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Immunomodulatory activity of an acidic polysaccharide isolated from Arrowhead

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Abstract

An acidic polysaccharide (AAP) was obtained from the aqueous extracts of the stems of arrowhead by hot water extraction followed ethanol precipitation. The immunomodulatory effect of the acidic polysaccharide (AAP) was investigated. The results showed that the high dose AAP(100 mg/ kg) has significant increased immune index of S180 mice, and strongly promoted the secretion of IL-2, TNF-α and IFN-γ. Therefore, the acidic polysaccharide AAP could be explored as a novel potential immunomodulatory drug.

Keywords: Immunomodulatory, Acidic polysaccharide, Arrowhead.

Introduction

Arrowhead, belongs to the Alismaceae family, is an erect stem less aquatic winter herb, flowering in November-December, and has been used in China medicinally during childbirth and for skin diseases[1], anti-cancer[2], antibacterial activity[3] *et al*. Polysaccharides exist widely in numerous plants and are identified as essential biomacromolecules in plant life, playing important roles in cell-cell communication, cell adhesion, and molecular recognition in the immune system.[4-7] Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects, which is a major problem associated with immunomodulatory polysaccharides. Thus, plant polysaccharides are ideal candidates for therapeutics with immunomodulatory and antitumor effects and low toxicity.[8]

There are abundant polysaccharides in arrowhead[9] and the polysaccharide from arrowhead exhibited strong hypoglycemic activity[10]. But the immunomodulatory of the polysaccharides from arrowhead has been no reported. Therefore, the purpose of the present investigation was to elucidate the isolation of acidic polysaccharide from the arrowhead, as well as to evaluate its immunomodulatory effect.

Materials and Methods

Drugs and reagents

The tumor necrosis factoralpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-2 (IL-2) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Senxiong Biotech Co. (Shanghai, China). Cyclophosphamide(CTX) was purchased from Hengrui medicine Co.(Jiangsu China). The plant materials were bought from the market of traditional Chinese medicinal materials in Chengdu (China), and

were identified according to the identification standard of the Pharmacopoeia of the People's Republic of China (PPRC). All the other chemical reagents were analytical reagent grade.

Isolation of the acidic polysaccharide

Isolation of the acidic polysaccharide from arrowhead was carried out according to the method of Fan *et al*[11]. with some modifications. Briefly, the arrowhead was triturated into power and boiled in petroleum ether for 2 h. After filtration to remove the petroleum ether, the residue was further extracted with 80% ethanol. After filtered, the residue was further extracted with 0.5M NaOH at 50°C for 3 h three times. Then all extracts were combined, concentrated and filtrated. The extract was deproteinized 4 times using the Sevag reagent[12], and the polysaccharide was free of proteins as scan by UV Spectra in 260 nm and 280 nm. After removal of the Sevag reagent, the extract was precipitated by adding ethanol (4 times the volume of aqueous extract), and the mixture was kept overnight at 4°C for the polysaccharide. The precipitate was collected by centrifugation at 4000 rpm for 20 min, and then dissolved in water and dialyzed against deionized water for 72 h, freeze-drying to yield the polysaccharide, which was named AAP.

Immunomodulatory test in vivo

Animals and treatment

Kunming mice (weight: 20.0±2.0 g) were purchased from the Experimental Animal Center of Sichuan Academy of Medical Sciences. The mice were housed under normal laboratory conditions, i.e., room temperature, 12/12 h light–dark cycle with free access to standard rodent chow and water. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Sarcoma 180 cells (S180) were generously donated by Sichuan Academy of Chinese Medicine Sciences. Sarcoma 180 cells were passed into mice ascites. Then, ascites was inoculated subcutaneously 0.2 ml (5.0×107 cells/ml) into the sword arm of each experimental mouse. Normal control mice were not inoculated Sarcoma 180. The mice were treated as following: normal control (normal saline), positive control (Cyclophosphamide, 12.5 mg/kg body weight), model control group (normal saline) and the polysaccharide (50 mg/kg, 25 mg/kg and 12.5 mg/kg body weight). All the groups were administered daily by intraperitoneal injection (0.2 ml).

The tested samples (25, 50, 100 mg/kg body weight of each polysaccharide) and CTX (25 mg/kg body weight) were dissolved in saline, and then injected intraperitoneally (i.p.) once a day for 14 days, starting 24 h after tumor inoculation. The normal control and model control mice received an equal volume of saline (0.2 ml). 24 h after last tested sample administration; all animals were weighted and sacrificed.

Analysis of immune index

After these mice were sacrificed by cervical dislocation, the spleens and thymus of these mice were recovered by anatomized. The spleen index was determined from the weight of the spleen and the results are expressed as the formula: weight of spleen (mg)/ body weight (g).[13] The thymus index and the tumor weights of the mice were measured by using the method.

Determination of IL-2, TNF-α and IFN-γ by the ELISA method

These mice were anatomized after 14 days, and the blood samples were obtained from eye orbitae of these mice and the serum was collected after the blood samples had been processed by centrifugation at 2500 rpm for 10 min at 10°C. The interleukin-2 (IL-2)14, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) concentration were measured with an enzyme-linked immunosorbent assay (ELISA kit, Shanghai Senxiong Biotech) according to the indication of the manufacturer.

Statistical analysis

The data were expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the results were processed by SPSS software.

Results and Discussion

Analysis of spleen index

To evaluate the effect of the acid polysaccharide on the immune system, the experiment evaluated the effects on spleen index of different samples. The results showed in Figure 1, which indicated that the different dose of acid polysaccharide could cause a significant increase in the spleen index compared with S180 control group, and the effect in a concentration-dependent manner. At 100 mg/kg, the ability was the strongest. On the other hand, there was significant decrease in CTX-treated animals.

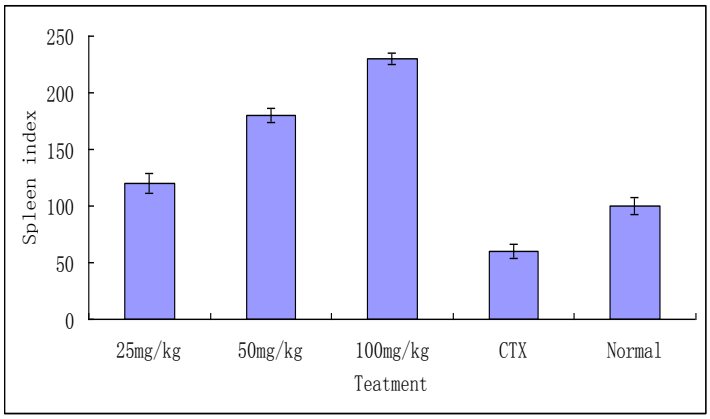

Figure 1: Spleen index was measured in the ratio of spleen. Values are means±S.D, (n=10).

Analysis of thymus index

The effects on spleen index of different acid polysaccharides were exhibited in Figure 2. As seen from the figure, the effects of all polysaccharide samples correlated well with increasing concentrations. Moreover, at 50 mg/kg, the effect got to 67, which closed to 100 mg/kg (70). The effects were much higher than that of the control. There was also no significant effect was observed in CTX-treated animals. The results indicated that the polysaccharide could significantly increase the immune system of S180 mice.

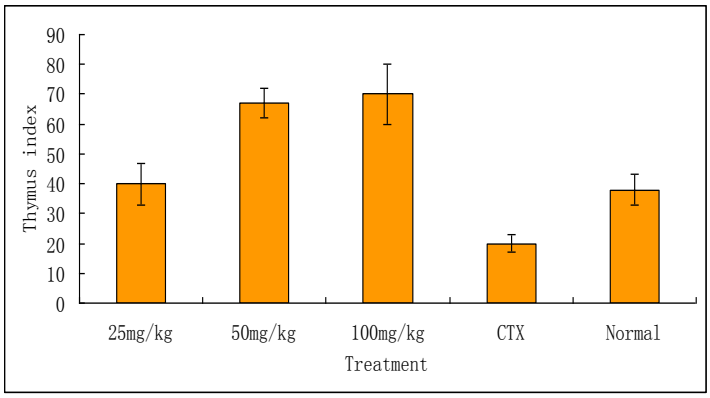

Figure 2: Thymus index was measured in the ratio of thymus. Values are means±S.D, (n=10)

Determination of IL-2, TNF-α and IFN-γ

IFN-γ is an important immunoregulatory molecule. It induces the generation of T cells, activates macrophages, and regulates crossly Th1 and Th2 cells. TNF-α and IFN-γ can enhance immunoregulatory ability each other towards tumor. The interleukin-2, tumor necrosis factor-alpha and interferon-gamma concentration were measured with an enzyme-linked immunosorbent assay according to the indication of the manufacturer. The serum from each groups were collected 4 h after administration of the drugs.

From the results of Figure 3-5, the concentrations of TNF-α, IFN-γ and IL-2 in the group with CTX-treated were significant low, which indicated CTX could not promote the secretion of the three cytokines.

As for IL-2, AAP-treated group exhibited strong promoted effect. Especially at the high dose of 100 mg/kg, the promoted effect got to the strongest. On the other hand, there was significant decrease in CTX-treated animals. Therefore, the results indicated that the level of different concentrations polysaccharides were significant increase compared with the control.

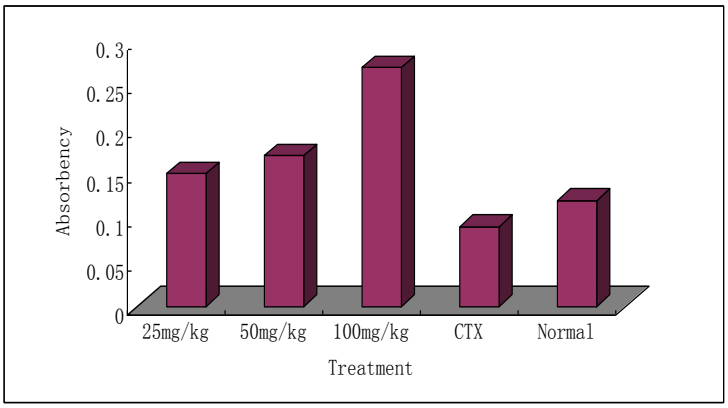

Figure 3: Effects of different concentrations polysaccharides and CTX on the secretion of IL-2

The concentrations of TNF-α with different doses polysaccharide-treated have different degrees increase. Especially at 50 mg/kg and 100 mg/kg of AAP have strongly promoted the secretion of the three cytokines ($p<0.01$). Compared with control, there were higher concentrations in the serum (620.5 mg/ml) in 50 mg/kg-treated group.

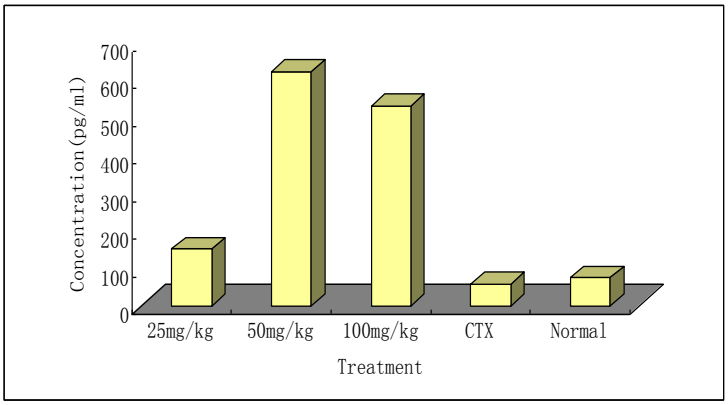

Figure 4: Effects of different concentrations polysaccharides and CTX on the secretion of TNF-α

The effects of different concentrations polysaccharides and CTX on the secretion of IFN-γ were exhibited in Figure 5. As seen from the figure, the effects of all polysaccharide samples correlated well with increasing concentrations. Moreover, at 100 mg/kg, the effect was the strongest. Also there was significant decrease in CTX-treated animals. Therefore, the results indicated that the level of different concentrations polysaccharides were significant promote the secretion of the three cytokines.

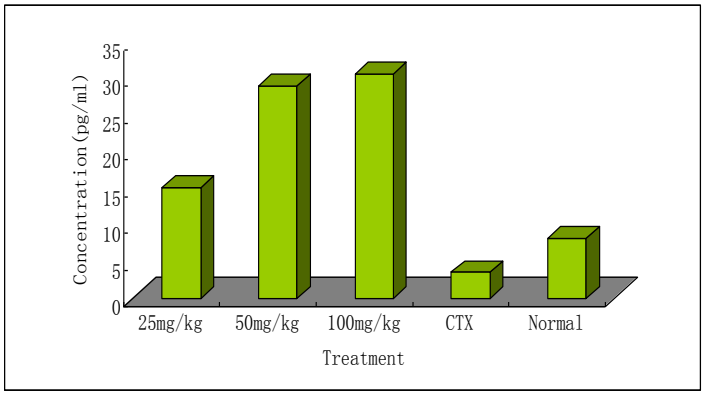

Figure 5: Effects of different concentrations polysaccharides and CTX on the secretion of IFN-γ

Conclusions

In the present study, the acidic polysaccharide was obtained from Arrowhead. Through immunoregulatory in vivo, AAP exhibited strong ability, meanwhile, AAP with 100 mg/ kg also significant increased immune index of S180 mice, and strongly promoted the secretion of IL-2, TNF-α and IFN-γ. IL-2 could promote the long-term proliferation of T cells, TNF-α and IFN-γ can enhance immunoregulatory ability each other towards tumor. Therefore, with the results above, the acidic polysaccharide AAP could be explored as a novel potential immunomodulatory drug.

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