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Inhibition on hepatitis B virus in vitro of lectin from Musca domestica pupa via the activation of NF-κB

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ABSTRACT

The present study reported that the secretions of HBsAg and HBeAg in HepG2.2.15 cells were significantly decreased under the treatment of lectin from Musca domestica pupa (MPL). Both the replication of hepatitis B virus (HBV) DNA and HBV cccDNA in cells, and the copies of extracellular HBV DNA were inhibited by MPL. The mRNA expressions of interleukin-2 (IL-2), gamma interferon (INF-γ) and MxA were up-regulated by MPL treatments, but down-regulated when nuclear factor-κB (NF-κB) signal pathway was blocked by pyrrolidine dithiocarbamate (PDTC). Subsequent investigation revealed that nuclear factor-κB inhibitory κB (IκB) in endochylema was inhibited and NF-κB was translocated into the nucleus. These findings indicate that MPL could inhibit HBV replication via the induction of the expression of IL-2, INF-γ and MxA through the activation of NF-κB.

1. Introduction

Hepatitis B virus (HBV) infection remains a major worldwide health problem, responsible for more than 350 million chronic HBV carriers and 1 million human death annually (Esteban, 2002; Ganem and Varmus, 1987). HBV is the smallest enveloped DNA virus ever found which usually compromises the host immune response (Jung and Pape, 2002; Van der Molen et al., 2004) and leads to a wide spectrum of diseases such as liver diseases, acute hepatitis, chronic active hepatitis, cirrhosis, and primary hepatocellular carcinoma (Szmuness, 1978; Beasley et al., 1981; Okuda, 1992; Lin et al., 2001). Although interferons and nucleoside analogs have been widely used as the best treatments for chronically infected patients, their rapidly developing resistance and undesirable side-effects are major disadvantages and therefore more effective approaches are needed (Chen et al., 2005; Fan et al., 2009).

Musca domestica are able to survive and rarely get infected in the most unfavorable surroundings from larvae to adults, largely due to its antibacterial and immunoactive substances in the immune system. These substances, such as prophenoloxidase, antibacterial protein, lysozyme and some other secretions from Musca domestica, have already been well studied. However, there have been few reports on the lectin purified from Musca domestica pupa (MPL), which is reported to play a significant role in strengthening the immune system of the insects via participating in cellular immunity and humoral immunity (Pendland et al., 1988; Yu and Kanost, 2000).

In our study, the galactose-specific lectins from Musca domestica pupae (MPLs) were extracted through affinity chromatography on Sepharose-4B. The innate immune system is the first line of host defense against viral infection. It was previously reported that the MPLs had anti-tumor activity and immunoregulation function (Cao et al., 2003, 2009, 2010). It was also demonstrated in our primary study that the MPLs played a role in regulating the immunity activity of macrophages and lymphocyte via activating the signal pathways of NF-κB (Cao et al., 2011a,b). Increasing numbers of cytokines could be induced when the signal pathway NF-κB was

Abbreviations: MPL, lectin from Musca domestica pupa; HBV, hepatitis B virus; NF-κB, nuclear factor-κB; HepG2.2.15, human hepatoma G2.2.15; PDTC, pyrrolidine dithiocarbamate; IL-2, interleukin-2; INF-γ, gamma interferon; IκB, nuclear factor-κB inhibitory κB.

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moderately activated, which largely helped activate the immune function of hepatocyte (Akira et al., 2006; Hiscott, 2007). However, it remains unknown whether the MPL show immunoregulation function on HepG2.2.15 via activation of NF-κB and then boost the cleanup of virus. In order to investigate the mechanism of antiviral actions of lectin, we observed the effects of MPL on the secretions as immune cell cytokines and antiviral proteins in HepG2.2.15 cell line. We also investigated the effect of lectins on the activation of certain signal pathways which regulate the antiviral genes expressions.

2. Materials and methods

2.1. Preparation for MPL

Musca domestica was supplied by Tianjin Sanitation and Epidemic Prevention Station, Tianjin, China, and MPL was purified according to Cao et al. (2010).

2.2. Cell culture

HepG2.2.15 cells were purchased from Saier Biomedtech Co., Ltd. (Tianjin, China), They were maintained in Dulbecco's modified Eagle's medium (Hyclone, Logan, UT, USA) plus 15% (v/v) fetal bovine serum (Gibco BRL, Grand Island, NY, USA) and 200 μg ml^{-1} G418 (Sigma, St. Louis, MO, USA), and were incubated at 37 °C in 5% (v/v) CO_2 humidified incubator.

2.3. ELISA analysis

HepG2.2.15 cells were treated with various concentrations of MPL (5–80 μg ml^{-1}) for 4, 6 and 8 days, respectively. Equal amounts of cell medium were harvested and analyzed for HBsAg and HBeAg antigen expression using the ELISA kit (Ke hua Biotech, Shanghai, China) according to the manufacturer's instructions.

2.4. Cellular toxicity

The cells (1×10^5 cells ml^{-1}) were cultured in 96-well microtiter plates in a final volume of 100 μl. MPL with various concentrations (5–320 μg ml^{-1}) were added in cells for 6 days. Before harvested, cell viability was measured by WST-1 Cell Proliferation and Cytotoxicity Assay Kit (Beyotime, Haimen, China) following the manufacturer's instructions. Relative cell numbers were calculated from three independent experiments and inhibition rate was determined as $(C - T)/C \times 100\%$, in which C stands for the average OD_{450} of control cells group and T stands for the average OD_{450} of MPL treated groups.

2.5. Real-time quantitative PCR

HBV DNA and cccDNA were isolated from HepG2.2.15 cells both untreated and treated with MPL, and were analyzed by real-time PCR using hepatitis B virus Fluorescence Quantitative Polymerase Chain Reaction Diagnostic Kit and hepatitis B virus cccDNA Fluorescence Quantitative Polymerase Chain Reaction Diagnostic Kit (Suoao Biomedtech Co., Ltd., Beijing, China), respectively, according to manufacturer's.

2.6. RNA preparation and RT-PCR

HepG2.2.15 cells were treated with 20 μl MPL ml^{-1} for 12 h. Pyrrolidine dithiocarbamate (PDTC) (Sigma, MO, USA), a known chemical inhibitor of NF-κB, was used to provide additional evidence on the role of MPL in the activation of NF-κB. For the PDTC inhibition group, 20 μg MPL ml^{-1} was added to cells which were treated with 100 μM PDTC for 1 h. Total RNA was obtained from 1×10^6 cells using TransZol reagent (TransGen Biotech, Beijing, China) following the manufacturer's instructions. The reverse transcription of 5 μg RNA was performed using EasyScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech). The PCR was carried out with the primers of 5′-actcctgccacaatgta-3′ (sense) and 5′-attgctgattaagtccct-3′ (antisense) for IL-2, 5′-tattcggtaactgacttg-3′ (sense) and 5′-gtaatcacatagccttgc-3′ (antisense) for INF-γ, 5′-ccagtgatgattctcttgagagc-3′ (sense) and 5′-ccccaaagcgtagaggtcca-3′ (antisense) for PKR, 5′-acaatcagcctggtggtggtc-3′ (sense) and 5′-cctcccctacagtttcctctcc-3′ (antisense) for MxA.

2.7. Western blot analysis

After treated with MPL for 6 h, proteins in cytoplasm were prepared using Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Jiangsu, China), respectively. The equal amount (30 μg) of protein was then subjected to the SDS-polyacrylamide gel electrophoresis (12% separating gel and 5% stacking gel) and was transferred onto a nitrocellulose filter membrane (BIO-RAD, CA, USA). After blocking the nonspecific site with 5% (w/v) skim milk in PBS containing 0.1% (v/v) Tween-20, a primary antibody (Beyotime, Haimen, China) and a secondary antibody–enzyme conjugate were applied to the membrane consequently. Immunoreactive bands were detected by DAB kit (Zhongshan Goldenbridge, Beijing, China).

2.8. Immunofluorescence imaging

Cells were fixed with 3% (v/v) formaldehyde for 20 min, and washed twice with PBS. Afterwards, 0.2% (v/v) Triton X-100 was used to permeabilize the cells, which were then blocked in 2% BSA. Then cells were washed twice with PBS, and incubated with the primary antibody (Santa Cruz) (dilution 1:100) with 2% (v/v) BSA for 1 h at 37 °C. The resulting cells were washed three times with PBS and incubated with fluorescein FITC-labeled polyclonal goat anti-mouse IgG antibody (Santa Cruz) (dilution 1:100) for 1 h at 37 °C. After washed with PBS, cells were stained with propidium iodide (Sigma) and scanned under the Nikon D-Eclipse C1 Confocal Laser Scanning Microscope (Tokyo, Japan).

2.9. Statistical analysis

The experiments were repeated three times and the mean values were analyzed by a two-tailed unpaired t-test. The level of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of MPL on the expression of viral antigen

To investigate the effect of MPL on the expression of viral antigen, the productions of HBsAg and HBeAg in the culture media were measured by ELISA. Both HBsAg and HBeAg were be inhibited simultaneously after treatment with MPL, and the effects were dose-dependent and time-dependent (Fig. 1). After 6-day treatment, the inhibition rate of HBsAg was 75.64% when the concentration of MPL was 20 μg ml^{-1} and the inhibition rate of HBeAg overtopped 50% with 10 μg ml^{-1} of MPL-treatment.

3.2. Cellular toxicity of MPL

Cell toxicity of MPL on HepG2.2.15 cells was detected by WST-1 assay. After 6-day treatment, MPL did not show significant toxicity