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Antiviral activities of *Radix Isatidis* polysaccharide against type II herpes simplex virus in vitro

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Abstract

This study investigated the antiviral activities of *Radix Isatidis* polysaccharide (RIP) against type II herpes simplex virus (HSV-2) in vitro. RIP was prepared from the *Radix Isatidis* root. The toxicity of RIP on Vero cells was detected. The direct killing effect of RIP on HSV-2, inhibitory effect of RIP on HSV-2 replication and inhibitory effect of RIP on HSV-2 adsorption were determined. Results showed that, RIP in concentration range of 25-800 mg/L had no toxic effect on Vero cells. RIP with different concentrations could not directly inactivate the HSV-2. The effective rates on inhibition of HSV-2 replication and adsorption in 800 mg/L RIP group were 71.57% and 48.37%, respectively, which were the highest among different groups. In conclusion, RIP has the antiviral effect against HSV-2 in vitro. This effect mainly occurs in inhibiting the virus duplication and adsorption.

Keywords: *Radix Isatidis*; polysaccharide; HSV-2.

Practical Application: *Radix Isatidis* polysaccharide may be applied to treating type II herpes simplex virus related diseases.

1 Introduction

Type II herpes simplex virus (HSV-2) is a virus which majorly causes the genital herpes. HSV-2 infection is one of the major problems that affect the health of adults, especially in patients with immune impairment (Schiffer & Corey, 2013). At present, the antiviral drug acyclovir is often clinically used to treat the HSV-2 infection. This drug exerts the antiviral action mainly by interfering with viral DNA replication. However, as the virus is easy to produce variation, the drug resistance still exists, so the treat outcome is not very satisfactory (Bacon et al., 2003). Therefore, it is urgent to develop a new mode of anti-HSV-2 drugs. In recent years, drug researchers have paid more and more attention to screening antiviral drugs from natural products. *Isatidis Radix* is a traditional herb in China. It is firstly collected in *Shennong's Classic of Materia Medica*. In traditional Chinese medicine (TCM), *Isatidis Radix* is bitter and cold, and has the efficacy of clearing away heat and toxicity and relieving sore throat. It is clinically used in treating viral and bacterial infection diseases (Ke et al., 2012). Polysaccharide is the main chemical component of *Radix Isatidis*. It is often obtained by water extraction from the root of *Radix Isatidis*. Many studies have shown that, *Radix Isatidis* polysaccharide (RIP) has the antioxidant and anti-inflammatory activities (Du et al., 2013), and can resist the viruses and enhance the body immunity (Zhao et al., 2008; Fang et al., 2009). Until now, the effect of RIP on HSV-2 is less reported. If it is confirm that RIP has antiviral activities against HSV-2, this will further broaden the range of therapeutic medicine for HSV-2 related diseases and the application range of RIP. In this study, it was the first time to investigate the antiviral activities of RIP against HSV-2. The objective of this study was to provide a basis for further studying the in vivo anti-HSV-2 activity of RIP and broadening the application scope of RIP.

2 Materials and methods

2.1 Preparation of RIP

Radix Isatidis root (500 g) was decocted (micro boiling) with 3000 mL distilled water for 4 h. After heat filtering, the filter residue was decocted with 2000 mL of distilled water for 2 h. The filtrates of two decoctions were merged, and were concentrated to volume of 200 mL. 800 mL anhydrous ethanol was added, and the final alcohol concentration was adjusted to 80%. After standing overnight, the supernatant was discarded, and the precipitate was dissolved in 100 mL of distilled water, and 400 mL anhydrous ethanol was added. The final alcohol concentration was adjusted to 80%. After standing overnight, the supernatant was discarded, and the precipitate was collected. After freeze-drying, and the crude polysaccharide product was obtained. After deproteinization using Sevage method (Yanhua et al., 2014), the refined RIP was obtained. The sulfuric acid-phenol method showed the content of polysaccharide was 80%.

2.2 Cell culture

Vero cells were cultured with Dulbecco's Modified Eagle's medium (DMEM) containing mL/L fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at condition of 37 °C and 5% CO_2. All the cell culture reagents were provided by Sigma-Aldrich Corp., MO (USA).

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2.3 Determination of virus virulence

HSV-2 (333 strains) was provided by Institute of Virology, Wuhan University (Wuhan, China). HSV-2 stock solution was diluted into 8 dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}) using cell maintenance medium containing 2% FBS. The virus fluid with each dilution was inoculated to the 96-well culture plate of monolayer Vero cells, 4 repeated wells for each dilution, followed by culture at 37 °C with 5% CO_2. The normal Vero cells were set as control. The cytopathy was observed under the inverted microscope. Two fields of vision were selected from each well using the eyepiece micrometer, and 100 squares of each field of vision were selected to calculate the percentage of normal and abnormal cells. The 50% tissue culture infection dose ($TCID_{50}$) was determined using the Reed-Muench method (Gustafsson et al., 2012).

2.4 Determination of toxicity of RIP on Vero cells

Vero cells were digested with trypsin, and the cell suspension (1×10^6 cell/mL) was prepared. The cell suspension was seeded into 96-well culture plates, 200 μL in each well. After culture for 24 h, RIP was added to the well. Based on the suitable dose range obtained in preliminary experiments, the concentration of RIP was set as 25, 50, 100, 200, 400, 800 mg/L, 6 wells for each concentration. The continued culture was performed for 48 h at 37 °C with 5% CO_2 and saturated humidity. At 4 h before the end of culture, 20 μL of methylthiazolyldiphenyl-tetrazolium bromide (MTT) was added to each well. At the end of culture, the supernatant was discarded and 200 μL of dimethyl sulphoxide was added to each well, followed by oscillation for 5 min. The optical density (OD) of each well was measured by the microplate reader (wavelength 570 nm). The higher OD value represented the higher cell activity.

2.5 Determination of direct killing effect of RIP on HSV-2

RIP with different concentration (25, 50, 100, 200, 400, 800 mg/L) was mixed with the same amount of $100TCID_{50}$ HSV-2 fluid, followed by standing at 37 °C for 4 h. The incubation fluid was added to the monolayer of Vero cells, 200 μL for each well, followed by standing at 37 °C for 1.5 h. The supernatant liquid was changed by 200 μL of 2% DMEM, followed by continued culture for 72 h. The cytopathic effect (CPE) was observed under inverted microscope, and the results were recorded as follows: 0% CPE: -; 1-25%: +; 25-50%: ++; 50-75%: +++; 75-100%: ++++. At the same time, the virus group and normal cell group were set, 6 wells for each group. The OD value of each well was measured by MTT method.

2.6 Determination of inhibitory effect of RIP on HSV-2 replication

$100TCID_{50}$ HSV-2 fluid was inoculated into monolayer of Vero cells, 100 μL for each well, followed by adsorption at 37 °C for 2 h. The virus fluid was discarded, and the 2% DMEM containing RIP with different concentration (25, 50, 100, 200, 400, 800 mg/L) was added, followed by continued culture for 72 h. The CPE was observed under inverted microscope, and the results were recorded. At the same time, the virus group and normal cell group were set, 6 wells for each group. The OD value of each well was measured by MTT method. The antiviral effective rate of RIP was calculated as follows: effective rate (%) = (average $OD_{RIP\ group}$ - average $OD_{virus\ group}$) / average $OD_{virus\ group}$ × 100%.

2.7 Determination of inhibitory effect of RIP on HSV-2 adsorption

RIP with different concentration (25, 50, 100, 200, 400, 800 mg/L) was mixed with the same amount of $100TCID_{50}$ HSV-2 virus, followed by direct inoculating into monolayer of Vero cells, 200 μL of mixture for each well, followed by adsorption at 37 °C of 2 h. The supernatant was removed, and the 2% DMEM was added, 200 μL for each well, followed by continued culture for 72 h. The CPE was observed under inverted microscope, and the results were recorded. At the same time, the virus group and normal cell group were set, 6 wells for each group. The OD value of each well was measured by MTT method. The antiviral effective rate of RIP was calculated.

2.8 Statistical analysis

All statistical analysis was carried out using SPSS20.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as mean ± SD, and were compared using y one-way ANOVA with SNK-q test. $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Virus virulence test result

The test of Reed-Muench method showed that, the $TCID_{50}$ of the virus was 10^{-5}. The virus concentration used in this study was $100TCID_{50}$.

3.2 Toxicity of RIP on Vero cells

Microscopy showed that, the morphology of Vero cells treated by RIP presented a tight and orderly arrangement, without shedding, fusion or round retraction, which was not significantly different from the morphology of normal cells. At the same time, MTT test showed that, there was no significant difference in cell proliferation between each concentration of RIP and normal cells ($P > 0.05$). This indicated that, RIP in the concentration range of 25-800 mg/L had no toxic effect on Vero cells (Table 1).

Table 1. Toxicity of RIP on Vero cells.

Group	Dose (mg/L)	OD_{570nm}
Norma cells	-	0.49 ± 0.06
RIP	25	0.50 ± 0.04
	50	0.51 ± 0.07
	100	0.53 ± 0.07
	200	0.51 ± 0.08
	400	0.52 ± 0.06
	800	0.53 ± 0.08

RIP: *Radix Isatidis* polysaccharide; OD: optical density.