Chemico-Biological Interactions 247 (2016) 30–38

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Three kinds of *Ganoderma lucidum* polysaccharides attenuate DDC-induced chronic pancreatitis in mice

Koukou Li [a], Min Yu [b], Yang Hu [a], Guangming Ren [a], Tingting Zang [a], Xiuhong Xu [a], Juanjuan Qu [a, *]

[a] College of Resources and Environmental Science, Northeast Agriculture University, Harbin 150030, PR China
[b] Drug Safety Evaluation Center, Heilongjiang University of Chinese Medicine, Harbin 150040, PR China

ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form
4 January 2016
Accepted 18 January 2016
Available online 27 January 2016

Keywords:
Ganoderma lucidum
Polysaccharides
Chronic pancreatitis
Antioxidant activity

ABSTRACT

Chronic pancreatitis (CP) is a progressive inflammation of pancreas characterized by irreversible morphologic change and dysfunction. Patients with chronic pancreatitis often present with abdominal pain, diarrhoea, jaundice, weight loss and the development of diabetes. Polysaccharides of *Ganoderma lucidum* strain S_3 ($GLPS_3$) possess antioxidative and immunomodulatory activities. This study was to characterize chemical structures of $GLPS_3$ and determine their effects on diethyldithiocarbamate (DDC)-induced CP in mice. The total sugar content of $GLPS_3$ from fermentation broth ($GLPS_3$-I), cultured mycelia ($GLPS_3$-II) and fruiting body ($GLPS_3$-III) was 90.4%, 92.2% and 91.8% respectively. $GLPS_3$-I, $GLPS_3$-II and $GLPS_3$-III were composed of Glu:Gal:Ara:Xyl, Glu:Gal:Ara:Xyl:Man:Rha, and Glu:Gal:Xyl:Man:Rha:Fuc, with molar ratio of 2.82: 1.33: 1.26: 0.87, 5.84: 2.23: 0.72:1.38: 1.40: 0.51 and 5.34: 2.72: 1.14: 1.10: 0.33: 0.38, respectively. The antioxidative activity of $GLPS_3$-IIfrom cultured mycelia in vitro is higher than other two polysaccharides. The superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in serum were increased while the malondialdehyde (MDA) levels were reversely decreased by $GLPS_3$ treatment. Serum amylase (AMS) and lactic dehydrogenase (LDH) changes indicated the therapeutic effects of $GLPS_3$. Moreover, interleukin-1beta (IL-1β) and interferon-gamma (INF-γ) contents were reduced most by $GLPS_3$-II. The results revealed that $GLPS_3$ especially $GLPS_3$-IIfrom cultured mycelia were effective for CP therapy and bioactivity difference might be attributed to monosaccharide composition.

1. Introduction

Chronic pancreatitis (CP) is a progressive inflammation characterized by the permanent destruction, insufficient exocrine and chronic disabling pain of pancreas [1]. In industrialized countries, the CP incidence ranges from 3.5 to 10 per 100,000 population. While people suffering from CP are more predisposed to pancreatic cancer with a 5-year cumulative incidence of 1.1% and a 10-year cumulative incidence of 1.7% [2]. So far, the aetiology for CP is mainly contributed to a combination of genetic and environmental factors, especially alcohol consumption, therefore no definitive medical treatment is used for CP [3]. The oxidative stress and excessive generation of reactive oxygen species (ROS) play a key role in the pathophysiology of acute and chronic pancreatitis as supported by clinical findings and experimental model [4]. Moreover, many participants with moderate intake of vitamins C, E and selenium reduced the risk to develop into pancreatic cancer [5].

Ganoderma lucidum (also known as *Reishi* or Lingzhi) is a

Abbreviations: CP, chronic pancreatitis; GLP, *Ganoderma lucidum* polysaccharides; $GLPS_3$, polysaccharides of *Ganoderma lucidum* strain S_3; $GLPS_3$-I, polysaccharides of *Ganoderma lucidum* strain S_3 from fermentation broth; $GLPS_3$-II, polysaccharides of *Ganoderma lucidum* strain S_3 from cultured mycelia; $GLPS_3$-III, polysaccharides of *Ganoderma lucidum* strain S_3 from fruiting body; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; AMS, amylase; LDH, lactic dehydrogenase; IL-1β, interleukin-1beta; INF-γ, interferon-gamma; Glu, D-glucose; Man, D-mannose; Ara, D-arabinose; Fuc, L-fucose; Gal, D-galactose; Rha, L-rhamnose; Xyl, D-xylose; DDC, diethyldithiocarbamate; HPGPC, high performance gel permeation chromatography; HPAEC, high performance anion exchange chromatography; PAD, pulse amperometic detector; FT-IR, Fourier transform infrared; OH^-, hydroxyl radical; VC, vitamin C; O_2^-, superoxide radical; DPPH, 1,1-diphenyl-2-picryl-hydrazyl radical; DMSO, dimethyl sulfoxide; DEAE, diethylethanolamine; EDTA, ethylene diamine tetraacetic acid; PC, positive control group; NC, normal control group; MC, model control group; H&E, hematoxylin and eosin; NBT, nitroblue tetrazolium; NF-κB, Nuclear Factor-Kappa B; GR, glutathione reductase; TBA, thiobarbituric acid; SE, mean ± standard error; ANOVA, analysis of variance.

* Corresponding author.
E-mail address: juanjuan4050234@163.com (J. Qu).

http://dx.doi.org/10.1016/j.cbi.2016.01.013
0009-2797/

popular medicinal basidiomycete belonging to the polyporaceae. The fruiting body of *G. lucidum* was once considered a panacea in ancient China and has been used for centuries as a folk medicine in oriental countries. Recently it has been successfully used for the prevention and treatment of various human disease, such as hepatitis, hypertension, chronic bronchitis, brochial asthma, cancer and others [6]. Polysaccharides as important biologically active components from *G. lucidum* have been reported for a long time. Up to now more than 200 polysaccharides have been isolated and purified from fruiting bodies, spores, mycelia and cultivated broth of *G. lucidum* [7]. These polysaccharides are composed of glucose, mannose, galactose, xylose, fucose and arabinose with different glycosidic linkage and molecular weigh ranging from thousands to millions Daltons [8]. As complex molecules, polysaccharides have no uniform structures and features, but their bioactivities could be greatly affected by specific glycosidic linkage, molecular weight and monosaccharide composition [9]. *G. lucidum* polysaccharides (GLP) are significant sources for anti-tumor, anti-oxidant, anti-inflammatory, anti-diabetic and immunoregulation [10]. It is believed that at least part of the health benefits of GLP may attribute to their significantly anti-oxidant features. Research indicated that GLP from fruiting body can effectively reduce the oxidative injury and inhibit the apoptosis by increasing anti-oxidant enzyme activity and eliminate reactive oxygen species [11]. However, GLP obtained from cultured mycelia and fermentation broth may differ from those from fruiting body in biological activity due to the monosaccharide composition and molar ratio [12], but their properties for CP therapy were unknown. In this study we aimed to compare the activity of GLP from fruiting body, cultured mycelia and fermentation broth of *G. lucidum* strain S_3 on DDC-induced CP in mice, and revealed the composition-activity relationship of GLP.

2. Materials and methods

2.1. Chemicals and reagents

Analytical reagents and double distilled water were used to prepare all solutions. Standard sugars including D-glucose (Glu), D-mannose (Man), D-arabinose (Ara), L-fucose (Fuc), D-galactose (Gal), L-rhamnose (Rha), D-xylose (Xyl) and diethyldithiocarbamate (DDC) were purchased from Sigma Chemical Co. (St. Louis, USA). The reagent kits to determine the levels of superoxide dismutase (SOD), glutathion peroxidase (GSH-Px), malondialdehyde (MDA), interferon-gamma (IFN-γ), interleukin-1beta (IL-1β), amylase (AMS), lactic dehydrogenase (LDH) and hydroxyproline were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Preparation of G. lucidum polysaccharides

The fruiting body of *G. lucidum* strain S_3 was provided by microbiology lab of Northeast Agricultural University, China. The strain was inoculated in the liquid fermentation broth (containing Lactose 3.04%, Peptone 0.28%, VB_1 0.0047%, KH_2PO_4 0.10%, $MgSO_4$ 0.10%) and cultivated at 25 °C for 5 days in a rotary shaker to acquire fermentation broth and cultured mycelia.

2.2.1. Polysaccharides from fermentation broth

The fermentation broth was concentrated to one fourth of its original volume with a rotary evaporator under reduced pressure at 60 °C. The concentrated liquid was treated with Sevag's regent (Chloroform:butanol = 5:1) several times to remove protein, then the liquid was fully mixed with 4 volumes of chilled 95% (v/v) ethanol and kept at 4 °C overnight. The precipitate was collected by centrifugation at 6500 g for 10 min and lyophilized.

2.2.2. Polysaccharides from cultured mycelia

The cultured mycelia was washed three times with distilled water and dried to constant weight at 60 °C. Then the dried mycelia was ground to powder and suspended in distilled water for 2–3 h heating at 70 °C for several times. The filtrate was concentrated and treated as described above to obtain the lyophilized crude polysaccharides.

2.2.3. Polysaccharides from fruiting body

The dried fruiting body was ground into fine powder and extracted with petroleum ether at 70 °C for 2–3 h to remove fatty and fat-soluble substances. The residue was then extracted with distilled water at 70 °C for 2–3 h. The filtrate was concentrated and treated as described above to obtain the lyophilized crude polysaccharides.

2.3. Purification

1 g of crude polysaccharides were dissolved in 40 mL distilled water and centrifuged at 4500 rpm for 15 min. Then the supernatant was purified by an anion-exchange diethylethanolamine (DEAE) Sephadex A-25 column equilibrated with 50 mM Tris–HCl buffer (pH 7.6). The polysaccharide solution was initially eluted with 1.5 column volume of distilled water, 0.2, 0.4 and 0.6 M NaCl solution at a flow rate of 3.0 mL/min, and 6 mL solution per tube was collected with a fraction collector. The polysaccharide content in the eluent was determined by the phenol–sulfuric acid method [13]. Then the main fraction was further purified in a Sepharose 6B column, and eluted with 50 mM Tris–HCl buffer (pH 7.6) at a flow rate of 1.0 mL/min. The purified fraction from fermentation broth, mycelia and fruiting body was named as $GLPS_3$-I, $GLPS_3$-II and $GLPS_3$-III, respectively. Finally, three fractions were concentrated and lyophilized to fine powder.

2.4. Homogeneity and molecular mass determination

The homogeneity and molecular mass of $GLPS_3$-I, $GLPS_3$-II and $GLPS_3$-III were determined by high performance gel permeation chromatography (HPGPC), which was performed on a high performance liquid chromatography system equipped with a two columns in series, G-5000 PW_{XL} and G-3000 PW_{XL} (Tosoh Biosep, Japan) and eluted with distilled water (1.0 mL/min). The samples were dissolved in distilled water at 4 mg/mL and centrifuged at around 15,000 rpm for 30 min before injection. The column was kept at 40 °C for the whole process and calibrated with the Dextran T-series standard (T2000, T500, T70, T40, T40, T10, T2.9) from Sigma–Aldrich. The molecular weights (MW) of $GLPS_3$-I, $GLPS_3$-IIand $GLPS_3$-III were estimated with reference to the calibration curve prepared above.

2.5. Monosaccharide composition

$GLPS_3$ (4 mg) was hydrolyzed with 1 mL of 4 M trifluoroacetic acid at 110 °C for 5 h, then the hydrolyzate was dried under vacuum and dissolved in ultrapure water. The obtained liquid sample was applied to high performance anion exchange chromatography (HPAEC) (Dionex, USA) fitted with a CarboPac PA10 analytical column (4 mm × 250 mm) according to the method of Obro et al. [14], then the column was eluted with 2.3 mM NaOH (0.5 mL/min). The monosaccharide composition was analyzed using a pulse amperometric detector (PAD) (Dionex, USA). Calibration was performed with monosaccharide standards including D-glucose (Glu), D-mannose (Man), D-arabinose (Ara), L-fucose (Fuc), D-galactose (Gal), L-rhamnose (Rha) and D-xylose (Xyl) [15].