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Bush Sophora Root polysaccharide and its sulfate can scavenge free radicals resulted from duck virus hepatitis

Yun Chen[a], Wen Xiong[a], Ling Zeng[a], Yu Wang[b], Shuaibing Zhang[a], Meiyun Xu[a], Meiyun Song[a], Yixuan Wang[a], Hongxu Du[a], Jiaguo Liu[a,*], Deyun Wang[a], Yi Wu[a], Yuanliang Hu[a]

[a] Institute of Traditional Chinese Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China
[b] BGI-Shenzhen, Shenzhen 518083, PR China

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ABSTRACT

In order to study the antioxidant effect of Bush Sophora Root polysaccharide (BSRPS) and its sulfate on anti-duck virus hepatitis (DVH), sulfated Bush Sophora Root polysaccharide (sBSRPS) was prepared by chlorosulfonic acid–pyridine method. Ducklings were fed with BSRPS and sBSRPS after challenged DHAV. Death was monitored, evaluation indexes of peroxidative and hepatic injury at the initial (4th and 8th hour) and later (54th hour) stages were detected. The results showed a fine treatment effect of BSRPS and sBSRPS. Visual hepatic pathological injury severities were less serious after the treatment. At the initial stage, free radical levels in all groups were the same, and BSRPS and sBSRPS reduced the hepatic injury through inhibiting virus replication. At the later stage, mass free radicals were detected in VC group while free radical levels in BSRPS and sBSRPS groups were significantly lower than VC group. The antioxidant effect of BSRPS and sBSRPS might alleviate the hepatic injury.

1. Introduction

Duck hepatitis A virus (DHAV) is a kind of hepatotropic virus infecting ducklings aged less than three weeks. DHAV can induce acute death and high mortality rate of ducklings and cause serious hepatic injury [1]. Oxidative stress plays a fatal role in the pathogenesis of viral hepatitis [2,3]. Larrea et al. discovered that hepatitis C virus can result in the production in free radicals of hepatocyte and the rise of malondialdehyde (MDA) [4]. Antioxidant supplementation may be of benefit to the treatment of hepatitis B virus [5]. Barraud et al. reported the peroxidative injury of duck virus hepatitis (DVH) caused by duck hepatitis B virus and aflatoxin B1 [6]. But no study has reported the changes of free radicals of DVH caused by DHAV so far.

Peterhans confirmed the rise of free radicals in splenocyte when infected by RNA virus [7]. Virus inhibits the activity of antioxidant including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), promotes the release of nitric oxide (NO), and destroys the balance of free radicals [8]. The imbalance would cause serious injury of the organism. Free radicals include reactive oxygen species (ROS), reactive nitrogen species (RNS), etc., in vivo. ROS would attack unsaturated fatty acids and induce lipid peroxidation. MDA is the main product of lipid peroxidation which is a typical chain reaction of free radicals. Adduct formed by MDA with DNA or protein brings about cytotoxicity and mutation [9]. Of cause, cell membranes are the target of attack. Numerous studies demonstrated that ROS would result in inactivation of protein [10,11] and injury of the DNA [12,13]. NO (primary RNS) would cause protein nitrification which results in changes of the structure and function [14–16], and also lead to the nitrated injury of DNA. NO is the main catalytic synthesized by nitric oxide synthase (NOS) widely distributed in the body. Inducible nitric oxide synthase (iNOS) is a type of NOS which synthesizes much more NO than any other types whose expression is regulated by many induction factors [17].

Toxic heat is the crux of DVH. Bush Sophora Root is a well-known traditional herbal medicine in China applied to clear away the heat-evil and detoxify [18]. Bush Sophora Root polysaccharide (BSRPS), a natural polysaccharide with a mean molecular weight

Abbreviations: DHAV, duck hepatitis A virus; MDA, malondialdehyde; DVH, duck virus hepatitis; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; NO, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; BSRPS, Bush Sophora Root polysaccharide; sBSRPS, sulfated Bush Sophora Root polysaccharide; CAT, catalase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TP, total protein; ALB, albumin; GLO, globulin; VC, virus control; BC, blank control.

* Corresponding author. Tel.: +86 25 84395203; fax: +86 25 84398669.
E-mail address: liujiaguo@njau.edu.cn (J. Liu).

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of 2.24×10^4 composed of fucose, rhamnose, arabinose, xylose, mannose, galactose and glucose, is the most important active ingredient of Bush Sophora Root. Researches proved that BSRPS could resistant to oxidation effectively [19,20], enhance the activities of SOD and GSH-Px, and reduce the level of MDA on BALB/c inbred mice (8 weeks old, 18–20 g). Sulfated Bush Sophora Root polysaccharide (sBSRPS) shows fine inhibition of hepatitis virus in vitro [21]. And in our previous study, BSRPS and sBSRPS also showed fine inhibition of DHAV both in vitro and in vivo [22]. The DHAV inhibitory rates of BSRPS and sBSRPS were 53.38% and 58.25% on duck embryonic hepatocytes, respectively. BSRPS and sBSRPS also had significant treatment effect of DVH on ducklings. In the present study, we monitored the changes of evaluation indexes of peroxidative injury including SOD, catalase (CAT), GSH-Px, MDA and iNOS and evaluation indexes of hepatic injury including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein (TP), albumin (ALB) and globulin (GLO) in DVH ducklings challenged by DHAV. The aim of this experiment is to analyze the correlation between oxidative stress and hepatic injury, to observe the protection of BSRPS and its sulfate against free radicals caused by DVH, and to investigate the importance of antioxidant effect in the treatment with BSRPS and sBSRPS.

2. Materials and methods

2.1. Reagents and virus

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. Heparin sodium was dissolved into 2 mg/mL with physiological saline.

Duck SOD ELISA kit (Lot no. 201310), GSH-Px ELISA kit (Lot no. 201310), CAT ELISA kit (Lot no. 201310), MDA ELISA kit (Lot no. 201310) and iNOS ELISA kit (Lot no. 201310) were bought from BioCalvin, China.

Alanine aminotransferase kit (Lot no. 130828), aspartate aminotransferase kit (Lot no. 130531), alkaline phosphatase kit (Lot no. 130425), lactate dehydrogenase kit (Lot no. 130917), total protein kit (Lot no. 131031) and albumin kit (Lot no. 130422) were the products of AusBio Laboratories Co., Ltd.

DHAV (LQ_2D_7 strain) for challenge experiment was supplied by the Shandong Institute of Poultry in China.

2.2. BSRPS and sBSRPS

BSRPS and sBSRPS were prepared and the structures were identified according to the methods described previously [22]. Briefly, BSRPS was extracted by water decoction and ethanol precipitation [23], purified through eliminating protein by Sevage method and column chromatography of Sephadex G-200 (2 cm × 100 cm) [24]. 4 mL chlorosulfonic acid was added mixed with 20 mL pyridine in an ice bath, with stirring. BSRPS (500 mg) dispersed in *N*,*N*-dimethylformamide (0.5 mL) was added to the mixtures then and stirred in a water bath at 95 °C for 2 h. The result solution was subsequently neutralized with NaOH, dialyzed, purified, and lyophilized to yield sBSRPS. The polysaccharide content of BSRPS was 83.77% determined by phenol-sulfuric acid method [25]. The content of sBSRPS was 89.18% calculated with the sum of its polysaccharide content determined by phenol-sulfuric acid method and sulfur content determined by barium chloride-gelatin method [26]. KBr pellets method was used to analyze the structure of BSRPS and sBSRPS [24]. The FT-IR spectrum of BSRPS and sBSRPS in a wave number range of 4000–400 cm^{-1} was recorded by a Nicolet 200 Magna-IR spectrometer (Nicolet Instrument Corp.). Specific absorption peaks of polysaccharides (3600–3200 cm^{-1}, 1400 cm^{-1}, 3020–2820 cm^{-1}, 1200–950 cm^{-1} and 1620 cm^{-1} and 1420 cm^{-1}) were found in both BSRPS and sBSRPS. And absorption peaks of sulfate (1248 cm^{-1} and 814 cm^{-1}) were found in sBSRPS.

2.3. Animals grouping and treatment

A total of 240 four-day-old cherry valley ducks (Purchased from Tangquan Poultry Farm, Jiangsu province, China) were randomly divided into four groups: BSRPS group (treated with BSRPS), sBSRPS group (treated with sBSRPS), virus control (VC) group and blank control (BC) group (separately reared). Ducklings of BSRPS, sBSRPS and VC groups were intramuscularly injected DHAV 0.2 mL per feather. The ducklings of BSRPS group was treated with aqueous solution at the dosage of 4 mg net BSRPS per feather, once a day for 5 days; while the ducklings of sBSRPS group at the dosage of 2 mg net sBSRPS per feather. The dosage of drugs was determined referencing to our previous studies [22]. At each stage (the initial (4th and 8th hour) and later (54th hour) after challenged virus) blood samples randomly taken from 5 feathers per group were collected, and the numbers of these ducklings (15 feathers per group) were not calculated in the mortality rate. Half of each sample was treated as heparin anticoagulation and the rest was not.

In order to monitor the dynamic deaths, the number of deaths of each group at 12th, 24th, 30th, 36th, 48th, 72nd, 96th and 120th hour after challenged virus was recorded. Once duck death was found, the pathological changes would be examined carefully and the number of the ducks without pathological changes would be eliminated. The clinical symptom was checked daily. The mortality rate of each group was calculated until no death was found. Surviving ducklings after determining rehabilitation were sent to Nanjing Tangquan Poultry Farm to be bred in accordance with National regulation. Dead duckings were executed bio-safety disposal according to local standard protocols.

2.4. Evaluation indexes of peroxidative injury

The plasma contents of SOD, GSH-Px, CAT, iNOS and MDA at 8th and 54th hour were tested by Duck SOD, GSH-Px, CAT, iNOS and MDA kit.

2.5. Evaluation indexes of hepatic injury

The serum levels of ALT, AST, ALP, LDH, TP and ALB at 4th, 8th and 54th hour were tested by enzymatic colorimetry used automatic biochemistry analyzer (7180 Automatic Analyzer, HITACHI, Japan) in Nanjing Shihuang Institute of Animal Science and Technology. The serum level of GLO was calculated with the subtraction of the serum level of ALB from the serum level of TP.

2.6. The correlation analysis of peroxidative injury and hepatic injury indexes

The correlation of peroxidative and hepatic injury indexes was analyzed by using Pearson coefficient by SPSS Software Package v.20.0.

2.7. Statistical analysis

Statistical analyses were performed by one-way analysis of variance and groups were compared by Duncan's multiple range test using SPSS Software Package v.20.0. Results were expressed as means ± S.E. for five ducklings in each group. The difference of the