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Prevention effects of Schisandra polysaccharide on radiation-induced immune system dysfunction

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ABSTRACT

In this study, we investigate the efficacy of SP (Schisandra polysaccharide) in prevention of radiation-induced immune dysfunction and discussed the underlying mechanisms with a Bal/bc mouse model. The data demonstrated that SP could reverse the decreases in the number of white blood cells and lymphocytes in peripheral blood. In addition, the immunoglobulin G (IgG) and complement C3 in blood serum were all decreased after radiation and SP could restore this radiation disorder. Furthermore, SP could reverse the deregulation of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cell subsets in peripheral blood and thymus of mice after radiotherapy. We also performed terminal dexynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) and Immunohistochemistry (IHC) to investigate the apoptosis and underlying mechanisms of SP in thymus. Data showed that radiation-induced apoptosis of thymocytes could be reversed by SP through inducing upregulation of Bcl-2 expression and downregulation of Fas and Bax levels. Furthermore, SP has no any side-effects on immunity of normal mice. In conclusion, our results indicated that SP could effectively prevent immune injury during radiotherapy by protecting the immune system. This valuable information should be of assistance in choosing a rational design for therapeutic interventions of prevention immune system damage in the radiation treatment.

1. Introduction

Schisandra chinensis (SC) is a traditional plant that is endemic and used as a tonic and sedative agent in the northeast of China, Korea, and the far east of Russia for centuries [1,2]. Accumulated studies have demonstrated that Schisandra polysaccharide (SP), which was extracted from SC, possesses a large variety of beneficial effects including anticancer [3,4], antioxidant [5], anti-aging [6]. It could also exert a variety of immune regulatory functions, for instance, activating lymphocytes [7], macrophages [8,9], and NK cells [10], as well as promoting cytokines release [11]. In consideration of the above, Schisandra polysaccharides might be regarded as a crucial immunomodulator to enhance host defense responses against infection, cancer and other immune-mediated disease.

Tumor radiotherapy has become one of the most dominant anti-tumor treatments because of its superiority in killing local tumor cells and reducing the tumor burden. Recently, at least 50% of patients with advanced tumors need to receive radiotherapy to slow tumor progression and improve the quality of life [12]. However, radiation can damage normal cells while killing tumor cells including circulating cells in peripheral blood, especially for immune cells, leading to tumor progression and recurrence [13,14]. Thus, appropriate immunomodulators which could reverse or prevent the damage to immunity could be potent adjuvant drugs to improve efficacy of tumor radiotherapy. Ghoneum et al. [15] reported that the pre-treatment with MGN-3, an arabinoxylan from rice bran, was able to protect the CBC (complete blood count) parameters from irradiation-induced damage in bone marrow and protection of the spleen indices changes, thus exerting protection of immunity function at the time of receiving radiation therapy.

According to the above effects of Schisandra polysaccharides (SP) on immune regulation, SP may be a new potent adjuvant drug able to protect immune cells from irradiation damage. In the present study, we evaluated the roles and the mechanisms of SP in preventing radiation-induced immune dysfunction through repairing radiation-induced cellular and humoral immunity disorder.

2. Materials and methods

2.1. Materials

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-X nick end labeling (TUNEL) kit was obtained from Roche

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Diagnostic (Manheim, Germany). Proteinase K was purchased from Takara (Otsu, Japan). IgG and C3 kits were all purchased from SUNBIO (Shanghai, China). Antibodies against Bax, Bcl-2 and Fas were supplied by Santa Cruz Biotechnology, Inc. (Dallas, USA).

2.2. Polysaccharide extraction and identification

The *Schisandra chinensis* (Turcz.) Baill was obtained from Lerentang Pharmacy of Shijiazhuang (Hebei province, China) and authenticated by Professor Ren Fengzhi (New Drug Research and Development Center of North China Pharmaceutical Group Corporation, Shijiazhuang, China). The crude polysaccharide extraction was obtained from *S. chinensis* (Turcz.) Baill by adding distilled water (3 h/time), then was concentrated under reduced pressure at 50 °C prior to precipitate the crude polysaccharide at 4 °C overnight by ethanol (final concentration 75% (v/v)). Then crude polysaccharide was purified by diethyl-aminoethanol (DEAE)-52 and SephadexG-100 by Dalian Meilun Biotech Co., Ltd. (Dalian, China). The molecular weight of the polysaccharide was determined by high performance gel permeation chromatography (HPGPC) according to the method of Li et al. [16]. Also, FT-IR analysis of SP was carried out using the potassium bromide (KBr) pellet method with a NEXUS 670 FT-IR (Thermo Nicolet, USA) spectrophotometer between 400 and 4000 cm^{-1}.

2.3. Mice

Forty-eight BALB/c mice (6-week-old, male) were purchased from Chinese Academy of Medical Science (Beijing, China) and were provided with a basal diet and free access to drinking water, according to the guidelines of the Animal Experiment Center of Hebei Medical University. The mice were randomly divided into four groups: Group-I received saline solution (N.S., 5 ml/kg) served as a normal control group (NC group). Group-II received only radiation (6 Gy) for 396 s with a cobalt-60 unit therapy everyday for 7 days, served as irradiation group (IR group). Group-III received SP at a dose of 4 g/kg or 10 g/kg gavage everyday for 7 days, served as SP group. Group-IV was pretreated with SP and irradiated as described above, served as (SP + IR) group. All mice were fasted for 30 min after intragastric administration. Mice were irradiated uniformly on their whole bodies while in an organic glass fixture.

2.4. Analysis of lymphocyte number and prop eral blood

Blood was harvested from the orbital sinus of mice into EDTA-K2 anti-coagulative tubes and mixed quickly for analysis of white blood cells (WBCs) and T lymphocyte subsets. The blood samples were analyzed by a blood cell counter (Coulter Act Diff2, Beckman Coulter, Kraemer Boulevard Brea, USA) to detect the numbers of WBCs and lymphocytes. For analyzing proportion of subsets, 100 μl peripheral bloods was placed in an Eppendorf tube, then 10 μl of each CD3-PC5, CD4-FITC, and CD8-PE immunofluorescence antibodies (Chemicon, Billerica, USA) at a dilution of 1:100 were added to the tubes. Ten μl of IgG1-FITC, IgG1-PE, and IgG1-PC5 (immunofluorescence antibodies) were added to the isotype control tubes respectively. After incubation in darkness for 30 min, tubes were placed in a FACScan flow cytometer (Beckman Coulter) to test the proportions of $CD3^+$ T, $CD3^+CD4^+$ T, and $CD3^+CD8^+$ T cells.

2.5. Analysis of IgG and C3

Immune turbidity technique was used to detect the release of immunoglobulin G (IgG) and complementary C3 according to manufacturer's instruction. In brief, after centrifugation, 20 μl of serum was required from each mouse. The serum samples were diluted as five different concentrations to form a standard curve, and then the contents of IgG and C3 were tested at absorbency of 340 nm with the immune turbidity method.

Table 1
The primer sequences of Bcl2, Bax, Fas and beta-actin genes for RT-PCR.

Genes	Primers	Lengths
Bcl2	5′-CGACTTCGCCGAGATGTCCAGCCAG-3′ 5′-ACTTGTGGCCCAGATAGGCACCCAG-3′	388 bp
Bax	5′-CGGCGAATTGGAGATGAACTG-3′ 5′-AGCAAAGTAGAAGAGGGCAACC-3′	188 bp
Fas	5′-GCTGCAGACATGCTGTGGATC-3′ 5′-TCACAGCCAGGAGAATCGCAG-3′	418 bp
beta-Actin	5′-AGAGGGAAATCGTGCGTGAC-3′ 5′-CAATAGTGATGACCTGGCCGT-3′	138 bp

2.6. Giemsa staining

Thymus paraffin sections were stained with the Giemsa solution and observed under a microscope. In brief, slides were stained with Giemsa (0.1% in glycerol and methanol) for 1 min, and then with 0.025% Giemsa (diluted in PBS) staining solution for 15 min, then the slides were washed with water three times, and dried in the air. Thymus morphological changes were detected under the microscope.

2.7. Detection and quantization of thymocyte apoptosis with TUNEL

TUNEL assay was used to detect the apoptosis of thymocytes according to the previous study [17]. Briefly, paraffin-embedded thymus tissue sections were rehydrated and treated with proteinase K solution for permeation. Then the slides were immersed in terminal deoxynucleotidyl transferase (TdT) labeling buffer followed by incubating them with anti-bromodeoxyridine (anti-BrdU) and sterptavidin-horse radish peroxidase (HRP) solution. Diaminobenzidine (DAB) was used as chromate, and tissue sections were counterstained with methyl green. The slides were investigated in a blinded fashion. Cells containing fragmented nuclear chromatin, the characteristic of apoptosis exhibited a brown nuclear staining, but negative controls did not have any brown staining.

2.8. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from the lymphocytes of thymususing TRIZOL Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. RT-PCR was conducted as previously described [18] with some modifications. In brief, cDNA was prepared using RNA samples. 2 μg was added in 1 μg oligo(dT), 0.5 mM dNTP and 200 units of Revert RT enzyme (MBI Fermentas, Ottawa, Canada) and mixed. PCR analysis was performed using primers described in Table 1 (synthesized at Sangon, Shanghai, China) 1 μl of RT product was incubated with 1 unit of Taq DNApolymerase in a 20 μl reaction mixture (MBI Fermentas). The amplified fragments were detected in 1.2% (w/v) agarose gel and analyzed using an IS1000 image analysis system (AlphaInnotech, San Leandro, USA).

2.9. Confirmation of Bcl-2, Bax, and Fas expression by immunohistochemistry

Immunohistochemical analysis was performed by the streptavidin-peroxidase (SP) method. Formalin-fixed, paraffin-embedded thymus tissues were cut into 4–5 μm thick sections. The sections were dewaxed and rehydrated with xylene and ethanol.