower than the model control group (P < 0.05 or P < 0.01); however, there was no significant difference between the model control group and the CSP-treated groups (P > 0.05). In addition, the body weight of the cisplatin-treated mice was significantly decreased compared with the tumor control mice (P < 0.05). No significant loss of body weight was observed among the animals after treatment with all doses of CSP. These data suggested that CSP had a beneficial effect on immune organ, body weight and caused no bone marrow suppression in H22 tumor-bearing mice.

3.5. Effect of CSP on cytokine levels

Serum cytokines play a pivotal role in fighting against the tumor growth [36]. The cytokines IL-2 and IL-6 are capable of inducing the proliferation of responsive T-cells. TNF-α has been proven to be an effective anticancer agent in in vitro and in vivo preclinical studies, by inducing apoptotic cell death and tumor necrosis. As shown in Table 3, the expression levels of IL-2, IL-6 and TNF-α were significantly higher in the CSP groups than in the tumor model group, particularly in the medium- and high-dose groups (P < 0.01). However, these parameters levels were lower in the cisplatin group than in the model group (P < 0.05 or P < 0.01). All the data implied that promoting cytokine secretion by CSP might be one of its adjunct anticancer mechanisms.

3.6. Effect of CSP on hepatic and renal function

An excellent chemotherapeutics include not only the virtue of depressing the growth of malignant cells but also the minimum toxicity to their normal organs. To examine the potential toxicological effects of CSP administration on the kidney and liver of the host, we evaluated their serum renal function markers such as BUN, UA, and CRE, and serum hepatic function markers including ALT and AST. As shown in Table 4, the increased levels of serum ALT, AST, BUN, UA, and CRE were obviously observed in cisplatin group as compared to the model group (P < 0.05). All these indexes were restored or downregulated after CSP treatment at three doses, although they
Table 3
Effects of CSP on serum cytokines (L-2, IL-6 and TNF-α) levels of transplanted H22 mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dosage (mg/kg)</th>
<th>IL-2 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>11.55 ± 3.25</td>
<td>26.47 ± 3.31</td>
<td>16.28 ± 2.38</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5.00 ± 0.02</td>
<td>13.05 ± 1.47</td>
<td>6.74 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>CSP</td>
<td>13.06 ± 3.58</td>
<td>36.51 ± 3.43</td>
<td>26.81 ± 3.05</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>19.98 ± 4.21</td>
<td>45.82 ± 5.52</td>
<td>32.41 ± 4.16</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>23.35 ± 4.62</td>
<td>53.95 ± 5.94</td>
<td>36.97 ± 4.82</td>
<td></td>
</tr>
</tbody>
</table>

Model: model control, in which received 0.9% normal saline.
Positive: positive control, in which received 5 mg/kg cisplatin.
CSP: test groups, in which received 50, 100, 200 mg/kg CSP.
Values are shown as mean ± SD (n = 10). Significance was determined using the Student’s t-test.
* P<0.05, vs. model group.
** P<0.01, vs. model group.

Table 4
The effects of CSP on liver and renal function indexes of transplanted H22 mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dosage (mg/kg)</th>
<th>Enzyme activity (1 U/L)</th>
<th>ALT</th>
<th>AST</th>
<th>BUN</th>
<th>UA</th>
<th>CRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>100.28 ± 7.74</td>
<td>346.21 ± 23.17</td>
<td>8.25</td>
<td>0.85</td>
<td>98.07 ± 8.63</td>
<td>41.28 ± 3.75</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>263.85 ± 15.84</td>
<td>625.08 ± 45.64</td>
<td>16.10</td>
<td>2.24</td>
<td>120.73 ± 9.42</td>
<td>56.25 ± 5.32</td>
<td></td>
</tr>
<tr>
<td>CSP</td>
<td>113.08 ± 8.97</td>
<td>374.04 ± 26.75</td>
<td>9.07</td>
<td>0.76</td>
<td>106.38 ± 7.53</td>
<td>43.56 ± 4.57</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>110.55 ± 9.09</td>
<td>370.85 ± 26.07</td>
<td>8.55</td>
<td>0.76</td>
<td>102.17 ± 7.67</td>
<td>41.02 ± 4.21</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>105.72 ± 9.15</td>
<td>351.40 ± 27.72</td>
<td>8.08</td>
<td>0.72</td>
<td>100.18 ± 8.76</td>
<td>40.25 ± 4.03</td>
<td></td>
</tr>
</tbody>
</table>

Model: model control, in which received 0.9% normal saline.
Positive: positive control, in which received 5 mg/kg cisplatin.
CSP: test groups, in which received 50, 100, 200 mg/kg CSP.
Values are shown as mean ± SD (n = 10). Significance was determined using the Student’s t-test.
* P<0.05, vs. model group.

4. Conclusions

At present, chemotherapy is usually adopted as one of the main measures for tumor therapy. However, the adequate dosage in a complete course may induce severe adverse effects, such as bone marrow suppression, liver and kidney function disorder and a lowered immunological function [37]. This may possibly limit the adequate dosage, and decrease the therapeutic effects. As the tumor developing, the tumor-derived factors may also have harmful impact on the immune system of hosts [38]. Therefore the immune system plays an important role in fighting tumorigenesis. Now, many reports about immuneenhancement, the body resistance-strengthening and antitumor activities of the polysaccharides from traditional Chinese herbs medicines have attracted more and more attention [39,40]. The results of the present investigation demonstrate CSP from corn silk significantly inhibited the tumor growth and extended the survival time of mice bearing H22 hepatocellular carcinoma. The antitumor activity of CSP was in a dose dependent manner, with no signs of toxicity to weight, kidney and liver. Furthermore, peripheral WBC count, thymus index and spleen index of H22 tumor-bearing were also improved. In addition, CSP administration could significantly decrease the production of serum IL-2, IL-6 and TNF-α in mice bearing H22 tumor. The increase may also explain the antitumor properties of this polysaccharide. Based on the results of the present study, it can be suggested that the antitumor activity of CSP might be at least in part due to immune function activation. But the molecular mechanism by which CSP imparts cancer chemopreventive effects has not been clearly defined and further studies are needed.

References