Journal of Traditional and Complementary Medicine 5 (2015) 56–61

HOSTED BY

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Short communication

Polysaccharide gel coating of the leaves of *Brasenia schreberi* lowers plasma cholesterol in hamsters

Hyunsook Kim [a, b], Qian Wang [c], Charles F. Shoemaker [d], Fang Zhong [c], Glenn E. Bartley [e], Wallace H. Yokoyama [e, *]

[a] *Department of Nutrition, University of California, Davis, CA, USA*
[b] *Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, South Korea*
[c] *Department of Food Science, Jiangnan University, Wuxi, China*
[d] *Department of Food Science and Technology, University of California, Davis, CA, USA*
[e] *USDA, ARS, Western Regional Research Center, Albany, CA, USA*

ARTICLE INFO

Article history:
Received 18 June 2013
Received in revised form
9 March 2014
Accepted 14 May 2014
Available online 17 December 2014

Keywords:
Brasenia
cholesterol
gene expression
hamster
polysaccharide

ABSTRACT

Brasenia schreberi (蓴菜 chún cài) is an invasive aquatic weed found in the USA, but the plant has economic value in Asia where it is cultivated for food. The young leaves of *B. schreberi* are coated with gelatinous water-insoluble mucilage. This mucilage is a polysaccharide composed of galactose, mannose, fucose, and other monosaccharides. Because some carbohydrate gels are hypocholesterolemic, we evaluated their cholesterol-lowering properties in male hamsters fed hypercholesterolemic diets containing 2% gel coat from *B. schreberi* (GEL), or 1% cholestyramine (CA), or 5% hydroxypropyl methylcellulose (HPMC), and compared them to 5% microcrystalline cellulose (control) for 3 weeks. We found that very-low-density lipoprotein-, low-density lipoprotein-, and total-cholesterol concentrations in plasma were significantly lowered by GEL, CA, and HPMC compared to control. High-density lipoprotein-cholesterol concentration was lowered by CA and HPMC. Body weights and abdominal adipose tissue weight of GEL and control group animals were greater than those of the CA and HPMC groups. Fecal lipid excretion was greater in the CA and HPMC groups than in the control group. Expression of hepatic CYP51 and CYP7A1 mRNA was upregulated by CA, HPMC, and GEL, indicating increased hepatic cholesterol and bile acid synthesis. Expression of low-density lipoprotein receptor mRNA was upregulated by all treatments. These results suggest that modulation of hepatic expression of cholesterol and bile acid metabolism-regulated genes contributes to the cholesterol-lowering effects of GEL.
Copyright © 2015, Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. All rights reserved.

1. Introduction

The perennial water plant *Brasenia schreberi* (蓴菜 chún cài), called the watershield in the USA and junsai in China and Japan, produces a mucilage coating that covers the underwater leaves and stems. In the USA, *B. schreberi* blocks waterways and is considered a nuisance invasive species, although plant specialists believe that it is not an imported or exotic plant.[1] In Asia, it is considered a vegetable, and is cultivated and traded. The mucilage coating has been reported to have antialgal and antibacterial properties, although studies of its allelopathic, antialgal, and antibacterial properties were conducted on extracts from the whole plant.[1] The mucilage consists of a gelatinous polysaccharide coating composed of about 32–40% D-galactose, 19–29% D-glucuronic acid, 13–16% L-fucose, 10–14% D-mannose, and other monosaccharides.[2] Mannose is believed to form the backbone of the polymer, and galactose forms its side chains. The acidic polymer can be extracted with hot water or alkali, but subsequently loses its ability to form gels. When used as a food, the leaves with the gel coating are served in cool soups to preserve its gel texture. Mannan-based polysaccharides such as glucomannan (konjac root) and galactomannan (fenugreek, guar gum, and locust bean gum) have been shown to lower blood cholesterol,[3] suggesting that *B. schreberi* polysaccharide may also lower plasma cholesterol.

Syrian hamsters are widely used as animal models of plasma and liver cholesterol modulation by diet.[4] Plasma cholesterol lowering by dietary intake of psyllium,[5] cholestyramine (CA),[6]

* Corresponding author. USDA, ARS, Western Regional Research Center, PFR, 800 Buchannan Street, Albany, CA 94710, USA.
E-mail address: Wally.Yokoyama@ars.usda.gov (W.H. Yokoyama).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2014.10.003
2225-4110/Copyright © 2015, Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. All rights reserved.

guar,[7] oat and barley beta-glucans,[8] and hydroxypropyl methylcellulose (HPMC)[9] have been studied in hamsters. Cholesterol synthesis in the livers of male hamsters and male humans are low compared to other rodents[10] or female hamsters and humans. The sensitivity to dietary cholesterol-lowering foods or food components is facilitated by high initial plasma cholesterol levels. Dietary cholesterol can increase plasma cholesterol levels but also lowers hepatic cholesterol synthesis. Decreasing hepatic cholesterol synthesis in male hamster has little effect on plasma cholesterol because of the lower hepatic cholesterol synthesis, whereas more dietary cholesterol is required to increase plasma cholesterol in rats or mice. Hamsters also have identical primary bile acids to humans. Therefore, we selected a male Syrian hamster fed a hypercholesterolemic diet as the animal model to determine the effects of feeding the gel from *B. schreberi*.

2. Materials and methods

2.1. Animal care

Male golden Syrian hamsters (50–60 g, LVG strain; Charles River, Wilmington, MA, USA) were housed individually in wire-bottomed cages in an environmentally controlled room (20–22°C, 60% relative humidity, 12-hour alternating light/dark cycle). The hamsters were fed 5001 rodent diet (LabDiet; PMI International, Redwood, CA, USA; protein, 239 g/kg; fat, 50 g/kg; non-nitrogenous substances, 487 g/kg; crude fiber, 51 g/kg; ash, 70 g/kg; energy, 17 MJ/kg; and sufficient amount of minerals and vitamins for healthy maintenance) *ad libitum* for 1 week prior to feeding the experimental diets to acclimatize them to the new environment. Hamsters were weighed and randomized into four groups of nine to 10 hamsters each and were fed high-fat diets *ad libitum* for 4 weeks. The total dietary fiber content and cholesterol content of all diets were 5% and 0.1%, respectively. Dietary fiber composition of the diets were as follows: 5% microcrystalline cellulose (MCC) in the control diet, 5% HPMC (The Dow Chemical Company, Midland, MI, USA) in the HPMC diet, 1% CA and 4% MCC in the CA diet, and 2% *B. schreberi* polysaccharide and 3% MCC in the *B. schreberi* polysaccharide (GEL) diet. Diets contained 17% of energy as protein, 43% as carbohydrate, and 39% as fat (Table 1). Body weights were recorded weekly, and food intake was monitored twice per week. The study was approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA, USA.

2.2. Polysaccharide composition of B. schreberi gel

B. schreberi leaves were collected from a lake in the Sierra Nevada foothills of California. The gel coating surrounding the leaves was stripped off by hand, centrifuged to separate leaf fragments from the gel and freeze-dried for the feeding study. The monosaccharide composition of the gels was analyzed by the method of gas chromatography. Briefly, a sample of the freeze-dried gel was hydrolyzed by 2M trifluoroacetic acid for 1.5 hours at 115°C. The hydrolysate was dried, and water was added to drive off any acidic residues once more. Inositol was added as the internal standard. The aldehyde hydrolysates were reduced to their respective alcohols with hydroxylamine in pyridine and reacted with acetic anhydride to form the acetate derivative. The derivatized monosaccharides were analyzed by injecting 0.8 μL into a 30M Agilent J&W DB-1701 column (Santa Clara, CA, USA) on a gas chromatograph (GC-14A; Shimadzu, Kyoto, Japan). The temperature program was 120°C (2 minutes), ramped to 175°C at 10°C/minute, and ramped to 240°C at 3°C/minute and held for 10 minutes. The flame ionization detector (FID) was kept at 260°C. Standard curves of peak area and monosaccharide standards were determined for quantitation. Molecular weight of the watergel polymer was about 1.7×10^6 Da, as determined by multiple-angle laser light scattering (Wyatt Technologies, Santa Barbara, CA, USA). Alduronic acids were determined by the sulfuric acid carbazole method. The dried gel contained 55.2% polysaccharides, 7.34% H_2O, 7.26% protein, and 13.94% ash.

Table 1
Diet composition.[a]

Ingredients/diet type	Control	Gel	CA	HPMC
MCC	52.6	31.6	42.1	0
HPMC	0	0	0	52.6
Watergel	0	21.0	0	0
Cholestyramine	0	0	10.5	0
Total fat (%)	20.0	20.0	20.0	20.0
Total protein (%)	20.0	20.0	20.0	20.0
Total carbohydrate (%)	49.8	49.8	49.8	49.8
HPMC (%)	0	0	0	5.0
Total dietary fiber (%)	5.0	5.0	5.0	5.0

CA = cholestyramine; HPMC = hydroxypropyl methylcellulose; MCC = microcrystalline cellulose.

[a] All diets contained anhydrous butter fat, 80.0 g; corn oil, 100.0 g; fish oil, 20.0 g; cholesterol, 1.0 g; casein, 221.9 g; DL-methionine, 3.0 g; choline bitartrate, 3.0 g; AIN-93 mineral mix, 35.0 g; AIN-93 vitamin mix, 10.0 g; and corn starch, 553.3 g.

2.3. Plasma and tissue collection

Hamsters were feed deprived for 12 hours and anesthetized with a mixture of Isoflurane (Phoenix Pharmaceutical, St. Joseph, MO, USA) and oxygen. Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% w/v), and plasma was separated after centrifugation at 2000 × *g* for 30 minutes at 4°C. Livers were excised, weighed, immediately frozen in liquid nitrogen, and stored at −80°C for analysis.

2.4. Plasma and liver lipid analysis

Cholesterol in plasma lipoproteins was determined by size-exclusion chromatography as previously described.[11] Plasma triglycerides were determined by enzymatic colorimetric assays (Genzyme Diagnostics PEI Inc., Charlottetown, PE, Canada). Lyophilized, ground liver samples were extracted using an automated solvent extractor (Dionex, Sunnyvale, CA, USA) at 100°C and ~13.8 MPa with 3:1 hexane:2-propanol (method A) or at 125°C and 6.7 MPa with 3:2 hexane:2-propanol (method B). The lipid extracts were analyzed for hepatic triglycerides (Genzyme Diagnostics PEI Inc.), total cholesterol, and free cholesterol by enzymatic methods using kits (Wako Chemicals, Richmond, VA, USA). Feces were collected for 3 consecutive days immediately prior to sacrifice, lyophilized, milled, and stored at −20°C. Fecal lipids were determined by weighing after solvent extraction at 100°C and 13.8 MPa with 3:1 hexane:2-propanol.

2.5. Real-time polymerase chain reaction

Total RNA from livers was extracted using a TRIzol Plus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA, USA), and cDNA was synthesized using GeneAmp RNA polymerase chain reaction (PCR) kit (Applied Biosystems, Foster City, CA, USA) as per manufacturer's protocol. Approximately 1 μL of diluted cDNA (1:10) was used in each real-time PCR reaction carried out using SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX, USA). The cycle conditions were as follows: 5 minutes at 95°C, followed by 20–35 cycles of incubation at 94°C for 15 seconds, and then at 55–60°C for 1 minute and 72°C for 30 seconds. Sequences of the primers used for

Download English Version:

https://daneshyari.com/en/article/3099711

Download Persian Version:

https://daneshyari.com/article/3099711

Daneshyari.com