Carbohydrate Polymers 137 (2016) 52–58

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Effects of *Marsdenia tenacissima* polysaccharide on the immune regulation and tumor growth in H_{22} tumor-bearing mice

Shuang Jiang[a,*,1], Limin Qiu[b,1], Yiquan Li[c], Lu Li[a], Xingyun Wang[a], Zhi Liu[a], Yan Guo[a,*], Haotian Wang[d,*]

[a] College of Basic Medical Sciences, Changchun University of Chinese Medicine, Changchun 130117, Jilin, PR China
[b] Qianwei Hospital, Changchun 130021, Jilin, PR China
[c] Animal Science and Technology College, Jilin Agricultural University, Changchun 130117, Jilin, PR China
[d] School of Pharmaceutical Sciences, Jilin University, Changchun 130021, Jilin, PR China

ARTICLE INFO

Article history:
Received 29 August 2015
Received in revised form 5 October 2015
Accepted 15 October 2015
Available online 17 October 2015

Keywords:
Marsdenia tenacissima polysaccharide
Immunity
Antitumor
Hepatic carcinoma
Antioxidant

ABSTRACT

One water-soluble polysaccharide (*Marsdenia tenacissima* polysaccharide, MTP), with an average molecular weight of 4.9×10^4 Da, was isolated from the dried rattan of *M. tenacissima*. MTP contained 93.8% carbohydrates, 5.6% proteins and 21.3% uronic acid, and were composed of arabinose, mannose, galactose, xylose, glucuronic acid at a molar ratio of 9.1, 17.7, 30.2, 22.4 and 20.6. The experiments on the animals showed that MTP could increase the serum hemolysin, promote the formation of antibody-forming cells and improve the phagocytosis of mononuclear macrophage in normal mice. Meanwhile, MTP could also inhibit the growth of tumor in H_{22} tumor-bearing mice dose-dependently, and increase the spleen index, thymus index and serum albumin level in the mice. In addition, MTP could elevate the serum level of TNF-α and IL-2, increase the activity of GSH-Px, CAT and SOD in the liver tissue, and reduce the content of VEGF and MDA. These results suggest that MTP can regulate the immune function in mice and suppress the growth of tumor in H_{22} tumor-bearing mice, and its antitumor activity may be related to its antioxidant and immunomodulatory effects.

1. Introduction

The incidence of primary hepatic carcinoma is only second to that of gastric cancer and esophageal cancer, ranking the third pace in all tumors, and one of the most common malignant tumors in China (Wang, Chen, Zhang, Tang, & Wu, 2015). The incidence of liver cancer in China ranks first in the world, the number of patients that die of liver cancer accounts for about 50% of that worldwide, and its incidence rate is about 10 times of that in European countries (Fan et al., 2013). The surgical resection of liver cancer is the best way to cure it, but liver cancer is almost found at its advanced stage, often accompanied by hepatic cirrhosis, so that it is difficult to remove it by the surgical resection and it is easy for it to relapse after the surgery (Cho, Han, Yoon, Choi, & Lee, 2014; Ni et al., 2013a,b). Studies have reported that after the radical surgery of liver cancer, there are still its metastasis and recurrence in 60–70% of the patients within 5 years (Zhou et al., 2015). Drugs commonly used for the treatment of liver cancer currently are fluorouracil and doxorubicin, etc., but they are not satisfactory since they can produce severe side effects, such as bone marrow suppression and gastrointestinal reactions, with a too low effective rate and without significant effect of prolonging survival especially for the treatment of patients with liver cancer not suitable for the surgical resection (Nouso et al., 2013; Song et al., 2015). Traditional Chinese medicine has a long history in the treatment of cancers, characterized by a low cost and less or smaller side effects, so that the development of active ingredients with antitumor effects from traditional Chinese medicine has become a hot research on the treatment of liver cancer (Li, Bao, Li, Zhang, & Shen, 2012; Zeng et al., 2013).

Marsdenia tenacissima (MT) is the climbing stem of *M. tenacissima* (Roxb.) Wight et Arn., a member of Asclepiadaceae herbs, primarily produced in Yunnan and Guizhou. MT, with bitterness in taste and slight cold, has functions such as clearing away heat and toxic material, relieving cough and asthma, and anti-inflammation and analgesia. Modern pharmacological studies have shown that MT has significant antitumor, immunomodulatory, hepatoprotective and diuretic effect (Han, Zhao, Zhou, Zhou, & Li, 2014; Huang et al., 2013; Zhu et al., 2014), and a definite effect on various tumor

* Corresponding authors at: Changchun University of Chinese Medicine, College of Basic Medical Sciences, Changchun 130117, Jilin, China. Tel.: +86 431 81967733; fax: +86 431 81967711.
E-mail addresses: jiangshuang_2000@163.com (S. Jiang), wht@jlu.edu.cn (H. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.carbpol.2015.10.056
0144-8617/

including esophageal cancer and lung cancer (Fan et al., 2015; Han et al., 2015). MT contain steroidal glycosides, polysaccharides, alkaloids, organic acids, resin and pigment, and other chemical components (Pang et al., 2015; Yao et al., 2014), of which steroidal glycosides are considered the main active substances with antitumor effect (Wang, Lai, Tian, & Yang, 2006; Zhang et al., 2010). Since the research on the activity of MT polysaccharides is still less to our best knowledge, and the antitumor effect of a variety of plant polysaccharides have been confirmed (Wu et al., 2012; Xiang et al., 2014), a polysaccharide with a uniform molecular weight (MTP) from MT was obtained by extraction, separation and purification, and its effects on immune regulation and H_{22} tumor-bearing mice were studied in this study, in order to lay the foundation for the research and development of MT polysaccharides.

2. Materials and methods

2.1. Materials

MT was purchased from a local medicine market and identified by Prof. Guangxin Yuan (College of Pharmacy, Beihua University) according to the identification standards of Pharmacopeia of the People's Republic of China; glucose, arabinose, mannose, galactose, xylose and glucuronic acid (Amresco Inc., OH, USA); T-series dextran (Sigma Chemical Co., MO, USA); DEAE Sepharose Fast Flow and Sepharose 6 Fast Flow (GE Healthcare Bio-Sciences, NJ, USA); detecting kits of vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) (Nanjing Jiancheng Biotechnology Co., Jiangsu, China). All the other chemicals used were of analytical grade.

2.2. Isolation and purification of the polysaccharide

The dried rattan of MT was extracted two times with 95% ethanol at 75 °C for 3 h under reflux to remove lipid, and the supernatant was removed. The residue was then extracted three times with eight volumes of distilled water at 100 °C for 2 h. After the centrifugation, the supernatant was concentrated, and precipitated with six times of 95% ethanol at 4 °C for 24 h. The precipitate collected by centrifugation was deproteinated by the Sevag method (Staub, 1965), and then exhaustively dialyzed with water for 48 h. The dialyzate was concentrated and precipitated with four volumes of 95% (v/v) ethanol at 4 °C for 24 h to precipitate the polysaccharides. The precipitate was washed with absolute ethanol, acetone and ether, respectively. Finally, the precipitate was suspended in water and lyophilized to yield crude polysaccharide, named as CMTPs.

The CMTPs was dissolved in distilled water, centrifuged, and then the supernatant was applied to a column of DEAE Sepharose Fast Flow equilibrated with 1.0% NaCl. After loading with sample, the column was eluted with continuous gradient concentrations of NaCl aqueous solution (0.1 → 1.5 M) stepwise at 2 mL/min. The eluted solution was collected and monitored with the phenol–sulfuric acid method assay at 490 nm, and then a main fraction was obtained. The fraction was purified by gel-permeation chromatography on a Sepharose 6 Fast Flow column (90 × 2 cm) with 0.15 M NaCl at a flow rate of 2 mL/min. One main fraction was collected, deproteinized, dialyzed and precipitated with ethanol to obtain a purified polysaccharide, named MTP.

2.3. Analysis of polysaccharide, protein and uronic acid contents

Total carbohydrate content in MTP was determined by phenol–sulfuric acid colorimetric method using glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The concentration of total protein was estimated by the folin–phenol method using BSA as standard (Lowry, Rosebrough, Farr, & Randall, 1951). The content of total uronic acid was measured by *m*-hydroxydiphenyl method (Filisetti-Cozzi & Carpita, 1991).

2.4. Analysis of monosaccharide compositions

The identification and the quantification of monosaccharides in MTP were achieved by gas chromatography (GC) analysis. MTP was hydrolyzed with 2 M TFA at 100 °C for 2 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 acetate was extracted with $CHCl_3$ and analyzed by GC. The following neutral monosaccharides were used as references: rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose.

GC was performed on a Shimadzu GC-14C instrument (Shimadzu, Japan) equipped with a DM-2330 capillary column (30 m × 0.32 mm × 0.20 μm) and flame-ionization detector (FID). The column temperature was kept at 120 °C for 2 min, and increased to 250 °C (maintained for 3 min) at a rate of 8 °C/min. The injector and detector heater temperature were 250 °C and 300 °C, respectively. The rate of N_2 carrier gas was 10 mL/min.

2.5. Molecular weight determination

The molecular weight of MTP was determined by high-performance size-exclusion chromatography (HPSEC), which was performed on a Shimadzu HPLC system fitted with one TSK-G3000PWXL column (7.8 mm ID × 30.0 cm) and a Shimadzu RID-10A detector. The mobile phase was 0.7% Na_2SO_4, and the flow rate was 0.5 mL/min. The sample was dissolved in the mobile phase and centrifuged, and 20 μL of the supernatant was injected in each run. Dextran standards with different molecular weights (T-2000, T-70, T-40, T-20, and T-10) were used to calibrate the column and establish a standard curve.

2.6. Experiments on the generation of serum hemolysin and antibody-forming cells

50 mice were randomly divided into 5 groups, namely, control group, low-, medium- and high-dose MTP (50, 100, and 200 mg/kg) groups, and lentinan (50 mg/kg) group. All the mice were administered the corresponding agents intragastrically (i.g) one time daily, in which the mice in the control group were given the same volume of saline. On the 7th day after the administration, mice in each group were given 0.2 mL of 20% SRBC in intraperitoneal injection for the sensitization of them; 7 d later, the same dose of SRBC was given to the mice again for the immunity for 12 d; at 2 h after the last administration, the mice were sacrificed and their eyeballs were removed for the collection of blood samples, the blood samples were left to stand for 1 h, and then centrifuged. The upper layer of serum was diluted with normal saline to a certain fold. 1 mL of the diluted serum was add 0.5 mL of 10% SRBC and 1 mL of a complement (the Guinea pig serum diluted with normal saline at a ration of 1:10), which was incubated at 37 °C in warm bath for 10 min, then placed into ice bath to stop the reaction, and centrifuged at 2000 rpm for 10 min. The supernatant was detected at 540 nm wavelength with colorimetry for the calculation of half value of hemolysin (HC_{50}) in the serum.

Two spleens of each mouse were homogenated together. The spleen cell suspension was prepared with cold Gey's solution at a concentration of 15 mg spleen tissue/mL, 0.5 mL of 0.2% SRBC and 0.5 mL of 1:10 Guinea pig serum were added to 0.5 mL of the suspension, which was mixed evenly, then incubated at 37 °C in