Hypocholesterolemic Activity of Desalted Miso in Rats Fed an Atherogenic Diet

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Effect of dietary miso on the cholesterol metabolism was examined in rats fed the diet containing 0.5% cholesterol and 0.15% sodium cholate. The inclusion of 20% desalted miso to the atherogenic diet significantly decreased the concentration of serum cholesterol throughout the 30 days experimental period. Serum triglyceride and phospholipid as well as hepatic lipid concentrations at the time of sacrifice (30 days) were in no way affected by dietary miso. The ingestion of the miso preparation doubled the fecal excretion of acidic steroids, while it increased only slightly that of neutral steroids. The dietary miso appeared to exert the potent hypocholesterolemic activity primarily through the enhancement of the catabolism of cholesterol to bile acids in rats fed an atherogenic diet.

It has been well demonstrated that soybean in addition to several kinds of cereal beans cause significant reduction of serum cholesterol in rats fed a high-cholesterol diet¹⁻⁴⁾. The components that exert the potent hypocholesterolemic activity have been isolated from soybean and thus accounted for the cholesterol lowering effect⁵⁻⁹⁾. Thus, the ingestion of soybean products hopefully reduce the incidence of atherosclerosis not only in experimental animals but also in humans.

There are several kinds of soybean fermentation products which have been consumed by Japanese population. The fermentation processes cause modification or degradation of the components included in soybean¹⁰⁻¹³⁾. However, little is known whether these changes modify the characteristic nature of soybean to affect the lipid metabolism in experimental animals. In this context, we currently examined the effect of dietary miso, a fermentation product of soybean, on the cholesterol metabolism in rats fed an atherogenic diet. From the

consideration that high contents of NaCl which is usually seen in miso may exert untoward effect on the lipid metabolism of the animals and thus make the interpretation of the results obtained difficult, the desalted product was employed in this trial.

Materials and Methods

Animals and diets

Male Sprague-Dawley rats (4 weeks of age) obtained from Charles River Japan Co., Kanagawa were fed a commercial non-purified diet (Type NMF, Oriental Yeast Co., Tokyo). After 7 days of acclimation to our housing conditions, the rats were randomly divided into two groups of 6 animals and fed the atherogenic purified experimental diets containing both 0.5% cholesterol and 0.15% sodium cholate. The ingredients and fatty acid compositions of the diets are summarized in Table 1. The diet for a control group contained 20% casein (purity 94.5%), 10% lard and α -corn starch in addition to sucrose as protein,

Table 1 Ingredients and fatty acid compositions of atherogenic experimental diets

Ingredients	Diets	
	Control	Miso
	(weight %)	
Miso (desalted)	—	20
Casein	20	14.78
α -Corn starch	15	15
Sucrose	41.65	32.47
Lard	10	4.4
Cellulose	8	8
Cholesterol	0.5	0.5
Sodium cholate	0.15	0.15
Mineral mixture	3.5	3.5
Vitamin mixture	1.0	1.0
Choline bitartrate	0.2	0.2
Fatty acid composition (weight %)		
14 : 0	1.51	0.92
16 : 0	21.82	17.66
16 : 1	4.06	2.37
18 : 0	10.95	7.74
18 : 1	46.52	34.55
18 : 2	12.40	30.41
18 : 3	1.94	5.82
P/S*	0.418	1.377

*The ratio of polyunsaturated to saturated fatty acids

lipid and available carbohydrate sources, respectively. Cellulose as a non-available carbohydrate (dietary fiber) source was added to the experimental diets at 8% levels. Vitamin and mineral mixtures, the compositions of which were the same as recommended by American Institute of Nutrition¹⁴⁾, were obtained from Nihon Nosan Kogyo K.K., Tokyo. The lyophilized preparation of desalted miso (a kind gift from Marudai, K.K., Niigata) containing less than 2% NaCl was added to the experimental diet at 20% level. As the miso preparation contained 26.1% protein and 28.1% lipid, the amounts of casein and lard in the diet were reduced and made 100% with sucrose. Thus, these experimental diets served similar amounts of protein (18.90% and 19.19% for the control and miso diets, respectively) and lipid

(10.00% and 10.02%, respectively). Although we have not analyzed contents and compositions of the materials other than protein and lipid of lyophilized miso, the preparation presumed to contain significant amounts of components other than available carbohydrate (starch and sugar) including dietary fiber. Thus, the dietary energy served from carbohydrate sources might be somewhat less in the miso diet compared to the control diet. As miso lipid contained considerable amounts of polyunsaturated fatty acids (linoleic and linolenic acids), the ratio of polyunsaturated to saturated fatty acids (P/S) in the diet containing miso was considerably higher than that in the control diet containing lard as a sole lipid source. Phytosterol content of the diet containing miso was 23.9 mg/100 g. Rats were fed these experimental diets for 30 days. Small volumes (less than 0.1 ml) of blood were withdrawn from tail vein periodically (at 7, 14 and 23 days) during the feeding period for the analysis of serum cholesterol. Feces were also collected for 2 days at 12 ~ 14, 21 ~ 23 and 28 ~ 30 days of the feeding period. At the termination of the experimental period, rats were lightly anesthetized with diethylether and blood was withdrawn from vena cava. Livers were quickly excised.

Lipid analyses

Hepatic and serum lipids were extracted and purified as described by FOLCH *et al.*¹⁵⁾. Cholesterol, triglyceride and phospholipid in the lipid extracts except for serum cholesterol were chemically determined by the methods reported by us previously¹⁶⁾. Serum cholesterol was enzymatically determined by a commercial assay kit (Cholesterol C-test, Wako Chemical, Osaka).

Fecal steroid analysis

Fecal neutral and acidic steroids were extracted with ethanol under reflux. Neutral steroids (cholesterol and coprostanol) and acidic steroids (bile acids) were determined by a gas-liquid chromatograph using 5 α -cholestane and nordeoxycholic acid as the internal standards, respectively¹⁷⁾.

Statistical analysis

Data were analyzed by a Student's *t*-test and significant differences of the means were inspected at $p < 0.05$.

Results

Dietary miso did not affect the daily food intake and growth of animals. The diet containing miso revealed a potent hypocholesterolemic activity (Fig. 1). The serum cholesterol concentrations in rats fed miso were approximately one-half those in the control group during the entire experimental period.

Serum and hepatic lipid concentrations at the time of sacrifice (30 days) were summarized in Table 2. Although dietary miso apparently reduced the serum cholesterol concentration, it was in no way effective to alter the triglyceride and phospholipid concentrations. Hepatic cholesterol concentration appeared to be reduced slightly by dietary miso. However, the difference was statistically insignificant. There were no significant differences in the hepatic triglyceride and phospholipid concentrations between the two groups of rats.

Feces were occasionally collected for 2 days during the 30 days experimental period and excretion of neutral and acidic steroids were determined (Table 3). The inclusion of miso

Fig. 1 Effect of desalted miso on the concentration of serum cholesterol in rats fed an atherogenic diet. ○, miso; ●, control. *Significantly different from the corresponding value in rats fed control diet at $P < 0.05$.

Table 2 Serum and hepatic lipid concentrations in rats fed desalted miso for 30 days

	Diets	
	Control	Miso
Serum lipids (mg/dl)		
Cholesterol	313±38	127±24*
Triglyceride	217±40	236±53
Phospholipid	222±17	202±20
Hepatic lipids (mg/g)		
Cholesterol	61.7±4.2	50.0±4.8
Triglyceride	87.8±6.0	90.4±6.6
Phospholipid	25.7±0.8	28.0±1.0

Values represent means±SE of 6 rats.

*Significantly different from the value in rats fed control diet at $p < 0.05$.

in the experimental diet apparently increased the amounts of feces excreted. Dietary miso doubled the daily excretions of acidic steroids during the entire experimental period. Although excretions of neutral steroids in rats fed miso also considerably increased, the significant difference of the parameter was only detectable in the early phase of the feeding period (12 ~ 14 days).

The steroid compositions of feces collected at 28 ~ 30 days of the experimental period are summarized in Table 4. The dietary miso considerably affected the composition of fecal acidic steroids. The percentage value for cholic acid considerably decreased in rats fed miso. The decrease in the value was compensated by the increases in those of lithocholic, hyodeoxycholic or ursodeoxycholic as well as β -muricholic acids. The occurrence of bacterial hydrogenation of cholesterol to coprostanol appeared to be minimal in the control group. On the other hand, dietary miso apparently increased the proportion of coprostanol in the fecal neutral steroid. The similar results were obtained in the feces collected at 12 ~ 14 and 19 ~ 21 days of the experimental period.

Discussion

It has been observed that soybean as well as several kinds of cereal beans caused significant

Table 3 Effect of desalted miso on the fecal excretions of acidic and neutral steroids

Feeding period (days)	Feces excreted (g/day)	Acidic steroids (mg/day)	Neutral steroids (mg/day)
12~14 days			
Control	2.02±0.08	16.8±0.6	19.3±2.3
Miso	2.96±0.12*	30.2±1.7*	29.6±2.9*
19~21 days			
Control	2.38±0.12	21.7±2.0	26.8±2.4
Miso	3.44±0.21*	40.0±3.3*	30.4±3.1
28~30 days			
Control	2.38±0.04	22.6±1.2	24.4±2.6
Miso	3.57±0.19*	40.0±0.19*	31.4±3.8

Values represent means±SE of 6 rats. *Significantly different from the value in rats fed control diet at $p<0.05$.

Table 4 Effect of desalted miso on the compositions of fecal acidic and neutral steroids

	Diets	
	Control (weight %)	Miso
Acidic steroids		
Lithocholic acid	0.96±0.44	2.82±0.61*
Deoxycholic acid	36.7±3.7	39.9±3.0
Hyodeoxycholic+		
Ursodeoxycholic acids	10.5±1.5	16.4±1.0*
Cholic acids	28.2±3.9	8.9±1.9
α -Muricholic+		
ω -Muricholic acids	9.3±1.4	10.0±0.4
β -Muricholic acid	14.4±1.1	22.0±2.3*
Neutral steroids		
Coprostanol	3.23±1.38	15.6±5.3*
Cholesterol	96.8±1.4	84.4±4.9*

Values represent means±SE of 6 rats. *Significantly different from the value in rats fed control diet at $p<0.05$.

reduction of serum cholesterol in rats fed a high-cholesterol diet¹⁾⁻⁴⁾. This study also demonstrated that miso, a fermentation product of soybean, retained the hypocholesterolemic activity in the experimental animal (Fig. 1 and Table 2). Dietary miso dependent reduction of serum cholesterol levels accompanied the increased fecal excretion of acidic

steroids (Table 3). Thus, dietary miso presumably accelerated the catabolism of cholesterol to bile acids and thus reduced the cholesterol concentration in the serum. As increases in the excretion of neutral steroids in rats fed miso were rather moderate and insignificant except for one occasion (Table 3), the reduced intestinal absorption of cholesterol could not be regarded as a determinant factor for the cholesterol lowering effect of miso observed currently.

Several components of soybean now have been demonstrated to exert potent hypocholesterolemic activities. Lipid components including triglyceride¹⁷⁾ and phospholipid⁸⁾ containing high levels of polyunsaturated fatty acid as well as phytosterols⁹⁾ could lower the serum cholesterol in rats as well as in humans. Fatty acid composition of miso was resembled with that reported for soybean (data not shown)¹⁷⁾. Thus, the fermentation process does not seem to modify the fatty acid moieties of soybean lipids significantly. As miso lipid retained the high proportions of n-6 (linoleic acid) and n-3 (linolenic acid) polyunsaturated fatty acids, the ratio of polyunsaturated to saturated fatty acid (P/S ratio) in the diet containing miso is considerably higher than that in the control diet containing lard as a sole source of dietary lipid (Table 1). The difference in the P/S ratio between the two

diets may account for the cholesterol lowering effect of miso observed currently. The phyto-sterol content of the miso preparation appeared to be too low to exert any significant effect on serum cholesterol⁹⁾.

There are a number of studies to demonstrate the cholesterol lowering effect of soy protein⁷⁾ and saponin⁵⁾⁶⁾. It is not clear whether the fermentation process for the production of miso modify the propensities of these components to alter the lipid metabolism in experimental animals. However, it is possible that these components in addition to lipid moieties in miso retain the meritorious propensities and thus contribute to the cholesterol lowering effect of the fermentation product.

Many kinds of dietary fiber have been demonstrated to decrease the serum cholesterol concentrations in experimental animals and humans¹⁸⁾¹⁹⁾. Increased excretion of feces in rats fed miso indicate the presence of considerable amounts of non-digestible fiber in the miso preparation employed currently. Although information regarding the effect of fiber contained in soybean on cholesterol metabolism is rather scanty, it would also be a candidate responsible for the cholesterol lowering effect of dietary miso.

The dietary miso affected the fecal bile acids not only quantitatively but also qualitatively. The percentages of bile acids derived from chenodeoxycholic acid either primarily through the hepatic metabolism (β -muricholic acid) or secondly through the metabolism by intestinal bacteria (lithocolic, hyodeoxycholic and ursodeoxycholic acids) except those for α - and ω -muricholic acids increased. On the other hand the percentage of cholic acid decreased and that of the secondary metabolite (deoxycholic acid) was unchanged. The results indicate that the catabolism of cholesterol to chenodeoxycholic acid rather than to cholic acid is enhanced in rats fed miso. The composition of fecal neutral steroids was also affected by dietary miso. Increase in the proportion of coprostanol, a bacterial hydrogenation product of cholesterol, in rats fed

miso indicated that the dietary miso modified the composition and (or) the activities of intestinal flora.

In conclusion, this study demonstrated a potent hypocholesterolemic activity of dietary miso in rats fed an atherogenic diet. The miso appeared to increase the catabolism of cholesterol to bile acids and thus reduced the serum cholesterol in the experimental animal.

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高コレステロール・胆汁酸摂取ラットにおける 脱塩みその血清コレステロール低下作用

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高コレステロール・胆汁酸摂取ラットのコレステロ

ール代謝に与える脱塩みその効果を調べた。雄 Sprague-Dawley 系ラットを 0.5% コレステロール、0.15% コール酸ナトリウムを含む食餌で 30 日間飼育した。飼育開始後 7, 14 および 23 日目に尾静脈より血液を採取し、血清コレステロール濃度を測定した。また糞を 12, 21, および 28 日目に 2 日間採取し、ステロイドの分析を行った。30 日目にラットを屠殺し血清、肝臓の脂質分析を行った。食餌中に脱塩みそを 20% 添加することにより血清コレステロール濃度の低下が認められた。みそ添加群の血清コレステロール濃度は全実験期間中対照群の約半分であった。屠殺時の血清トリグリセリド、リン脂質濃度および肝臓脂質濃度には、みその効果は認められなかった。糞中への胆汁酸排泄は、みその食餌への添加により約二倍に増加した。中性ステロイドの排泄もみそにより増加するがその程度は小さく有意差は認められなかった。以上のことからみその食餌への添加はコレステロールの胆汁酸への異化とその体外への排泄を増加させることにより血清コレステロール濃度を低下させるものと思われた。

新 刊 紹 介

食物のバイオテクノロジー

貝沼圭二編

本書は、農畜水産食品の生産から加工にいたる過程で進められているバイオテクノロジー研究の最新の現状とその将来性について分かりやすく解説した好著である。その内容は、先端技術、稲、果実・野菜、畜産、水産、発酵食品、甘味料、食品加工、夢の植物の各項目毎に、その分野で実施されているバイオテクノロジーが解説されている。(H)

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