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Full Length Research Paper

Chemical composition and antitumor activity of polysaccharide from *Inonotus obliquus*

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The alkaline soluble polysacchride AIOPA isolated and purified from *Inonotus obliquus* using gel filtration was subjected to composition analysis and determined for the antitumor and immunomodulatory activitives. Based on the results of high performance size-exclusion chromatography (HPSEC), gas chromatography (GC) and infra-red (IR) spectrum, AIOPA consisted of rhamnose, xylose, manose, galactose, glucose and galacturonic acid in a molar ratio of 3.09:1.61:2.06:4.45:19.7:1 with a molecular weight of 6.3 kDa. In the present study, it was found that AIOPA remarkably enhanced spleen and thymus index in mice bearing S180 sarcoma, and also stimulated LPS-induced splenocyte proliferation. Immunomodulatory activity assay *in vitro* indicated AIOPA could significantly enhance cellular lysosomal enzyme activity, nitric oxide (NO) formation and tumor necrosis factor-a (TNF-α) secretion in macrophages. Furthermore, AIOPA dose-dependently stimulated macrophages to produce NO through the up-regulation of inducible NO synthase (iNOS) activity and the maximal effect occurred at a concentration of 300 ug/ml by AIOPA. These data suggest that the antitumor activity of AIOPA may be associated with its potent immunostimulating effect.

Key words: *Inonotus obliquus*, polysaccharide, immunomodulatory, antitumor.

INTRODUCTION

Recent studies have revealed the presence of natural bioactive materials in many different plant species worldwide and extracts from these materials are currently under wide application. In particular, medicinal mushroom polysaccharides have received special attention due to their potential biological and pharmacological activities including antitumor (Myung et al., 2009), immunostimulation (Wasser, 2002), anti-oxidation (Yamada, 1994), cardiovascular (Li et al., 2003), hypocholesteric, antiviral, antibacterial, antiparastic, antifungal, antidiabetic and hepatoprotective activities (Liu et al., 1997).

Inonotus obliquus (Pers.: Fr.) Pilat is well known as one of the most popular medicinal species for its therapeutic effect. It is a white rot fungus that belongs to the family Hymenochaetaceae. This fungus is usually found as a sterile conk (sclerotia) called Chaga on *Betula* species (Saar, 1991). Since the sixteenth century, Chaga has been used as a folk medicine for tumors and stomach ulcers in Russia. Moreover, many studies have shown anti-tumor effects of the water extracts from *I. obliquus in vitro* (Mizuno et al., 1999; Wasser and Weis, 1999), as well as antimicrobial activities (Kahlos et al., 1989). A recent study showed that a hot water extract of *I. obliquus* suppressed cellular proliferation of human stomach cancer cells in a time-dependent manner (Hwang et al., 2003) and significantly inhibited the growth of mouse sarcoma S180 cells (Chen et al., 2007) and HepG2 cells (Youn et al., 2008). However, it is relatively little knowledge of cancers mechanism of action (Shashkina et al., 2006). Especially, little research has been conducted on the antitumor of the alkaline extraction from *I. obliquus*. In our previous study, we isolated and analyzed an alkaline soluble polysaccharide, named AIOPA from *I. obliquus.* The anti-tumor activity of AIOPA and the mechanism were

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investigated.

MATERIALS AND METHODS

Materials and chemicals

The fruiting body of *I. obliquus* used in this experiment was purchased from Jilin drugstore. A voucher specimen identified by Prof. Hongxing Xiao, a faculty member of School of Life Science, Northeast Normal University, Changchun, China. Sephadex G-100, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), concanavalin A (ConA) and lipopolysaccharide (LPS) were from Sigma Chemical Co. Medium RPMI-1640 and cyclophosphamide were purchased from Gibco Invitrogen Co. The complete RMPI-1640 medium, used for immunological tests, was supplemented with penicillin 100 IU/ml, streptomycin 100 µg/ml, and 10% newborn bovine serum, pH 7.4. All other reagents were of analytical grade made in China.

Isolation and purification of polysaccharide fractions

The fruiting body of *I. obliquus* (1 kg) were extracted with 70% ethanol at 80°C for 2 h, and distilled water three times at 100°C for 1 h. Then the water unextractable solid was washed, dried and extracted with 0.5 M NaOH solution at 80°C for 2 h twice. After centrifugation (3500 rpm for 10 min, at 20°C). The suspension was neutralized with hydrochloric acid (0.1 M) and filtered. The filtrate was concentrated 10-fold, and precipitated with 95% EtOH (1:4, v/v) at 4°C for overnight. The crude polysaccharide AIOP (47.9 g) was obtained by centrifugation (3500 rpm for 10 min, at 20°C), was suspended in distilled water to remove the protein by the Sevag method (Alum and Gupta, 1986). The polysaccharide was separated with nominal molecular weight cut-off (NMWCO) of 6.3 kDa. The main fraction mostly containing the higher molecular weight polysaccharide was loaded on sephadex G-100 (2.5 x 90 cm), and eluted with 0.9% sodium chloride. The eluate was detected using phenol-sulfuric acid (Dubios et al., 1956) and the main polysaccharide fraction (AIOPA 0.6 g) was collected, dialyzed and lyophilized. AIOPA was used for chemical components analysis and activity assessment.

Chemical components analysis

Homogeneity and molecular weight

The homogeneity and the molecular weight distribution of AIOPA were determined by high-performance size-exclusion chromatography (HPSEC), which was performed on a SHIMADZU HPLC system fitted with one TSK-G3000 column (7.8 mm ID x 30.0 cm) and a SHIMADZU RID-10A detector. The eluent was 0.7% Na_2SO_4, and the flow rate was 0.5 ml/min at 40°C, with 1.6 mPa.

Molecular mass was estimated using a reference against calibration curve made from a set of standards Dextran (T 130, 80, 40, 20, 10, Sigma).

Carbohydrate, uronic acid content and protein contents analysis

Total sugar and uronic acid content were determined by phenol-sulfuric acid method and m-hydroxydiphenyl analysis (Tullia and Filisetti, 1991) using D-glucose and D-glucuronic acid as standard, respectively. The protein content was measured by Lowry method (Crueger and Crueger, 1990) with bovine serum albumin as the standard.

Monosaccharide and FT-IR spectrum analysis

Polysaccharide was also analyzed for monosaccharide by gas chromatography. After hydrolysis with 2 M trifluoroacetic acid and conversion of hydrolysate into alditol-acetates as previously described method (Alum and Gupta, 2006), the resulting alditol-acetates were analyzed by GC using a Vavian 3400 instrument (Varian, Component, USA), equipping with DM2330 column (30 m x 0.32 mm x 0.2 µm). The temperature of column was kept at 170°C for 2 min and then was increased to 250°C for 20 min at the rate of 10°C /min. The FT-IR spectra were obtained from 1 mg of with a SPECORD IR spectrometer in a range 400 to 4000 cm^{-1}. The samples were analyzed as KBr pellets.

Animals

Male ICR mice (Grade II, 5–6 weeks old) weighing 18 to 22 g, were purchased from Pharmacology Experimental Center of Jilin University and acclimatized for 1 week prior to use. All mice were housed under standard conditions at 24 ± 1°C, with humidity of 50 ± 10%, and a 12/12 h light/dark cycle. Rodent laboratory chow pellets and tap water were supplied *ad libitum*. All the procedures conducted by Institute for Experimental Animals of Jilin University were carried out in strict accordance with the PR China legislation on the use and care of laboratory animals and were approved by the university committee for animal experiments.

Treatment

The Sarcoma 180 cells were purchased from Pharmacology Experimental Center of Jilin University. Sixty mice were randomly divided into six groups, each group consisting of 10 animals. One group was normal control. And seven-day-old Sarcoma 180 (S180) ascites (0.2 ml, 2 x 10^6 cells) were transplanted subcutaneously into the right axilla of each mouse of the rest groups. The mice were treated as following: normal control group (normal saline); model control group (normal saline); three AIOPA groups (30, 100 and 300 mg/kg body weight); positive control group (cyclophosphamide, 15 mg/kg body weight). All the groups were administered by intragastric infusion in 0.2ml every day for ten days, starting 24 h after tumor implantation.

Cell culture

The Sarcoma 180 cells were suspended in complete RPMI 1640 medium with penicillin 100 IU/ml, streptomycin 100 ug/ml, and 10% newborn bovine serum, pH 7.4.

In vivo antitumor activity

Twenty-four hours after last drug administration, all mice were sacrificed and the spleen, thymus and tumor were dissected and weighted from every living mouse. The anti-tumor activity of the polysaccharide *in vivo* was expressed as an Inhibitory rate calculated as [(A-B)/A] x 100%, where A and B were the average tumor weight of the model control and experimental groups, respectively. Serum collected from the tumor-bearing mice were measured using murine enzyme-linked immunosorbent assay (ELISA) kit for tumor necrosis factor-alpha (TNF-α) according to the indication of the manufacturer.

Cytotoxicity on sarcoma 180 cells *in vitro*

Sarcoma 180 cells were suspended in complete RPMI1640 medium. The proliferation was determined using the colorimetric MTT assay as described previously (Page et al., 1988). Briefly, cells were seeded at a density of 5 x 10^3 cells/well in 100 µl volume of the medium in 96-well plates and allowed to attach 24 h. The different dosages of AIOPA on the selected cell lines were in the range of 3, 10, 30, 100 and 300 µg/ml while the negative control was treated with the complete RPMI1640 medium only. MTT (5 mg/ml) 20 µl was added 44 h later. After incubated at 37°C for 4 h, the supernatant was aspirated, and 150 µl dimethyl sulphoxide was added to each well. Absorbance was measured at 570 nm by a 96-well microplate reader (BIO-RAD 550, American). All tested samples were carried out in triplicates.

Lymphocyte proliferation assay

Splenocyte (1 x 10^6 cells/ml) was suspended in a solution of complete RPMI-1640 medium. The purity and viability of splenocyte (tested by trypan blue dye exclution) was always over 90%. The cells 100 µl were plated in 96-well plates and incubated with or without concanavalin A (ConA, 5 µg/ml) or lipopolysaccharide (LPS, 20 µg/ml), and various concentrations of the polysaccharides (AIOPA) were added. Cells were kept for 72 h at 37°C in a humidified incubator which maintained a constant atmosphere of 5% CO_2. Cell concentration was checked by MTT assay (Chien et al., 2004). The absorbance A570 nm of the culture was measured in an ELIAS reader (BIO-RAD 550, American).

***In vitro* activation of peritoneal macrophage**

Phagocytosis of macrophages assay: Every male ICR mouse was injected intraperitoneally with 2 ml of sterile thioglycollate medium daily. After 3 days, the resident macrophages were harvested by peritoneal lavage and centrifugation (4°C, 1000 rpm, 5 min). Then peritoneal macrophages were cultured in complete RPMI 1640 medium in 96-well plate for 2 h at 37°C in a 5% CO_2 cell incubator. Non-adherent cells were removed by washing the plate with warm PBS (0.01 M, pH 7.4). Macrophages were cultured with different concentrations of AIOPA (10, 30 and 100 µg/ml) for 48 h, LPS 20 µg/ml was used as positive control, 0.075% aseptic neutral red solution 100 µl was added and then cultured again for 1 h. Then the cultures were washed three times with PBS, and were added 150 µl cell lysate a mixture of 100% ethanol and 99.9% acetic acid (1:1 v/v). The mixtures were mixed fully and evaluated at 550 nm in an ELIAS reader (BIO-RAD 550, American). The absorbance represented ability of phagocytosis of macrophages (Weeks et al, 1987). All determinations were conducted in triplicate.

Assays for nitric oxide

Peritoneal macrophages (2 x 10^6 cells/ml) obtained as above were cultured in complete RPMI 1640 media in 48-well plate with sample in different concentrations, and LPS 20 µg/ml was used as positive control. The cells were cultured for 48 h at 37°C in humidified 5% CO_2 incubator. Production of nitric oxide was estimated by measuring nitrite levels in cell supernatant with Greiss reagent (1% sulfanilamide in 2.5% phosphoric acid, 0.1% napthyl ethyl diamine dihydrochloride in 2.5% phosphoric acid). Absorbance was read at 540 nm (BIO-RAD 550, American).

TNF-α secretion

The prepared Peritoneal macrophages (2 x 10^6 cells/ml) were cultured in 48-well plates. After incubated for 48 h, TNF-α secretion was measured using enzyme-linked immunosorbent assay according to the indication of the manufacturer. LPS (20 µg/ml) was used as positive control.

Measurement of enzyme activity

Inducible NO synthase (iNOS) activity was determined using a diagnostic reagent kit from Jiancheng Bioengineering (Nanjing, China) and the assay procedure was based on the instructions of the kit and optical density was measured at 530 nm (wavelength). One activity unit of iNOS was defined as the production of 1 nmol NO per 1 x 10^6 cells/min and expressed as U/ml.

Statistical analysis

The results were reported as means±S.D. Statistical difference between tested groups and control was analyzed by Student's *t*-test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Characteristic of the polysaccharide

The GPC profile (Figure 1) showed a single and symmetrically sharp peak, which indicated that AIOPA was a homogeneous polysaccharide, with an average molecular weight of 6.3 kDa. The total sugar content of AIOPA was determined to be 92.6%. The protein content was 7.2%. As determined by m-hydroxydiphenyl colorimetric method, the content of uronic acid was 3.1%. GC analysis showed that AIOPA was composed of L-rhamnose, D-xylose, D-manose, D-galactose, D-glucose and D- galacturonic acid in a molar ratio of 3.09:1.61:2.06:4.45:19.7:1 (Figure 2). The IR spectrum of AIOPA is shown in Figure 3. The attribution of the main absorption characteristics of polysaccharide structures were related to O-H stretching between 3500 and 3000 cm^{-1}. C–H stretching between 3000 and 2800 cm^{-1}. A band in the region of 1645 cm^{-1}could be due to associated water. A peak between 1200 and 1000 cm^{-1} was found in association with the C–O–H and C–O–C link band positions. Moreover, the band corresponding at 839 cm^{-1} displayed stretching absorption, suggesting that α-glycosidic bonds should be present in AIOPA.

Tumor weight, relative spleen and thymus weight and TNF-α secretion

The anti-tumor activity of AIOPA was shown in Table 1. The ICR mice were administered the sample orally and continuously with the same dose for 10 days. Tumor weights of polysaccharides-treated groups were 0.67, 0.56 and 0.42 g for the three doses (30, 100 and 300 mg/kg body weight) of AIOPA, while the tumor weight of the negative control group was 1.73 g. Therefore, the fraction AIOPA showed excellent inhibitory activity against solid tumor formation of Sarcoma 180, with

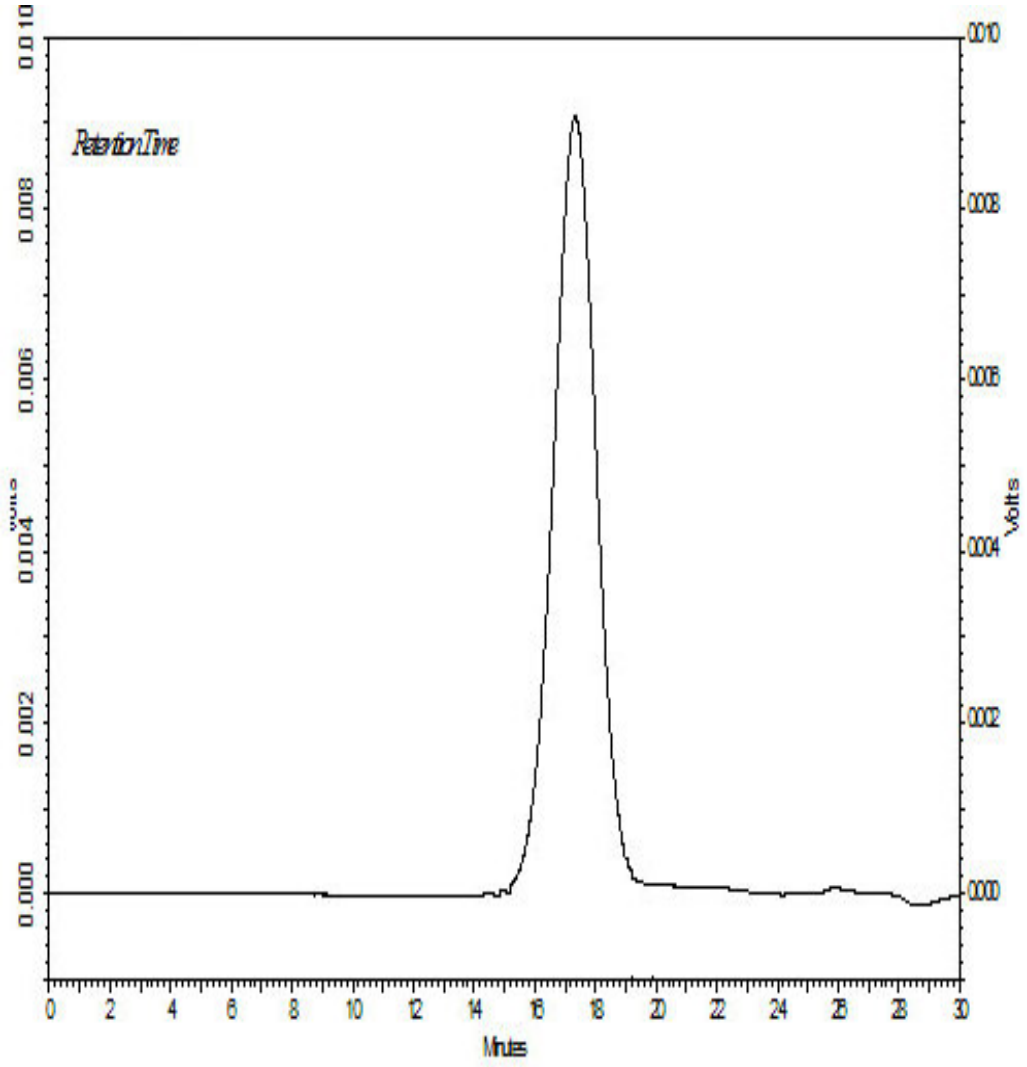

Figure 1. The HPLC prolife of the polysaccharide AIOPA.

inhibitory ratios of 56.29, 65.74 and 70.56%, respectively. A significant increase ($P<0.01$) in relative spleen and thymus weight was also observed (Table 1).

Mushroom polysaccharides are the best known and most potential mushroom-derived substances with antitumor properties. These polysaccharides have shown widely inhibitory effects towards many kinds of tumors including Sarcoma 180 solid cancers, Ehrlich solid cancer, B16-F10 melanoma cells, Yoshida sarcoma and Lewis lung carcinoma (Wasser and Weis,1999). The antitumor activity of the polysaccharide was usually believed to be a consequence of the stimulation of the cell-mediated immune response (Ooi and Liu, 2000). For instance, immunostimulatory activities were found in the polysaccharides from *Panax ginseng*, *Ganoderma lucidum*, *Coriolus versicolor*, etc., which suggested that immunostimulatory effects might be the main mechanism for the anti-tumor activities of polysaccharides (Shin et al., 2002; Cao and Lin, 2004; Ho et al., 2004). But some polysaccharides, such as polysaccharides from Phellinus linteus (Li et al., 2004), and Cordycepssinensis (Chen et al., 1997), could directly inhibit the proliferation of cancer cell *in vitro*. In the present study, we isolated a polysaccharide AIOPA from the alkaline extract of the fruiting body of *I. obliquus* and showed that AIOPA inhibits tumor cell growth of Sarcoma 180 cells *in vivo*. It improved the weight of immune organs of tumor-bearing mice. A significant increase in the relative thymus and spleen weight was observed compared to the model control group. So far as we know, thymus and spleen were important immune organs so thymus and spleen index reflect the immune function of the organism. Immunopotentiator could increase the weight of thymus and spleen.

The level of TNF-α in serum was increased in a dose-dependent manner at three doses of AIOPA treated mice (Figure 4). Furthermore, the appetite, activity and coat

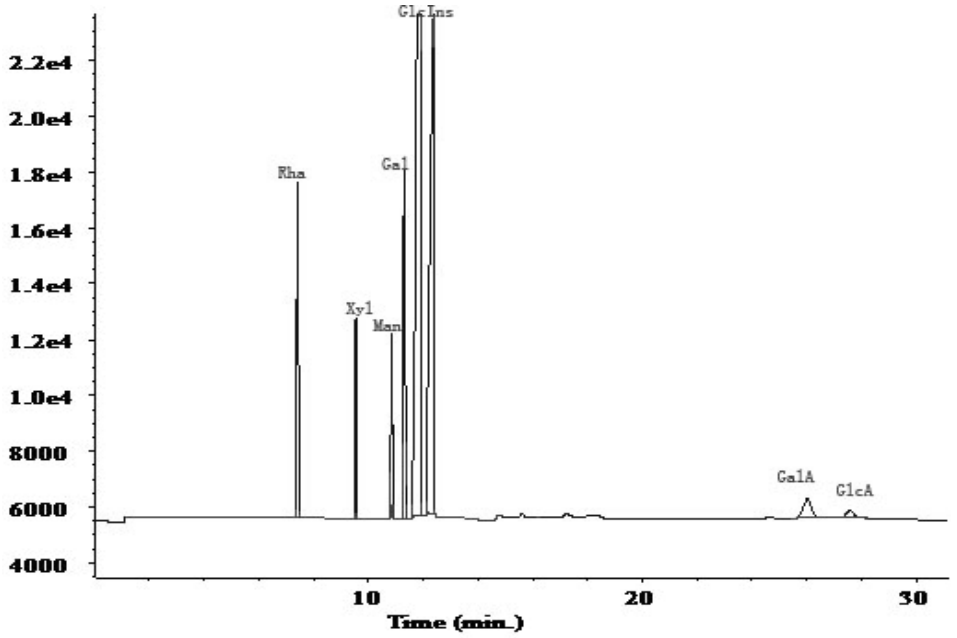

Figure 2. GC profile of the polysaccharide AIOPA of *I. obliquus.* Peaks from left to right: rhamnose, xylose, mannose, galactose, gluctose, Inositol, galacturonic acid and glucuronic acid.

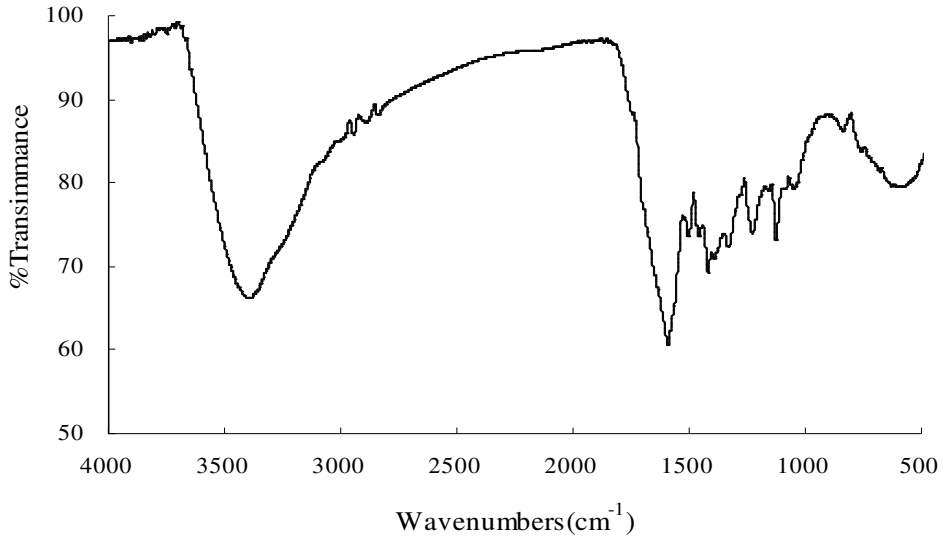

Figure 3. The IR spectra of the polysaccharide AIOPA of *I. obliquus.*

luster of each animal in AIOPA groups were better than the tumor-bearing controls.

In vitro anti-tumor activity

Sarcoma 180 tumor cells were incubated with different concentrations (3, 10, 30, 100 and 300 μg/ml) of AIOPA for 48 h, then measured by MTT method. The highest inhibition on Sarcoma 180 cell was 7.1%, suggesting that the polysaccharide AIOPA had no significant cytotoxicity to Sarcoma 180 cell *in vitro*, even at the highest concentration of 1000 μg/ml (data not shown). Inasmuch as the fraction AIOPA could not kill tumor cell *in vitro*. The result indicated that the antitumor effect of this polysaccharide was probable related to immunomodulation.

Table 1. Antitumor activity of polysaccharide AIOPA on tumor growth and relative spleen and thymus weight of tumor-bearing mice.

Group	Dose (mg/kg)	Tumor weight(g)	Relative thymus weight (mg/g)	Relative spleen weight (mg/g)	Inhibitory rate of tumor (%)
Normal control			3.39 ± 0.34	5.69 ± 0.55	
Model control		1.73 ± 0.31	2.54 ± 0.49	6.06 ± 0.63	
Cys	15	0.32 ± 0.15	1.51 ± 0.36	4.87 ± 0.31	81.31
	30	0.67 ± 0.05 [a]	3.06 ± 0.19 [a]	17.23 ± 1.01 [a]	56.29
AIOPA	100	0.56 ± 0.07 [a]	3.23 ± 0.26 [a]	18.17 ± 0.63 [a]	65.74
	300	0.42 ± 0.08 [a]	3.29 ± 0.39 [a]	18.80 ± 1.08 [a]	70.56

Normal control and model control group received saline, positive control group received cyclophosphamide (Cys), and polysaccharide groups received the polysaccharide AIOPA. Relative thymus weight was measured in the ratio of thymus weight (mg) to body weight (g). Relative spleen weight was measured in the ratio of the spleen weight (mg) to body weight (g).Values are shown as means±S.D. Significance was determined using the Student's t-test. a P<0.01 vs. model control.

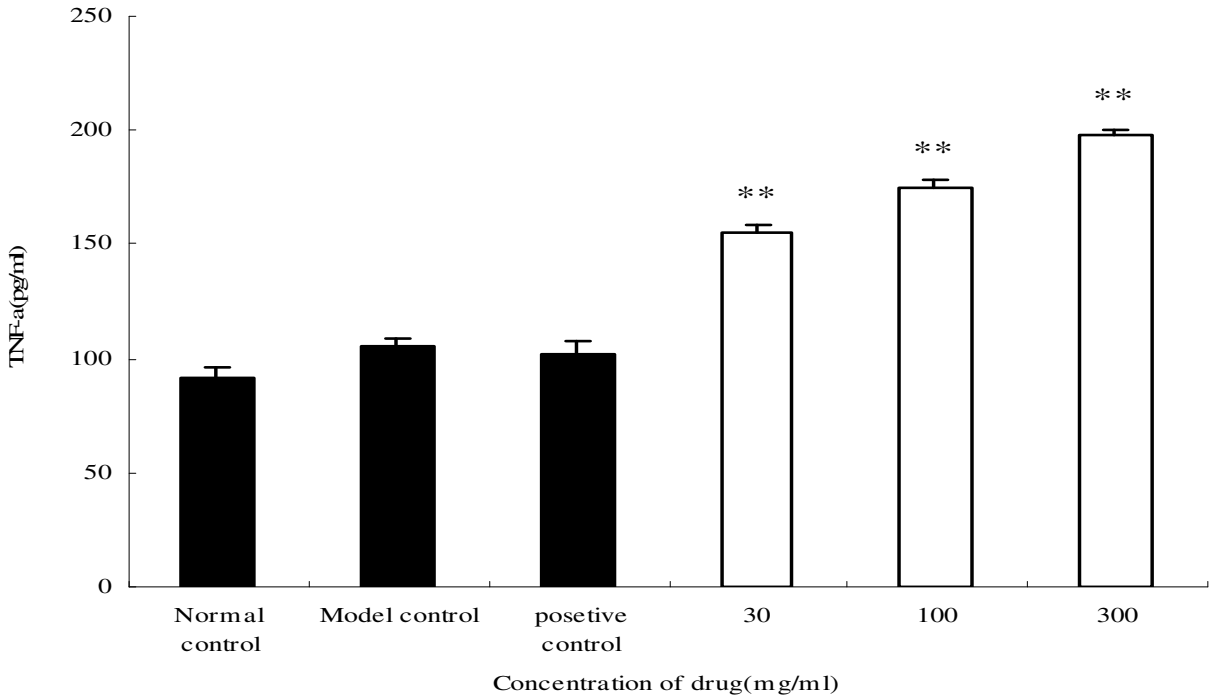

Figure 4. Concentration of TNF-α in serum of Sacroma 180 tumor-bearing mice. Normal control and model control received saline, positive control received cyclophosphamide, and AIOPA groups received polysaccharide AIOPA (30, 100, and 300 mg/kg). The dose volume was 0.2 ml. Values are means±S.D. **P<0.01 *vs.* model group.

Lymphocyte proliferation assay

When stimulating alone, the polysaccharide AIOPA could cause a dose-dependent proliferation of the mouse spleen cells, with the significant difference compared with negative control, reaching the highest stimulation index (Figure 5). AIOPA could evidently increase LPS-induced lymphocyte proliferation *in vitro*. In addition, it had effect on ConA-induced lymphocyte proliferation at 10, 30, 100 and 300 μg/ml, but had no obvious difference (Figure 6).

Splenocyte proliferation was an indicator of immuno enhancement. Extensive experimentation demonstrated that polysaccharides display immunomodulating function by stimulating both cellular and humoral immunity (Hase et al., 1997; Liu et al., 1999; Wang et al., 1997). The immunologic action of polysaccharides may begin with activating effector cells such as lymphocytes, macrophages, natural killer (NK) cells. Ishizaka et al. (1980) found that λ-carrageenan could enhance lymphocyte activation *in vitro*. Our results also demonstrated that the polysaccharide AIOPA could augment splenocyte proliferation, especially LPS-induced splenocyte proliferation.

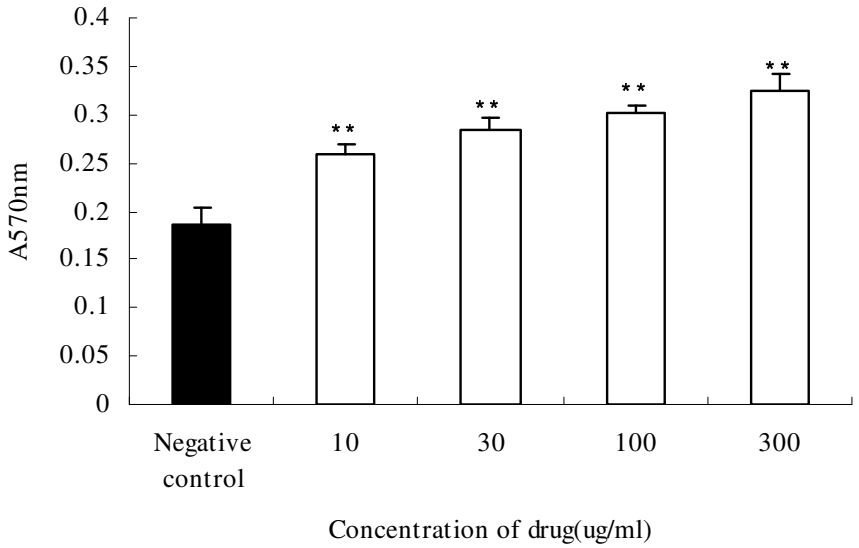

Figure 5. Effect of polysaccharide AIOPA on lymphocyte proliferation. Spleen cells were stimulated by different concentration of the polysaccharide AIOPA. Proliferation activity was expressed at 570 nm. Values are means±S.D. **P<0.01 *vs.* negative control.

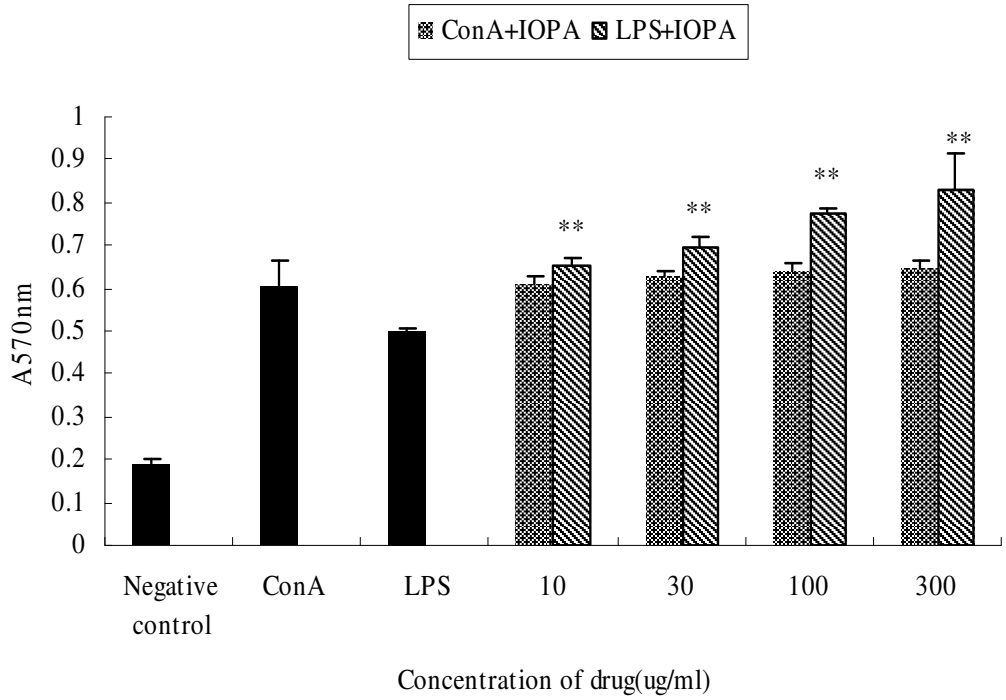

Figure 6. Effect of polysaccharide AIOPA on ConA-induced or LPS-induced lymphocyte proliferation. Proliferation activity was expressed at 570 nm. Values are means±S.D. **P<0.01 *vs.* ConA or LPS, respectively.

Activation on peritoneal macrophages

In this study, peritoneal macrophages were cultured with the polysaccharide AIOPA from 10 to 300 μg/ml, and LPS (20 μg/ml) was used as positive control. The results showed that each concentration of AIOPA could enhance the peritoneal macrophages phagocytosis compared with LPS control (P<0.01, Figure 7). The production of nitric oxide by stimulated macrophages was shown in Table 2. The fraction AIOPA markedly induced production of NO

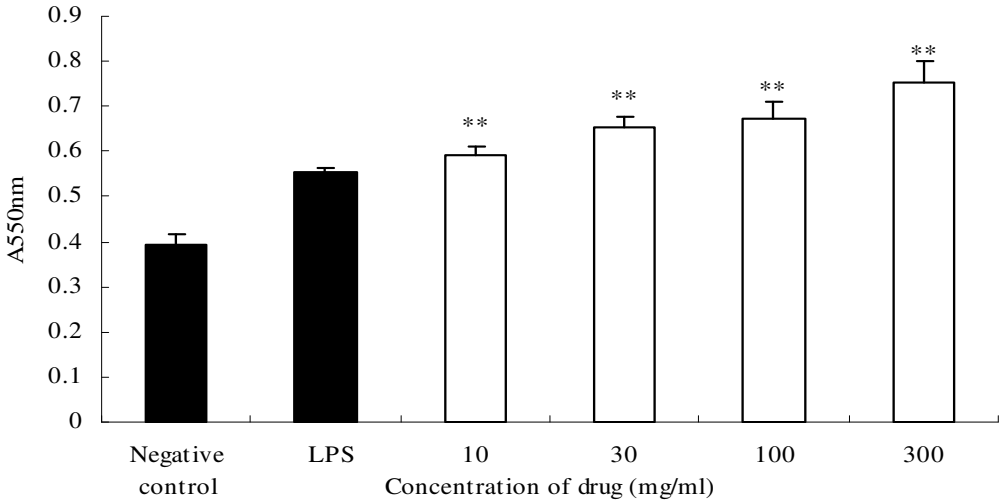

Figure 7. Effect of the polysaccharide AIOPA on phagocytosis of macrophages. The phagocytotic activity was expressed by the absorption at 550 nm. Values are means±S.D. **P<0.01 *vs*. LPS control (20 µg/ml).

Table 2. Amount of NO, iNOS activity and TNF-α produced by AIOPA stimulated macrophages.

Group	Nitrite production (µM)	iNOS activity ($U/10^6$)	TNF-α (pg/ml)
Negative control	10.18 ± 1.75	11.13 ± 2.32	121.68 ± 5.31
LPS	28.72 ± 1.35**	22.32. ± 2.57**	281.44 ± 4.59**
10	12.38 ± 0.91	17.53 ± 3.46	142.21 ± 27.54
30	18.41 ± 1.50**	21.95 ± 2.39**	174.22 ± 13.10
100	23.57 ± 0.37**	19.61 ± 3.19**	216.76 ± 26.79**
300	25.68 ± 1.29**	18.74 ± 2.37**	259.59 ± 6.16**

In vitro activation of peritoneal macrophage stimulated by different concentrations of the samples in terms of NO, iNOS activity and TNF-α production. The fraction AIOPA in the concentration range from10 to 300 µg/ml was investigated in the test. The amount of NO, iNOS activity and TNF-α was measured after 48 h of incubation. Values were compared against negative control using Student's *t* -test **P<0.01 *vs*. negative control.

in macrophages at the concentration 30 µg/ml or above. With an increase of the concentration of AIOPA, the production of NO increased. It was proved to be the most active when the polysaccharide was 300 µg/ml, and the amount of NO produced by macrophages was close to that of LPS 20 µg/ml.

To elucidate the underlying mechanisms of the regulation of NO synthesis by AIOPA, the change of iNOS activity in AIOPA-induced macrophages was evaluated. The results from Table 2 show that AIOPA also remarkably improved iNOS activity of macrophages in the increasing order of 10, 30, 100 and 300 ug/ml. This increased effect on iNOS activity showed that the augmentation of NO formation in macrophages by AIOPA was the result of the improvement of iNOS activity.

TNF-*α* played an important role in tumoricidal and immune response, and it was measured by sandwich ELISA (Table 2). Peritoneal macrophages were stimulated by different concentrations of AIOPA or LPS. When the doses were 100 and 300 µg/ml, the level of TNF-*α* was significantly increased compared with negative control level (P<0.01).

Macrophage was one of the research foci of the immunology community and exhibits cytotoxicity by phagocytosis, direct cellular cytotoxicity through cell-to-cell contact and the secretion of cytotoxic cytokines (IL-1, TNF-a) and NO (Remer et al., 2005). Activated macrophages were also considered to be one of the important components of the host defense against tumor growth (Wasser, 2002). Especially NO, an effector of macrophages, not only participate in killing tumor cells, but also in defense infection of bacteria, fungi, and parasite. The research (Kim et al., 2006) also demonstrated that the *in vitro* immunostimulating activity of water

soluble endo-polysaccharide isolated from *I. obliquus* mycelia could stimulate macrophage secretion of NO. In the experiment, AIOPA induced a significant increase in cell lysosomal enzyme activity and increased the NO production of macrophages at the doses of 30 to 300 µg/ml, and we also found that TNF-α secretion in AIOPA (100 to 300 µg/ml) treated macrophages was relatively high compared with negative control and both the maximal effect occurred at 300 µg/ml. Thus, the polysaccharide may indirectly play the role of anti-tumors activity through the releases of effect or molecules such as TNF-α, NO produced by macrophages.

Conclusions

In summary, the results from experiment in vivo showed that AIOPA could not only significantly inhibit the growth of sarcoma 180, but also remarkably increased thymus, spleen and lymphoid organ weight and raised the level of TNF-a. The results *in vitio* of study showed that AIOPA could not only promote lymphocyte transformation and multiplication and enhance phagocytic function of macrophage, but also remarkably promoted the level of NO, iNO and TNF-a. These results suggested that AIOPA could improve both specific and non-specific cellular immune response. This study also provided evidences to support the therapeutic effects of AIOPA for treatment of cancer in China. At present, detailed work on structure-function relationship are in progress.

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