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Role of plant stanol derivatives in the modulation of cholesterol metabolism and liver gene expression in mice

Wen-Sen He, Mei-Gui Wang, Xiao-Xia Pan, Jing-Jing Li, Cheng-Sheng Jia *, Xiao-Ming Zhang, Biao Feng *

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, Jiangsu, China

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ABSTRACT

The present study was to evaluate the cholesterol-lowering effect of two novel plant stanol derivatives and its potential molecular mechanism in hyper-cholesterol mice induced by a high-cholesterol diet. Results showed that oral administration of plant stanyl hemisuccinate (2×, 5×) and plant stanyl sorbitol succinate (2×, 5×) effectively attenuated the serum total cholesterol and low density lipoprotein cholesterol levels, while had no effect on the serum triacylglycerol and high density lipoprotein cholesterol. And plant stanol derivatives decreased liver cholesterol concentration and increased faecal cholesterol output. Meanwhile, both plant stanyl hemisuccinate and plant stanyl sorbitol succinate could remarkably promote liver X receptor alpha (LXRα) expression, and increased cholesterol 7α-hydroxylase (CYP7A1) expression and faecal total bile acid output to varying degrees. These results suggested two novel plant stanol derivatives possessed hypocholesterolemic effect, and the cholesterol-lowering action of plant stanol derivatives may be through activating the potential LXRα-CYP7A1-bile acid excretion pathway.

1. Introduction

Atherosclerosis and its related complications, such as cardiovascular diseases, were the major causes of morbidity and mortality worldwide. Hypercholesterolemia was a major risk factor for atherosclerosis and cardiovascular diseases (He et al., 2011; Smet, Mensink, & Plat, 2012). Therefore, the development of strategies for reducing the serum cholesterol concentrations has generated considerable interest in combatting hyperlipidemia-associated cardiovascular diseases.

Plant sterols were generally extracted from the deodorizer distillates produced during vegetable oil refining. Recently, the research and development of plant sterols and its derivatives has been attracting much attention due to their strong beneficial properties, mainly including cholesterol-lowering effects (Brufau, Canela, & Rafecas, 2008), anti-cancer property (Bradford & Awad, 2007), anti-inflammatory (Rudkowska, 2010) anti-atherogenic (Tan, Le, Moghadasian, & Shahidi, 2012), antipyretic (Rudkowska, 2010) and antioxidant activities (Tan & Shahidi, 2012). Plant stanols (phytostanols), consisting of sitostanol and campestanol, were the less abundant hydrogenated counterparts of plant sterols and had similar biological activities to plant sterols. In contrast, plant stanols were more resistant to oxidation than plant sterols. However, the practical application of plant stanols was greatly restricted by their poor solubility in oil and insolubility in water. Currently, numerous studies have been focused on the chemical modification of plant sterols/stanols in the presence of enzymes (He et al., 2010), chemical catalysts (Zhou et al., 2012) or ionic liquids (Yang et al., 2012) to improve their solubility in oil. Lately, hydrophilic plant stanol derivatives were firstly prepared via chemo-enzymatic way in our previous study (He et al., 2012), which opened an emerging area in the research and development of plant sterols.

The cholesterol-lowering effects of plant sterols and stanols were well established in both human and animal models and shown to be safe for half a century (Calpe-Berdiel, Escolà-Gil, & Blanco-Vaca, 2009; Chen, Jiao, & Ma, 2008; Smet et al., 2012). Our previous studies have demonstrated that plant steryl/stanyl laurate and β-sitosteryl conjugated linoleate prepared in our lab possessed cholesterol-lowering effects in mice (He et al., 2011; Li et al., 2010). Other researchers also reported that plant sterol and stanol esters supplementation reduced serum cholesterol in apoE deficient mice (Tan et al., 2012). However, the cholesterol-lowering mechanism of plant sterols and their derivatives was poorly understood. There were several suggested mechanisms for cholesterol-lowering of plant sterols and stanols over the years (Smet et al., 2012). In the 1960s, it was believed that plant sterols and stanols competed with dietary cholesterol for incorporation into mixed micelles. Since then, it has also been suggested that plant sterols and stanols interfered with the incorporation of cholesterol into chylomicrons. Currently, it was supposed that multiple genes involved cholesterol homoeostases were regulated by plant sterols and stanols.

* Corresponding authors. Tel.: +86 510 85913669; fax: +86 510 85884496.
E-mail addresses: chshjia@126.com (C.-S. Jia), bfeng@jiangnan.edu.cn (B. Feng).

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Cholesterol homoeostases was affected by several factors in different metabolic pathways that involved absorption, synthesis, clearance and excretion (Wang et al., 2010). The processes of cholesterol synthesis and clearance mainly involved five proteins and enzymes, sterol regulatory element binding protein 2 (SREBP-2), liver X receptor alpha (LXRα), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), low density lipoprotein receptor (LDLR) and cholesterol 7α-hydroxylase (CYP7A1), which was rich in liver (Liang et al., 2011). In detail, HMGCR was the rate-limiting enzyme in cholesterol synthesis and LDLR was responsible for the removal of LDL cholesterol from blood whereas CYP7A1 was the rate-limiting enzyme in bile acid synthesis for cholesterol elimination (Chen et al., 2008; Zhang et al., 2009). SREBP-2 governed the activation of the transcription for LDLR and HMGCR, whereas LXRα regulated the transcription of CYP7A1 (Liang et al., 2011). The objective of the present study was to evaluate the cholesterol-lowering effect of two novel plant stanol derivatives and the potential cholesterol-lowering mechanism by exploring their effects on the gene expression of hepatic SREBP-2, LXRα, HMGCR, LDLR and CYP7A1.

2. Materials and methods

2.1. Materials

Mice, experimental diets and sawdust bedding were purchased from Slac Animal Co., Ltd. (Shanghai, China). Total cholesterol (TC) enzymatic kits, triacylglycerol (TAG) enzymatic kits, high density lipoprotein cholesterol (HDL-C) kits, low density lipoprotein cholesterol (LDL-C) kits, alanine aminotransferase (ALT) kits and aspartate aminotransferase (AST) kits were purchased from FengHui Medical Science & Tech. Co., Ltd. (Shanghai, China). Lipozyme RM IM (lipase from *Rhizomucor miehei*, immobilised on an anionic exchange resin) was obtained from Novo Nordisk Co., Ltd. (Shanghai, China). Plant sterols were generous gifts from Jiangsu Spring Fruit Biological Products Co., Ltd. (Taixing, China). Sucrose esters (HLB 13) were generously provided by Hangzhou Jinhelai Food Additive Co., Ltd. (Hangzhou, China). Cholesterol and all other chemicals and solvents used were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade. Pork oil was obtained from local markets.

2.2. Preparation and separation of plant stanol derivatives

Plant stanols and plant stanyl hemisuccinate (PSH) were prepared as previously described (He et al., 2010, 2012). Plant stanyl sorbitol succinate (PSS) was prepared by esterification of PSH with D-sorbitol in the presence of lipozyme RM IM in *tert*-butanol (He et al., 2012). PSH and PSS were purified by silica gel column chromatography. The isolated PSH and PSS were used as test compounds. The compounds were analysed by high performance liquid chromatography prior to use. The purity of PSH was above 99% (76% sitostanyl hemisuccinate and 23% campestanyl hemisuccinate). And the purity of PSS was above 99% (69% sitostanyl sorbitol succinate and 30% campestanyl sorbitol succinate).

2.3. Diets, animals and groups

Regular rodent chow was provided by the Slac Animal Co., Ltd. (Shanghai, China). The control diet was prepared by mixing all powdered ingredients (g/kg): flour, 302; corn powder, 231; soybean meal, 218; pork oil, 100; fish meal, 45; premix, 36; clover fodder, 27; plant oil, 18; wheat bran, 13; cholesterol, 10. The control diet contained a trace amount of plant sterols and plant stanols (<0.01%), which were insignificant compared with the given dose of plant stanol derivatives.

Eighty-four male 6-week-old Kun Ming mice (Slac Animal Co., Ltd., Shanghai, China), weighing 20–22 g, were housed in polypropylene cages (6 per cage) in a room controlled at 25 ± 1 °C and 50 ± 10% humidity with a 12-h light/dark cycle. Experiments were approved (permission number: 2120936) and conducted in accordance with the guidelines set by the animal experimental ethical committee, Jiangnan University.

All animals were given regular rodent chow with free access to food and water. After 1 week of adaptation, the mice were weighed and randomly divided into seven groups (n = 12/group). And each group was fed the control diet. In the control group, the mice were treated with sucrose esters solutions. In the treatment group, the mice were orally administered with different doses of PSS (PSS 1×, 221.2 mg/kg d; PSS 2×, 442.5 mg/kg d; PSS 5×, 1106.2 mg/kg d) and PSH (PSH 1×, 167.9 mg/kg d; PSH 2×, 335.8 mg/kg d; PSH 5×, 839.4 mg/kg d) dissolved in sucrose esters solutions respectively, once a day for four consecutive weeks.

2.4. Animal experiment and sample collection

During the 4-week experimental period, the mice were allowed free access to food and drinking water. The fresh water and diets were given to the mice daily, and uneaten food was discarded. Food intakes were measured daily, body weight was recorded and sawdust bedding was changed twice a week. The faeces from each group were also collected once a week and saved. At the end of experimental period, the mice were kept fasting overnight, then weighed and sacrificed. The fasting blood glucose levels were measured in blood collected from the tip of the tail using a glucose metre (OneTouch Ultra; Johnson & Johnson, Shanghai, China). Blood was obtained from retro-orbital sinus, centrifuged at 8000 rpm for 15 min, and serum was isolated. Liver was dissected, washed with saline, blotted on filter paper, weighed and frozen in liquid nitrogen. Heart, kidney, spleen and lung were also collected. All samples were stored at −80 °C prior to analysis.

2.5. Serum lipids and hepatic biochemical parameters

Serum lipids were measured as we previously described (He et al., 2011). In brief, serum TC, HDL-C, and LDL-C were measured with the corresponding test kits (FengHui Co., Ltd., Shanghai, China) using an automatic FH-400 biochemical analyser (FengHui Co., Ltd., Shanghai, China). The toxicity of two novel plant stanol derivatives was evaluated by measuring serum concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with the corresponding test kit (FengHui Co., Ltd., Shanghai, China) using an automatic FH-400 biochemical analyser (FengHui Co., Ltd., Shanghai, China).

2.6. Liver cholesterol, liver and serum stanols

Liver cholesterol, liver and serum stanols were extracted using the previous method with minor modification (He et al., 2011). Briefly, liver samples were firstly homogenised in saline. The liver homogenate or serum was extracted with chloroform/ethanol (2/1, v/v) to a final volume of 20 times (0.5 g in 10 mL of solvent mixture) in a 15-mL screw-capped vial. The vial was fully shaken and placed in a water-bath at 60 °C for 2 h. The extract was shaken, then centrifuged at 5000 rpm for 10 min, and the supernatant was collected. A portion of supernatant was taken out, evaporated under a nitrogen stream in an Eppendroff tube, saponified at 70 °C for 2 h in ethanolic potassium hydrate and evaporated under a nitrogen stream. The samples were re-dissolved in water, neutralised by adding hydrochloric acid and extracted with chloroform.