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Seleno-lentinan prevents chronic pancreatitis development and modulates gut microbiota in mice

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ABSTRACT

The ability of seleno-lentinan in preventing chronic pancreatitis was examined and its prebiotic-like efficacy on gut microbiota in mice induced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine was also investigated. Seleno-lentinan more effectively impeded the progress of inflammation and fibrosis than selenite and lentinan through the enhancement of superoxide dismutase and glutathione peroxidase activities, inhibiting lipid peroxidation and decreasing the levels of serum tumour necrosis factor-α and interleukin-1β, as well as pancreatic hydroxyproline. The high throughput sequencing analysis showed that seleno-lentinan increased the relative abundance of Bacteroidetes, whereas it decreased that of Firmicutes. At the genus level, seleno-lentinan increased the proportion of beneficial bacteria such as *Lactobacillus*, *Bacteroides*, *Prevotella*, and *Roseburia*. Besides, the supplementation of selenium had a beneficial impact on the proliferation of lactic acid bacteria. Thus, seleno-lentinan availably prevents chronic pancreatitis development by elevating antioxidant status and has a prebiotic-like efficacy on the modulation of gut microbiota in mice.

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1. Introduction

Chronic pancreatitis (CP) is a progressive inflammatory disorder of pancreas with clinical symptoms of pain, vomiting, malnutrition, fatty diarrhoea and pancreatogenic diabetes. Pancreatic fibrosis is a major pathological feature of CP, resulting from the endocrine and/or exocrine dysfunctions of pancreatic parenchyma (Braganza, Stephen, Rory, & Michael, 2011). Imbalance between reactive oxygen species (ROS) production and antioxidant defence response in vivo is supposed to be the pathogenesis of CP (Santos, Gois, Mendonça, & Freire, 2014). Excessive ROS could convert the pancreatic acinar cells into a "factory" for chemotactic cytokines through the activation of signalling cascades (Leung & Chan, 2009). These inflammatory mediators activate pancreatic stellate cells (PSCs), subsequently promote pancreatic fibrogenesis and consequently induce CP (Erkan et al., 2012). Clinical approaches, such as surgery and pancreatic enzyme replacement therapy, are normally used to alleviate CP (Leung & Chan, 2009). Currently, the consumption of functional foods or dietary supplements to prevent CP has become more and more intriguing.

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Lentinula edodes (*Berk*.) Pegler is one of the most valuable and popular edible mushrooms in the world, particularly in China, Japan and other Asian countries. Regular consumption of *L. edodes* enhances the immune function of an organism due to the immunostimulating or therapeutic properties of polysaccharides (lentinan), which can be potentially used as the ingredients of novel functional foods and nutraceuticals (Giavasis, 2014). For instance, polysaccharides from *L. edodes* have been successfully used as a partial replacement of wheat flour in baked foods (Kim et al., 2011) and noodles (Kim, Chung, Nam, & Kang, 2009) for their antioxidant and hypocholesterolaemic effects. *Agaricus* extracts have been made into snacks with antioxidant capacity and free radical scavenging activity (Richu, Moushumi, & Abhijit, 2009). Glucans from chestnut mushroom (*Agrocybe aegerita*) have been applied in the snack foods for its low glycaemic effect after consumption (Brennan, Derbyshire, Tiwari, & Brenna, 2012). Moreover, polysaccharides can be utilized as gelling, thickening, stabilizing and emulsifying agents in food processing (Venugopal, 2012).

Selenium is an essential trace element for organisms. Organic selenium, as a health-promoting ingredient, is thought to be supplemented by food fortification for lower toxicity and more actively absorbed by organisms than inorganic selenium (Ahn et al., 2006; Qin et al., 2013; Reilly, 1998; Wang, Zhan, Zhang, Wu, & Yuan, 2011). Because of the scarcity of selenium source, selenylation of polysaccharide, a chemical process to combine selenium with polysaccharides, becomes alluring in food fortification industry. It is not only conducive to the development of new selenium source, but also potentiates the physiological and pharmacological activities of selenium and polysaccharide in immunomodulation, hypoglycaemic, antibacterial and anticancer effects (Fan et al., 2006). Several researchers have reported that selenized polysaccharide could potentially serve as a dietary supplement of selenium or an ingredient for the formulation of nutraceuticals (Malinowska et al., 2009; Sanmartín, Plano, Sharma, & Palop, 2012). For instance, selenized *Hericium erinaceum* polysaccharide (Malinowska et al., 2009) and selenized *Artemisia sphaerocephala* polysaccharide (Wang, Zhao, Wang, Yao, & Zhang, 2012) might be novel selenium sources in dietary supplements due to their potent antioxidant properties. Besides, Mao et al. (2014) demonstrated that selenized *Grifola frondosa* polysaccharide could be explored as an antioxidant health food because of its strong antioxidant activity. The selenized lentinan (seleno-lentinan) is more protective towards oxidative damage in liver than lentinan (Ren et al., 2015). However, whether seleno-lentinan exerts a similar influence on CP is still unknown.

Gut microbiota is a complex community responsible for health and disease of a host. It affects the immune-regulation and nutrient metabolism by acting on the metabolic reaction and energy balance (Everard et al., 2014). Aberrant variation of gut microbiota could cause a broad range of chronic inflammatory diseases including pancreatitis, esoenteritis, diabetes, asthma, atherosclerosis and thrombosis (Sachdev & Pimentel, 2013). On the contrary, diet and health status can alter gut microbiota, disturb intestinal homeostasis and influence anti-bacterial response (Chen, Tai, & Hsiao, 2015). For example, dietary selenium may be biotransformed by lactic acid bacteria (LAB), which plays a role in decreasing stress factors affecting LAB (Palomo, Gutiérrez, Pérez-Conde, Cámara, & Madrid, 2014). Polysaccharides derived from plant and fungi cannot be digested by human enzymes (Chen et al., 2011), but can be utilized by gut microbiota (Flint, Scott, Louis, & Duncan, 2012). Polysaccharide (lentinan) could significantly change the populations of Proteobacteria and Bacteroidetes, which was helpful in regulating the host immune responses (Xu & Zhang, 2015). These good performance and health benefits have led to more attentions to exploit a novel organic selenium compound (selenized polysaccharide) that can be used as a functional ingredient or food. This study aims to examine the ability of seleno-lentinan in preventing CP, and further investigate its prebiotic-like efficacy on gut microbiota modulation in mice.

2. Materials and methods

2.1. Materials

3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and selenite were purchased from Sigma Chemicals (St. Louis, MO, USA). Assay kits for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), thiobarbituric acid reactive substances (TBARS), hydroxyproline (Hyp), mouse tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Faecal DNA Isolation Kit and DNA Purification Kit were purchased from Tiangen Biotechnology Co. Ltd. (Beijing, China). All other chemicals are analytical grade products purchased from Tianjin Zhiyuan Chemical Reagent Co. Ltd. (Tianjin, China).

2.2. Preparation of seleno-lentinan

Seleno-lentinan was prepared with the nitric acid–sodium selenite (HNO_3–Na_2SeO_3) method based on a previous study (Ren et al., 2015). Briefly, certain amount of sodium selenite and barium chloride were added into lentinan solution, then the solution was adjusted to proper pH value and continuously stirred for 6 h. After removing Ba^{2+}, supernatant was collected, dialysed and lyophilized. The purified seleno-lentinan was obtained after application with DEAE-cellulose anion-exchange column (2.5 × 30 cm) and Sephadex G-200 gel filtration column (1.6 × 40 cm). Lentinan was also prepared with the above extraction and purification procedure. Both lentinan and seleno-lentinan are soluble in water and insoluble in ethanol, acetone and ether. Total content of soluble sugar percentage and selenium content of lentinan and seleno-lentinan were 93.27% and 0.55 $\mu g \cdot g^{-1}$ and 90.01% and 0.32 $mg \cdot g^{-1}$ respectively.

2.3. Animals

Specific pathogen-free ICR mice (male) with body weights of 25 ± 3 g were obtained from the Experimental Research Center of Animal (Heilongjiang University of Chinese Medicine, Harbin, China). Mice were kept in home cages at 25 ± 1 °C and 55 ± 5% relative humidity with a regular 12 h light/dark cycle. They all had free access to sterilized water and standard mouse chow.

All animal experiments were approved by the Ethical Animal Care and Use Committee of Heilongjiang University of Chinese Medicine (Approval No.SCXK-2013-012). The protocols

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