Carbohydrate Polymers 90 (2012) 1671–1676

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Purification and antitumor activity of two acidic polysaccharides from the roots of *Polygala tenuifolia*

Tao Xin[a], Fubin Zhang[b,*], Qiuying Jiang[a], Chunhong Chen[a], Dayong Huang[a], Yanju Lv[a], Weixi Shen[a], Yinghua Jin[a]

[a] *Department of Medical Oncology, The Second Affiliated Hospital, Harbin Medical University, Harbin 150081, China*
[b] *Department of Gynecology, The Third Affiliated (Tumor) Hospital, Harbin Medical University, Harbin 150040, China*

ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form 8 July 2012
Accepted 19 July 2012
Available online 27 July 2012

Keywords:
Polygala tenuifolia
Polysaccharide
Antioxidative
Antitumor
Human lung adenocarcinoma A549

ABSTRACT

Two acidic polysaccharide fractions (PTPa and PTPb) extracted from the roots of *Polygala tenuifolia*, were obtained by DEAE-Sephacel anion-exchange, and Sephadex G-100 gel-permeation chromatography. High-performance liquid chromatography (HPLC) identified that PTPa and PTPb was composed of Ara, Glc, Gal, Man and GlcUA in the proportion of 2.4:1.2:0.6:0.4:1.1 and 2.1:1.7:0.5:0.6:1.7, respectively. Their molecular weight was evaluated to be 5.9×10^4 (PTPa) and 2.5×10^4 Da (PTPb) as determined by high performance size exclusion chromatography (HPSEC). Pharmacological studies revealed PTPa and PTPb significantly inhibited the growth of A549 cells in vitro and exhibited significantly higher antitumor activity against solid tumor A549 in vivo than did a blank control. Moreover, treatment with two acidic polysaccharides caused an enhancement of superoxide dismutase (SOD) and catalase (CAT) activities in tumor-bearing mice and a reduction in thiobarbituric acid reactive substances (TBARS) level. Taken together, these results indicated that two acidic polysaccharides from the roots of *P. tenuifolia* may be useful as potent antitumor agents for the prevention of lung tumorigenesis.

1. Introduction

"Yuan Zhi" (the roots of *Polygala tenuifolia* Willd., Polygalaceae) is an important herb prescribed in traditional Chinese medicine to mediate sedative, antipsychotic, cognitive improving, neuron protective, and anti-inflammatory therapeutic effects on the central nervous system (Chung et al., 2002; Chung, Kim, & Kim, 1992; Jang et al., 1997; Kim et al., 1998; Park et al., 2002; Zhang et al., 2008). It has also been used for insomnia, neurasthenia, amnesia, palpitations with anxiety, restlessness, and disorientation, and to prevent dementia and memory failure. Pharmacognosy study had revealed the presence of various xanthones, saponins, and oligosaccharide esters in this plant (Cheng et al., 2006; Lin, Chen, Ko, Ko, & Wu, 2008; Liu et al., 2007). In another unpublished report, we had made a systemic extraction and purification for the water-soluble polysaccharides from the roots of *P. tenuifolia*, and tested the antitumor activities of the neural water-soluble polysaccharide (PTP) in vitro and in vivo. Our results clearly demonstrated that the antitumor mechanism of PTP might be mediated by GSH depletion and the increase of intracellular ROS in cancer cells, thus resulting in the apoptosis. Supporting this fact, the tumor growth was significantly suppressed in SKOV3 bearing-mice after 7 weeks of PTP treatment. As a subsequent research of our previous work, we intend to isolate the acidic polysaccharides from this plant for further discovery of new anticancer drugs.

2. Experimental

2.1. Materials and chemicals

P. tenuifolia was harvested in October from a farm in Northeast China. It was dried in sunlight, cut into small pieces and then dried at an oven for 16 h before use. The material, which passed through 160-mesh screen and was retained on an 80-mesh screen, was collected for subsequent experiment. DEAE-Sephacel and Sephadex G-100 were purchased from Amersham Pharmacia Biotech. Dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), CF_3COOH (TFA), 1-phenyl-3-methyl-5-pyrazolone (PMP), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and monosaccharide standards, including mannose, glucosamine, ribose, rhamnose, glucuronic acid, galacturonic acid, galactosamine, glucose, galactose, xylose, arabinose, and fucose, were purchased from Sigma. All other reagents were of the highest available quality.

* Corresponding author. Tel.: +86 0451 86641537; fax: +86 0451 86641537.
E-mail address: fubinzhang_hrbmu@hotmail.com (F. Zhang).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.07.048

2.2. Extraction, isolation and purification of acidic polysaccharides

The dried small pieces of *P. tenuifolia* (200 g) was sufficiently extracted with toluene–ethanol (1:1, v/v) by refluxing in a Soxhlet apparatus for 6 h to remove fats and waxes. The resulting residue (178 g) was dried and then extracted two times with 1000 mL of warm water (80 °C) each time for 2 h. All water extracts were combined, filtered, concentrated and precipitated with 95% ethanol until the end concentration of 50% (v/v) and was kept for 1 h. Then 10% $CaCl_2$ was added and kept overnight to precipitate the tannin. The supernatant was obtained by centrifugation at 20,000 rpm for 5 min and successively ultrafiltrated with membrane (MWCO: 1k) and membrane (MWCO: 10k) on an ultrafiltration apparatus. The ultrafiltrate (outside of membrane of 10k) was concentrated by heating and lyophilized to obtain crude polysaccharide (cPTP).

The crude polysaccharides were redissolved in distilled water, forced through a filter (0.45 μm), and then applied to a DEAE-Sephacel column (2.6 cm × 30 cm) and eluted first with deionized water, then a linear gradient of 0 → 2 M NaCl in distilled water at a flow rate of 1.0 mL/min. A 0.2 mL sample collected from each eluted fraction (6 mL/tube) was mixed with sulfuric acid and phenol to produce color reaction and the fractions with rose color were combined together. In this procedure, two main fractions contained polysaccharide were obtained in deionized water and gradient NaCl eluants. The fraction containing polysaccharide eluted by NaCl solution was further applied to a column (2.5 cm × 100 cm) of Sephadex G-100 equilibrated with 50 mM acetate buffer (pH 5.2), and were eluted with the same buffer at a flow rate of 1 mL/min. The relevant fractions were collected, concentrated, dialyzed (MWCO: 1k), deproteinated by the Sevag method (Staub, 1965) and lyophilized for further study, respectively.

2.3. Analysis of acidic polysaccharides

2.3.1. Chemical characteristics analysis

Total sugar content was determined as anhydroglucose by the modified phenol–sulfuric acid method as D-glucose equivalents (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Uronic acid contents were determined by measuring the absorbance at 523 nm using the m-hydroxybiphenyl colourimetric procedure and with D-glucuronic acid as the standard (Filisetti-Cozzi & Carpita, 1991). Proteins were estimated by the Bradford assay (Bradford, 1976), using BSA as the standard.

2.3.2. Monosaccharide composition analysis by high-performance liquid chromatography (HPLC)

The monosaccharide composition of acidic polysaccharide was determined using HPLC method (Honda et al., 1989), with some modifications. Briefly, the polysaccharide sample (5 mg) was completely hydrolyzed with 0.5 mL of 2 M TFA to monosaccharide at 105 °C for 4 h, derived with 1-phenyl-3-methyl-5-pyrazolone (PMP), and subsequently analyzed by an HPLC (ZORBAX Eclips XDB-C18, 5 μm, 250 mm × 4.6 mm column) analysis with detection by absorbance monitoring at 245 nm. The standard monosaccharide mixture (mannose, glucosamine, ribose, rhamnose, glucuronic acid, galacturonic acid, galactosamine, glucose, galactose, xylose, arabinose, and fucose) were derivatized to be PMP derivatives and subjected to HPLC system in the same conditions. The mobile phase was 0.1 M NaCl buffer with flow rate of 0.5 mL/min and the column temperature was 50 °C.

2.3.3. Molecular weight determination

The homogeneity and molecular weight of acidic polysaccharide was determined by high-performance size-exclusion chromatography (HPSEC) (Liu et al., 2011), which was performed on a Agilent 1100 HPLC system (USA) fitted with TSK-GEL G3000PWxl 7.8 cm (i.d.) × 30.0 cm gel filtration columns and monitored by a refractive index detector. The samples were dissolved in 0.1 mol/L Na_2SO_4 to reach a final concentration of 5 mg/mL and the sample solution was filtered through 0.45 μm pore size filter membrane before injection. A sample solution (20 μL) was injected in each run and eluted with 0.1 mol/L Na_2SO_4 as the mobile phase at the flow rate of 0.5 mL/min. The column temperature was 25 °C. The standard curve was established with T-series dextrans of known MW (T-130, T-80, T-50, T-25 and T-10). Calculation of the molecular weights of samples was carried out using the Angilent GPC software (USA).

2.3.4. Infrared spectrum analysis

The major functional groups of acidic polysaccharides were measured using Fourier-transformed infrared (FT-IR) spectroscopy (Sun et al., 2011). Pellets for FT-IR analysis were obtained by grinding a mixture of 2 mg acidic polysaccharides with 200 mg dry KBr, followed by pressing the mixture into a 16-mm diameter mold. The FT-IR spectra were recorded with a Nicolet 170SX FT-IR (Spectrum One, PerkinElmer Co., USA) spectrometer in the region of 4000–400 cm^{-1}.

2.4. Cell lines and culture

Five common human tumor cell lines, A549 (human lung adenocarcinoma), SK-N-AS (human neuroblastoma), MCF-7 (human breast cancer), HT-29 (human colon cancer) and HepG2 (human hepatocellular carcinoma) were obtained from American Type Culture Collection (Rockville, MD, USA). A549, SK-N-AS, and HT-29 cancer cells were maintained and propagated in 90% Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. MCF-7 and HepG2 cancer cells were maintained in Eagle's minimum essential medium with 10% heat-inactivated FBS, 100 units/mL penicillin and 100 mg/mL streptomycin. Cells were cultured as an adherent monolayer and maintained at 37 °C in a humidified atmosphere of 5% CO_2 and were fed with fresh cultured medium every 2–3 times per week and subcultured when 80% confluent. Cells were harvested after subjecting them to brief trypsinization.

2.5. Cell proliferation assay

To evaluate the cytotoxicity of two acidic polysaccharides, a MTT colorimetric assay was performed to determine the cell viability (Mosmann, 1983). Cells were seeded in 24-well plates at a density of 1×10^4 cells per well and treated with various concentrations of samples for 48 h at 37 °C. At the end of the exposure period, cells were washed with PBS and then incubated with 20 μL MTT (5 mg/mL) for 4 h. The viable cell number is directly proportional to the production of formazan following solublization with 150 μL of dimethyl sulphoxide (DMSO), which can be measured spectrophotometrically at 570 nm by a microplate ELISA reader BIO-RAD 680, American). The results were determined by three independent experiments and the IC_{50} values were calculated according to the inhibition ratios.

2.6. Antitumor activity in vivo

Female congenital athymic BALB/c nude (nu/nu) mice were maintained in microinsulator cages under pathogen-free conditions on a 12 ± 1 h light–dark cycle and provided with normal mouse chow and water ad libitum. The experiments were performed according to the procedures of the Committee on Animal Research (Harbin Medical University). All experiments were carried out using 6–8-week-old mice weighting 18–22 g. Tumors were induced by subcutaneous (s.c.) injection of A549 cells (2×10^6 cells

in 0.1 mL of PBS) at one site of the right flank. When the tumor appeared as small nodules at the sites of injection (80–120 mm^3) 14 days after injection, 30 animals were randomly distributed into five groups ($n = 6$): a control group, which received vehicle (PBS); and four drug-treated group, which received a high dose (100 mg/kg) and a low dose (50 mg/kg) of PTPa or PTPb. Daily administration of vehicle or two polysaccharides (PTPa or PTPb) via intraperitoneally (i.p.) injection continued for additional 40 days. On the 40th day, six animals from each group were anaesthetized slightly with anesthetic ether and blood was collected from retro-orbital puncture. Before and after the experiment, the body weight of mice was measured on the balance. Immediately after collecting the blood samples, the mice were killed by cervical dislocation. Tumor volume (TV) was calculated by measurement of the length (L) and width (W) of the tumor mass with the following formula:

$$\text{TV}\ \ (\text{mm}^3) = \frac{L \times W^2}{2}.$$

Tumor inhibition rate (TIR, %) in mice was expressed as follows: TIR (%) = (1 − mean TV of treated group)/(mean TV of control group) × 100.

2.7. In vivo antioxidative assay

Blood samples (3 mL) form each mouse were obtained on the last day of the experiment and then centrifuged at 3000 × g for 10 min at 4 °C to obtain serum for the estimation of the antioxidant parameters, such as catalase (CAT), superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) by spectrophotometric methods. The TBARS level in the serum is expressed as the malondialdehyde (MDA) equivalent (nM/mL). The SOD and CAT activity in the blood were assayed by using a commercially available colorimetric kit according to the manufacturer's protocol (Beyotime Biotechnology, Haimen, China). The activity of SOD and CAT in the serum is expressed in units per 1 mL of serum.

2.8. Statistical analysis

Statistical analysis is performed using Student's unpaired t-test (SPSS release 12.0; SPSS Inc.). Data were expressed as (mean ± SD) and considered significant for $p < 0.05$.

3. Results and discussion

3.1. Isolation and purification of acidic polysaccharides

The crude polysaccharides (cPTP) with a yield of 5.27% (w/w) of the crude material were extracted and isolated from the roots of *P. tenuifolia* by hot-water extraction, ultrafiltration and EtOH precipitation. After the cPTP was separated and sequentially purified through DEAE-Sephacel and Sephadex G-100 column, the fractions contained large amount of polysaccharide were collected as detected by phenol-sulfuric acid assay and deproteinated by the Sevag method and lyophilized for further study. The cPTP was fractioned by chromatography on DEAE-Sephacel column into two parts: cPTP-I and cPTP-II, and fraction cPTP-II eluted with gradient NaCl solution was subsequently loaded into Sephadex G100 and the results were shown in Fig. 1. Two acidic polysaccharides (PTPa and PTPb) with different molecular weight and uronic acid content were purified by gel-filtration on Sephadex G100 column. In the current study, these two acidic polysaccharides would be used for further physicochemical and biological analysis.

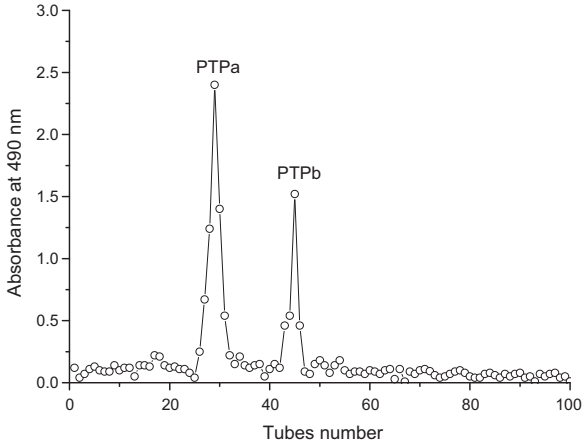

Fig. 1. Elution profiles of two acidic polysaccharides on Sephadex G-100 column. The crude polysaccharide cPTP was purified on a DEAE-Sephacel column with deionized water and a linear gradient of 0–2 M NaCl solution at a flow rate of 1.0 mL/min. Then the fraction cPTP-II from NaCl eluate was further purified on a Sephadex G-100 column and eluted with 50 mM acetate buffer (pH 5.2) at a flow rate of 1 mL/min.

3.2. Analysis of physico-chemical characteristics of acidic polysaccharides

The polysaccharide contents of PTPa and PTPb were found to be 59.54%, and 46.25%, respectively, as determined by the phenol–sulfuric acid method. It had a negative response to the Bradford test and no absorption at 280 nm in the UV spectrum, indicating the absence of protein. The uronic acid contents evaluated in PTPa and PTPb were 14.42% and 23.16%, respectively (Table 1).

After two acidic polysaccharides were hydrolysed, the released monosaccharides were separated, derived by PMP and identified by comparing with the chromatogram of the mixture of standard monosaccharides by an HPLC method. As seen in Table 1, PTPa was composed of Ara, Glc, Gal, Man and GlcUA in the proportion of 2.4:1.2:0.6:0.4:1.1 and PTPb contained the same monosaccharide but in the ratio of 2.1:1.7:0.5:0.6:1.7.

The GPC profile showed two single and symmetrically sharp peaks, indicating that two acidic polysaccharides were homogeneous polysaccharides (Fig. 2). The estimated average molecular weights of PTPa and PTPb were approximately 5.9×10^4 and 2.5×10^4 Da, respectively, which were obtained based on the calibration of using dextran (Table 1).

The FT-IR spectra of carbohydrates are used for determination of their structural features. The IR spectra of PTPa and PTPb were basically indistinguishable only with some difference in the intensity of bands recorded from 4000 to 400 cm^{-1}. The absorption bands within the range of 3600–3200 cm^{-1} (stretching vibration of O—H), 3000–2800 cm^{-1} (stretch vibration of C—H) and 1200–1000 cm^{-1} (stretching vibration of C—O) were the characteristic absorption peaks of polysaccharides (Sun et al., 2011; Wang, 2011; Wu et al., 2010; Zhang et al., 2010) (Table 1). In addition, the absorption peak at about 1640 cm^{-1} were due to asymmetric stretch vibration of COO^- of uronic acids, and a weak one at near 1440 cm^{-1} were attributed to symmetric stretch vibration of COO^- and stretch vibration of C—O within COOH, indicating the existence of uronic acids in two acidic polysaccharides (Wu et al., 2012; Zhu et al., 2010). Moreover, the characteristic absorption at around 830 and 890 cm^{-1} in the FTIR spectra indicated that α- and β-configurations were simultaneously present in two acidic polysaccharides (Xie et al., 2010).

More importantly, PTPa and PTPb were soluble in water but not soluble in organic solvents such as ethanol, diethyl ether, acetone, and chloroform. Results of carbazole assay, Coomassie Blue

Table 1
The summary for physicochemical properties of two acidic polysaccharides (PTPa and PTPb), including chemical content (carbohydrate, protein and uronic acid), molecular weight, monosaccharide composition and IR spectrum characterizations.

Sample	Carbohydrate content (%)	Protein content (%)	Uronic acid content (%)	Molecular weight (Da)	Monosaccharide composition	IR spectrum (cm^{-1})
PTPa	59.54	–	14.42	5.9×10^4	Ara, Glc, Gal, Man and GlcUA (2.4:1.2:0.6:0.4:1.1)	3453, 2931, 1640, 1440, 1068, 892, 831
PTPb	46.25	–	23.16	2.5×10^4	Ara, Glc, Gal, Man and GlcUA (2.1:1.7:0.5:0.6:1.7)	3452, 2934, 1640, 1441, 1065, 890, 830

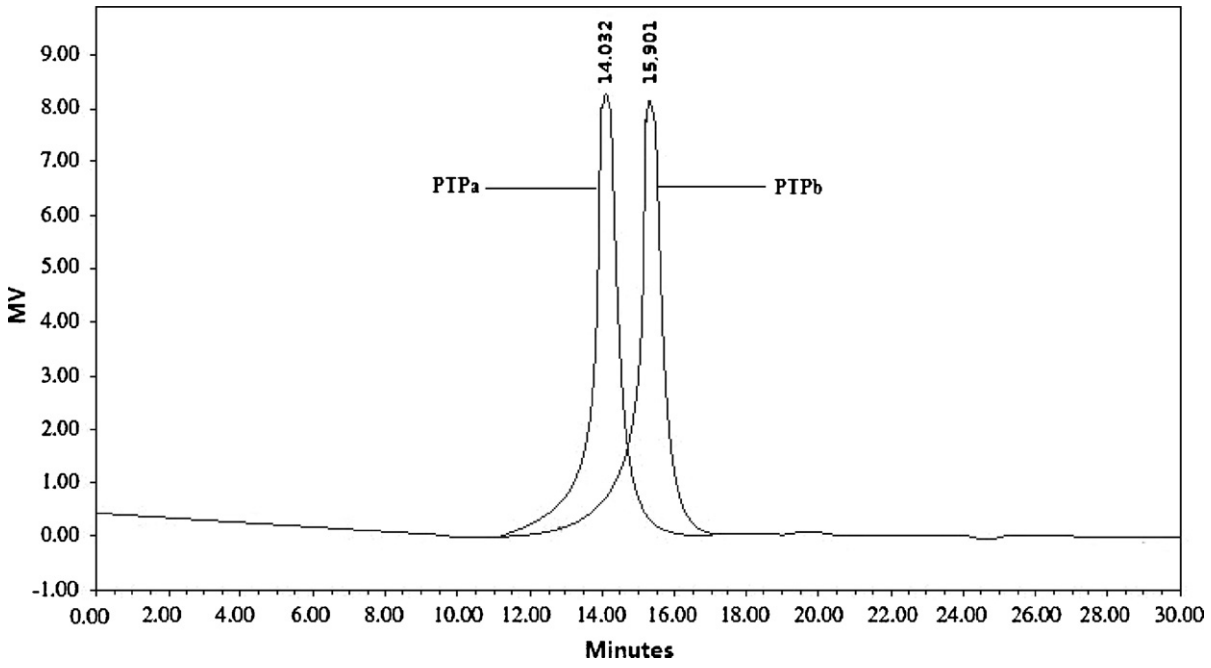

Fig. 2. The elution profile of two acidic polysaccharides (PTPa and PTPb) on high-performance size-exclusion chromatography (HPSEC). A sample solution (20 μL) was injected in each run and eluted with 0.1 mol/L Na_2SO_4 as the mobile phase at the flow rate of 0.5 mL/min.

assay and iodine assay suggested that the polysaccharides were non-starch acidic polysaccharides.

3.3. Antiproliferative activity of acidic polysaccharides in vitro

The growth inhibitory effects of two acidic polysaccharides on various human cancer cell lines were determined by MTT assay. Cells were treated with PTPa or PTPb at different concentrations (0–200 mg/mL) for 48 h. As shown in Table 2, the IC_{50} values of PTPa and PTPb tested in these cancer cells were ranged from 40 to 90 μg/m, except for HepG-2 and SK-N-AS cancer cells treated by PTPa or SK-N-AS cancer cells by PTPb. Particularly, PTPa and PTPb showed maximum cytotoxicity to human lung adenocarcinoma A549 cancer cells than others, with the IC_{50} value of 47.8 and 41.2, respectively. Importantly, the IC_{50} value of PTPb in all cancer cells were low than those of PTPa, which may be influenced by the difference of the structure, conformation and chemical composition in two acidic polysaccharides. Briefly, these results showed that two acidic polysaccharides could induce high cytotoxicity to various human cancer cells in a descending order: A549 > MCF7 > HT-29 > HepG-2 > SK-N-AS. Therefore, the special potent inhibitory effect of two acidic polysaccharides on human lung adenocarcinoma A549 cancer cells would be initiated and explored in the following experiments.

Table 2
The IC_{50} of different tumors by two acidic polysaccharides (PTPa and PTPb) treatment.

Cell lines	Tumor type	IC_{50} (μg/mL)	
		PTPa	PTPb
A549	Human lung adenocarcinoma	47.8	41.2
MCF7	Huamn breast carcinoma	67.2	65.9
HT-29	Human colon carcinoma	78.3	71.4
HepG-2	Human hepatocellular carcinoma	>100	83.2
SK-N-AS	Human neuroblastoma	>100	>100

Cells were seeded in 24-well plates at a density of 1×104 cells per well and treated with various concentrations of PTPa and PTPb for 48 h at 37 °C. Cytotoxicity was measured with the MTT assay. IC_{50} = extract concentration causing a 50% decrease in the survival curve (mean ± SD from three independent experiments).

3.4. Anti-tumor activity of acidic polysaccharides in vivo

To further explore the anti-tumor function of two acidic polysaccharides in vivo, we evaluated their effect on the growth of xenografted A549 tumors in nude mice. Mice were treated with vehicle (PBS), low-dose extract (50 mg/kg) or high-dose extract (100 mg/kg) of two acidic polysaccharides via i.p. injection from 14 days after inoculation of tumor cells and continuously received treatment for additional 40 days. After daily i.p. administration of PTPa or PTPb to tumor-bearing mice, the tumor volumes were statistically lower in PTPa or PTPb-treated mice at low and high doses ($P < 0.05$ or $P < 0.01$) compared with the vehicle-treated group (Table 3). Interestingly, the better tumor growth suppression was found on PTPb-treated A549 xenografts with the high TIR value

Table 3
Effects of two acidic polysaccharides (PTPa and PTPb) on tumor growth and body weight in A549 tumor-bearing mice.

Group	Dose (mg/kg, BW)	Tumor volume (TV, cm^3)	Tumor inhibition rate (TIR, %)	Body weight (BW, g)	
				Before experiment	After experiment
Control	PBS	0.92 ± 0.15	–	21.3 ± 0.31	30.4 ± 0.22
PTPa	50	0.63 ± 0.09	31.5[a]	20.5 ± 0.23	28.8 ± 0.18
	100	0.41 ± 0.09	55.4[b]	20.2 ± 0.21	29.3 ± 0.20
PTPb	50	0.57 ± 0.08	38.0[a]	20.7 ± 0.22	29.0 ± 0.21
	100	0.35 ± 0.05	62.0[b]	21.1 ± 0.21	30.1 ± 0.23

Female BALB/c nude (nu/nu) mice were treated with PTPa or PTPb (50 or 100 mg/kg body weight) everyday 14 days after an initial subcutaneous (s.c.) injection of A549 cells at one site of the right flank. 40 days post implantation, tumor volume (TV) of xenograft tumors were measured using vernier calipers to measure the length (L) and width (W) of the tumor mass, calculated as the following formula: TV (mm^3) = ($L \times W^2$)/2. Throughout the experiment, the body weight of tumor-bearing mice was measured before and after experiment. Significant difference versus control group was analyzed by Student's *t*-test.
[a] $P < 0.05$.
[b] $P < 0.01$.

Table 4
Effect of two acidic polysaccharides (PTPa and PTPb) on antioxidant status of A549 tumor-bearing mice in serum.

Parameters	Control	PTPa treatment (mg/kg, BW)		PTPb treatment (mg/kg, BW)	
		50	100	50	100
CAT (U/mL serum)	0.35 ± 0.012	0.36 ± 0.016	0.41 ± 0.013	0.42 ± 0.021	0.46 ± 0.015
SOD (U/mL serum)	0.42 ± 0.015	0.67 ± 0.022[a]	0.87 ± 0.033[c]	0.65 ± 0.041[a]	1.04 ± 0.053[c]
TBARS (MDA nM/mL)	5.32 ± 0.35	3.24 ± 0.242[b]	2.45 ± 0.268[b]	3.34 ± 0.317[b]	2.14 ± 0.239[c]

The serum collected from the mice was stored at −30 °C for analyzing the level of TBARS and the activity of SOD/CAT by spectrophotometric methods. Significant difference versus control group was analyzed by Student's *t*-test.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.01$.

of 38.0 (50 mg/kg) and 62.0 (100 mg/kg), but a lower rate was observed on PTPa-treated mice with 31.5 for 50 mg/kg and 55.4 for 100 mg/kg. In addition, no signs of toxicity were observed in the mice treated with the polysaccharide on the basis of body weight (Table 1). These results demonstrated that PTPa and PTPb had antitumor activity in nude mice bearing A549 lung cancer xenografts.

3.5. Antioxidative activity of acidic polysaccharides in vivo

In order to investigate the antioxidant status change in tumor-bearing mice post PTPa or PTPb treatment, we measured SOD and CAT activities, as well as TBARS level using a commercially available colorimetric kit. As shown in Table 4, treatment with two acidic polysaccharides at doses of 50 and 100 mg/kg induced SOD level increases of 59% and 107% for PTPa, and 79% and 148% for PTPb, respectively. Moreover, two acidic polysaccharides also dose-dependently increased CAT activity, although this effect was not as robust as the effect on SOD activity. To the best of our knowledge, the increase of TBARS indicates enhanced lipid peroxidation, which leads to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals (Zhao, Li, Yue, Zhang, & Dou, 2012). Upon PTPa or PTPb administration, the blood serum TBARS levels were significantly decreased in two dose-treated groups, indicating that two acidic polysaccharides could effectively scavenge superoxide radicals and inhibit the generation of lipid peroxidation products in the tumor-bearing mice.

4. Conclusions

In summary, the present study describes the isolation and biological activities of the two acidic polysaccharides found in the roots of *P. tenuifolia*. PTPa and PTPb had the similar sugar composition (Ara, Glc, Gal, Man and GlcUA), but varied in the content. The molecular weights of PTPa and PTPb were approximately 5.9×10^4 and 2.5×10^4 Da, respectively. In vitro screening test for discovering potential antiproliferation agent, the results indicated that the two polysaccharide fractions showed different antiproliferation activities on various cancer cells, especially with a low value of IC_{50} of 47.8 and 41.2 on the A549 cancer cell by PTPa and PTPb, respectively. In vivo experiment, both acidic polysaccharides dramatically inhibited the tumor growth in nude mice bearing A549 lung cancer xenografts and no significant change was observed in body weight. Moreover the activities of SOD and CAT in the serum of tumor-bearing mice were improved after polysaccharides treatment, accompanied by a reduction of TBARS. Therefore, it is reasonable to hypothesize that two acidic polysaccharides prompt tumor regression at least partly through its antioxidative and radical scavenging properties. From above data, we can clearly discover an interesting fact that PTPb treatment is superior to the PTPa in most cases. This difference of anti-tumor and antioxidant activities of two acidic polysaccharides might be related to the physicochemical difference mentioned above. However other structural characteristics, such as configuration and position of glycosidic linkages, could also influence the biological activities. Therefore, further studies are needed to elucidate the structures of two acidic polysaccharides and the relationship between structure and antitumor activity is also needed.

Acknowledgements

We wish to thank Jianping Cai at Harbin Medical University for the positive suggestions in the manuscript preparation. This study was financially supported by Research Fund of Health Department of Heilongjiang Province (No. 2009-022) and Scientific Research Foundation of The Third Affiliated Hospital of Harbin Medical University (No. JJ2009-09).

References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. *Analytical Biochemistry*, *72*, 248–254.
Cheng, M. C., Li, C. Y., Ko, H. C., Ko, F. N., Lin, Y. L., & Wu, T. S. (2006). Antidepressant principles of the roots of *Polygala tenuifolia*. *Journal of Natural Products*, *69*, 1305–1309.

Chung, I. W., Kim, S. R., & Kim, E. G. (1992). Dopamine-2 and serotonine-2 receptor bindings in antipsychotic medicines from natural products. *Journal of Korean Neuropsychiatric Association*, *31*, 856–867.

Chung, I. W., Moore, N. A., Oh, W. K., O'Neill, M. F., Ahn, J. S., Park, J. B., et al. (2002). Behavioural pharmacology of polygalasaponins indicates potential antipsychotic efficacy. *Pharmacology Biochemistry and Behavior*, *71*, 191–195.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, *197*, 157–162.

Honda, S., Akao, E., Suzuki, S., Okuda, M., Kakehi, K., & Nakamura, J. (1989). High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl-5-pyrazolone derivatives. *Analytical Biochemistry*, *180*, 351–357.

Jang, K. J., Lee, K. H., Kim, S. L., Choi, D. Y., Park, B. K., Im, D. H., et al. (1997). Chongmyungtang attenuates kainic acid-induced seizure and mortal effect in the mouse. *Archives of Pharmacal Research*, *20*, 375–378.

Kim, H. M., Lee, E. H., Na, H. J., Lee, S. B., Shin, T. Y., Lyu, Y. S., et al. (1998). Effects of *Polygala tenuifolia* root extract on the tumor necrosis factor-1 secretion from mouse astrocytes. *Journal of Ethnopharmacology*, *61*, 201–208.

Lin, Y. L., Chen, W. P., Ko, H. C., Ko, F. N., & Wu, T. S. (2008). Norepinephrine transporter inhibitors from *Polygala tenuifolia*. *Journal of Food and Drug Analysis*, *16*, 26–30.

Liu, J., Yang, X., He, J., Xia, M., Xu, L., & Yang, S. (2007). Structure analysis of triterpene saponins in *Polygala tenuifolia* by electrospray ionization ion trap multiple-stage mass spectrometry. *Journal of Mass Spectrometry*, *42*, 861–873.

Liu, Y., Sheng, Y., Yuan, G. X., Wang, Y., Wei, H. F., Guan, M., et al. (2011). Purification and physicochemical properties of different polysaccharide fractions from the water extract of *Boschniakia rossica* and their effect on macrophages activation. *International Journal of Biological Macromolecules*, *49*, 1007–1011.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55–63.

Park, C. H., Choi, S. H., Koo, J. W., Seo, J. H., Kim, H. S., Jeong, S. J., et al. (2002). Novel cognitive improving and neuroprotective activities of *Polygala tenuifolia* Willdenow extract, BT-11. *Journal of Neuroscience Research*, *70*, 484–492.

Staub, A. M. (1965). Removal of protein-Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.

Sun, Y. X., Liu, J. H., Yue, L. L., Zhang, C. J., Yu, H. T., Wu, Q., et al. (2011). One proteoglycan from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O.K. Miller: Purification and structural features. *Carbohydrate Polymers*, *86*, 1381–1384.

Wang, B. (2011). Chemical characterization and Ameliorating effect of polysaccharide from Chinese jujube on intestine oxidative injury by ischemia and reperfusion. *International Journal of Biological Macromolecules*, *48*, 386–391.

Wu, Q., Tan, Z. P., Liu, H. D., Gao, L., Wu, S. J., Luo, J. W., et al. (2010). Chemical characterization of *Auricularia auricula* polysaccharides and its pharmacological effect on heart antioxidant enzyme activities and left ventricular function in aged mice. *International Journal of Biological Macromolecules*, *46*, 284–288.

Wu, Y., Cui, S. W., Wu, J. H., Ai, L. Z., Wang, Q., & Tang, J. (2012). Structure characteristics and rheological properties of acidic polysaccharide from boat-fruited sterculia seeds. *Carbohydrate Polymers*, *88*, 926–930.

Xie, Y., Jiang, S. P., Su, D. H., Pi, N. N., Ma, C., & Gao, P. (2010). Composition analysis and anti-hypoxia activity of polysaccharide from *Brassica rapa* L. *International Journal of Biological Macromolecules*, *47*, 528–533.

Zhang, H., Han, T., Zhang, L., Yu, C. H., Wan, D. G., Rahmanc, K., et al. (2008). Effects of tenuifolin extracted from radix polygalae on learning and memory: A behavioral and biochemical study on aged and amnesic mice. *Phytomedicine*, *15*, 587–594.

Zhang, S., He, B., Ge, J. B., Zhai, C. L., Liu, X., & Liu, P. G. (2010). Characterization of chemical composition of *Agaricus brasiliensis* polysaccharides and its effect on myocardial SOD activity, MDA and caspase-3 level in ischemia–reperfusion rats. *International Journal of Biological Macromolecules*, *46*, 363–366.

Zhao, W., Li, J. J., Yue, S. Q., Zhang, L. Y., & Dou, K. F. (2012). Antioxidant activity and hepatoprotective effect of a polysaccharide from Bei Chaihu (*Bupleurum chinense* DC). *Carbohydrate Polymers*, *89*, 448–452.

Zhu, M. Y., Mo, J. G., He, C. S., Xie, H. P., Ma, N., & Wang, C. J. (2010). Extraction, characterization of polysaccharides from *lycium barbarum* and its effect on bone gene expression in rats. *Carbohydrate Polymers*, *80*, 672–676.