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Anti-duck virus hepatitis mechanisms of Bush Sophora Root polysaccharide and its sulfate verified by intervention experiments

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ABSTRACT

In our previous study, Bush Sophora Root polysaccharide (BSRPS) and its sulfate (sBSRPS) exhibited anti-duck virus hepatitis (DVH) abilities as well as anti-oxidative and immuno-enhancement effects. The aim of this paper was to ulteriorly investigate the exact anti-DVH mechanisms of BSRPS and sBSRPS by intervention experiments. Hinokitiol and FK506 were used as the pro-oxidant and immunosuppressant, respectively. The dynamic deaths, oxidative and immune evaluation indexes and hepatic pathological change scores were detected. When was intervened by hinokitiol, sBSRPS still possessed therapeutic effect while BSPRS was useless. Under the condition of immunosuppression, BSRPS lost a part role in treating DVH; however such a role of sBSRPS completely exhausted. These results suggested both anti-oxidative and immuno-enhancement effects of BSRPS played roles in healing DVH, and the former was more crucial; unlike BSRPS, only immuno-enhancement ability of sBSRPS was imperative for its curative effect on DVH.

1. Introduction

Virus hepatitis is a kind of disease which endangers humans' and animals' health seriously. The established human hepatitis viruses include five species which belong to five distinct virus families: hepatitis A (Baptista et al., 1993), B (Du et al., 2012), C (Bhargava et al., 2011), D (Hsu et al., 1988) and E (Ohnishi et al., 2006) virus. All these viruses are RNA viruses except the hepatitis B virus. The animal hepatitis viruses including mouse hepatitis virus A59 (Aparicio et al., 2011), woodchuck hepatitis virus (Lu et al., 2008) and duck hepatitis virus (Ma et al., 2011) also belong to infectious viruses. Duck hepatitis A virus (DHAV) is one serotype of duck hepatitis virus which is the most widely distributed and virulent one. DHAV belongs to family *Picornaviridae*, genus *Avihepatovirus*. It mainly infects ducklings aged less than 3 weeks and induces a high mortality rate (Tseng et al., 2007). To date, there is no therapeutic drug in clinic, hence the damage to the duck industry is still huge.

For thousands of years, traditional Chinese veterinary medicine is widely used to treat animal diseases in China and even the whole East Asia. It has been proven anti-oxidative (Xiong et al., 2014) and immuno-enhancement (Zhou and Zhang, 2013) abilities play important roles in antiviral effect of traditional Chinese veterinary medicine. Bush Sophora Root polysaccharide (BSRPS) is a main effective component of Bush Sophora Root which belongs to family *Leguminosae*, species *Sophora tonkinensis* Gagnep. It also shows anti-oxidative (Chen et al., 2007) and immuno-enhancement (Shuai et al., 2010) abilities. 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). The main chain of this polysaccharide is (1 → 4) linked α-D-glucan to which are attached two glucosyl side chains at 3-O and 6-O of the glucosyl residues in every 12 repeating unit of the main chain (Shuai et al., 2010). In our previous study, BSRPS and its sulfate, sulfated Bush Sophora Root polysaccharide (sBSRPS), exhibited excellent antiviral effects against duck virus hepatitis (DVH) caused by DHAV (Chen et al., 2014a,b). The anti-DVH effects of BSRPS and sBSRPS were closely related to their anti-oxidative (Chen et al., 2014b) and immuno-enhancement (unpublished results) abilities, and it turned out that both BSRPS and sBSRPS owned anti-oxidative and immuno-enhancement abilities. So now comes the question: Do both the two abilities play the important roles in anti-DVH effect or only

Abbreviations: DHAV, duck hepatitis A virus; BSRPS, Bush Sophora Root polysaccharide; sBSRPS, sulfated Bush Sophora Root polysaccharide; DVH, duck virus hepatitis; SOD, superoxide dismutase; MDA, malondialdehyde; CAT, catalase; GSH-Px, glutathione peroxidase; IL, interleukin; IFN-γ, interferon-gama; BC, blank control; VC, virus control; APS, Astragalus polysaccharide.

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Table 1
The animal grouping and treatment.

Group	Treated from Day 1 to 3	Treated at the 4th day	Treated from Day 4 to 11
Group 1 (G1)	Injected solvent	Injected normal saline	Water
Group 2 (G2)	Injected hinokitiol	Injected normal saline	Water
Group 3 (G3)	Injected FK506	Injected normal saline	Water
Group 4 (G4)	Injected solvent	Injected DHAV	Water
Group 5 (G5)	Injected hinokitiol	Injected DHAV	Water
Group 6 (G6)	Injected FK506	Injected DHAV	Water
Group 7 (G7)	Injected solvent	Injected DHAV	BSRPS
Group 8 (G8)	Injected hinokitiol	Injected DHAV	BSRPS
Group 9 (G9)	Injected FK506	Injected DHAV	BSRPS
Group 10 (G10)	Injected solvent	Injected DHAV	sBSRPS
Group 11 (G11)	Injected hinokitiol	Injected DHAV	sBSRPS
Group 12 (G12)	Injected FK506	Injected DHAV	sBSRPS

one of them plays the role? We therefore researched the single anti-oxidative or immuno-enhancement role in the anti-DVH effect of BSRPS or sBSRPS by the intervention method.

In this paper, the anti-oxidative or immuno-enhancement ability of BSRPS or sBSRPS was intervened by hinokitiol or FK506. The mortality rates, oxidative evaluation indexes including superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GSH-Px) and immune evaluation indexes including interleukin (IL) -2 and interferon-gama (IFN-γ) were detected. The scores of hepatic pathological changes were recorded. And the exact anti-DVH mechanisms of BSRPS and sBSRPS were eventually analyzed.

2. Materials and methods

2.1. Reagents

Hinokitiol (Lot no. MMKWF-BR) used as the pro-oxidant which is also known as 4-isopropyltropolone was brought from Tokyo Chemical Industry Co., Ltd.; FK506 (Lot no. A1422027) used as the immunosuppressant which is also known as tacrolimus was brought from Aladdin Industrial Corporation. The solvent used to dissolve hinokitiol and FK506 as well as treat ducklings was normal saline containing 0.3% Tween-80, 0.5% glycerinum and 0.1% dimethyl sulfoxide. Chlorsulfonic acid (Lot no. 130622) and pyridine (Lot no. 20130220) were the products of Shanghai Ling Feng Chemical Company and Sinopharm Group Chemical Company, respectively.

2.2. Drugs and virus

BSRPS and sBSRPS were prepared according to the method mentioned previously (Chen et al., 2014a). In brief, a mean of methanol-reflux was utilized to edulcorate Bush Sophora Root powder. Water-extraction and alcohol-precipitation method was used to extract BSRPS. Sevag method was used to eliminate proteins. And the product was dialysed for 3 days. It was precipitated with three times volume of the absolute ethyl alcohol again. This precipitate was then dehydrated with the absolute ethyl alcohol, acetone and diethyl ether. The crude BSRPS was obtained after the desiccation. Hereafter, the main polysaccharide was acquired by DEAE cellulose column chromatography. And the BSRPS was purified by column chromatography of Sephadex G-100. Its final polysaccharide content was 93.15% determined by phenol–sulfuric acid method (Hsieh et al., 2005). Chlorosulfonic acid–pyridine method was applied to prepare sBSRPS. The polysaccharide content of sBSRPS was determined by phenol–sulfuric acid method while the sulfur content was determined by barium chloride–gelatin method (Dodgson and Price, 1962). The content of sBSRPS was 96.27% which was calculated with the sum of its polysaccharide and sulfur contents.

DHAV (LQ_2 strain) was supplied by Shandong Institute of Poultry in China. It was diluted to $10LD_{50}$ (2.5×10^{-1}) with normal saline and used for the challenge test.

2.3. Preliminary experiments

2.3.1. Dosage of hinokitiol and FK506

Fifty-five 2-day-old ducklings (Nanjing Tangquan Poultry Farm) were randomly divided into 11 groups. Five groups were injected with hinokitiol at dosages of 20, 40, 60, 80 and 100 mg/kg intramuscularly, respectively (Cho et al., 2011); five groups were injected with FK506 at dosages of 1, 3, 5, 7 and 9 mg/kg intramuscularly, respectively (Vannaprasaht et al., 2013); meanwhile one group was injected with the solvent (mentioned in Section 2.1) intramuscularly being treated as blank control (BC) group. All ducklings were treated for 3 days, once per day. The blood of each duckling was taken after that, the serum was isolated for testing.

2.3.2. Intervention effect

In order to verify the pro-oxidative effect of hinokitiol and the immunosuppressive effect of FK506 on ducklings, the oxidative evaluation indexes of hinokitiol treated ducklings at five doses were determined, meanwhile the immune evaluation indexes of FK506 treated ducklings at five doses were determined. Then the ideal doses of hinokitiol and FK506 were taken consideration.

Afterwards, the oxidative evaluation indexes of FK506 at the ideal dose and the immune evaluation indexes of hinokitiol at the ideal dose were also measured, respectively. This aim was to make sure hinokitiol do not affect the immune level and FK506 do not affect the oxidative level. The final doses of hinokitiol and FK506 for formal experiments were then ascertained.

2.4. Formal intervention experiment

2.4.1. Animal grouping and treatment

A total of 720 two-day-old ducklings (Nanjing Tangquan Poultry Farm) were randomly divided into 12 groups. The treatment of each group was listed in Table 1. G1–G12 were the abbreviation of Groups 1–12, respectively. G1 and G4 were equivalently BC and virus control (VC), respectively. The dosages of BSRPS and sBSRPS were, respectively, 4 and 2 mg per feather, once per day.

2.4.2. Dynamic detections

Since the challenge of DHAV, five blood samples of each group were taken at the 4th, the 8th and the 54th h (Chen et al., 2014b, 2015). And the ducklings were monitored at the 24th, the 48th, the 72nd, the 96th, the 120th, the 144th h and the 168th h (Chen et al., 2014b, 2015). The mortality rate of each group was calculated