Amaranth squalene reduces serum and liver lipid levels in rats fed a cholesterol diet

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ABSTRACT

In this study, the hypocholesterolaemic effect of amaranth grain, oil and squalene are examined. In experiment 1, rats are given a semi-purified diet containing 1% (w/w) cholesterol for four weeks and either amaranth grain (AG; 300 g/kg) or amaranth oil (AO; 90 g/kg) substituted in experimental groups. Both AG and AO lowered serum and hepatic cholesterol and triglyceride levels. Faecal excretion of cholesterol and bile acid in the AO group increased, while AG affected only bile acid excretion. In experiment 2, rats were fed the cholesterol diet for four weeks and injected (i.p.) with saline (control), amaranth squalene (AS) or shark liver squalene (SS, 200 mg/kg) for seven days. The hypolipidaemic effects of AS were evident in both serum and liver. In addition, AS markedly increased faecal excretions of cholesterol and bile acid, and slightly inhibited 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. In contrast, none of these effects were observed in the SS group. This preliminary study suggests that the cholesterol-lowering effect of AS may be mediated by increased faecal elimination of steroids through interference with cholesterol absorption, and that different sources of squalene (plant versus animal) may affect cholesterol metabolism differently.

KEY WORDS: Amaranth. Bile acids and salts. Cholesterol.

Introduction

Amaranth is an old crop from south and central Asia that has exceptional nutritional value. Its protein content includes a high concentration of essential amino acids (especially lysine and sulphur amino acids) that are limited in other crops.[1] In addition, compared to other cereals, amaranth contains high levels of calcium, iron and sodium, [2] and also more soluble fibre.[3-5] Importantly, it is reported to exhibit a hypocholesterolaemic effect.[4,6]

Amaranth has been suggested as an alternative to marine sources of squalene. The amaranth oil (AO) contains extremely high amounts (6--8%) compared to other cereals (0.01–0.3%) or olive oil (0.1–0.7%) – a good source of plant squalene.[3]

Squalene, a precursor of cholesterol, is absorbed in humans and can enhance cholesterol synthesis and plasma cholesterol concentrations.[7] However, since it is also thought to inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase,[8] the effect of squalene is controversial. The aim of the present study is to determine the cholesterol-lowering effect of amaranth oil and squalene in hypercholesterolaemic rats.

Materials and methods

Extraction of amaranth squalene

Amaranth grain (AG) was extracted with hexane, then degummed by storing at 0°C for 72 h. Squalene was obtained by silicagel column chromatography using a method described previously,[9] and purity was confirmed by thin-layer chromatography (TLC; data not shown) with shark liver squalene (Sigma, St. Louis, MO, USA) as a standard. The structure of the amaranth squalene was analysed by $^{1}H/^{13}C$ nuclear magnetic resonance (NMR) and electron impact mass spectrometry (EI-MS).

Animals and diets

Male Sprague-Dawley rats (110–130 g) were maintained in standard environmental conditions and fed a semipurified diet containing 1% cholesterol for four weeks to induce hypercholesterolaemia.

In experiment 1, AG (300 g/kg diet) and amaranth oil (AO; 90 g/kg diet) were substituted for a portion of control diet, and other ingredients were modified according to the composition of AG[10] to provide the same percentages of carbohydrate, fat, protein, fibre and mineral between the diet groups (Table 1). In experiment 2, rats were fed the cholesterol diet for four weeks, and saline (control), amaranth squalene (AS) or shark liver squalene (SS) were injected (200 mg/kg, i.p.) for seven days prior to sacrifice.

Diet and water were given *ad libitum*. Faecal samples were collected for the last three days for analysis of steroid excretion. The experimental procedures followed the guidelines provided by the Animal Care and Use Committee of Korea University.

Serum and liver lipids

Serum concentrations of total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglyceride were measured enzymatically using commercially available kits (Yeongdong, Korea). Hepatic lipids were extracted using a procedure described previously.[10] The dried lipid residues were dissolved in 1 mL ethanol for cholesterol and

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Table 1. Composition of diets.

Ingredients	Group		
	Control (g/kg)	Amaranth grain (g/kg)	Amaranth oil (g/kg)
Casein	200	160	200
DL-Methionine	3	3	3
Corn starch	457	240	457
Sucrose	150	150	150
Corn oil	90	64	–
Cholesterol	10	10	10
Cellulose	40	30	40
Mineral mix*	35	28	35
Vitamin mix*	10	10	10
Choline bitartarate	2	2	2
Na-taurocholate	3	3	3
Amaranth	-	300	-
Amaranth oil	-	-	90

*AIN-76 diet (American Institute of Nutrition 1977)

triglyceride determination. Hepatic total cholesterol and triglyceride were measured using the kits described above.

HMG-CoA reductase activity

Liver microsomes were isolated using a method described previously,[11] with slight modification. HMG-CoA reductase activity was assayed from the measurement of released coenzyme A during the reduction of HMG-CoA to mevalonate.[11] Microsomal protein concentrations were determined using Lowry's method.[12]

Faecal steroids

Faecal steroids were assessed by cholesterol and bile acid excretion in faeces. After extracted with ethyl ether, cholesterol was measured by the same method used for the liver samples. Bile acid was assayed from the enzymatic method described by Bruusgaard[13] in ethanol extracts of faeces.

Statistical analysis

Data were expressed as mean ± SE. One-way ANOVA was used to determine treatment effects. Differences among means were inspected using Duncan's multiple range test and were considered to be significant at $P < 0.05$.

Results

Hypocholesterolaemic effect of amaranth grain and oil

Body weight gain of rats fed AG showed a decrease of 66%, with only a 16% decrease in food intake. This resulted in a 50% decrease in the food efficiency ratio (FER). Consumption of AG significantly ($P<0.05$) reduced the levels of serum cholesterol and triglyceride compared with control group, and the effects observed in the AO group were more pronounced.

Concentration of serum HDL-cholesterol was not affected by AG consumption whereas AO consumption significantly increased (56%) HDL-cholesterol level. The ratios of HDL to total cholesterol showed similar increases in both groups. Therefore, greater hypocholesterolaemic and anti atherogenic effects were observed in the AO group than in the AG group.

HMG-CoA reductase activity was not altered significantly by either AG or AO intake (data not shown). Hepatic cholesterol and triglyceride levels were reduced in both the AG and the AO group. Faecal dry weight was greater in the AO group compared with AG and control groups. In addition, faecal excretions of cholesterol and bile acid were increased with AO consumption, but only bile acid excretion was affected by AG ($P<0.05$).

Effect of amaranth squalene on hypercholesterolaemia

The structure of extracted AS was confirmed by $^{1}H/^{13}C$-NMR and EI-MS, by comparison with SS (Sigma). Standard squalene showed m/z at 410, and major peaks were seen at 69 and 137. AS showed m/z at 410 and major peaks were seen at 69 and 136 (data not shown). From the spectral data, extracted component was confirmed as 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane (squalene; $C_{30}H_{50}$).

Treatment with AS had a significant effect on blood and hepatic lipid profiles. Serum concentrations of cholesterol and triglyceride were decreased by 22% and 14%, respectively, and hepatic levels were decreased by 27% and 26%, respectively. Serum HDL-cholesterol levels in the AS group were significantly increased, resulting in a 45% reduction in atherogenic index.

HMG-CoA reductase activities were 333.8 ± 46.6, 295.4 ± 60.7 and 339.3 ± 97.3 pmol/min per mg protein in the control, AS and SS groups, respectively. Faecal dry weight, cholesterol and bile acid excretions were all increased by AS treatment. However, shark liver squalene failed to affect these parameters.

Discussion

This study examined the lipid-lowering potential of amaranth, especially the effect of amaranth squalene in rats. To our knowledge, it is the first study to show the hypocholesterolaemic effect of amaranth squalene. Although hypocholesterolaemic effects of amaranth seed and oil have been reported in rats and chicken,[4,5] the effect of amaranth squalene was not tested.

Consumption of AO produced a greater effect on serum lipid profile and faecal steroid excretion than was seen in the AG group. As the only dietary difference between the control and AO groups was the lipid source (corn oil versus amaranth oil), this suggests that the potential effect is due to the lipid fraction in amaranth. The fatty acid composition of AO is similar to that of corn oil, with linoleic acid predominating.[3] Moreover, corn oil contains slightly higher levels of unsaturated fatty acid (especially oleic acid) than does AO, [3] which suggests that unsaturated fatty acid in AO is not the cholesterol-lowering element. Therefore, the effect of non-fatty acid component(s) should be considered and the possible regulatory role of squalene, which is present in markedly higher amounts in AO compare to corn oil, in hypocholosterolaemic action was examined.

Of particular interest was the finding that AS treatment significantly reduced serum and hepatic cholesterol, leading to a 45% decreases in atherogenic index compared with control group. Therefore, our data support a clear

Table 2. Effect of amaranth grain and oil in experimental rats.

	Group		
	Control	Amaranth grain	Amaranth oil
Body weight gain (g/4 weeks)	143 ± 27[a]	63 ± 10[b]	130 ± 7[a]
Food intake (g/4 weeks)	381.4 ± 17[a]	322.0 ± 12[b]	392.6 ± 18[a]
FER*	0.37 ± 0.01[a]	0.20 ± 0.01[b]	0.33 ± 0.02[a]
Serum			
Triglyceride (mmol/L)	5.64 ± 1.09[a]	2.98 ± 0.44[b]	2.38 ± 0.29[b]
Total cholesterol (mmol/L)	8.40 ± 0.58[a]	6.63 ± 0.54[b]	6.10 ± 0.28[b]
HDL cholesterol (mmol/L)	0.98 ± 0.12[a]	1.03 ± 0.03[a]	1.53 ± 0.05[b]
HDL/total cholesterol	0.12 ± 0.02[a]	0.18 ± 0.01[b]	0.24 ± 0.03[c]
Liver			
Cholesterol (mmol/g)	71.6 ± 3.2[a]	57.6 ± 2.3[b]	61.9 ± 2.8[b]
Triglyceride (mmol/g)	46.7 ± 1.5[a]	32.8 ± 2.1[b]	36.7 ± 0.9[b]
Faecal dry weight (g/3 days)	21.7 ± 2.0[a]	27.3 ± 4.3[ab]	30.6 ± 2.0[b]
Faecal steroid			
Cholesterol (mmol/3 days)	180.6 ± 9.4[a]	225.3 ± 27.9[a]	424.6 ± 61.5[b]
Bile acid (mmol/3 days)	79.7 ± 5.6[a]	133.7 ± 11.4[b]	170.8 ± 19.9[c]

Values are means ± SE (n=8); Means in same row with different superscript are significantly different ($P < 0.05$)

* Body weight gain (g) / food intake (g) x 100

Table 3. Effect of amaranth squalene in experimental rats.

	Group		
	Control	Amaranth squalene	Shark liver squalene
Body weight gain (g/4 weeks)	111 ± 6	80 ± 13	98 ± 9
Food intake (g/4 weeks)	413.5 ± 15.2	346.4 ± 36.2	348.0 ± 17.3
Serum			
Triglyceride (mmol/L)	3.51 ± 0.15[a]	3.03 ± 0064[b]	3.63 ± 0.07[a]
Total cholesterol (mmol/L)	6.08 ± 0.54[a]	4.72 ± 0.09[b]	5.18 ± 0.15[ab]
HDL cholesterol (mmol/L)	0.67 ± 0.08[a]	0.91 ± 0.07[b]	0.55 ± 0.03[a]
HDL/total cholesterol	0.12 ± 0.02[a]	0.20 ± 0.02[b]	0.10 ± 0.01[a]
Atherogenic index*	9.05 ± 1.38[a]	4.45 ± 0.47[b]	8.93 ± 0.41[a]
Liver			
Cholesterol (mmol/g)	35.91 ± 0.56[a]	26.54 ± 1.41[b]	37.42 ± 1.99[a]
Triglyceride (mmol/g)	81.6 ± 1.27[a]	60.27 ± 3.22[b]	85.00 ±4.55[a]
Faecal dry weight (g/3 days)	8.1 ± 0.7[ab]	10.2 ± 0.8[b]	6.2 ± 1.5 [a]
Faecal steroid			
Cholesterol (mmol/3 days)	169.7 ± 34.3a	283.9 ± 71.1b	154.9 ± 35.9a
Bile acid (mmol/3 days)	125.4 ± 20.1a	182.3 ± 15.9b	85.3 ± 14.0c

Values are means ± SE (n=6). Means in same row with different superscript letters are significantly different (P<0.05)

*Total cholesterol – HDL-cholesterol) / HDL-cholesterol

cholesterol lowering action for AS. However, results for the cholesterol-lowering effect of squalene were less conclusive.

Oral administration of shark squalene has no effect on serum and hepatic cholesterol and triglycerides concentrations in rat.[15] Nine weeks of 1 g squalene administration produced an increase in serum cholesterol concentrations in humans.[7] Nonetheless, it is suggested that squalene reduces serum cholesterol by inhibition of HMG CoA reductase,[3] but, as these reports do not indicate the source of squalene (plant versus animal), it is difficult to make a comparison with the results presented here.

Several hypotheses of the mechanism(s) by which squalene elicits a hypocholesterolaemic effect have been proposed. The most frequently suggested mechanism is

interference with intestinal cholesterol and bile acid absorption, leading to an increase in faecal neutral sterol and bile acid excretion. In the present study, excretion of faecal cholesterol and bile acid was significantly greater in the AS group than in the control group, suggesting lower intestinal absorption of cholesterol following AS injection. These results coincide with the serum and hepatic effect in AS group.

Cholesterol homeostasis is the result of a delicate balance between dietary intake, synthesis and catabolism. Serum total cholesterol can be also lowered if cholesterol synthesis is inhibited. HMG-CoA reductase mediates the first committed step in the *de novo* synthesis of cholesterol. The inhibition of this rate-limiting enzyme by plant sterols and hypocholesterolaemic drugs is an example of reducing serum cholesterol.[16] In present study, HMG-CoA reductase activity was decreased only in the AS group, suggesting that inhibition of this enzyme might be part of the cholesterol lowering mechanism of AS.

In summary, the present study demonstrated that the cholesterol-lowering effect of amaranth is associated, at least in part, with its squalene content. The effect of squalene may be attributed to enhanced excretion of faecal steroids through interference of cholesterol absorption. On the basis of this preliminary study, it would be of considerable interest to further elucidate the cholesterol metabolism of amaranth squalene in larger studies and in other species using a range of amaranth doses. □

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