Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(3):507-511

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Influence of polysaccharides from *angelica* and *astragalus* on H22 hepatocarcinoma mice

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ABSTRACT

The present study was carried out to evaluate influence of polysaccharides from angelica and astragalus（AAP）on H22 hepatocarcinoma mice. The built H22 hepatocarcinoma models mice were treated with AAP, astragalus membranaceus polysaccharide (AMP), angelica sinensis polysaccharide (ASP) (50 mg/kg bw/day) and 5-FU (25 mg/kg bw/day) for 10 days, once a day, 0.2 mL/per. The major criteria for evaluating their antitumor effects including tumor inhibition rate, serum ALT and AST, spleen index and thymus index of mice. The AAP showed significant suppression effect on the growth of H22 tumor cell and serum ALT and AST (P<0.01). AAP could increase thymus index and resist the tumor-induced splenomegaly. And that these functions of AAP were more effective than AMP and ASP. These results indicated that AAP had an obvious antitumor activity in vivo.

Key words: Polysaccharides from a*ngelica* and a*stragalus* (AAP); A*stragalus membranaceus* polysaccharide (AMP); A*ngelica sinensis* polysaccharide (ASP); 5-FU; H22 tumor model mice

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INTRODUCTION

Cancer is as a major public health problem worldwide. Surgery, radiation, chemotherapy, and endocrine therapy are the standard cancer therapies [1]. Although chemotherapy is effective, it is associated with severe adverse events and drug resistance, especially multidrug resistance (MDR) [2].

Recently, more and more attention is being placed on plant polysaccharides due to their various biological activities that could be applied to health-care food or medicine, especially its antioxidant, antitumor effects and immunostimulatory [3-5]. A*stragalus membranaceus* polysaccharide (AMP) has received an extensive attention, mainly with respect to its immunopotentiating properties, its ability to counteract the side effects of chemotherapeutic drugs, and its anticancer activity [6,7]. *Angelica sinensis* (AS) polysaccharide (ASP) owns anti-oxidative, antiinflammatory, and immunomodulatory and so on pharmacological activities [8,9].

However, little has been investigated with respect to the antitumour efficacy of their synergistic polysaccharides from a*ngelica* and a*stragalus* (AAP), except for the anti-oxidation activity of AAP *in vitro* conducted in our laboratory [10].

The purpose of this work was to examine whether AAP has more effective antitumor activity than ASP and AMP in H22 hepatocarcinoma mice. For this purpose, KM model mice infected with murine H22 hepatocarcinoma cell were used to study the antitumor efficacy of AAP through analyzing the inhibition rate of tumor, serum ALT, AST and the immune organs index. To our knowledge, this may be the first report of the antitumour activity of AAP *in vivo*.

EXPERIMENTAL SECTION

Medical herbs and reagents

Angelica sinensis and *Astragalus membranaceus* were purchased from Minxian Shunfa Medicinal Material Co. (Gansu Minxian City, China). Water extraction, ethyl alcohol deposition and Sevag method [11] were used to extract AAP, ASP and AMP, whose total carbohydrate content were respectively assayed to be 87.6%, 64.3% and 75.1% by the phenol-sulfuric acid method [12]. Fluorouracil (5-FU) was obtained from ShangHai XuDong Pharmaceutical Ltd. Co. (ShangHai, China). All other reagents or drugs were of analytical grade and commercially available.

Tumor cell line

Murine H22 hepatocarcinoma cell line was obtained from Gansu Tumor Hospital (Lanzhou, China).

Animals

Specific pathogen-free KM mice weighing 20 ± 2 g (male and female in equal numbers) were purchased from Experimental Animal Center of Lanzhou University, China. Mice with clinical signs of disease or abnormalities were not used in the study. All the mice were allowed free access to water, were fed a non-medicated ration and were acclimated for at least 2 days prior to experimentation. All studies involving animals were performed in accordance with local institutional and governmental regulations on the use of experimental animals.

Establishing solid tumor mouse model

Antitumor activity against a solid tumor mass was evaluated in KM model mice. Murine H22 hepatocarcinoma cells were dissociated into single cell suspensions by trypsin and the cell concentration was adjusted to 1×10^6 cells/mL by PBS. Then, 0.2 mL of the cell suspension was injected into abdominal cavity of specific pathogen-free mice weighing 20±2 g. These mice were sacrificed after 10 days, H22 cells in ascites were collected and washed, and resuspended in PBS. And H22 cells viability was determined through trypan blue exclusion test. Only these cells with >90% cell viability were applied to building the solid tumor mouse model. 100 mice were respectively injected subcutaneously (s.c.) into the right oxter with 0.2 mL H22 tumor cell suspension (1×10^6 H22 cells/mL) to establish solid tumor model mice according to the reported experimental procedure [13].

Antitumor efficacy of AAP in solid tumor mice

Above the 100 models mice were randomly divided into 5 groups, each group comprising 20 mice. They were respectively AAP-treated groups, ASP-treated groups, AMP-treated groups, the H22 solid model control group (untreated group) and 5-FU group. In addition, a control group of 20 normal (uninjected, untreated) mice was run in parallel to the other groups in order to study the influence of AAP on immune organs, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum in solid tumor mice.

24 hours after inoculation of H22 tumor cells, all mice received corresponding treatment. Briefly, the mice of the AAP, AMP and ASP groups were respectively orally administered AAP AMP and ASP (50 mg/kg/day body mass) for 10 days. 5-FU (25 mg/kg/day body mass) was infused to the stomach once a day for 10 days, from the day after implantation. At the same time, the mice of the model control group and the normal group were orally administered distilled water (10 mL/kg/day). Parameters for determining the anti-tumor efficacy of AAP, ASP and AMP in solid tumor mice included the tumor inhibition rate of AAP, serum ALT, AST and spleen and thymus indexes.

24 hours after the last administration, all animals were sacrificed according to the local institutional and governmental regulations on the use of experimental animals. Blood samples were collected, and the serum was separated by centrifugation at 5000 rpm for 10 min at a low temperature for assay of AST and ALT according to the kit instructions from Nanjing Jiancheng Bioengineering Insititute, China. At the same time, the solid tumors, spleens and thymuses were excised and weighed.

The tumor inhibition rate (%) was calculated according to the following formula:

$$\text{Inhibition rate}\ (\%) = \frac{\text{Average tumor weight of the model group} - \text{Average tumor weight of the treatment group}}{\text{Average tumor weight of the model group}} \times 100\%$$

The spleen index and the thymus index were calculated respectively according to the following formula:

$$\text{Spleen index} = \frac{\text{Average spleen weight}\ (\text{mg})}{\text{Average mouse body weight}\ (\text{g})} \qquad \text{Thymus index} = \frac{\text{Average thymus weight}\ (\text{mg})}{\text{Average mouse body weight}\ (\text{g})}$$

Statistical analysis

The data were analyzed using the statistical software SPSS 18.0 for windows. Analysis of variance (ANOVA) was used to analyze the data. Value of $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

***In vivo* effects of AAP on the inhibition of tumor cell growth**

The inhibition effects of AAP on H22 tumor cell growth in mice were investigated, which were shown in Table 3. We found that the tumor weight in the model group (3.48 ± 0.41) was the biggest in the all groups (Table. 1). However, they were significantly decreased on day 10 in all of the treatment groups ($p < 0.05$). The tumor weight of AAP, AMP and ASP groups were 1.39 ±0.14, 1.63 ±0.25 and 1.97 ±0.38, respectively. While the tumor weight of 5-FU group was only 1.34± 0.17, whose antitumor effect was superior to that of AMP and ASP groups ($p < 0.05$). And the AAP group had no significant differences as compare with that of the 5-FU group ($p > 0.05$).

The tumor inhibition rates of AAP, AMP and ASP on H22 tumor were 60.1%, 53.2% and 43.4%, respectively. At the same time, 5-FU showed it's the most antitumor efficiency, whose tumor inhibition rate reached to 61.5%.

Table 1 *In vivo* effects of AAP on the inhibition of tumor cell growth

Groups	Dose(mg/kg/day)	N/end	Tumor weight（g±S.D）	Inhibition rate（%）
Model control group	—	20/18	3.48 ± 0.41	—
5-FU group	25	20/19	1.34± 0.17★★	61.5
AAP group	50	20/20	1.39 ±0.14★★	60.1
AMP group	50	20/20	1.63 ±0.25★★▲	53.2
ASP group	50	20/19	1.97 ±0.38★★▲	43.4

★★P<0.01, compared with model control group; ▲P<0.05，compared with 5-FU group

Influence of AAP on the serum enzymes and immune organs in H22 solid mice

The experiment was also conducted to investigate the influence of AAP on serum enzymes and immune organs in H22 solid mice.

The results showed that the activity of ALT and AST of the model group were significantly higher than that of the normal control group ($p < 0.01$) (Table 2). It indicated that the growth of H22 tumor cell led to liver injury of mice, which caused the higher activity of ALT and AST. However, the serum ALT and AST activities were changed dramatically after using AAP, AMP, ASP and 5-FU. For example, the serum ALT concentrations of AAP, AMP and ASP groups were dropped dramatically, which dropped from 55.24±2.17 to 21.12±1.18, 27.32±2.06 and 34.45±2.41, respectively. The serum AST activity showed the same change trendency as ALT. But, interestingly, they obviously increased in 5-FU group ($p < 0.01$). And there were significant difference between these treatment groups and the H22 solid model group ($p < 0.01$) (Table 2).

Table 2 Influence of AAP on serum ALT, AST, Spleen and thymus indexes of H22 solid tumor mice

Groups	ALT(U/L)	AST(U/L)	Spleen index (mg/g)	Thymus index (mg/g)
Normal control group	20.33±1.04★★▲▲	36.14±1.21★★▲▲	8.46±0.04★★▲▲	2.87±0.09★★▲▲
H22 solid model group	55.24±2.17▲▲	87.56±2.49▲▲	13.87±1.17	1.49±0.12
5-FU group	110.12±1.15★★	149.07±2.16★★	15.31±1.44★	1.02±0.11★
AAP group	21.12±1.18▲▲★★	37.45±3.34★★▲▲	8.72±0.53★★▲▲	2.73±0.18★★▲▲
AMP group	27.32±2.06★★▲▲#	46.61±1.56★★▲▲#	10.84±0.19★★▲▲#	1.91±0.34★★▲▲#
ASP group	34.45±2.41★★▲▲##	52.23±1.44★★▲▲##	11.45±1.36★★▲▲##	1.55±0.16★★▲▲#

★★:Compared with the H22 solid model group p <0.01; ★:Compared with the H22 solid model group p <0.05；
▲▲:Compared with the 5-FU group p <0.01;
Compared with the AAP group p <0.01; # Compared with the AAP group p <0.05;

On the other hand, the results showed that the spleen index of H22 solid tumor model group was higher than that of the normal control group ($p < 0.01$) (Table 2). But it was lower than that of 5-FU group ($p < 0.01$). After treatment of AAP, AMP and ASP, the spleen indexes of the model group and 5-FU group dramatically decreased ($p < 0.01$). It indicated that the proliferation of H22 tumor cell led to splenomegaly, which was aggravated by 5-FU. However, AAP AMP and ASP could significantly resist the splenomegaly resulting from H22 tumor cell and 5-FU. Simultaneously, thymus index of the model group and 5-FU group showed a reverse change trend. It decreased from 2.87 to 1.49 and 1.02 in the model group and 5-FU group ($p < 0.05$). Nevertheless, AAP, AMP and ASP enhanced from 1.49 to 2.73, 1.91 and 1.55. The significant differences between AAP, AMP and ASP groups and the H22 solid

model group was showed ($p < 0.05$). And the AAP group showed significant differences compared with the AMP and ASP group ($P < 0.05$; $P < 0.01$) in spleen and thymus indexes (Table 2).

The antitumor activity of the polysaccharide was usually believed to be a consequence of the stimulation of the cell-mediated immune response [14]. For instance, immunostimulatory activities of the polysaccharides from Panax ginseng and Ganoderma lucidum [15,16].

The aim of the study was evaluate whether the antitumor activities of AAP were resulted from stimulation of the cell-mediated immune response or directly inhibit the proliferation of cancer cell *in vivo*. The experiment was performed using H22 solid tumor model mice. As shown in Table 1, AAP, AMP and ASP significantly inhibited the growth of H22 tumor cell in mice, compared with the model controls ($p < 0.01$). Moreover, AAP had a more significant antitumor activity than AMP and ASP on the growth of H22 hepatocarcinoma cells ($p < 0.05$).

At the same time, the experiment was also conducted to investigate the influence of AAP on serum enzymes and immune organs in H22 solid mice. The serum biomarkers of liver injury may be changed in response to proliferative H22 tumor cell in animals, therefore, the activity of serum enzymes, such as ALT and AST in H22 solid tumor mice were measured. The results showed 5-FU increased liver damage in H22 transplantable tumors mice (the activity of ALT and AST in 5-FU group obviously higher than the H22 tumor model group). While AAP, AMP and ASP reduced the activity of ALT and AST in serum in tumor-induced mice. It indicated that AAP, AMP and ASP relieved liver injury and improved liver function resulting from 5-FU and H22 transplantable tumors in mice.

Some reports [17,18] thought that tumor inoculation alone caused leukemoid reactions in experimental animals as characterized by splenomegaly. The present data (Table 2) was in agreement with previous findings, as animals inoculated with H22 tumor cells presented an increase in spleen weight (the spleen index was increased from 8.46±0.04 to 13.87). At the same time, we found that AAP, AMP and ASP inhibited the tumor-induced splenomegaly (the spleen index was decreased from 13.87 to 8.72, 10.84 and 11.45). Furthermore, as is well-known, thymus is one of the important immune organs of the organisms. In this paper, the weight of thymus as a parameter was also determined to evaluate body immune responses. The results implied that the AAP, AMP and ASP enhanced thymus index in H22 solid tumor mice. These results further demonstrated that AAP, AMP and ASP influenced immuno-regulating properties, which may be involved in its antitumor activity *in vivo*. However, precise mechanism of immune system and its effect on antitumor activity requires further investigation.

CONCLUSION

AAP could relieve liver injury and improve immune function resulting from 5-FU and H22 transplantable tumors mice. And that these anticancer effects were more effective than the AMP and ASP. AAP may play a major anticancer and immunomodulatory effects during the treatment of hepatocarcinoma. AAP is considered to be less toxic than 5-FU, and may warrant further evaluation as a possible agent in the treatment of cancer.

Acknowledgments
This study was supported by research grants from The National Natural Science Foundation of China (NO 81260070).

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