HYPOCHOLESTEROLEMIC EFFECTS OF CHITOSAN IN CHOLESTEROL-FED RATS

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ABSTRACT

Hypocholesterolemic activity of some brown algae and chitosan, that had been found to be capable of binding bile acid in vitro, was examined in rats. When the diets contained cholesterol and cholate in combination, feeding chitosan, compared with cellulose, at the 5% level for 20 days resulted in a reduction of liver, but not plasma, cholesterol. Whilst in rats fed the diets containing cholesterol alone, chitosan caused a marked decrease in plasma and liver cholesterol, the magnitude of the reduction being comparable with that observed on cholestyramine. This aminosugar showed no demonstrable effects on food intake and growth of rats, but increased fecal excretion of neutral sterols accompanied with a conspicuous depression of microbial transformation of cholesterol to coprostanol. Three brown algae examined herein, *Sargassum ringgoldianum*, *Hijikia fusiforme* and *Sargassum thunbergii*, showed no plasma cholesterol lowering effects. So far as the present data indicate, chitosan appears to be an effective hypocholesterolemic agent.

INTRODUCTION

A number of nonnutritive dietary fibers have been found to be effective in lowering plasma cholesterol levels of man and the experimental animals (l-6). These fibers appear to exert their hypocholesterolemic activity primarily by interfering with intestinal absorption of bile acids. The mechanism of binding probably differs with different types of fibers. Since most dietary fibers have no ionizable groups, they may act either as nonionic absorbers or by imbibing water in which bile acids and perhaps neutral sterols are trapped, rather than act as anion-exchangers (7,8).

Because of abundant resources and relatively high contents of non-digestible materials, and in view of geographical location and dietary habits, hypocholesterolemic properties of seaweeds have been examined extensively by several Japanese investigators (9-12). However, most of nonnutritive fibers present in seaweeds have no functional groups that are capable of actively binding bile acids, but rather have hygroscopic properties (13). Since the structural skeleton of chitosan, a polymer of glucosamine, resembles that of neomycin (14), it seems likely that this compound sequesters bile acids. In fact, chitosan is readily soluble in dilute acids (15), and the resulting quaternary ammonium ions possible serve as anion-exchangers.

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The results of the present studies show that this amino-sugar has demonstrable cholesterol lowering activity in rats fed an atherogenic diet.

METHODS

Animals and diets

Male Wistar rats (Kyudo Co., Kumamoto) weighing approximately 180g were housed individually in an air-conditioned room (22-24°C), and fed the diets *ad libitum* for 20 days. Body weight and food consumption were recorded every other day. The animals were sacrificed by decapitation after fasting overnight (12 hr.). The composition of the basal diet was (0%): casein, 20; lard, 10; mineral mixture, 4; vitamin mixture (water soluble), 1; cellulose powder, 2; choline chloride, 0.15 and sucrose to 100 (16). Vitamin A palmitate, 600 IU; vitamin D, 60IU and *dl-a*-tocopheryl acetate, 10 mg were added per 100 g of the diet. In trial I, 5% test materials, 0.5% cholesterol and 0.125% sodium cholate were added at the expense of sucrose. In trial II, rats were maintained similarly as in the preceding trial, except that the diets containing cholesterol alone (cholate excluded) were used. Feces were collected for 2 days beginning 8 days and 3 days before killing the rats in trials I and II, respectively.

Materials

Seaweeds were harvested in late Spring at Tsuyazaki, Fukuoka. Three brown algae, Ohbamoku *(Sargassum ringgoldianum),* Hijiki *(Hijikia fusiforme)* and Umitoranoo (*Sargassum thunbergii)* were tested herein. After washing with water, they were air-dried and reduced to powder (20 mesh through). Chitosan, purchased from Tokyo Chemical Industry Co., Tokyo, was powdered and sieved (20 mesh through). Cholestyramine (Dowex 1 x2, Cl-, 50-100 mesh) was obtained from Muromachi Kagaku Kogyo Ltd., Tokyo.

Lipid analyses

Plasma and liver lipids were extracted with chloroform-methanol (2: 1, v/v). Cholesterol and triglyceride were determined as described elsewhere (16). Feces were lyophilized and treated with ethanol in a micro Soxhlet apparatus for 24 hr. Gas-liquid chromatographic analysis of neutral sterols was done as reported previously (16).

RESULTS

Effect on growth, food intake and liver and feces weight

As Table I shows, in trial I, weight gain of rats fed chitosan was to some extent lower than that of the animals fed cellulose, but the difference was not statistically significant. Food intake was the same in all groups. Feeding chitosan prevented an enlargement of the liver due to dietary cholesterol, the difference in liver weight between the cellulose and chitosan groups was significant. Fecal weights tended to increase slightly by chitosan.

In trial II, there were no differences in weight gain and food intake in all groups. Cholestyramine and chitosan reduced liver weight significantly in comparison with that observed on cellulose. Weight of the feces was clearly greater on chitosan than on cellulose either on the wet basis (Table I) or on the dry basis (1.8 +/- 0.1 vs. 2.3 +/- 0.1 g/day, $p<0.05$).

TABLE I. GROWTH, FOOD INTAKE AN-D WEIGHT OF LIVER AND FECES

Dietary regimens'	Weight gain (g)	Food intake (g/day)	Liver weight (g/100g bd.wt.)	Fecal weight (g/day)
Trial I				
Cellulose	130 ± 3.3"	20.4 ± 0.3"	4.46 ± 0.07"	3.4 ± 0.3"
Chitosan	116 ± 8.4[ab]	19.5 ± 0.8[ab]	3.77 ± 0.11"	3.8 ± 0.5"
Ohbamoku	116 ± 4.0[a]	19.1 ± 0.3"	4.22 ± 0.04'	3.4 ± 0.2"
Hijiki	141 ± 4.6"	21.3 ± 0.4"	4.38 ± 0.07"	3.8 ± 0.3"
Trial II				
Cellulose	131 ± 6.0"	18.5 ± 0.5"	4.18 ± 0.13"	2.2 ± 0.1"
Cholestyramine	131 ± 3.2"	19.5 ± 0.5"	3.76 ± 0.08"	2.1 ± 0.3[ab]
Chitosan	129 ± 7.1"	19.5 ± 0.6"	3.81 ± 0.17'	2.7 ± 0.1'
Umitoranoo	138 ± 5.1"	19.4 ± 0.5"	4.23 ± 0.08"	2.2 ± 0.2[ab]

'The test materials were added at the 5% level. Rats weighing 179g (trial I) and 178g (trial II) were fed the diets for 20 days. In trial I, diets contained 0.5% cholesterol and 0.125% sodium cholate, and in trial II, 0.5% cholesterol. See text for details. Three brown algae were tested; Ohbamoku (*Sargassum ringgoldianum),* Hijiki (*Hijikia fusiforme*) and Umitoranoo *(Sargassum thunbergii).*

[ab]Values represent the mean ± SEM of 5 rats (trial I) and 6 rats (trial II) per group. Values within same column not sharing a common super-script letter are significantly different at $p<0.01$-0.05.

Plasma and liver lipid concentrations

In trial I, plasma cholesterol and triglyceride levels were similar between the cellulose and chitosan groups. Seaweeds rather increased the levels of plasma cholesterol. The concentration of liver cholesterol was significantly lower in rats fed chitosan than in the animals fed cellulose. Liver triglyceride also tended to decrease by chitosan. Two brown algae examined showed no alleviating effects on liver lipids (Table II).

In trial II, cholestyramine and chitosan caused a significant decrease in plasma cholesterol, the magnitude of the decrease being the same in these two groups. Plasma triglyceride levels increased slightly on feeding cholestyramine, but not on chitosan. The concentration of liver cholesterol was the lowest on cholesty- ramine. Chitosan also decreased hepatic cholesterol significantly, but the level attained was twice that on cholestyramine. These two polymers lowered liver triglyceride. The alga showed no effects on plasma and liver lipids.

Fecal sterol excretion

Table III summarizes the content and composition of neutral sterols in feces. In trial I, feeding chitosan resulted in a significant increase in sterol excretion compared with that on cellulose. Seaweeds examined were not effective on this parameter. Even when cholate was excluded from the diet (trial II), chitosan again increased considerably fecal output of sterols. The excretion of cholesterol in rats fed cholestyramine was to some extent higher than that in the animals on cellulose, but the difference was insignificant. The composition of fecal sterols was markedly modified by feeding chitosan regardless the presence or the absence of cholate in the diets. The changes were characterized by decrease in the rate of microbial transformation of cholesterol and coprostanol. In contrast, the brown alga (Umitoranoo) and cholestyramine appeared to stimulate formation of coprostanol in the intestine.

TABLE II. PLASMA AND LIVER LIPID CONCENTRATIONS

Dietary regimens'	Plasma lipids (mg/dl)		Liver lipids (mg/g)	
	Cholesterol	Triglyceride	Cholesterol	Triglyceride
Trial I				
Cellulose	162 ± 13[ab]	81.4 ± 9.7"	70.0 ± 3.9"	54.9 ± 6.8[ab]
Chitosan	143 ± 10"	97.2 ± 10.7"	37.9 ± 3.3[b]	39.5 ± 7.7"
Ohbamoku	235 ± 30[b]	98.0 ± 8.0"	69.6 ± 2.8"	52.0 ± 9.9[ab]
Hijiki	226 ± 40[ab]	96.4 ± 16.0"	67.2 ± 3.4"	61.9 ± 2.4[a]
Trial II				
Cellulose	162 ± 11"	110 ± 6[a]	33.3 ± 2.6"	42.2 ± 1.7"
Cholestyramine	107 ± 7[b]	146 ± 13"	5.8 ± 0.7[b]	24.3 ± 1.8[b]
Chitosan	94 ± 12[b]	123 ± 9"	11.0 ± 1.7'	30.3 ± 2.5[b]
Umitoranoo	143 ± 16"	125 ± 7"	36.0 ± 1.9"	49.5 ± 3.0"

'See Table I.

[a, b, c]Values represent the mean ± SEM of 5 rats (trial I) and 6 rats (trial II) per group. Values within same column not sharing a common superscript letter are significant at $p<0.01$ - 0.05.

TABLE III. FECAL OUTPUT OF NEUTRAL STEROLS

Dietary regimens'	Cholesterol excreted (mg/day)	Composition of fecal sterols (percent of total)		
		Coprostanol	Cholesterol	Phytosterols
Trial I				
	27.4 ± 2.7"	44.4 ± 6.8"	50.2 ± 7.6"	5.4 ± 0.9"
Chitosan	53.3 ± 3.8[b]	23.5 ± 2.7[a]	70.6 ± 3.0[a]	5.9 ± 0.7"
Ohbamoku	28.7 ± 1.4"	35.4 ± 5.5[ab]	57.9 ± 5.7[ab]	6.7 ± 0.9"
Hijiki	30.3 ± 3.9"	34.9 ± 2.1"	58.1 ± 3.3"	6.9 ± 1.3"
Trial II				
Cellulose	45.3 ± 2.2"	28.6 ± 3.2"	68.4 ± 3.5[b]	3.0 ± 0.8"'
Cholestyramine	50.7 ± 4.1"	39.0 ± 3.1[b]	54.4 ± 2.7[a]	6.6 ± 1.4[a]
Chitosan	68.7 ± 2.4[a]	18.3 ± 3.0'	79.2 ± 3.3'	2.5 ± 0.7[a]
Umitoranoo	44.5 ± 5.3"	45.1 ± 2.0[bc]	49.9 ± 2.4"	5.0 ± 0.8""

'See Table I.

[a, b, c, d]Values represent the mean ± SEM of 5 rats (trial I) and 6 rats (trial II) per group. Values within same column not sharing a common superscript letter are significantly different at $p<0.01$ -0.05.

DISCUSSION

In a preliminary experiment, we have studied the capacity of seaweeds and polysaccharides of marine origin to absorb taurocholate *in vitro.* Among 36 seaweeds and 10 polysaccharides tested, some brown algae, Hijiki, Ohbamoku, Umitoranoo and Fushisujimoko (*Sargassum confusum*) showed detectable absorbing activity. The binding ability of chitosan has been found to be roughly comparable with that of cholestyramine. Judging from its chemical structure, chitosan seemed to act as an anion-exchanger like neomycin (14) or synthetic resins (17) with bile acid binding activity. Although chitosan

showed hypocholesterolemic activity, three algae examined herein were ineffective in lowering plasma cholesterol (11).

Growth rate and food intake were not apparently influenced by dietary chitosan, but fecal weights tended to increase. The feces were in no way wet and appeared normal. In contrast, feeding cholestyramine resulted in weight reduction of feces excreted. It seems therefore likely that chitosan may act in a non-specific manner by shortening the time of transit down the intestine and thus reducing the time available for reabsorption of bile acids and perhaps cholesterol (18).

Cholestyramine did not significantly increase the daily fecal excretion of neutral sterols (but in accordance with the available data (17), it increased excretion of bile acids very markedly, unpublished observations in a similar type of experiment). This was partly due to a decrease in weight of feces excreted, since the sterol contents per unit weight of feces were only slightly lower than those observed on feeding chitosan. In contrast, chitosan markedly stimulated fecal excretion of neutral sterols. The excretion of bile acids was also enhanced by chitosan but to a considerably lesser extent than that on cholestyramine. Thus, chitosan may affect cholesterol absorption both by binding bile acids and by binding cholesterol, presumably by disrupting micelle formation in the intestine. Alternately, the mechanism of hypocholesterolemic action of chitosan appears to be somewhat different from that of the synthetic resin. Of particular interest was the observation that chitosan caused a repression of microbial transformation of cholesterol to coprostanol. It is, however, not apparent at present whether chitosan influences microbial flora of the intestine or sterols absorbed on chitosan are minimally attacked by bacteria.

Although chitosan showed a conspicuous hypocholesterolemic potency and it was regarded as an usable agent, we should focus attention on the fact that this compound is soluble in acidic media (15). Perhaps, this aminosugar at least partly dissolves in gastric hydrochloric acid. Polycations thus formed may be responsible for its hypocholesterolemic activity, they may, however, in turn cause interaction with gastric mucus and may lead to an unknown or unfavorable side-effect, if any (19). It is also not clear whether the change in microbial transformation of ‘cholesterol to the coproderivative causes noxious effects on intestine physiology. Because of these considerations and of the limited length of the experimental periods employed in the present studies, more extended information regarding the hypocholesterolemic activity of chitosan awaits further works. This also includes effects of differences in molecular weights, acetyl contents and particle size of the chitosan preparation commercially available. In addition, interaction of chitosan in cholesterol absorption and metabolism requires consideration in overall study of cholesterol dynamics. However, the data presented here indicated possible usefulness of chitosan for treatment of hypercholesterolemia.

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