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Characterization and antitumor activities of the water-soluble polysaccharide from *Rhizoma Arisaematis*

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ABSTRACT

A water soluble polysaccharide (RAP-W1) was purified from *Rhizoma Arisaematis* and its antitumor activity was evaluated in BALB/c mice bearing human breast cancer MCF-7. RAP-W1 had the following physicochemical properties: total carbohydrate content (95.9%); no protein; molecular weight (≈57 kDA); monosaccharides composition (rhamnose:fucose:arabinose:mannose:galactose:glucose = 0.4:0.5:0.3:0.6:0.9:5.3). After 14 days' treatment to tumor-bearing mice, RAP-W1 could significantly inhibit the growth of tumor transplanted in mice and increase the body weight and the spleen index. Moreover, RAP-W1 could significantly stimulate Con A- or LPS-induced splenocyte proliferation in tumor-bearing mice, as well as enhance the CTL activity. The level of Th1 cytokines (INF-γ and IL-2) in the serum of tumor-bearing mice was increased by RAP-W1 treatment, whereas the Th2 cytokine (IL-10) secretion displayed a dramatic reduction. All the data implied that RAP-W1 can activate T cells by up-regulating Th1/Th2 cytokine ratio, which might partially cause the inhibition of tumor growth.

1. Introduction

Rhizoma Arisaematis (RA) is come from the rhizome of *Pinellia pedatisecta* Schott, which has bitter, warm, pungent, and toxic properties (Sun, Ding, & Qian, 1995). It distributes widely in northwest and southwest of China and has be recorded in Chinese Pharmacopoeia as a traditional Chinese medicine (TCM), displaying sedative, stomachic, analgesic, anticoagulant, anti-inflammatory, antiemetic, and antitumor activities (Mao, Cheng, & Wu, 2002; Mao, Wu, & Cheng, 1994; Zhu, Zhou, & Ding, 1999). In TCM books, it was recorded to possess efficacy in dispelling wind and relieving convulsion, drying dampness to eliminate phlegm, and eliminating stagnation. The pharmacognostical study identified the probable active components of RA includes beta-sitosterol, total alkaloids, guanosine, γ-aminobutyric acid and dipeptides (Qin, Kong, Fan, Su, & Li, 1984; Wang, Wen, Yang, & Qin, 1997; Wu, Su, Cai, Zhang, & Ye, 1995). Since the 1970s, Li, Xu, and Sun (1981) had used RA to treat 247 cases of cervical cancer and the total effective rate was 81.5% and also proved the lipid-soluble fraction of this plant was responsible for its antitumor activity in vitro studies. Thereafter, another research group prepared a novel lipid-soluble extract (PE) from RA and tested its cytotoxicity and apoptosis induction effects on CaSki, HeLa and HBL-100 cells. The results showed that PE can significantly inhibit human cervical cancer cells proliferation by inducing apoptosis, but it has little side effect on normal cells. It probably took effect via mitochondria-dependent and death receptor-dependent apoptotic pathways (Li et al., 2010). As we know, RA is very toxic and must be processed carefully. As documented in Chinese Pharmacopoeia, the crude RA should be processed with an optimum amount of alumen in combination with ginger juice or bile juice to lower its toxicity (He, Wu, & Wang, 1997; Wu et al., 1995; Wu, Ye, Diao, & Cai, 1996). It is claimed that these different processes not only enhance the pharmacological efficacy but also attenuate the toxic side effects of the crude RA (Wu, Lu, Shun, Dong, & Ye, 1998). However, this processing drug with reduced toxicity is still not very safe to human body especially at an improper dosage.

In recent years, much attention has been focused on polysaccharides isolated from natural sources such as bacteria, fungi, algae and plants (Leung, Liu, Koon, & Fung, 2006; Sun, 2011). Their wide range of biological activities and a relatively low toxicity are

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the main reasons for the increase in interest toward this class of molecules (Paulsen, 2001; Tzianabos, 2000; Wasser, 2002). The antitumor activities of RA are mainly limited to its small molecules, and evaluated in an in vitro study. There are not sufficient evidences to support its efficacy and action mechanism. On the other hand, until now there is not any research published on the polysaccharide from RA and to evaluate its antitumor effect in vivo. In view of this, the present study was undertaken to purify the water-soluble polysaccharide from this plant and further elucidated its underlying mechanisms of the anti-tumor activities in vivo. In addition, the inhibition effect of RA polysaccharide on the tumor transplanted in BALB/c mice was also examined by magnetic resonance imaging (MRI). The results will make for better understanding of the anticancer efficacy of the plant and the active constituents in it, which would be beneficial to expand the resources of the medicinal plants.

2. Materials and methods

2.1. Materials

The crude RA (the rhizome of *P. pedatisecta* Schott) was purchased from a local drugstore of Xi'an city in China. Ovalbumin (OVA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), concanavalinA (ConA) and lipopolysaccharide (LPS), standard sugars, bovine serum albumin (BSA), dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DEAE-cellulose and Sephacryl S-400/HR were from Amersham (Sweden). Cyclophosphamide (CTX) was purchased from Jiangsu Hengrui Co. (Lianyungang, China). RPMI 1640 medium, penicillin and streptomycin (cell culture grade) were purchased from Gibco (Grand Island, NY, USA). Fetal calf serum (FCS) was provided by Hangzhou Sijiqing Corp. (Hangzhou China). All other chemicals were of grade AR.

2.2. Extraction and purification of polysaccharides

The dried of RA (1.0 kg), previously cut into small pieces, were soaked with 95% EtOH for 6 h to remove lipids, followed by through Whatmann filter paper (4 mm) and the filtrate centrifuged. The residue was dried in air and then extracted with boiling water for three times under stirring (6 h for each). The filtrate was concentrated, dialyzed, and centrifuged to remove insoluble material and small molecular compounds. By precipitation with 3 volumes of EtOH (12 h stirring and 24 h standing at 4 °C), a resulting precipitate was obtained following centrifugation and subsequent drying in vacuo at 45 °C. This precipitate dissolved in 200 mL distilled water was excessively mixed with organic working solvent (chloroform: n-butanol = 3:1) on a rotary-shaking apparatus according to the Sevag method (Staub, 1965). The protein-free solution was subjected to dialysis against the tap water for 24 h, and a further 24 h against distilled water again. After centrifugation, the supernatant was added with 3 volumes of 95% EtOH to precipitate crude polysaccharides. The precipitate was recovered by centrifugation and washed successively with absolute EtOH and acetone, followed by drying in vacuo at 45 °C, yielding RA crude polysaccharide (yield: 40.02 g, 4.0% of the starting material). RA crude polysaccharide (10 g) was subjected to a DEAE-cellulose column (2.5 cm × 50 cm) and eluted stepwise with distilled water, 0.2, 0.4, and 1.0 M NaCl. The eluate was monitored by the phenol–sulfuric acid method. The distilled water eluate was concentrated, dialyzed, and lyophilized. The resulting polysaccharide was purified on a Sephacryl S-400/HR column (2.6 cm × 80 cm) and eluted with 0.1 M NaCl. The yield rate of RA polysaccharide (RAP-W1) was 0.29% (0.734 g) for the starting material.

2.3. Molecular weight determination, monosaccharide composition and chemical properties

The molecular weight of the fractions was determined by gel-permeation chromatography, in combination with a high-performance liquid chromatography instrument (Angilent1100, USA). Sample (2.0 mg) was dissolved in distilled water (2 mL) and passed through a 0.45-lm filter, applied to a gel-filtration chromatographic column of TSK-G3000 PWXL, maintained at a temperature of 50 °C, eluted with 0.003 MNaOAc as the mobile phase at a flow rate of 0.5 mL/min and detected by a refractive index detector. The molecular weight was calculated by the calibration curve obtained by using various standard dextrans (50 KD, 25 KD, 12 KD, 5 KD, 1 KD) using linear regression.

The polysaccharides were hydrolyzed with 1 M TFA at 100 °C for 8 h, followed by evaporation to dryness and successive reduction with $NaBH_4$ and acetylation with Ac_2O–NaOAc at 120 °C for 1 h. The Ac_2O was destroyed with ice-water, and the resulting alditol acetates extracted with $CHCl_3$ (Whiton, Lau, Morgan, Gilbart, & Fox, 1985) and analyzed by gas chromatography (GC).

Total carbohydrate and protein of these polysaccharides were determined by the phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and (Bradford, 1976), respectively.

2.4. Cell lines

Human breast cancer cell line MCF-7 was obtained from Shanghai Institute of Cell Biology in the Chinese Academy of Sciences. MCF-7 cells were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2 incubator with saturated humidity.

2.5. Experimental animal and experimental design

BALB/c mice (5 weeks old) weighing 18–22 g was purchased from the Pharmacology Experimental Center of the Fourth Military Medical University and acclimatized for 1 week before use. Half of them were male and the others were female. Mice were randomized and housed in a cage and received standard mouse chow and water. At room temperature, a 12/12-h light–dark cycles were maintained. Animal experiments were performed according to the protocols of the Fourth Military Medical University.

MCF-7 cells growing exponentially were harvested and inoculated into the oxter of mice by subcutaneous injection at a density of 2×10^6 cells/mL. Soon after tumor cell injection, the mice were randomly assigned to the following five groups (15 per group): the negative control group (normal saline, for 14 days once daily, intragastrically); the RAP-W1 oral administration group (100 and 200 mg/kg, for 14 days once daily, intragastrically); the positive group (CTX, 40 mg/kg, for 2 day once daily, intraperitoneally); the normal control group (normal saline, for 14 days once daily, intragastrically). Treatment began when tumors were palpable and no less than 100 mm^3 in volume calculated from caliper measurements and was continued for 14 days.

Ten mice in each group were sacrificed and the weight of mice was measured on the last day of the experiment after sacrifice of animals and excision of the tumor mass and spleen. The tumor inhibition rate (%) was expressed according to the following formula: (the mean tumor weight of control group − the mean tumor weight of treated group)/the mean tumor weight of control group × 100. Relative spleen weight was expressed as the ratio of the spleen weight to body weight (mg/g).

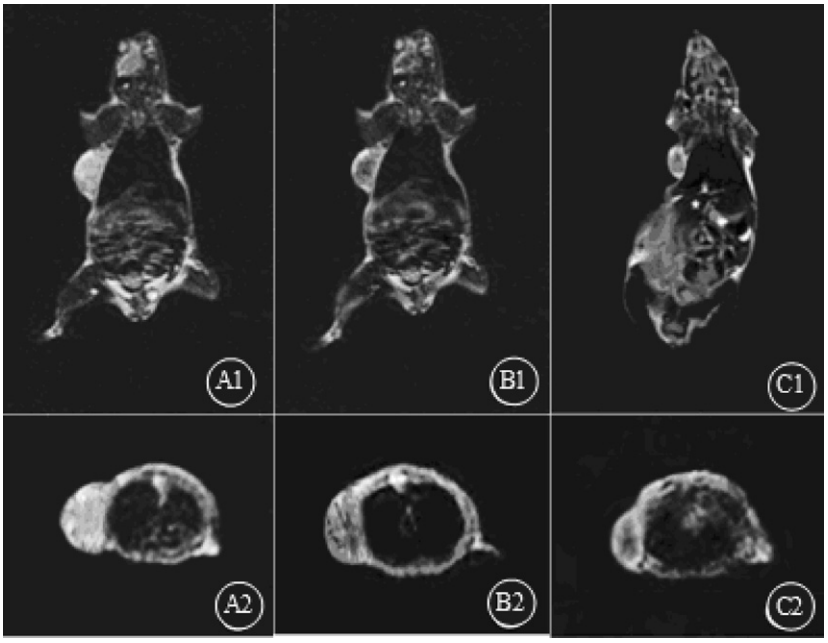

Fig. 1. MRI images for tumor mass in tumor-bearing mice. (A1 and A2) coronal and axial T1WI + C MRI images for model control; (B1 and B2) coronal and axial T1WI + C MRI image for RAP-W1 (200 mg/kg) treatment group; (C1 and C2) coronal and axial T1WI + C MRI image for CTX treatment group.

2.6. Magnetic resonance imaging (MRI) examination and histological analysis

The remaining 5 mice in each group were observed for MRI and histological analysis. The specimens from the excised tumors were rinsed with PBS, fixated with 4% formaldehyde in PBS, dehydrated with ethanol, embedded in paraffin blocks, sectioned (5 μm) and stained with hematoxylin–eosin for light microscopic analysis.

We used the SIEMENS Trio Tim 3.0T Magnetic Resonance Imaging system for MRI scanning with a knee coil. Chloral hydrate solution (6 mL/kg) was injected into the peritoneum of the mice for anesthesia, which were then fixed on the retaining plate in supine position and underwent head-first sequence scans in coronal T1WI, T2WI + FS and axial T1WI, T2WI + FS positions, as well as enhanced scan. After injection of contrast agent GD-DTPA (0.2–0.3 mL/kg) into the tail vein, the mice were administered with coronal and axial T1WI + FS sequence scans in the following conditions: thickness: 2 mm, interlamellar spacing: 0 mm, FOV: 10 × 10 mm, matrix: 256 × 160, NEX: 2; T1WI: TR/TE = 400 ms/26 ms, and T2WI: TR/TE = 3600 ms/80 ms.

2.7. Splenocyte proliferation assay

Spleen cells of mice obtained under aseptic conditions was minced using a pair of scissors and gently teasing the organ in RPMI-1640 medium and centrifuged at 2000 rpm for 10 min at room temperature. The red blood cells were removed by hemolytic Gey's solution. After centrifugation (380 × *g* at 4 °C for 10 min), the pelleted cells were washed three times in PBS and resuspended in RPMI 1640 complete medium. Cell numbers were counted with a haemocytometer by trypan blue dye exclusion technique. Cell viability exceeded 95%. Cell proliferation was assessed using MTT-based colorimetric assay as previously described (Sun & Liu, 2008). The cell concentration was adjusted to 4×10^6 cells/mL. The cell suspension (100 μL) was plated in a 96-well culture plate with concanavalin A (ConA, 5.0 μg/mL), lipopolysaccharide (LPS, 10.0 μg/mL) or RPMI1640 medium, giving a final volume of 200 μL. After incubation for 44 h at 37 °C in a humidified 5% CO_2 incubator, 50 μL MTT (2 mg/mL) was add to each well. The plate was further incubated for another 4 h. Then the plates were centrifuged (1400 × *g*, 5 min) and the untransformed MTT was carefully removed by pipetting. 100 μL of dimethylsulfoxide (DMSO) was added to dissolve formazan crystals. The absorbance of each well was read at 570 nm. The stimulation index (SI) was calculated based on the following formula: SI, the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

2.8. Assays of cytotoxic T lymphocyte (CTL) activity

The CTL activity was analyzed using MTT method as described above. Tumor cells and splenocytes were used as target cells and effector cells, respectively. The ratio of effector cells to target cells was 50:1. To determine the percentage of

Table 1
Effect of RAP-W1 from the rhizome of *Pinellia pedatisecta* Schott on body weight, tumor growth and spleen index in mice.

Group	Initial body weight (g)	Final body weight (g)	Final tumor weight (g)	Tumor growth inhibition (%)	Spleen index (mg/g)
The normal control	19.34 ± 0.53	25.05 ± 0.53	–		10.21 ± 0.31
The negative control	20.31 ± 0.45	25.10 ± 0.55	2.35 ± 0.32		7.34 ± 0.43
RAP-W1 (100 mg/kg)	18.96 ± 0.46	26.76 ± 0.61	1.55 ± 0.41[a]	34.0	9.45 ± 0.40[a]
RAP-W1 (200 mg/kg)	19.04 ± 0.36	27.42 ± 0.46	1.13 ± 0.34[b]	52.0	11.95 ± 0.73[b]
CTX (40 mg/kg)	19.11 ± 0.52	24.32 ± 0.530	0.83 ± 0.30[b]	64.7	3.61 ± 0.14[b]

Values are expressed as mean ± SD ($n = 10$).
[a] $P < 0.05$.
[b] $P < 0.01$ vs. the negative control.

Fig. 2. Histopathological examination of MCF-7 tumor tissue cell-xenografted mice treated with RAP-W1 or CTX. (A) The model control (100×); (B) the 200 mg/kg RAP-W1 group (100×); (C) the CTX group (100×).

target cells killed, the following equation was used: CTL activity (%) = (ODT − (ODS − ODE))/ODT × 100, where ODT, optical density value of target cells control, ODS, optical density value of test samples, ODE, optical density value of effector cells control.

2.9. Determination of Th1/Th2 cytokines in serum

Levels of serum INF-γ, IL-2 and IL-10 were analyzed using a commercially available ELISA kit (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions.

2.10. Statistical analysis

Statistical analysis was performed using SPSS, version 11.0 (SPSS, Chicago, IL, USA). The data were expressed as the mean ± SD, and significant differences were assessed using Student's *t* test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Preparation of RA polysaccharide and its chemical properties

The crude polysaccharide was extracted from RA by hot water and precipitation by 3 volumes of EtOH with a yield of 40.02 g, accounting for 4.0% of the starting materials. And then it was purified on a DEAE-cellulose column and eluted stepwise with distilled water, 0.2, 0.4, and 1.0 M NaCl, respectively. The fractions eluted with distilled water contained abundant total sugar and were pooled, dialyzed and were further fractionated on a Sephacryl S-400/HR column based on molecular mass, giving RAP-W1 (0.734 g, 0.29%). On high-performance gel-permeation chromatography (HPGPC), RAP-W1 showed a symmetrical peak (data not shown), indicating a homogenous polysaccharide. Its molecular weight was estimated to be 57 kDa according to the calibration curve. According to GC analysis, RAP-W1 was composed of rhamnose, fucose, arabinose, mannose, galactose and glucose, with molar ratios of 0.4:0.5:0.3:0.6:0.9:5.3. The results indicated that glucose was the predominant monosaccharide. Total carbohydrate content was determined to be 95.9% and no protein was observed with the absence of absorption in 280 nm and a negative response to Bradford test.

3.2. Effect of RAP-W1 on the growth of human breast cancer MCF-7 in mice

The growth-inhibitory effect of RAP-W1 on human breast cancer MCF-7 cells was evaluated in vivo by inoculating tumor cells to BALB/c mice. As seen in Table 1, RAP-W1 powerfully inhibited growth of MCF-7 tumors with the highest growth-inhibitory rate of 52.0% at the dose of 200 mg/kg and 34.0% at the dose of 100 mg/kg After 2 weeks of therapy. Also, the relative spleen weights of the RAP-W1-treated groups were much higher than those of the control groups. Consistent with the antitumor activity, the relative spleen weight increased to a maximum at the dose of 200 mg/kg. As expected, frequently used chemotherapy drug, cyclophosphamide (CTX), exhibited a high antitumor activity (64.7%) and simultaneously considerably decreased the body and spleen weight of tumor-bearing mice, indicating the strong side effect and immunological suppression to the body. However, the body weights of polysaccharide-treated groups increased moderately than that of the negative control group after the 14 day-experimental period. It was notable that the body and spleen weights of the mice treated with RAP-W1 were higher than those of the mice treated only with CTX, which implied that RAP-W1 would be a valuable immunoregulator for anticancer therapy.

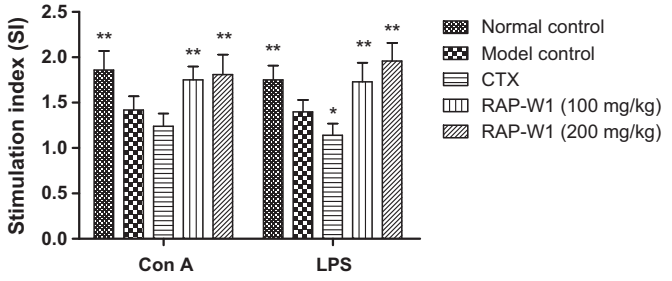

Fig. 3. Effect of RAP-W1 from the rhizome of *Pinellia pedatisecta* Schott on Con A- and LPS-stimulated splenocyte proliferation in mice. The values are presented as mean ± SD ($n = 10$). Significant differences compared to model control group (MC) are designated as $^{*}P < 0.05$ or $^{**}P < 0.01$.

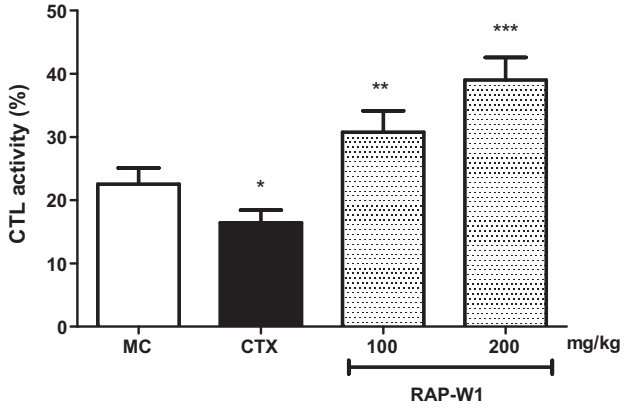

Fig. 4. Effect of RAP-W1 from the rhizome of *Pinellia pedatisecta* Schott on CTL activity in mice. The values are presented as mean ± SD ($n = 10$). Significant differences compared to model control group (MC) are designated as $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$.

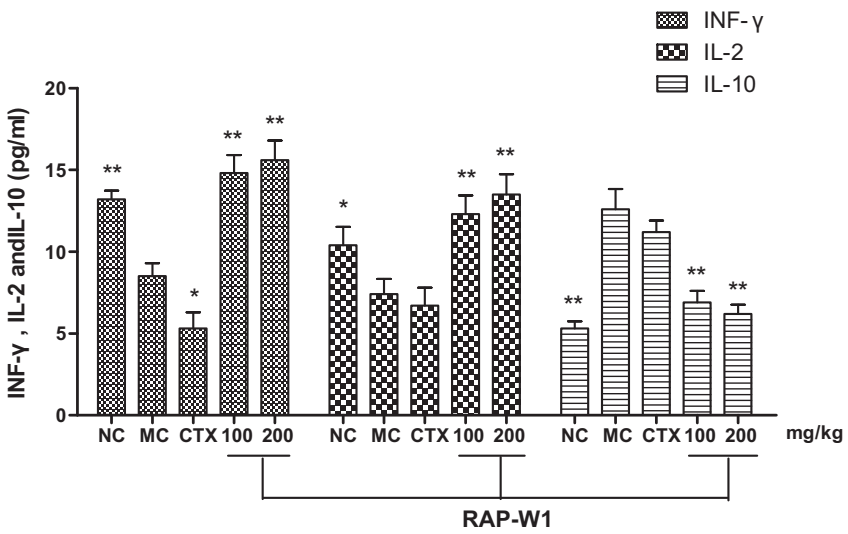

Fig. 5. Effect of RAP-W1 from the rhizome of *Pinellia pedatisecta* Schott on serum INF-γ, IL-2 and IL-10 production in mice. The values are presented as mean ± SD ($n = 10$). Significant differences compared to model control group (MC) are designated as $^{*}P < 0.05$, or $^{**}P < 0.01$.

3.3. MRI results

Apart from the healthy mice group, other groups of mice inoculated with breast cancer cells developed evident tumor lesions in axillary subcutaneous tissue, which showed moderate hypo-intensity in T1WI sequence, moderate hyper-intensity in T2WI + FS sequence and obvious homogeneous or heterogeneous enhancement in enhanced sequences, with boundary obscurity observed. For negative control group(drug-free), the tumor had the largest size with homogeneous intensity in plain and enhanced scans and no necrosis inside (Figs. 1(A1), (B1) and 2A); For the polysaccharide drugs (200 mg/kg) groups, the tumor shrunk slightly in size, with little necrosis inside and relatively heterogeneous enhancement (Figs. 1(A2), (B2) and 2B); For the cyclophosphamide (40 mg/kg) group, compared with results of the negative control one, the tumor shrunk significantly in size with obvious internal necrosis and manifested obvious heterogeneous enhancement (Figs. 1(A3), (B3) and 2C).

3.4. Effect of RAP-W1 on splenocyte proliferation

Lymphocytes are the key effecter cells of the mammalian immune system. Therefore the effect of RAP-W1 on mitogen-stimulated splenocyte proliferation in mice bearing human breast cancer MCF-7 xenografts was evaluated. In Fig. 3, ConA- and LPS-induced splenocyte proliferation in the tumor-bearing mice was significantly enhanced by RAP-W1 at two doses ($P < 0.01$). However, mitogen-stimulated splenocyte proliferations, especially LPS induced, in the CTX-treated group were significantly lower than those of the model control ($P < 0.05$). Moreover our experiment results also proved that immune function of model control mice was markedly lower than that of normal mice ($P < 0.01$).

3.5. Effect of RAP-W1 on CTL activity in mice

CTL, as major populations of cytotoxic lymphocytes (Kos & Engleman, 1996; Medzhitov & Janeway, 1997), are important in the defense against tumors and viruses (Boon, Cerottini, Van den Eynde, van der Bruggen, & Van Pel, 1994; Moretta, Bottino, Cantoni, Mingari, & Moretta, 2001), which are able to kill autologous cells infected with intracellular pathogens, as well as tumor cells. There is hence a need to measure the CTL activity against tumor cell and the results were shown here. As shown in Fig. 4, CTL activity in tumor-bearing mice was markedly decreased by CTX compared with model control group ($P < 0.05$). However, CTL activity in tumor-bearing mice treated with ARP-W1 (100 and 200 mg/kg) was significantly higher than that of model control group ($P < 0.01$ or $P < 0.001$).

3.6. Effects of RAP-W1 on the production of Th1/Th2 serum cytokines in mice

Since cytokines play a pivotal role in the development of immune response, we investigated the effect of RAP-W1 on serum INF-γ, IL-2 and IL-10 production in mice by ELISA. As shown in Fig. 5, in tumor-bearing mice, the level of Th2 cytokine, IL-10, were significantly higher than those of normal mice ($P < 0.01$), whereas the expression of Th1 cytokines, INF-γ and IL-2, were remarkably lower than that of normal mice ($P < 0.05$ or $P < 0.01$). After RAP-W1 treatment to tumor-bearing mice, the serum level of INF-γ ($P < 0.01$) and IL-2 ($P < 0.01$) was increased and the serum level of IL-10 ($P < 0.01$) was decreased. In addition, CTX treatment led to a reduction in the production of INF-γ and IL-2 and an increment in IL-10 secretion compared with model control ($P < 0.001$). All the data implied that RAP-W1 can activate T cells by up-regulating Th-1 response and that Th-1 cells might be the main target cells of the RAP-W1.

4. Conclusion

Many chemotherapeutic agents against cancer are immunosuppressive agents. Because they kill many immune cells in addition to tumor cells, the discovery and identification of new antitumor drugs capable of potentiating immune function has become an important research objective in immunopharmacology and oncotherapy (Jiao, Jiang, Zhang, & Wu, 2010). A recent study showed that 45% of breast cancer patients used complementary and alternative medicine (CAM), including the consumption of dietary supplements utilization (Baum, Ernst, Lejeune, & Horneber, 2006; Pasanisi et al., 2008). In this respect, the polysaccharides and polysaccharide–protein complexes from natural plants should be ideal because they are nontoxic and their anti-cancer effects are mainly host mediated.

In present study we purified and preliminarily characterized a water soluble polysaccharide from the rhizome of *P. pedatisecta* Schott and demonstrated antitumor activity of RAP-W1 against human breast cancer MCF-7 in mice. RAP-W1 contained

total carbohydrate content of 95.9% and no protein. Its molecular weight was estimated to be 57 kDa determined by HPGPC. According to GC analysis, RAP-W1 was composed of rhamnose, fucose, arabinose, mannose, galactose and glucose, with molar ratios of 0.4:0.5:0.3:0.6:0.9:5.3. The anti-tumor and immunostimulatory activities of RAP-W1 have been demonstrated in mice models. RAP-W1 could significantly inhibit the growth of tumor transplanted in mice compared with model controls. Simultaneously the body weight and spleen index was increased by RAP-W1. What's more, RAP-W1 significantly stimulated splenocyte proliferation in tumor-bearing mice, suggesting immunomodulation might be the mechanism of the anti-tumor activity of RAP-W1. Therefore, we further investigated the effect of this polysaccharide on the cellular and humoral immunity in tumor-bearing mice to analyze the underlying mechanism of their antitumor activity.

Protective immunity against tumors comprises both cellular and humoral immunity. These immune systems play an important role in the elimination of both locally growing and circulating tumor cells and thus result in the inhibition of both growth and metastasis of tumors. The capacity to elicit an effective T- and B-cell immunity can be shown by the stimulation of lymphocyte proliferation response (Marciani et al., 2000). It is generally known that Con A stimulates T-cells and LPS stimulates B-cell proliferation. Among the T lymphocytes, helper T cells induce B lymphocytes to secrete antibodies. Helper T cells can be divided into two subsets of effector cells, namely Th1 and Th2 cells. The Th1 cells secrete cytokines, such as IL-2, TNF-β and IFN-γ. The Th2 subset produces cytokines, such as IL-4, IL-5 and IL-10. Th1 cytokines IL-2 and IFN-γ are immunostimulatory and capable of limiting tumor growth. Conversely, the Th2 cytokines IL-4 and IL-10 are immunoinhibitory and thus capable of stimulating tumor growth (Clerici et al., 1997). The Th1 response against intracellular pathogens and malignant cells is superior to the Th2 response. Moreover, Th1 immune response is a requisite for cytotoxic T lymphocyte (CTL) production. As we have shown, RAP-W1 enhances lymphocyte proliferation and CTL activity, as well as stimulates the production of Th1 cytokines (INF-γ and IL-2) and reduction of Th2 cytokines (IL-10) in vivo. All the data implied that RAP-W1 can activate T cells by up-regulating Th1/Th2 cytokine ratio in serum in the tumor bearing mice, which might partially cause the inhibition of tumor growth. Therefore, it is reasonable to deduce that RAP-W1 inhibits MCF-7 tumors in mice in part through activating the Th1 immune response system. Nevertheless, our experimental results support the conclusion that RAP-W1 has antitumor activity. The structural domains or features related to the activities and the mechanism by which this complex induces these effects needs to be further studied.

Acknowledgments

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