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Characterization and immunomodulating activities of polysaccharide from *Lentinus edodes*

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Abstract

The polysaccharide L-II was isolated and purified from the fruiting body of *Lentinus edodes*, which consisted of D-glucopyranose and had the molecular weight of 2.03×10^5 Da. We evaluated the effects of the polysaccharide L-II on the cellular immune response of Sarcoma 180-bearing mice. Mice were treated with three doses of the polysaccharide L-II (1, 5, and 10 mg/kg body weight) for 10 days. Tumor weight, relative spleen and thymus weight, delayed-type hypersensitivity (DTH) response, phagocytosis of macrophage, splenocytes proliferation were studied. Concentration of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-2 (IL-2) in mice serum were measured in control and polysaccharide groups. At the dose of 1, 5 and 10 mg/kg, a significant increase ($p<0.05$) in relative spleen and thymus weight, DTH, phagocytosis of macrophage was observed, as well as a significant decrease in tumor formation. The concentration of TNF-α, IFN-γ in serum increased significantly in the polysaccharide groups compared with the model control group, but IL-2 not. Moreover, the polysaccharide L-II could increase NO production and catalase activity in macrophages. Results of these studies demonstrated the antitumor activity of the polysaccharide L-II on mice-transplanted sarcoma 180 was mediated by immunomodulation in inducing T-cells and macrophage-dependent immune system responses.

Keywords: *Lentinus edodes*; Polysaccharide; Macrophage; Immunity; Antitumor

1. Introduction

During the past 30 years, many polysaccharides and polysaccharide–protein complexes have been isolated from mushrooms, fungi, yeast, algae, lichens, and plants. The biological activities of these polysaccharides have attracted more attention recently in the biochemical and medical fields because of their

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immunomodulatory and antitumor effects [1]. Mushrooms have recently become attractive as foods (physiologically functional) and as a source material for the development of drugs. Several investigators have isolated and purified immunomodulating polysaccharides as a biological response modifier (BRM) from mushrooms [2]. Recently, lentinan, schizophyllan, and krestin have been accepted as immunoceuticals in several oriental countries [3,4]. Augmentations of NK, cytotoxic T lymphocytes (CTL) and delayed-type hypersensitivity (DTH) responses against tumor antigen were observed after administration of Lentinan [5,6]. Having been prepared by ethanol precipitation of the hot water extract of culture mycelia of *Lentinus edodes* KSLE 007, KS-2 suppressed the growth of Sarcoma 180 tumors and induced an interferon in mice, when given either orally or intraperitoneally [7]. Administration of lentinan, schizophyllan, and PSK was known to inhibit the growth of various transplantable tumors in experimental animals and increase the survival rate [8].

However, the mechanisms of the inhibition of tumor retain unclear [9]. The enhancement or potentiation of host defense mechanisms has been recognized as a possible means of inhibiting tumor growth without harming the host. We will evaluate the effects of the polysaccharide L-II isolated from *L. edodes* on tumor-bearing mice.

2. Materials and methods

2.1. Preparation for polysaccharide

The fruiting body of *L. edodes* used in this study is commercially available in Wuhan, China. It was triturated and boiled in distilled water for 4 h at 100 °C. After filtration to remove debris fragments, the filtrate was concentrated in a rotary evaporator. Protein was removed with the sevag method [10]. Then the solution was precipitated with three volumes of 95% ethanol for 24 h at 40 °C. The precipitates was collected by centrifugation and washed with acetone. It was dissolved with distilled water. Then it was applied to a DEAE-cellulose (2.5×50 cm) equilibrated with distilled water. The solution was collected and freeze-dried. It was applied to DEAE-Sephadex A-25 with 0.2 M NaCl. The polysaccharide was dialyzed with a dialysis tube for 48 h. Only one peak appeared. After freezing, its corresponding fraction was white powder (polysaccharide L-II). The polysaccharide L-II was endotoxin free with Limulus amebocyte lysate (LAL) test.

2.2. Animals and treatment

2.2.1. Animals

Female Kunming mice (18–20 g, 7–9 weeks old) were purchased from the Animal Research Center, Center for Disease Control and Prevention of Hubei province, China. The mice were housed under normal laboratory conditions, i.e., room temperature, 12/12-h light–dark cycle with free access to standard rodent chow and water.

2.2.2. Treatment

Sarcoma 180 cells (donated by Professor Zeng Fanbo, Tongji Medical College, Huazhong University of Science and Technology, Wuhan) were passed into mice ascites. Then, ascites was inoculated subcutaneously 0.2 ml (1×10^6 cells) into the right axilla of each mouse.

Normal control mice were not inoculated Sarcoma 180 (group I). The mice inoculated Sarcoma 180 was divided into five groups (group II–VI). The mice were treated as following: (Group I) normal control, received normal saline; (Group II) model control, received normal saline; (Group III), the polysaccharide L-II (1 mg/kg body weight); (Group IV), the polysaccharide L-II (5 mg/kg body weight); (Group V), the polysaccharide L-II (10 mg/kg body weight); (Group VI), positive control, received Cyclophosphamide (Cy, 20 mg/kg body weight).

Normal and model control mice received the saline intraperitoneally (i.p.), while positive control mice cyclophosphamide. The polysaccharide L-II was dissolved in saline and was administered (i.p.) for 10 days. The dose volume was 0.2 ml.

2.3. Chemical and structural analysis

2.3.1. Assay for molecular weight

The weight average molecular weight of the polysaccharide L-II was determined by gel permeation chromatography (GPC; Agilent 1100, USA). Plaquagel-OH column was used. The eluent was 0.2 mol