



Antitumor activity of polysaccharides from *angelica* and *astragalus in vitro*

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ABSTRACT

The present study was carried out to evaluate antitumor activity of polysaccharides from *angelica* and *astragalus* (AAP) *in vitro*. Inhibition rate, percentage of viability and IC₅₀ were served as target to evaluate its antitumor activity on human tumor cell lines (Hela cell, HepG2 cell, A549 cell, SPCA-1 cell and BGC-823 cell) *in vitro* through MTT method. The results showed that AAP exhibited greater *in vitro* cytotoxicity on BGC-823 cell and Hela cell than on HepG-2, A549, SPCA-1 cells. And the inhibition rates of 5-FU on BGC-823 cell had no a significant difference from that of AAP at a concentration of 80 µg/mL. These results implied that AAP had an obvious antitumor activity *in vitro*.

Key words: Polysaccharides from *angelica* and *astragalus* (AAP), 5-FU; Human tumor cell lines, Antitumor activity

INTRODUCTION

Cancer is as a major public health problem worldwide. The World Health Organization predicts that, by 2030, an estimated 21.4 million new cases of cancer and 13.2 million cancer deaths will occur annually [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 strong antioxidant, antitumor effects and immunostimulatory [3-5]. And these polysaccharides are often identified as biological response modifiers (BRMs). The roots of *astragalus membranaceus* (AM) are amongst the most popular health-promoting herbs in China, their use dates back more than 2000 years. One of the most active ingredients is polysaccharide (AMP), which has shown its immunopotentiating properties and its anticancer activity [6-7]. *Angelica sinensis* (AS) is one of the most important traditional Chinese herbs, whose polysaccharide (ASP) has 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 *angelica* and *astragalus* (AAP) *in vitro*, 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 vitro*. For this purpose, methyl thiazolyl tetrazolium (MTT) assay method was used to evaluate antitumour activities *in vitro* of AAP, AMP and ASP on human cervical cancer cell (Hela cell), liver hepatocellular cell (HepG2 cell), human lung cancer cell (A549 cell), human lung adenocarcinoma cell (SPCA-1 cell) and human gastric carcinoma cell (BGC-823 cell). To our knowledge, this may be the first report of the antitumour activity of AAP *in vitro*.

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 (Sun and Wang 2008) 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 [11]. Before this experiment, AAP, ASP and AMP was respectively diluted in DMEM or RPMI-1640 medium to a concentration of 2 mg/mL and filtered through sterile 0.22 μm filters.

Dimethyl sulfoxide (DMSO), methyl thiazolyl tetrazolium (MTT) were obtained from Sigma Chemical Co. (St. Louis MO, USA). Fetal bovine serum (FBS), RPMI-1640 medium and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO BRL (Auckland, New Zealand). 96-well cell plates were obtained from Corning Incorporated (USA). 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 lines

Five kinds of human tumor cell lines used for the assay, Hela cell, HepG2 cell, A549 cell, SPCA-1 cell and BGC-823 cell were obtained from the Central Laboratory of School of Life Science and Engineering, Lanzhou University of Technology.

These cell lines were respectively maintained in DMEM medium (Hela and HepG2 cells) and RPMI-1640 medium (A549, SPCA-1 and BGC-823 cells) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin in a humidified incubator 5% CO_2 atmosphere at 37°C.

Anti-proliferative activity of AAP *in vitro* on human tumor cells

Inhibition activities *in vitro* of AAP, ASP and AMP on Hela cell, HepG2 cell, A549 cell, SPCA-1 cell and BGC-823 cell proliferation were evaluated as described by Mosmann using MTT based colorimetric method [12]. The cells (5000 cells/well/100 μL) were respectively seeded into the 96-well plates. After 24 h of incubation at 37°C/5% CO_2 incubator, non-adherent cells were removed by washing three times with corresponding medium. Then, 100 $\mu\text{L}/\text{well}$ of varying concentration of AAP (10, 20, 40, 80 $\mu\text{g}/\text{mL}$), AMP (10, 20, 40, 80 $\mu\text{g}/\text{mL}$) and ASP (10, 20, 40, 80 $\mu\text{g}/\text{mL}$) were respectively added to wells to be further incubate at 37°C for 48 h in order to study their effects on cell proliferation. Each concentration was repeated 4 wells.

At the same time, positive control wells (5-FU 50 $\mu\text{mol}/\text{L}$) and negative control wells (only treated with the medium) were run in parallel to the experiments wells. After 48 h, MTT (20 $\mu\text{L}/\text{well}$) (5 mg/mL) was added to each well, and the plate was incubated again at 37°C for 4h. The supernatant was discarded and 150 μL of DMSO was added in, respectively. The suspension was placed on micro-vibrator for 10 min and the absorbance was measured at 490nm by a Microplate Reader (MK3, Finland).

The percent viability was calculated by comparing the absorbance of the AAP with that of the normal cell in the same incubation period. The percent viability of AAP and the percentage of inhibition were calculated as follows:

$$\text{Percentage of viability (\%)} = \frac{A_{490} \text{ of treated cells}}{A_{490} \text{ of untreated cells}} \times 100\%$$

$$\text{Inhibition rate (\%)} = \frac{1 - A_{490} \text{ of treated cells}}{A_{490} \text{ of untreated cells}} \times 100\%$$

Triplicate experiments were performed in a parallel manner for each concentration point. At the same time, the IC50 value was determined as the concentration that caused 50% inhibition of cell proliferation [13].

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

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

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 the present study, the anti-proliferative activities *in vitro* of AAP on the several human tumor cell lines were investigated. When these cells treated with different concentrations of AAP, AMP and ASP cultured up to 48 h, there was an increase in the percentage of cells being shrunk observed with inverted microscope.

The results of inhibitory proliferation activity of AAP, AMP and ASP on several human tumor cell lines were listed *in vitro* in Table 1. As shown in Table 1, with the concentration increasing from 10 to 80 $\mu\text{g/mL}$, the inhibition rate of AAP, AMP and ASP on these cells was significantly enhanced. Especially, AAP exhibited the best inhibition activity on BGC-823 cell (inhibitory rate, 75.1%) at a concentration of 80 $\mu\text{g/mL}$, followed by on HeLa cell, HepG-2 cell, SPCA-1 cell and A549 cell. The corresponding inhibition rates were respectively 56.8%, 41.1%, 23.8% and 19.1%, respectively. These results indicated that the inhibition activities of AAP against BGC-823, HeLa and HepG-2 cells were more effective than on A549 and SPCA-1 cells. And anti-proliferative effect of AAP on these human tumor cell lines was markedly superior to AMP and ASP.

Meanwhile, we found that 5-FU (50 $\mu\text{mol/L}$) showed the most powerful anti-proliferative effect on above human tumor cell lines, which inhibition rates reached to more than 50%. The inhibition rates of 5-FU on BGC-823 cell had no a significant difference from that of AAP at a concentration of 80 $\mu\text{g/mL}$. That is, the inhibit effect of AAP against BGC-823 cells (80 $\mu\text{g/mL}$) were comparable to that of 5-FU (shown in Table 1).

As shown in Table 2, the IC₅₀ values of AAP and AMP against BGC-823 were respectively 53.4 $\mu\text{g/mL}$ and 71.2 $\mu\text{g/mL}$, while the IC₅₀ values of AAP against HepG2, A549 and SPCA-1 (AMP against the other 4 tumor cells; ASP against 5 tumor cells) were more than 80 $\mu\text{g/mL}$. These results implied that the sensitivities of these tumor cells to AAP, AMP and ASP were different from cell to cell. Among these, BGC-823 cell (IC₅₀, 45.9 $\mu\text{g/mL}$) and HeLa cell (IC₅₀, 53.4 $\mu\text{g/mL}$) were more sensitive to AAP than the other three tumor cells *in vitro*.

Table 1 *In vitro* inhibition rate of AAP, AMP and ASP on human tumor cell lines

groups	Inhibition rate (%)					
	HeLa	HepG2	A549	SPCA-1	BGC-823	
AAP	10 $\mu\text{g/mL}$	18.9	12.7	8.6	9.5	20.1
	20 $\mu\text{g/mL}$	29.7	21.2	10.7	15.1	29.4
	40 $\mu\text{g/mL}$	41.5	29.8	14.3	21.4	46.3
	80 $\mu\text{g/mL}$	56.8	41.1	19.1	23.8	75.1
AMP	10 $\mu\text{g/mL}$	15.3	11.2	9.1	10.5	18.4
	20 $\mu\text{g/mL}$	22.1	16.7	10.4	14.3	26.5
	40 $\mu\text{g/mL}$	29.4	24.3	12.1	18.7	39.1
	80 $\mu\text{g/mL}$	37.5	31.3	14.2	22.4	54.7
ASP	10 $\mu\text{g/mL}$	10.4	8.9	6.3	6.4	11.8
	20 $\mu\text{g/mL}$	16.9	12.4	8.7	7.5	14.1
	40 $\mu\text{g/mL}$	21.7	18.1	11.1	9.1	16.5
	80 $\mu\text{g/mL}$	29.1	25.5	13.8	11.3	20.7
5-FU	50 $\mu\text{mol/L}$	72.1	69.4	54.7	59.1	77.5

Table 2 IC₅₀ of AAP, AMP and ASP against 5 human tumor cell lines

Cells	HeLa	HepG2	A549	SPCA-1	BGC-823
AAP	53.4 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	45.9 $\mu\text{g/mL}$
AMP	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	71.2 $\mu\text{g/mL}$
ASP	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$	>80 $\mu\text{g/mL}$

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]. But some polysaccharides could directly inhibit the proliferation of tumor cell *in vitro*, such as polysaccharides from *phellinus linteus* [17] and *cordyceps sinensis* [18].

The aim of the study was to evaluate whether AAP has more effective antitumor activity than ASP and AMP *in vitro*. In this study, we have extended the investigation to antitumor properties of AAP, AMP and ASP by testing their cytotoxicity on various human tumor cell lines. The *in vitro* antitumor activities of the AAP, AMP and ASP were tested on the tumor cell lines HeLa, HepG2, A549, SPCA-1 and BGC-823 cells by MTT method. The result showed that AAP, AMP and ASP influenced the proliferation of above 5 the cell lines. AAP inhibited the growth of BGC-823 cells with an IC₅₀ value of 45.9 $\mu\text{g/mL}$. At concentrations of 10, 20, 40 and 80 $\mu\text{g/mL}$, AAP suppressed

the growth of Hela cells with the inhibitory rates of 18.9%, 29.7%, 41.5% and 56.8%, respectively. Its IC₅₀ value was only 53.4 µg/mL on Hela cell. Simultaneously, AAP at concentrations of 80 µg/mL suppressed the growth of HepG-2, A549, SPCA-1 cells with the inhibitory rates of 41.1%, 19.1% and 23.8%, respectively. These results demonstrated AAP exhibited stronger *in vitro* cytotoxicity on BGC-823 cell and Hela cell than on HepG-2, A549, SPCA-1 cells. And the inhibition rates of 5-FU on BGC-823 cell had no a significant difference from that of AAP at a concentration of 80 µg/mL. Although AMP and ASP showed their inhibitory effects against above 5 human tumor cells in a concentration-dependent manner, IC₅₀ values were more than 80 µg/mL. It implied that AMP and ASP showed the weaker cytotoxicity on the above 5 tumor cell lines.

CONCLUSION

AAP at concentrations of 10–80 µg/mL exhibited better anticancer effects on the BGC-823 cell and Hela cell than on HepG-2, A549, SPCA-1 cells *in vitro*. And AAP exerted its antitumor efficiency through directly inhibiting the proliferation of cancer cells *in vitro* in this study. AAP is considered to be less toxic than 5-FU, and may warrant further evaluation as a potential anticancer agent.

To our knowledge, this is the first time, AAP was extracted from *angelica* and *astragalus* and the first report of antitumor properties. Further studies on the mechanism of the cytotoxicity of AAP are currently underway.

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REFERENCES

- [1] The World Health Report **2008**—primary health care: Now more than ever. http://www.who.int/whr/2008/whr08_en.pdf.
- [2] Kelloff, G.J. *Adv Cancer Res*, **2000**, 78, 199–334.
- [3] Chen, L.R., Wang, C.W., Tian, P., Y, W., & Zhang, X.G. *Carbohydr Polym*, **2009**, 78, 738–742.
- [4] Qiao, D.L., Ke, C.L., Hu, B., Lou, J.G., Ye, H., Sun, Y., et al. *Carbohydr Polym*, **2009**, 78, 199–204.
- [5] Sun, Y.X., & Liu, J.C. *Int J of Biol Macromol*, **2008**, 43, 279–282.
- [6] Balch, P.A. Prescription for Nutritional Healing, A Practical A-to-Z Reference to Drug-Free Remedies Using Vitamins, Minerals, Herbs and Food Supplements. 5th edition. New York, NY, USA: Avery Penguin Putnam Inc, **2006**.
- [7] Rittenhouse, J.R., Lui, P.D., Lau, B.H. *J Urol*, **1991**, 146(2), 486–490.
- [8] Wu, D.Z., Song, C.Q., Hen, Z.F., Kong, J.L., Fan, Y., Hu, Z.B. *Pharmacol. Clin. Chin. Mater. Med*, **1999**, 15, 3–6.
- [9] Zhang, S., He, B., Ge, J.B., Zhai, C.L., Liu, X., Liu, P.G. *Int J Biol Macromol*, **2001**, 46, 363–366.
- [10] Pu, X.Y., Li, Y., Wang, P. *Food and Beverage Industry*, **2011**, 1(11), 65–67.
- [11] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. *Analytical Chemistry*, **1956**, 28, 350 – 356.
- [12] Mosmann, T. *J Immunol Methods*, **1983**, 65(1–2), 55–63.
- [13] Peng, Y.R., Li, Y.B., Liu, X.D., Zhang, J.F., Duan, J.A. *Phytomedicine*, **2008**, 15, 1016–1020.
- [14] Ooi, V.E., & Liu, F. *Curr Med Chem*, **2001**, 7, 715–729.
- [15] Cao, Q.Z., Lin, Z.B. *Acta. Pharm. Sin*, **2004**, 25, 833–838.
- [16] Ho, J.C., Konerding, M.A., Gaumann, A, Groth, M., Liu, W.K. *Life Sci*, **2004**, 75, 1343–1356.
- [17] Li, G., Kim, D.H., Kim, T.D., Park, B.J., Park, H.D., Park, J.I., et al. *Cancer Lett*, **2004**, 216, 175–181.
- [18] Chen, Y.J., Shiao, M.S., Lee, S.S., Wang, S.Y. *Life Sci*, **1997**, 60, 2349–2359.