Immunostimulating effects of polysaccharides extracted from Schisandra chinensis fruits

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Abstract

Schisandra chinensis is considered useful to maintain homeostasis in human body. In our previous report, Ginsan, a polysaccharide isolated from the root of Panax ginseng, was administrated to mice and could be adapted as an immunostimulator. In this study, S. chinensis polysaccharides were isolated, and their effects were analyzed on Acanthamoeba castellanii and a bacterium. There were no obscure changes to A. castellanii trophozoites (e.g., membrane shrinkage, bigger vacuoles, motility). However, a higher concentration of the S. chinensis polysaccharides used to treat A. castellanii trophozoites induced a higher increase in the numbers of the amoebae. When the macrophages were pretreated with the polysaccharides, the polysaccharides induced a greater increase of the association of Escherichia coli. Interestingly, treatment of the macrophages with 250 mg of the polysaccharides induced about a two fold greater association than polysaccharide-untreated macrophages. If S. chinensis polysaccharides are used commercially, they will be helpful as supplements for health improvement.

Keywords: Schisandra chinensis, polysaccharide, Acanthamoeba castellanii, Escherichia coli, Macrophage

1. Introduction

Schisandra chinensis is a deciduous woody vine native to forests of northern China and the Russian far east [1], and is naturally cultivated for health improvement in Korea. It is commonly used for the treatment of chronic cough and dyspnea, nocturnal emission, spermatorrhea, enuresis, frequent urination, protracted diarrhea, spontaneous sweating, night sweating, impairment of body fluids with thirst, shortness of breath and feeble pulse, diabetes and wasting-thirst caused by internal heat, palpitation and insomnia [2, 3]. Overall, S. chinensis is considered useful to maintain homeostasis in human body. There are a few reports of its immunological functions. According to Lin et al., report, two major lignans, schizandrin and gomisin A, were identified and shown to induce interleukin (IL)-8, macrophage inflammatory...
immunosstimulating effects of polysaccharides extracted from Schisandra chinensis fruits

protein-1β and granulocyte-macrophage-colony stimulating factor release by THP-1 cells [4].

Polysaccharides isolated from various traditional medicinal plants had been shown to profoundly affect the immune system both in vivo and in vitro through their ability to modulate immune function, including cytokine/chemokine production, reactive oxygen species (ROS) production, and cell proliferation [5].

In our previous report, Ginsan, polysaccharide isolated from the root of Panax ginseng was administrated into mice and its effects showed increasing cytotoxicity of natural killer cells, increase of TNF-α and IFN-γ, suggesting that it can be adapted as an immunostimulator that requires a relatively short oral administration [6]. In addition, a water-soluble polysaccharide named SCP-IIa was isolated from the water extract of the fruit of S. chinensis by means of ethanol precipitation, deproteination, anion-exchange and gel-permeation chromatography [7]. SCP-IIa was involved in immunomodulatory effects, e.g., splenocyte proliferation leading to the exploration for SCP-IIa as a potential immunostimulant [7]. It is obvious that S. chinensis have a potential to stimulate immune cells, but it's not yet reported to analyze its effect on free-living amoebeae. Acanthamoeba spp. are single-celled protozoan organisms that are widely distributed in the environment [8-10]. They cause cutaneous lesions and sinus infections, vision-threatening keratitis and chronic granulomatous encephalitis [8, 11]. In particular, A. castellanii is an agent of human keratitis caused by mainly exogenous trauma and contaminated cleaning solutions. In this study, polysaccharides were isolated and their effects were analyzed on A. castellanii.

2. Materials and Methods

2.1 Culture and A. castellanii and a macrophage

A clinical isolate of A. castellanii belonging to T4 genotype, isolated from a amoebic keratitis patient was used in the present study. A. castellanii was grown without shaking in 12 ml of PYG medium [proteose peptone 0.75% (w/v), yeast extract 0.75% (w/v) and glucose 1.5% (w/v)] in 75T tissue culture flasks at 30°C as a previous report [12]. Murine macrophages (ATCC No. TIB-71) were cultured in Dulbecco's minimal essential medium (DMEM, Gibco BRL) containing 10% fetal bovine serum (Gibco BRL), and incubated at 37°C in a 5% CO₂ incubator as a previous report [13]. A. castellanii trophozoites adherent to flasks and macrophages grown in flasks over 2 days were applied for all subsequent experiments.
2.2 Preparation of *S. chinensis* polysaccharides

Protocol of the polysaccharides extraction from *S. chinensis* was briefly modified from a previous report [6]. Briefly, to extract polysaccharides of *S. chinensis* 4 to 8 volumes of water were added to its dried fruits, and then the oncoming mixture was extracted at 75°C to 80°C for 36 to 72 hours. The extracts were filtered with a 0.22 μm-sized filter and slowly cooled. The chilled extracts were filtered again with the filter above and concentrated at 60°C in a vacuum. The concentrated extracts were mixed with absolute ethanol and were precipitated in a cold condition until polysaccharides were completely precipitated. The resulting polysaccharides were dissolved with water and were concentrated in a vacuum. The final stabilized polysaccharides were autoclaved and freeze-dried.

2.3 Treatment of *S. chinensis* polysaccharides to *A. castellanii*

*S. chinensis* polysaccharides were treated to *A. castellanii* trophozoites, which is described at Table 1. Twenty to two hundred microliter of its polysaccharides were applied in a 96-well cell culture plate up to 24 hr and the morphology of *A. castellanii* was periodically observed. Moreover, cell counting assay was performed using a haemacytometer.

[Table 1. Treatment of *S. chinensis* polysaccharides to *A. castellanii* in 96-well plate]

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* A. *A. castellanii*

* Treatment of *S. chinensis* polysaccharides was shown with volume and brackets indicated 10-times dilution of its polysaccharides.
2.4 Phagocytosis of macrophages to Escherichia coli by S. chinensis polysaccharides

To analyze phagocytic ability of macrophage, association assays were applied because macrophages possess similar effects with Acanthamoeba [6]. Briefly, macrophages were cultured in 96-well plates in DMEM. Macrophages were incubated with Escherichia coli (2 x 10^6 cfu/well/0.5 mL of PBS) and plates incubated for up to 30 min at room temperature. To eliminate E. coli outside A. castellani membrane, macrophage were lysed by adding sodium dodecyl sulfate (SDS) (0.5% final concentration) to each well for 30 min and the numbers of macrophage were enumerated by plating on LB agar plated as previously described [6]. The percentage of macrophage association was calculated as follows: recovered E. coli (cfu)/total E. coli (cfu) x 100 = % E. coli associated with macrophage.

3. Results

3.1 Effect of S. chinensis polysaccharides to A. castellanii

To analyze the effect of polysaccharides extracted from S. chinensis to A. castellanii trophozoites, they were treated into the amoebae together. The amoebal morphology was periodically observed (Fig. 1A) under a microscope with 200-times magnification power. As a result, there were no obscure changes e.g., membranes shrinkage, bigger vacuoles, motility, etc. Thus, only one photo showed at figure 1A, which was no obscure changes. Higher concentration of the S. chinensis polysaccharides treated to A. castellanii trophozoites induced higher increase of the numbers of the amoebae shown figure 1B and 1C, which showed 10-times dilution of the polysaccharides.
[Fig. 1] Effect of S. chinensis polysaccharides to A. castellanii trophozoites. Data showed proliferation numbers of the amoebae by the natural product. Panel A indicates morphological findings of the amoebae (×2000). Panel B and C indicate the treatment of polysaccharides to the amoebae and bracket represents 10-times dilution of the polysaccharides. 'A' of an abbreviation indicates the amoebae trophozoites.

3.2 Phagocytosis of macrophages treated with S. chinensis polysaccharides to a bacterium, E. coli

The macrophages can ingest bacteria and kill them inside and/or outside the wall of the macrophages. To analyze the effect of S. chinensis polysaccharides to a immune cell, the phagocytic ability of the macrophages was analyzed using a bacterium of E. coli (Fig. 2). Here, association, that is the adherence of E. coli to the macrophages, was measured. When the polysaccharides were pre-treated to the macrophages, they induced more increase of the association. Interestingly, 250 mg of the polysaccharides induced about two-times more association than polysaccharides-unreated macrophages. Taken together, it suggested that the S. chinensis polysaccharides let an immune cell increase the killing more.
4. Discussion

S. chinensis is known to improve health status and widely used for cough, diarrhea, sweating, thirst, etc as mentioned in Introduction. In particular, S. chinensis polysaccharides must play a role in the effects above due to a composition of the S. chinensis. Ginsan called with the polysaccharides of P. ginseng is the constituent of P. ginseng. Saponin is well known as the constituent of P. ginseng. It has been also observed in other plants and used as adjuvants in vaccines, e.g., Quil A [14], isolated from the bark of Quillaja saponaria Molina, to stimulate both the Th1 immune response and the production of cytotoxic T-lymphocytes (CTLs) against exogenous antigens make them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens as well as for therapeutic cancer vaccines [15]. A constituent but not a whole body of the plants can be highly used as concentrated powder such as a saponin. Saponins as a kind of natural stabilizer, widely distributed in the plant kingdom, include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains [16]. Saponins have been widely used in food and other industrial applications mainly as surface active and foaming agents [17]. These many articles suggested that the saponin would be helpful to improve health conditions. This
study removed the saponin and/saponin-like molecules and only S. chinensis polysaccharides were applied to analyze their effect to cells. In other example of the polysaccharides-application, Rhizoma gastrodiae is considered a top grade medicine described to enter liver channel in classic literatures of traditional Chinese medicine [18]. Tests of the immunological activity in vitro showed that the two Rhizoma gastrodiae polysaccharides (RGP-1 and RGP-2) could significantly stimulate macrophages to release NO and enhance phagocytosis in a dose-dependent manner [19].

In this study, higher concentration of the S. chinensis polysaccharides treated to A. castellanii trophozoites induced higher increase of the numbers of the amoebae. When the polysaccharides were pre-treated to the macrophages, they induced more increase of the association. If S. chinensis polysaccharides is commercially used, they are helpful for health improvement as health supplement.

References


