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Apple Polysaccharide inhibits microbial dysbiosis and chronic inflammation and modulates gut permeability in HFD-fed rats

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ABSTRACT

The saying "An apple a day keeps the doctor away" has been known for over 150 years, and numerous studies have shown that apple consumption is closely associated with reduced risks of chronic diseases. It has been well accepted that dysbiosis is the reflection of various chronic diseases. Therefore, this study investigates the effects of apple polysaccharides (AP) on gut dysbiosis. High-fat diet (HFD) fed rats were treated for 14 weeks with AP. The microbiota composition, microbiota-generated short chain fatty acids (SCFAs), gut permeability and chronic inflammation were analyzed. AP treatment showed higher abundance of *Bacteroidetes* and *Lactobacillus* while lower *Firmicutes* and *Fusobacteium*. AP significantly increased total SCFAs level that contributed by acetic acid and isobutyric acid. Moreover, AP dramatically alleviated dysbiosis-associated gut permeability and chronic inflammation with decreased plasma LBP, up-regulation of Occludin, down-regulation of tumor necrosis factor a (TNF-a), monocyte chemotactic protein 1 (MCP-1), chemokine ligand 1 (CXCL-1) and interleukin 1 beta (IL-1β). The potential mechanism is due to the fact that AP reduces gut permeability, which involves the induction of autophagy in goblet cells. Therefore, AP exerts health benefits through inhibiting gut dysbiosis and chronic inflammation and modulating gut permeability in HFD-induced dysbiosis rats.

1. Introduction

An increasing number studies have shown that apple may decrease the risk of chronic diseases such as cardiovascular disease, cancer and neurodegenerative disorders [1]. In addition, epidemiological studies have linked the consumption of apple with reduced risk of some chronic diseases such as cancer, cardiovascular disease, asthma and diabetes [1,2]. Our previous results showed that apple polysaccharide (AP) suppresses chronic colon carcinogenesis in Azoxymethane/dextran sodium sulfate (AOM/DSS) treated mice *iin vivo*, and inhibits the migration and invasion of colorectal cancer cells induced by LPS *in vitro* [3–5]. However, the scientific basis for the usage of AP has not been well established and underlying mechanisms of action were still not clear.

In recent years, the studies of gastrointestinal microbiota in diseases have been gaining more attention. A well-balanced gut microbiota benefits host health involving energy harvest and absorption, as well as producing short chain fatty acids (SCFAs), maintaining suitable intestinal pH and developing structural and functional gastrointestinal tract (GIT) [6–8]. More importantly, gut microbiota interacts with the host immune system, promoting the maturation of immune cells and the development of the local and systemic immunity [9,10]. However, dysbiosis, dysregulation of the

Abbreviations: AP, apple polysaccharide; HFD, High-fat diet; SCFAs, short chain fatty acids; Bte, Bacteroidetes; Bde, Bacteroides; Lac, Lactobacillus; Fus, Fusbacteium; Fir, Firmicutes; TNF-a, tumor necrosis factor a; MCP-1, monocyte chemotactic protein 1; CXCL-1, chemokine ligand 1; IL-1β, interleukin 1 beta; LPS, lipopolysaccharide; MUC2, Mucin2; RELMβ, resistin-like molecule β; LBP, LPS-binding protein.

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intestinal microbiota homeostasis, leads to a multitude of chronic diseases, including obesity, metabolic syndrome, diabetes, inflammatory bowel disease (IBD), liver disease and colorectal cancer (CRC) [11]. Systematic epidemiologic comparisons between CRC patients and control subjects suggested that CRC case subjects had decreased overall microbial community diversity, with a lower relative abundance of Clostridia and increased carriage of Fusobacterium [12]. As mentioned above, dysbiosis plays an important role in the development of CRC. Thus, we hypothesized that the AP suppresses the colonic carcinogenesis may be due to modulation of the gut dysbiosis. Some evidence supports this hypothesis. Firstly, unlike the human genome, the composition of the commensal gut microbiota is readily changeable. The plasticity of the microbiome may allow a specific or systematic manipulation of certain gut microbiota associated with host diseases [13]. Secondly, the microbiota of the mammalian intestine depends largely on dietary polysaccharide as energy sources, most of which are not degradable by the host. In addition, dietary polysaccharides that reach the colon affect the gut microbial ecology and balance greatly [14].

A growing body of evidence suggests that high-fat diet (HFD) plays an important role in shaping gut microbiota and transforming healthy gut microbiota into a disease-inducing entity [15]. The development of dysbiosis-induced chronic maladies is a complex process involving perturbations of host-microbe interactions. Firstly, there is no doubt that the structure and composition of intestinal microbiota are altered in numerous chronic maladies and growing evidence suggests that these alterations play important roles in disease onset and progression [13,16]. Secondly, SCFAs as the microbial product, exerting important effects on the intestinal barrier integrity and function, are decreased in chronic dysbiosis [17,18]. Thirdly, alterations in the microbiota may disrupt the balance of the host immune system, leading to a chronic state of low-level inflammation and impaired metabolic activities [19–21]. Finally, changes in gut flora composition often correlate with disruptions in the epithelial and mucosal barrier, allowing the translocation of microbial product lipopolysaccharide (LPS) and pathogenic bacteria from the intestinal lumen into circulation [22,23]. Of note, the function of goblet cells directly manages the gut permeability because of the principal component of the intestinal mucus layer, Mucin2 (MUC2), which is mainly produced by goblet cells. A growing body of evidence indicates that MUC2 expression is regulated either at transcriptional or epigenetic level [24]. Activation of transcription factor NF-KB upregulates MUC2 expression *via* MAPK pathway in colon epithelial cells, while PGE2 induces MUC2 expression through the activation of transcription factors CREB/ATF1 mediated by MAPK and P38 pathways [25]. In addition, recent studies have shown that MUC2 expression is regulated by another goblet cell product resistin-like molecule β (RELMβ), which regulates macrophage and adaptive T cell responses during inflammation and induces goblet cell hyperplasia [26–28]. Given the important function of autophagy in numerous secretory pathways, a recent study showed that autophagic process was also required for the secretion of MUC2 granules from goblet cells [29,30]. Overall, the alteration of MUC2 can lead to the destructive colon barrier, which allows the translocation of microbiota and microbial products and systemic chronic inflammation. In this study, we evaluated the composition of microbiota, gut permeability and chronic inflammation to examine the effects of AP on modulating gut flora dysbiosis in HFD-fed rats. In addition, the underlying mechanisms were further explored to interpret the specific beneficial effects of AP.

2. Materials and methods

2.1. Preparation and analysis of AP

Apple polysaccharide was extracted as previously described in our previous paper [3]. Fuji apples (Rosaceae Malus pumila), were purchased from Xianyang (Shanxi, China). Fuji apples with skin were squeezed, then the pomace was used to extract crude AP. Firstly, the pomace was extracted with 98% methanol under reflux for three times to remove alcohol-soluble components, including polyphenols and glycosides, and the supernatant was removed. The residue was then extracted three times with 20 vols of distilled water at 100 °C for 3 h. Subsequently, the three filtrates were combined, concentrated, and precipitated with 70% ethanol for 5 times at 4 °C for 24 h. Secondly, the crude AP was deproteinated by repeated freezing and thawing, and then dialyzed for 72 h (molecular weight cutoff, 5000 d) with distilled water (yield: 5%). The purity of AP was characterized by detecting the content of the total carbohydrate and protein. The total carbohydrate was determined by phenol-H_2SO_4 method using glucose as standard. Protein content was determined with Coomassie brilliant blue reagent and bovine serum albumin as the standard. FT-IR spectra were recorded with a FT-IR spectrometer (FTIR8400S, Shimadzu, Japan). The homogeneity and molecular weight distributions were evaluated by gel filtration chromatography equipped with Ohpak SB-804 chromatographic column (Shodex, Japan), eluting with water at a flow rate of 0.6 ml/min at 30 °C. The column was calibrated by standard dextrans (180, 2500, 7100, 10000, 21400, 41100, 84400, 133800, 2000000 Da) using linear regression. Sample concentration upon injection was 1 mg/ml and 20 μl was injected. The monosaccharide was concluded from the component analysis obtained after acid hydrolysis. The hydrolyzed products were derivatized to be 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives and subsequently analyzed by HPLC with Inertsil® ODS-3 (250 × 4.6 mm, 5 um) column. The mobile phases are 15% acetonitrile containing 20 mM ammonium acetate (mobile phase A) and 40% acetonitrile containing 20 mM ammonium acetate (mobile phase B). The conditions for gradient elution are as follows: 0–10 min, 0–18% mobile phase B, 10–40 min, 18%-50% mobile phase B, with flow rates of 1 ml/min and monitored by UV absorbance at 250 nm and 20 μl was injected.

2.2. Treatments of rats

All experiments were performed in male Sprague–Dawley (SD) rats, weighing 180–220 g, provided by the Animal Center of Shanghai Institute of Medical Sciences. Twenty-four male SD rats received randomly standard chow or high-fat diet (containing 45% fat). After two weeks' feeding, rats fed with HFD were randomized to receive different doses of AP (5% and 10%, [wt/vol], 1 ml/100 g weight) and vehicle (water) by gavage daily for 14 weeks, six rats in each group (Fig. 1A). In addition, HFD was continued during AP treatment. Fecal samples were collected after AP treatment for 2, 8,10,14 weeks, and blood was collected after AP treatment for 10 weeks. Finally, the rats were sacrificed and colon tissues were collected.

2.3. Ethics statement

Animal welfare and experimental procedures were performed according to the international guidelines (Guide for the Care and Use of Laboratory Animals, Commission on Life Sciences, USA). The