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Bamboo-shaving polysaccharide protects against high-diet induced obesity and modulates the gut microbiota of mice

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ABSTRACT

Increasing evidence has shown that gut microbiota plays a critical role in regulating pathogenesis of low-grade inflammation and obesity. Bamboo-shaving polysaccharide (BSP, purity: 80–85%, Mw ≈ 10,000 g/mol) is well known for its immunomodulatory capacity. However, no study has previously investigated the anti-obesity activity of BSP. After high fat diet-fed mice were treated with BSP for 8 weeks, we showed that BSP not only improved community richness and diversity of gut microbiota, but also regulated the composition of gut microbiota. Specifically, mice treated with BSP showed lower ratio of *Firmicutes/Bacteroidetes*, lower relative abundance of harmful bacteria (*Enterobacter* and *Desulfovibrio*) and higher relative abundance of beneficial bacteria (*Akkermansia muciniphila* and *Lactobacillus*). Moreover, BSP meliorated intestinal barrier integrity, reduced low-grade inflammation, improved lipid metabolism and ameliorated insulin resistance in obese mice. Our results indicated that BSP could be exploited as prebiotic to protect against obesity and insulin resistance in obese individuals.

1. Introduction

As a major kind of chronic disease today, obesity is prevailing all over the world, and linked to numerous health problems and a reduced life expectancy. Growing epidemiological evidence indicates that obesity is closely linked with type 2 diabetes mellitus, insulin resistance, fatty liver disease, cardiovascular disease and cancer (Sonnenburg & Bäckhed, 2016). For these reasons, obesity has become a research focus in recent years and a growing body of novel treatment strategies have been developed. Dietary interventions, including plant-derived foods or functional components, offer a promising therapy to ameliorate obesity and its complications (Chang et al., 2015; Zhuang et al., 2017).

Increasing evidence suggests that chronic low-grade inflammation is crucial to the pathogenesis of obesity. Remarkably accumulated macrophages in visceral adipose tissue of obese individuals elevate circulating diverse pro-inflammatory cytokines levels, such as interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1). Pro-inflammatory adipokines will be further together linked to abnormal metabolism (pancreatic β cells damage, insulin resistance and glucose intolerance) (Lackey & Olefsky, 2016). Thus, control of pro-inflammation cytokine expression is critical to reduce chronic low-grade inflammation and insulin resistance in obese individuals.

Gut microbiota, composed of trillions of microorganisms, is associated with the development of obesity, and has been accepted as an "environmental factor", which exerts a prominent influence on host metabolism, nutrient digestion, energy utilization and storage. In obese mice, strong evidence for this argument is that ratio of the major phyla *Firmicutes/Bacteroidetes* increased significantly in fat ones as compared with lean ones. In addition, several specific bacterial species can alter the development of obesity in both dietary and genetic obese mice models. For instance, a single endotoxin-producing species *Enterobacter*

Abbreviations: BSP, bamboo-shaving polysaccharide; IL, interleukin; TNF-α, tumor necrosis factor alpha; MCP-1, monocyte chemoattractant protein-1; LPS, lipopolysaccaride; SCFAs, short chain fatty acids; NC, normal chow diet; HFD, high fat diet; T-CHO, total cholesterol; TG, triacylglycerol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; FFA, free fatty acid; H&E, Hematoxylin-Eosin; cDNA, complementary DNA; OTUs, operational taxonomic units; ITT, insulin tolerance test; GTT, glucose tolerance test; AUC, area under curve; Pcoa, principal coordinate analysis

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cloacae, isolated from the gut in a severely obese human, caused obesity and insulin resistance in germ-free mice (Fei & Zhao, 2013). Moreover, mounting studies confirmed that an increase in the *Akkermansia* spp. population could relieve obesity and intestinal barrier integrity in obese mice (Plovier et al., 2017). Other studies in obese animal models suggest that obesity-induced gut dysbiosis impairs intestinal integrity and further induces low-grade inflammation (Bleau, Karelis, St-Pierre & Lamontagne, 2015). High-fat diet increases intestinal permeability, thus elevating endotoxin lipopolysaccharide (LPS) from some intestinal Gram-negative bacteria (such as *Enterobacter* and *Desulfovibrio*) gets into the blood circulation and leads to metabolic inflammation in obese animal models, which is so called "metabolic endotoxemia" hypothesis. Therefore, efforts to improve obesity have been directed to reduce dysbiosis of the gut microbiota via one of the most effective strategies being added dietary intervention with functional food ingredients to reduce insulin resistance and increase systemic anti-inflammatory activities of intestine.

Polysaccharide, as a kind of abundant and active natural products, exhibited various biological activities, including anti-tumor, anti-inflammatory and blood lipid regulation effects (Chang et al., 2015; Hoang, Kim, Ji, You & Lee, 2015; Liu, Xie, Sun, Meng & Zhu, 2017). When the polysaccharide was absorbed into the large intestine (colon and cecum) in body, it would be converted to short chain fatty acids (SCFAs) (mainly including acetic acid, propionic acid and butyric acid) and other metabolites under the action of carbohydrate-active enzymes. Studies have shown that SCFAs were beneficial to the body with many biological functions, such as promoting colon epithelial cell proliferation and growth, participating in the body's energy metabolism, regulating the inflammatory response and inhibiting the intestinal pathogenic bacteria colonization, etc. (Canfora, Jocken & Blaak, 2015). Meanwhile, other studies have confirmed that polysaccharide with complex structure could promote the growth of beneficial microbes in the large intestine, such as *Bifidobacterium* and *Lactobacillus*, maintained the stability of intestinal microecology and improved the symptom of disordered intestinal flora (Everard et al., 2014).

In a previous study, we acquired a kind of active polysaccharide isolated from bamboo shavings (*Caulis Bambusae In Taenia*). The bamboo-shaving polysaccharide (BSP) is the arabinoxylan with a main carbohydrate chain of β-1,4-D-pyranoid xylose residues and a main substituent of Arab furanose base. It belongs to the hemicellulose polysaccharide (Mw ≈ 10,000 g/moL) and contains neutral and acidic structure (Fig. S1). Accordingly, BSP has a significant immunomodulatory effect on immunocompromised mice (Huang, et al., 2017). In the present study, we hypothesized that oral administration of BSP (200 mg/kg BW and 400 mg/kg BW) could positively modulate gut microbiota and alleviate intestinal inflammation in HFD-induced obese mice, which may contribute to its anti-obesity activity under dietary intervention. To test this hypothesis, C57BL/6 mice were orally administrated with high-fat diet contained two different concentrations of BSP for 8 weeks, then lipid and glucose profile, LPS, inflammatory markers and gut microbiota were then measured.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.08.015.

2. Materials and methods

2.1. Preparation of animal diet

BSP was prepared by our laboratory as described previously (Huang, et al., 2017). Major parameters of preparation of BSP were as follows: steam pressure was 2.2 MPa, time of steam pressure was 1 min, and the sample contains 80–85% polysaccharide. Normal chow diet: SLACOM breeding fodder was obtained from Shanghai Pluton Biotechnology Co., Ltd. (Shanghai, China). D12492 High-fat diet (60 kcal %) was obtained from Research Diets, Inc. Co., Ltd. (New Brunswick, NJ, USA). The ingredients and energy densities of the diets are listed in Tables S1 and S2.

2.2. Animals and experimental design

Forty-five eight-week-old C57BL/6J male mice, average body weight of 23.61 ± 1.0 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Laboratory animal license number: SCXK 2012–0001, Beijing, China). The mice were randomly assigned to five groups (nine animals each group) with free access to food and water in a controlled environment (12:12 h light–dark cycle, constant temperature 22 ± 2 °C with a relative humidity of 55 ± 5%, SPF level of barrier system). After one week of adaptation, five groups of mice were fed on different diets, as follows: (1) normal chow diet (NC; with 10% of energy from fat as food and distilled water as drinking water); (2) normal chow diet with 400 mg/kg BSP (NC + BSP_{400}, with 10% of energy from fat as food and 400 mg/kg BW, BSP infusions as drinking water); (3) high-fat diet (HFD; with 60% of energy from fat as food and distilled water as drinking water); (4) high-fat diet with 200 mg/kg BSP (HFD + BSP_{200}, with 60% of energy from fat as food and 200 mg/kg BW, BSP infusions as drinking water); (5) high-fat diet with 400 mg/kg BSP (HFD + BSP_{400}, with 60% of energy from fat as food and 400 mg/kg BW, BSP infusions as drinking water). The mice were adopted food and distilled water (or BSP infusions) *ad libitum*. During the experiments, body weight and food intake were recorded weekly. With 8th week, insulin tolerance test (ITT) and glucose tolerance test (GTT) were performed. The mice used for GTT were fasted overnight and given glucose intraperitoneally (1.5 g/kg BW), blood glucose was measured with tail vein blood at 0, 30, 60, 90 and 120 min using a GA-3 glucose meter (Sinocare, Changsha, China). Similarly, the mice used for ITT were fasted for 6 h before intraperitoneal injection of human insulin (0.75 units/kg; Novo Nordisk, Bagsvaerd, Denmark). All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University (Approval No: ZJU 20160460).

2.3. Sample collection

At the end of the experiment, faeces were collected and stored at -80 °C for the following gut microbiota analysis. After 12 h of food deprivation, animals were anaesthetized with 5% chloral hydrate, sacrificed by decapitation to collect blood. Serum was obtained using 3K-15 centrifugal machine (Sigma, Germany) at 2500*g* at 4 °C for 15 min, and stored at −80 °C for subsequent biochemical analysis. Epididymal fat, perirenal fat, liver, heart, kidney and spleen were carefully dissected and weighed. A section of liver and epididymal fat were filled in 10 mL 4% neutral formaldehyde at room temperature for Hematoxylin-Eosin (H&E) staining. Specimens of colon (≈5 mm) were enclosed in 10 mL 2.5% glutaraldehyde fixation fluid at room temperature for subsequent transmission electron microscope. The rest of adipose tissue was prepared at −80 °C for inflammatory cytokine determination.

2.4. Biochemical analysis

Plasma insulin concentration was measured using the mouse insulin ELISA kits (JYM0351Mo, Jiyinmei, Wuhan, China), LPS in plasma was determined by commercial ELISA-kits, (Cloud-Clone, Katy, USA), and plasma levels of total cholesterol (T-CHO), triacylglycerol (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and free fatty acid (FFA) were determined using commercially available kits (Jiancheng, Nanjing, China), based on the manufacturer's instructions.

2.5. Histological analysis

Small pieces of epididymal adipose tissue and liver of mice were selected and fixed in 4% neutral formaldehyde solution for 24 h. Then