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Effects of Bush Sophora Root polysaccharide and its sulfate on immuno-enhancing of the therapeutic DVH

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ABSTRACT

Bush Sophora Root polysaccharide (BSRPS) and its sulfate, sulfated Bush Sophora Root polysaccharide (sBSRPS), possess the antiviral activities against duck hepatitis A virus. However their antiviral mechanisms are still not clear. This paper reported their immuno-enhancing roles in the therapeutic effects for duck virus hepatitis (DVH). The effects of BSRPS and sBSRPS on stimulating lymphocyte proliferation were investigated by MTT methods. After that, ducklings were challenged with DHAV and treated with BSRPS and sBSRPS. Meanwhile, the total antibody (Ab), cytokines including interferon gamma (IFN-γ), hepatocyte growth factor (HGF), interleukin (IL)-2, IL-6 and IL-8 were determined by enzyme-linked immuno sorbent assay methods. The results showed that BSRPS owned a fine hepatoprotective effect with stable HGF producing ability. Sulfated modification was able to increase the proliferation rates of B and T lymphocytes and the secretions of total Ab, IFN-γ and IL-2, as comparison with those of BSRPS group. In summary, both of them exhibited immuno-enhancing effects on the therapeutic effects for DVH, and the capacity of sBSRPS was stronger than that of BSRPS.

1. Introduction

Polysaccharides composed of ten or more monosaccharides are distributed ubiquitously in the natural environment. The earliest study of biological polysaccharides can traced back to the antitumor activity research about bacterial polysaccharide in the 1930s [1]. In recent years, it is reported that many kinds of polysaccharides extracted from traditional Chinese herbal medicine play a part in antiviral [2] and immuno-enhancing [3] activities. And sulfated modification which can improve such antiviral [4] and immuno-enhancing [5] activities is a common modified method. To date, biological polysaccharides have been extensively applied in clinic because of their high biological activity and low toxicity.

Immuno-enhancing is an important way for individual to resist against virosis. Antibodies (Ab) secreted by B cells can neutralize viruses. Interferon gama (IFN-γ) pruduced by helper T (Th) or natural killer (NK) cells can remove virus indirectly via immuno-enhancing effect. It is reported that accessory cells are critical for the production of IFN-γ from NK cells and the regulation of immunity during hepatitis C virus infection [6]. Interleukin (IL)-2 and other cytokines are also closely related to virus cleaning [7,8].

It is reported that Bush Sophora Root polysaccharide (BSRPS) can stimulate the proliferation of murine splenic lymphocytes at the concentrations of 50, 100, 200 or 400 mg/L in vitro [9]. The analysis of performance liquid chromatography and gas chromatograph indicates that BSRPS is only comprised of D-glucose. Its molecular weight is 2.24×10^4 and the specific rotation $[\alpha]_D^{20} = +68^\circ$ (C 0.75, H_2O). According to the oxidation of perchloric acid, Smith degradation, methylation analysis, infrared spectroscopy assay, ^1H and ^{13}C NMR analysis, the results showed that the main chain of polysaccharide was (1 → 4) linked α-D-glucan which was attached two glucosyl side chains at 3-O and 6-O of the glucosyl residues in every 12 repeating unite of the main chain. The structural formula of BSRPS was showed in Fig. 1 [9].

Sulfated Bush Sophora Root polysaccharide (sBSRPS) which has an ability of inhibiting hepatitis virus in vitro [10] is the sulfated product of BSRPS. The infrared spectroscopy assay of BSRPS and

Abbreviations: Ab, antibodies; IFN-γ, interferon gamma; Th, helper T; NK, natural killer; IL, interleukin; BSRPS, Bush Sophora Root polysaccharide; sBSRPS, sulfated Bush Sophora Root polysaccharide; DVH, duck virus hepatitis; DHAV, duck hepatitis A virus; HGF, hepatocyte growth factor; DMEM, dulbecco's modified eagle medium; DEHs, duck embryonic hepatocytes; MM, maintenance medium; D-Hank's, dulbecco's Hanks balanced salt solution; PHA, phytohemagglutinin; LPS, lipopolysaccharide; CC, cell control; VC, virus control; BC, blank control.

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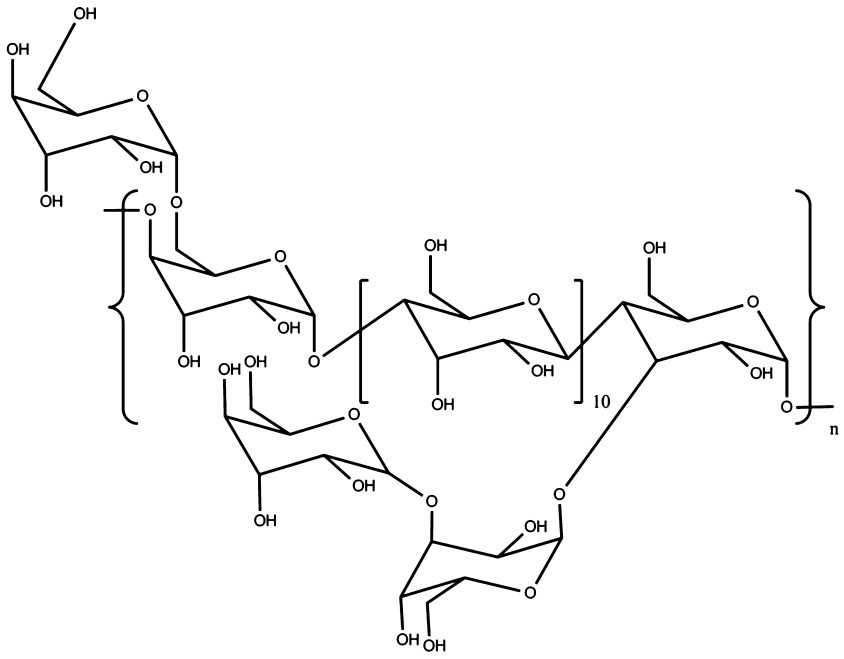

Fig. 1. The structural formula of BSRPS.

sBSRPS shows that two new absorption peaks are exhibited at 1248 cm^{-1} and 814 cm^{-1} in sBSRPS. These two specific absorption peaks represent the S=O asymmetry stretching vibration and C—O—S symmetry stretching vibration, respectively. And it proves the sulfuric acid group has been added into BSRPS in our previous study [11].

We ever studied the anti-DHAV abilities of BSRPS and sBSPRS as well as their antiviral mechanism in vitro [11]; and the results showed that their antiviral mechanisms were related to the inhibition of virus replication and release, what was more, the antiviral ability of sBSRPS was stronger than that of BSRPS. Then we researched their anti-oxidative abilities on the therapeutic duck virus hepatitis (DVH) caused by DHAV in vivo [12], which indicated the anti-oxidative roles of BSRPS and sBSRPS were important to their curative effects, the roles of them were similar but the anti-DVH ability of sBSRPS was better. In order to study the detailed anti-DVH mechanisms of BSRPS and sBSRPS in addition to their difference, we focused on exploring their immunologic mechanisms in this paper.

As yet, the roles of immuno-regulation in antiviral activities of BSRPS and its sulfate have still not been studied. As a series report of the anti-DHAV activities of BSRPS and sBSRPS, the purpose of the present study was to analyze their immuno-enhancing effects. The BSRPS was modified by the chlorosulfonic acid-pyridine method. The B and T lymphocyte proliferation rates were determined. The DHAV content, total duck-DHAV-Ab, duck-IFN-γ, duck-hepatocyte growth factor (HGF), duck-IL-2, duck-IL-6 and duck-IL-8 in serum were monitored.

2. Materials and methods

2.1. Reagents and virus

Dulbecco's modified eagle medium (DMEM) (Gibco) supplemented with penicillin 100 IU/mL, streptomycin 100 IU/mL, glutamine 0.75 mg/mL and 10% fetal bovine serum, was used for culturing the duck embryonic hepatocytes (DEHs); 1% fetal bovine serum, maintenance medium (MM) for diluting BSRPS or sBSRPS and maintaining DEHs. RPMI-1640 (Gibco) supplemented with penicillin 100 IU/mL, streptomycin 100 IU/mL and 10% fetal bovine serum, was used for diluting BSRPS or sBSRPS, re-suspending the lymphocytes and culturing the lymphocytes. Dulbecco's Hanks balanced salt solution (D-Hank's) was used for washing the embryo tissue fragments, DEHs and lymphocytes. The pH of D-Hank's, DMEM, MM and RPMI-1640 were adjusted to 7.4 by using 5.6% $NaHCO_3$. Trypsin (Amresco) was dissolved into 0.20% with D-Hank's. Phytohemagglutinin (PHA, Sigma), as the T-cell mitogen, and lipopolysaccharide (LPS, Sigma), as the B-cell mitogen, were dissolved into 100 μg/mL with RPMI-1640, respectively. These reagents were filtered through 0.22 μm syringe filters. DMEM, MM, RPMI-1640 and D-Hank's were stored at 4 °C and MTT solution was stored at 4 °C in dark bottles. Trypsin, PHA and LPS were stored at −20 °C.

Pyridine (Lot no. 20130220) and N,N-dimethylformamide (Lot no. 20130202) were bought from Sinopharm Group Chemical Company. Chlorsulfonic acid (Lot no. 130622) was the product of Shanghai Ling Feng Chemical Company. Lymphocyte separation medium (G20111118) was bought from Tianjin Hao Yang biological manufacture Company. Heparin sodium was dissolved into 2 mg/mL with physiological saline.

RNAiso Plus Reagent (Lot no. 9182), PrimeScript™ RT Master Mix Kit (Lot no. AK4202) and SYBR® Premix Ex Taq™ (Tli RNaseH Plus) Kit (Lot no. AK3802) were the products of Takara. Other chemicals used in the experiment were analytical grade.

DHAV (LQ_2 strain) was supplied by the Shandong Institute of Poultry in China.

2.2. BSRPS and sBSRPS

BSRPS was extracted and purified in our laboratory; sBSRPS was prepared by the chlorosulfonic acid-pyridine method [11].

A mean of methanol-reflux was utilized to edulcorate the Bush Sophora Root powder. The residue was then decocted three times for 8 h per time. And the supernatant was extracted by concentration and centrifugation. Three times volume of the absolute ethyl alcohol was added into the subsequent supernatant subsequently. Afterwards, Sevag method was used to eliminate protein. And the product was dialysed for 3 days. It was precipitated with three