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Pectin penta-oligogalacturonide reduces cholesterol accumulation by promoting bile acid biosynthesis and excretion in high-cholesterol-fed mice

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ABSTRACT

Haw pectin penta-oligogalacturonide (HPPS) has important role in improving cholesterol metabolism and promoting the conversion of cholesterol to bile acids (BA) in mice fed high-cholesterol diet (HCD). However, the mechanism is not clear. This study aims to investigate the effects of HPPS on cholesterol accumulation and the regulation of hepatic BA synthesis and transport in HCD-fed mice. Results showed that HPPS significantly decreased plasma and hepatic TC levels but increased plasma high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (apoA-I) levels, compared to HCD. BA analysis showed that HPPS markedly decreased hepatic and small intestine BA levels but increased the gallbladder BA levels, and finally decreased the total BA pool size, compared to HCD. Studies of molecular mechanism revealed that HPPS promoted hepatic ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1 (ABCG1), and scavenger receptor BI (SR-BI) expression but did not affect ATB binding cassette transporter G5/G8 (ABCG5/8) expression. HPPS inactivated hepatic farnesoid X receptor (FXR) and target genes expression, which resulted in significant increase of cholesterol 7α-hydroxylase 1 (CYP7A1) and sterol 12α-hydroxylase (CYP8B1) expression, with up-regulations of 204.2% and 33.5% for mRNA levels, respectively, compared with HCD. In addition, HPPS markedly enhanced bile salt export pump (BSEP) expression but didn't affect the sodium/taurocholate co-transporting polypeptide (NTCP) expression. In conclusion, the study revealed that HPPS reduced cholesterol accumulation by promoting BA synthesis in the liver and excretion in the feces, and might promote macrophage-to-liver reverse cholesterol transport (RCT) but did not liver-to-fecal RCT.

1. Introduction

Pectin is a complex hetero-polysaccharide founded in the cell wall of many plants, and is comprised of linear chain of α-1, 4 glycosidic linked galacturonic acid residues [1]. Natural pectin had been extracted from a variety of sources including Brazilian cupuassu [2], broccoli, tomatoes and carrots [3], citrus peel and apple pomace [4], hawthorn [5], etc. Extracted pectin had been widely used as a thickener in pastries, a gelling agent in jams and jellies, as well as a stabilizer in fruit juices and milk drinks in the food industries [6,7]. Furthermore, as a soluble dietary fiber, pectin had shown various biological activities. It could prevent diarrhea as a prebiotic, prevent constipation against colon cancer by promoting

Abbreviations: HPPS, haw pectin penta-oligogalacturonide; HCD, high-cholesterol diet; SD, standard diet; TG, Triglyceride; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; BA, bile acids; CYP7a1, Cholesterol 7α-hydroxylase 1; CYP8b1, sterol 12α-hydroxylase; FXR, farnesoid X receptor; SHP, short heterodimer partner; LRH-1, liver receptor homolog-1; LXRα, liver X receptor alpha; NTCP, sodium/taurocholate co-transporting polypeptide; solute carrier family 10, member 1; BSEP, bile salt export pump; RCT, reverse cholesterol transport; ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; SR-BI, scavenger receptor BI; ApoA-1, apolipoprotein A-I; ABCG5/8, ATP-binding cassette transporters.

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intestinal peristalsis, and also reduce the risk of cardiovascular disease by binding cholesterol and BA to promoting their excretion in the feces [8,9]. In our previous study, the pectin was extracted from the hawthorn (*Crataegus pinnatifida Bunge*) fruit, which belongs to the rose family, and found that the pectin content in hawthorn was higher than other cultivated fruits. Meanwhile, the haw pectin possess a superior viscosity, which is 4–6 times higher than that of commercial available lemon and apple pectin [10]. Functional research found that haw pectin exhibited good effects in antioxidant and improving lipid metabolism [11,12]. However, the larger molecular mass and superior viscosity of haw pectin restricted its application.

The pectin-derived oligosaccharides were considered "novel functional carbohydrates" [13]. In our previous study, treated by pectinase for 4 h, the crude haw pectin hydrolysates could significantly lower the total serum cholesterol and triglyceride levels in hyperlipidemic mice [14]. The results showed that the haw pectin oligosaccharides had great prospects in the development of functional food that would benefit human health. Further, haw pectin oligosaccharides were fractionated and yielded 10 oligosaccharides with DP 2 to 11 on a DEAE-Sephadex A-25 column chromatograph. Finally, the pure and highest yield fraction, haw pectin penta-oligogalacturonide (HPPS), was isolated [15]. Animal experiments showed that HPPS significantly reduced cholesterol accumulation in blood and tissue of high-cholesterol-fed mice, but the mechanism was unclear.

The conversion of cholesterol to bile acids plays a vital role for elimination of cholesterol, which is one of the main factors regulating cholesterol homeostasis in the body [16]. There are two pathways in bile acid biosynthesis, one is the classic pathway (main pathway) and CYP7A1 is the rate-limiting enzyme in regulating the conversion of cholesterol to bile acid, and the other is alternative pathway which is regulated by CYP8B1 [17]. Moreover, reverse cholesterol transport (RCT) is also an important pathway for cholesterol homeostasis, in which cholesterol is transported from peripheral macrophages to the liver for conversion to bile acids or excretion into bile and eventually feces [18,19].

Therefore, in this study, we adjusted the diet composition and animal experimental design on the basis of our previous experiments, and investigated the effect of HPPS on RCT-related transporters, BA synthesis and transport in the liver of HCD-fed mice. This could include the gene expression of RCT-related transporters, and the nuclear receptors that regulate BA synthesis, such as FXR, SHP, LRH, LXRα, together with BA synthesis genes CYP7A1 and CYP8b1, and BA transporters NTCP and BSEP.

2. Materials and methods

2.1. Materials

Fresh haw fruit (*Crataegus pinnatifida* Bunge. var. Major) was purchased from a farm on the outskirts of Shenyang (Liaoning, China). Haw pectin prepared by hot water extraction from haw fruit was treated by pectinase for 2 h, and then the hydrolysate was subjected to ultra- and nanofiltration to obtain HPPS [15].

2.2. Animals management

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Shenyang Pharmaceutical University (SYXK-L-2010-0009).

A total of 36 male Kunming mice (six weeks old and initial body weight 25–30 g) were obtained from the HFK Bioscience Co., Ltd. (Beijing, China). Upon arrival in the animal laboratory, the animals were housed individually in screen-bottomed, stainless steel cages and fed a standard commercial diet (Beijing HFK Bioscience Co., Ltd.) with controlled temperature (23 ± 1 °C) and humidity (60± 5%), under a 12-h light-dark cycle. After an adaptation period of 7 days, animals were divided into normal control, high-cholesterol control and HPPS groups (n = 12 each) according to their average body weight. The normal control group was fed a standard diet (SD) and the high-cholesterol control group was fed the high-cholesterol diet (HCD) for 6 weeks. The HPPS group was fed the HCD for 2 weeks, and then was fed HCD and additional daily alimentary supplementation with HPPS (300 mg/kg body weight) for 4 weeks. The composition of the experimental diet was shown in Table 1. All groups were treated by oral infusion with the same volume of water (SD and HCD groups) or HPPS solution daily. Food and drinking water were supplied ad libitum in the whole experimental period. Food intake and body weight were recorded once per day. Feces were collected daily and freeze-dried into powder for further analysis.

At the end of the experiment, animals were sacrificed under deep anesthesia with isoflurane (AMRESCO, USA) after fasting for 12 h. Blood was collected from orbital sinus, placed in EDTA K3 tubes at 37 °C for 30 min, and then centrifuged at 3000 rpm for 15 min at 4 °C. After laparotomy, gallbladder, liver and small intestine were removed, snap-frozen in liquid nitrogen, and stored at −80 °C for analysis.

2.3. Lipids and BA analysis

TC, TG and HDL-C levels in plasma, liver or feces were determined as previously described [20,21].

The total BA pool size was determined as the total BA content of the intact gallbladder, the whole liver tissue and small intestine tissue. Gallbladder, Liver and small intestine tissue were minced and BA were extracted in ethanol as described by Song et al. [22] with some slight modifications. Briefly, freeze-dried tissues were immediately ground to powder with liquid nitrogen in a mortar. Powder samples (0.2 g) were placed in a glass breaker filled with 100 ml of ethanol. The BA were extracted in breakers on a multi-position hot plate and covered with a watch glass until the volume of ethanol was reduced to approximately 30 ml. After removed and cooled breakers, the samples were filtered into 100-ml volumetric flasks. After rinsed 3 times with ethanol, each volumetric flask was diluted with ethanol to 100 ml. The total BA were determined with a SmartSpec Plus spectrophotometer (Bio-Rad) using a Total Bile Acid Assay Kit according to the manufacture's instructions (GenMed Scientifics Inc. USA; GMS70019).

2.4. Isolation of total RNA and real-time PCR

The total RNA from the mice liver samples was isolated using TRIZOL reagents following manufacturer's instruction (Invitrogen, Carsbad, CA, USA), and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. RNA concentration was determined by spectrophotometry at 260 nm, and the 260/280 nm absorbance ratios of all samples ranged from 1.9 to 2.0, indicating a satisfactory

Table 1
Composition of the experimental diets.

Ingredients	Standard diet (%)	High-cholesterol diet (%)
Chow diet	97.6	82.6
Lard	5	5
Cholesterol	0.4	2
Yolk powder	0	5
Sodium cholate	0	0.2
Propylthiouracil	0	0.2