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The Mechanism Underlying the Synergetic Hypocholesterolemic Effect of Sesamin and α-Tocopherol in Rats Fed a High-Cholesterol Diet

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Abstract. Sesamin is a major lignan in sesame seed. We confirmed that ingestion of sesamin and α-tocopherol synergistically reduced the concentration of blood cholesterol in rats given a high-cholesterol diet. To elucidate the molecular mechanism behind this effect, we analyzed the gene-expression profiles in rat liver after co-ingestion of sesamin and α-tocopherol. Six-week-old male Sprague-Dawley rats were fed a 1% cholesterol diet (HC) or HC containing 0.2% sesamin, 1% α-tocopherol or sesamin + α-tocopherol for 10 days. Blood samples were collected on days 1, 3, 7, and 10 and livers were excised on day 10. The gene expressions of ATP-binding cassette, sub-family G (WHITE), members 5 (ABCG5) and 8 (ABCG8) were significantly increased, while the gene expression of apolipoprotein (Apo) A4 was significantly decreased. ABCG5 and ABCG8 form a functional heterodimer that acts as a cholesterol efflux transporter, which contributes to the excretion of cholesterol from the liver. ApoA4 controls the secretion of ApoB, which is a component of low-density-lipoprotein cholesterol. These studies indicate that the cholesterol-lowering mechanism underlying the effects of co-ingestion of sesamin and α-tocopherol might be attributable to increased biliary excretion of cholesterol and reduced ApoB secretion into the bloodstream.

Keywords: sesamin, hypocholesterolemic activity, α-tocopherol, ATP-binding cassette transporter, apolipoprotein

Introduction

Sesamin, one of the major lignans in sesame seed and oil, has been identified as a natural inhibitor of Δ5-desaturase, which catalyzes the conversion of dihomo-γ-linolenic acid to arachidonic acid in both microorganisms and animals (1, 2). Sesamin is epimerized during the refining process of non-roasted sesame seed oil to form episesamin (Fig. 1) (3). Therefore, non-roasted sesame seed oil contains both sesamin and episesamin at a ratio of about 1:1. The sesamin isomers (a mixture of sesamin and episesamin) have been tested extensively for physiological activity in animals and humans by many investigators. Studies have demonstrated that the sesamin isomers have physiological effects, acting as an antioxidant (4), anti-carcinogen (5), and anti-hypertensive (6 – 11) and in reducing serum lipid (12 – 15). Yamashita et al. also indicated that the sesamin isomers enhanced the plasma levels of α- and γ-tocopherol in rats (16, 17). Furthermore, our previous microarray analysis showed that the sesamin isomers induced fatty acid oxidation and alcohol-metabolizing enzymes and decreased fatty acid synthesis enzymes in rat liver (18). Meanwhile, several studies have shown that sesamin alone exerts antihypertensive and neuroprotective effects (19 – 21). Ide et al. demonstrated that sesamin alone stimulated hepatic fatty acid oxidation by affecting the gene expression of various proteins regulating hepatic fatty acid metabolism in the rat liver using a microarray (22, 23).

Sesamin is known to exhibit antioxidative activities in the living body (3), although it has no antioxidative activity in vitro (24). When orally administered, sesamin is metabolized sequentially by cytochrome P-450 and catechol-*O*-methyltransferase (COMT) (25). Sesamin is initially metabolized by P-450 to SC-1 {2-(3,4-methylenedioxy-phenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabi-

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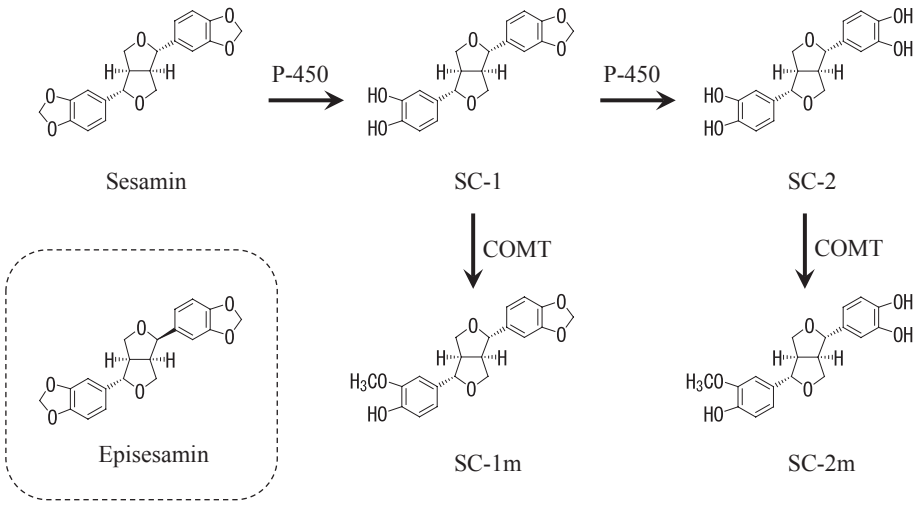

Fig. 1. Chemical structures of sesamin and its derivatives. Sesamin is sequentially metabolized by P-450 to SC-1 and SC-2, which are then metabolized by COMT to SC-1m and SC-2m, respectively. P-450, cytochrome P450; COMT, catechol-*O*-methyltransferase. Sesamin and episesamin are the *S* and *R* epimers at the C2 position, respectively.

cyclo-[3.3.0]octane}, which is then metabolized to SC-2 {2,6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo-[3,3,0] octane} (Fig. 1). SC-1 and SC-2 are further metabolized by COMT to *O*-methylated metabolites (SC-1m and SC-2m). Catechol metabolites from sesamin were found to exert strong antioxidative activity on the liver (25), suggesting a beneficial physiological role for ingested sesamin in the body (16, 26).

Hypercholesterolemia is a major risk factor for cardiovascular disease. Research on natural substances that affect cholesterol metabolism for the prevention of hypercholesterolemic atherosclerosis has therapeutic importance. In particular, the investigation of dietary components that can be added to foods to lower or regulate cholesterol levels is of special interest. In a previous study, it was reported that the sesamin isomers reduced the blood cholesterol level in rats fed a cholesterol-enriched diet owing to increased fecal excretion of cholesterol and inhibition of the activity of liver microsomal 3-hydroxy-3-methyl-glutaryl CoA reductase (12). Total cholesterol (T-CHO), low-density-lipoprotein cholesterol (LDL-C), and apolipoprotein (Apo) B were significantly lower in the sesamin isomers– and α-tocopherol–treated hypercholesterolemic patients (14). In addition, co-ingestion of the sesamin isomers and α-tocopherol synergistically reduced the concentration of blood cholesterol in rats given a high-cholesterol diet (15). However, the mechanism of cholesterol-lowering activity remains unclear.

In this study, we used not sesamin isomers but instead sesamin as a test substance to more easily understand this cholesterol-lowering mechanism. Then, we analyzed gene expression in rat liver to elucidate the cholesterol-lowering mechanism underlying the effects of co-ingestion of sesamin and α-tocopherol.

Materials and Methods

Materials

Sesamin was purified from a sesame lignan fraction that was prepared from refined sesame oil by high-performance liquid chromatography (HPLC) as described previously (25). The purity of sesamin exceeded 96.0%. DL-α-tocopherol acetate was purchased from Nacalai Tesque Co., Kyoto. All other reagents used were of analytical grade.

Animals and diets

Male Sprague-Dawley rats, aged 5 weeks, were purchased from CLEA Japan, Inc. (Tokyo) and maintained on a control diet (Table 1) for 6 days before starting the experiment. Three rats were housed in one cage in an

Table 1. Compositions of diets

	C	HC
Ingredients	Control	High-Cholesterol
	g/kg diet	
Casein	200	200
Beef tallow	100	100
Granular sugar	580	567.5
Cellulose	40	40
Cholesterol	–	10
Cholic acid	–	2.5
Vitamin mix[1]	10	10
Mineral mix[2]	70	70

[1]Vitamin mixture for Basic-Purified Diet designed by CLEA Japan, Inc. [2]Mineral mixture for Basic-Purified Diet designed by CLEA Japan, Inc.

animal laboratory under controlled ambient conditions: temperature, 23.0 ± 2°C; humidity, 55 ± 10%; and a 12-h light/dark cycle (7:00 – 19:00 and 19:00 – 7:00). Six days after acclimation, rats were divided into the following five groups by body weight. One group (C group, n = 6) received a control diet as a control group, while the other 4 test groups were fed high-cholesterol (1.0%) powdered diets. Furthermore, rats in the 4 test groups were fed a 1.0% α-tocopherol diet (V group, n = 6), 0.2% sesamin diet (S group, n = 6), 0.2% sesamin + 1.0% α-tocopherol diet (SV group, n = 6), or a diet without these supplements (HC group, n = 6). Control and high-cholesterol diets were modified by employing Basic-Purified Diet designed by CLEA Japan, Inc. The composition of these diets is shown in Table 1. Sesamin and/or α-tocopherol were subsequently added to the high-cholesterol diet to prepare experimental diets. Rats were fed a powdered experimental diet ad libitum and allowed free access to tap water for 10 days. Body weight (days 1, 3, 7, and 10) and food consumption (days 1, 3, 7, and 10) were recorded. Experimental protocols were approved by the Animal Care and Use Committee of Suntory Holdings, Ltd., and we followed the Guidelines for Animal Care and Use of Suntory Holdings, Ltd.

Biochemical profiles in plasma and liver

For all groups on days 0, 1, 3, and 7 after initiation of the experimental diets, blood was drawn from the tail vein. At day 10, the rats were anesthetized and killed. Blood samples were withdrawn from the abdominal aorta. Livers were excised and stored in RNAlater (Ambion, Inc., Foster City, CA, USA) until quantitative real-time PCR analyses. Plasma T-CHO and LDL-C were measured enzymatically using an automatic biochemical analyzer (Model 7180; Hitachi Ltd., Tokyo).

Quantitative real-time polymerase chain reaction (QRT-PCR)

To determine gene expression, differences in the liver between the control and test groups, total hepatic RNA was extracted from the liver using Isogen (Nippon Gene Co., Ltd., Toyama) and purified with an RNeasy mini kit (Qiagen GmbH, Hilden, Germany). Total RNA (2.0 μg) was reverse-transcribed with random primers using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) according to the recommendations of the manufacturer. To quantify gene expression, cDNA was amplified for various gene targets by QRT-PCR using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems). All primers and probes used were purchased as TaqMan Gene Expression Assays: ATP-binding cassette, sub-family G (WHITE), member 5 (ABCG5, Rn00587092_m1), ATP-binding cassette, sub-family G (WHITE), member 8 (ABCG8, Rn00590367_m1), ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1, Rn00710172_m1), ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1, Rn00585262_m1), ATP-binding cassette, sub-family B (MDR/TAP), member 4 (ABCB4, Rn00562185_m1), ATP-binding cassette, sub-family B (MDR/TAP), member 11 (ABCB11, Rn00582179_m1), hepatocyte nuclear factor 4α (HNF4α, Rn00573309_m1), liver receptor homolog 1 (LRH1, Rn00572649_m1), liver X receptor α (LXRα, Rn00581185_m1), ApoA4 (Rn00562482_m1), low-density-lipoprotein receptor (LDLR, Rn00598442_m1), ApoB (Rn01499050_g1), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR, Rn00565598_m1), sterol regulatory element binding transcription factor 2 (SREBF2, Rn01502638_m1), SREBF chaperone (SCAP, Rn01446560_m1), insulin induced gene 1 (INSIG1, Rn00574380_m1), insulin induced gene 2 (INSIG2, Rn00710111_m1), CYP3A2 (Rn00756461_m1), CYP4F2 (Rn00571492_m1), and α-tocopherol transfer protein (α-TTP, Rn00564885_m1) (Applied Biosystems). The PCR results were analyzed with ABI SDS software (Applied Biosystems). The relative expression levels of the genes in each sample were determined by the Comparative Ct Method. Expression assays for each gene were normalized to 18SrRNA (Hs99999901_s1) and expressed as fold change relative to that of the control group.

Measurement of concentrations of α- and γ-tocopherol, sesamin, and sesamin metabolites

The measurement of concentrations of α- and γ-tocopherol in serum and liver samples was carried out by Mitsubishi Chemical Medience Co., Ltd., Tokyo. Sesamin and sesamin metabolites were extracted from plasma samples using Oasis HLB cartridges (Waters Corp., Milford, MA, USA) and injected into an ultra performance liquid chromatography-MS/MS system (ACQUITY UPLC system, Waters Corp. and Quattro Micro; Waters/Micromass, Manchester, UK). Detection of ions was performed in the multiple reaction monitoring mode, monitoring the transition (m/z) of the precursor ion to the product ion as follows: SC-2, 329.3 to 137.1; SC-2m, 343.3 to 151.2; SC-1, 341.2 to 176.2 in negative ion mode; SC-1m, 374.2 to 233.2; sesamin, 372.2 to 233.2; and internal standard, 369.2 to 298.2 in positive ion mode.

Statistical analyses

All values are expressed as the mean ± S.E.M. Differences in the measurements were analyzed using SPSS 11.5.1 J for Windows (SPSS Japan Inc., Tokyo). Data were analyzed with one-way ANOVA to establish

whether the effects were significant. When one-way ANOVA revealed $P < 0.05$, the data were further analyzed using Tukey's multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

Results

Biochemical profiles in plasma

No significant differences in food intake (23.0 – 24.9 g/day) or growth (89.3 – 94.5 g/10 days) were seen among the groups. Liver weights were significantly higher in rats given diets containing cholesterol than in animals fed a cholesterol-free diet. As shown in Fig. 2A, the blood T-CHO level in HC group, V group, or S group began to rise from day 3 and peaked at day 7; and high T-CHO levels in comparison with that of the C group were maintained until day 10. No significant difference was noted in the blood T-CHO level among these three groups. On the other hand, the blood T-CHO level in the SV group was maintained at a low level until day 3, and began to rise from day 7, reaching a steady state at day 10. The blood T-CHO level was significantly lower in the SV group than in the HC, V, and S groups. The blood LDL-C level showed similar time course responses to the T-CHO variation profile in all groups (Fig. 2B). Thus, the reduction in the serum cholesterol level in the SV group was due to the marked reduction in LDL-C.

Gene expression involved in hepatic cholesterol excretion

Since the blood T-CHO level was significantly lowered in rats fed the sesamin and α-tocopherol diet, we examined changes in the abundance of gene transcripts involved in hepatic cholesterol excretion (Fig. 3). We used six TaqMan gene expression assays related to cholesterol efflux transport, including ABCG5, ABCG8, ABCA1, ABCG1, ABCB4, and ABCB11, for QRT-PCR. All groups on diets containing cholesterol exhibited significantly increased gene expressions of ABCG5 and ABCG8 relative to those of the group on the control diet. In addition, ABCG5 and ABCG8 were distinctly upregulated in the SV group compared with those in the HC, V, and S groups combined (Fig. 3A). The gene expressions of ABCA1, ABCG1, ABCB4, and ABCB11 were also increased in the rats fed the diets containing cholesterol. ABCA1 gene expression was decreased in the SV group compared with that in the HC group. The gene expression of ABCG1 was also decreased in the SV group relative to that in the HC group. No differences were seen among the other groups in the gene expressions of ABCA1 and ABCG1. ABCB4 and ABCB11 gene expressions showed no differences among the HC, V, S, and SV groups (Fig. 3B). Furthermore, we analyzed the gene expression of nuclear receptors that regulate ABCG5 and ABCG8, including HNF4α, LRH1, and LXRα. However, no significant changes were observed among all groups in terms of these three gene expressions (Fig. 3C).

Gene expression involved in hepatic cholesterol circulation

To characterize the effects of sesamin and α-tocopherol on the cholesterol circulation in rats fed the diet containing cholesterol, the gene expressions were analyzed using the following TaqMan gene expression assays: ApoA4, LDLR, and ApoB (Fig. 4). The ApoA4 gene was downregulated in the SV group compared with that in the HC group, while the C, HC, and S groups showed the same level of this gene expression. The decrease in expression level tended to be greater in the SV group than in the V group. The gene expression of LDLR in the SV group was lower than that in the C group, but the rats in the HC, V, S, and SV groups exhibited the same expression level of this gene. No differences were observed in the ApoB gene expression among all the groups.

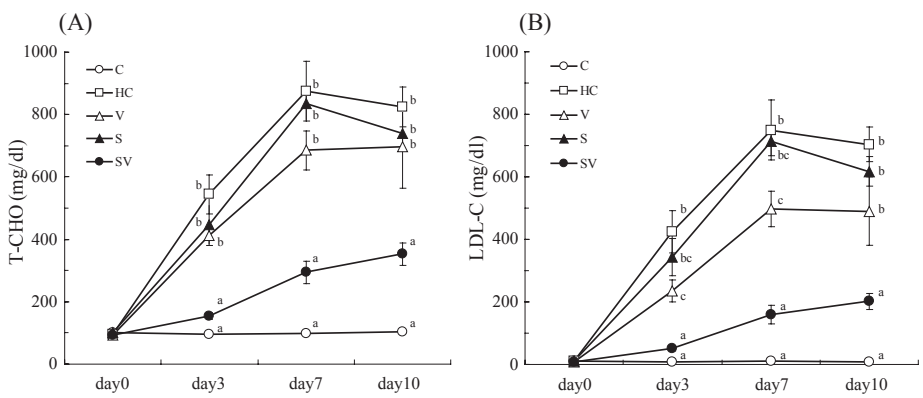

Fig. 2. Effects of sesamin and α-tocopherol on the blood total cholesterol (T-CHO) (A) and low-density-lipoprotein cholesterol (LDL-C) (B) levels. Changes in the blood T-CHO and LDL-C levels were measured during the experimental diet intake. Data are expressed as the mean ± S.E.M. (n = 6). Values at each time point not sharing a common letter are significantly different by Tukey's multiple comparison test ($P < 0.05$).

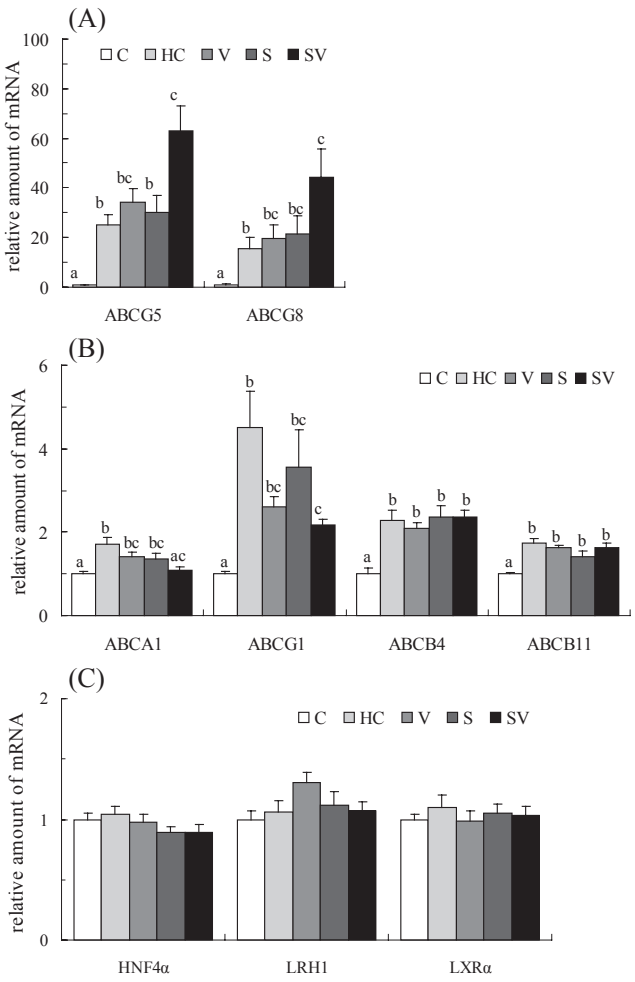

Fig. 3. Effects of sesamin and α-tocopherol on the gene expression involved in hepatic cholesterol excretion. Induction of cholesterol efflux transport genes was analyzed by QRT-PCR in liver total RNA (A, B); ABCG5, ATP-binding cassette, sub-family G (WHITE), member 5; ABCG8, ATP-binding cassette, sub-family G (WHITE), member 8; ABCA1, ATP-binding cassette, sub-family A (ABC1), member 1; ABCG1, ATP-binding cassette, sub-family G (WHITE), member 1; ABCB4, ATP-binding cassette, sub-family B (MDR/TAP), member 4; and ABCB11, ATP-binding cassette, sub-family B (MDR/TAP), member 11. The gene expression of the nuclear receptors that regulate ABCG5 and ABCG8 was analyzed by QRT-PCR in liver total RNA (C); HNF4α, hepatocyte nuclear factor 4α; LRH1, liver receptor homolog 1; and LXRα, liver X receptor α. Values are expressed as the mean ± S.E.M. (n = 6). Labeled means without a common letter are significantly different by Tukey's multiple comparison test ($P < 0.05$).

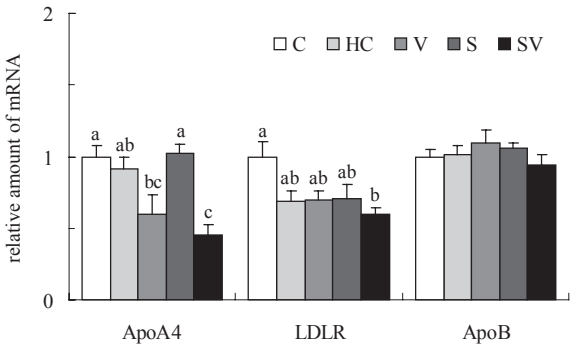

Fig. 4. Effects of sesamin and α-tocopherol on the gene expression involved in hepatic cholesterol circulation. QRT-PCR was performed to measure expression levels of apolipoprotein (Apo) A4, low-density-lipoprotein receptor (LDLR), and ApoB mRNA in the liver of each rat. Values are expressed as the mean ± S.E.M. (n = 6). Labeled means without a common letter are significantly different by Tukey's multiple comparison test ($P < 0.05$).

Gene expression involved in hepatic cholesterol synthesis

We also analyzed the expressions of genes including HMGCR, SREBF2, SCAP, INSIG1, and INSIG2 (Fig. 5). The gene expression of HMGCR in rats fed diets containing cholesterol became nearly less than one-third of that in the animals fed a control diet. However, the values were comparable among the HC, V, S, and SV groups. The gene expressions of SREBF2 and SCAP were decreased in the rats fed the diets containing cholesterol, but there were no significant differences in these gene expressions among the HC, V, S, and SV groups. Although the gene expression level of INSIG1 tended to be lower in the rats fed the diets containing cholesterol than in those in the C group, this gene expression showed no differences among all the groups. In contrast to the situation observed for INSIG1, the gene expression of INSIG2 tended to be increased in the rats fed the diets containing cholesterol, but there were no significant differences in this gene expression among the HC, V, S, and SV groups.

Gene expression involved in α- and γ-tocopherol metabolism

Figure 6 shows the gene expression of enzymes involved in α- and γ-tocopherol metabolism including CYP3A2, CYP4F2, and α-TTP. The gene expression of CYP3A2 was increased in the V and SV groups compared with that in the HC group. No significant differences were found in the CYP4F2 gene expression among the HC, V, S, and SV groups. The gene expression of α-TTP was increased in the V group compared with that in the S group.

α- and γ-tocopherol, sesamin, and sesamin metabolite levels in blood and liver

The concentrations of α- and γ-tocopherol in serum and liver on day 10 are shown in Fig. 7. Both V and SV groups showed higher values of α-tocopherol concentration in serum and liver than those in the C, HC, and S groups, which remained at a low level. In liver, the con-

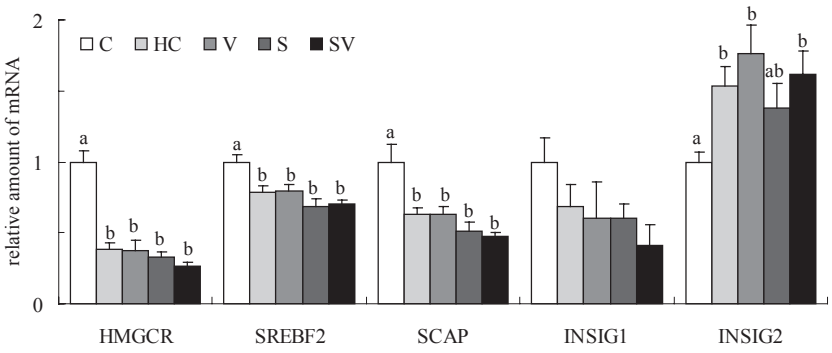

Fig. 5. Effects of sesamin and α-tocopherol on the gene expression involved in hepatic cholesterol synthesis and catabolism. QRT-PCR was performed to measure expression levels of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), sterol regulatory element binding transcription factor 2 (SREBF2), SREBF chaperone (SCAP), insulin induced gene 1 (INSIG1), and insulin-induced gene 2 (INSIG2) mRNA in the liver of each rat. Values are expressed as the mean ± S.E.M. (n = 6). Labeled means without a common letter are significantly different by Tukey's multiple comparison test ($P < 0.05$).

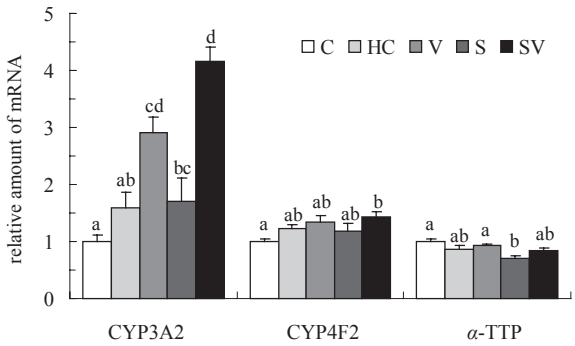

Fig. 6. Effects of sesamin and α-tocopherol on the gene expression involved in α- and γ-tocopherol metabolism. QRT-PCR was performed to measure expression levels of CYP3A2, CYP4F2, and α-tocopherol transfer protein (α-TTP) mRNA in the liver of each rat. Values are expressed as the mean ± S.E.M. (n = 6). Labeled means without a common letter are significantly different by Tukey's multiple comparison test ($P < 0.05$).

centration of α-tocopherol in the SV group was almost twice as high as that in the V group. In serum and liver, the γ-tocopherol concentration of the S group was higher than those of the other groups, although the γ-tocopherol concentrations in the diets of every group were the same.

The concentrations of sesamin and sesamin metabolites in plasma on day 10 are shown in Fig. 8. Each sesamin metabolite was detected in rat plasma at concentrations over 200 ng/ml and the concentrations of SC-1 and SC-2 were higher than those of their metabolite in plasma. No significant differences in the concentrations of sesamin and sesamin metabolites in plasma were seen between the S group and the SV group.

Discussion

In the present study, it was confirmed that the ingestion of sesamin together with α-tocopherol synergistically reduces the concentration of blood cholesterol in rats given a high-cholesterol diet. To investigate the molecular mechanism involved in this cholesterol lowering, we analyzed the gene-expression profiles in liver.

First, we examined the gene expression of several cholesterol efflux transporters. This revealed that co-ingestion of sesamin and α-tocopherol synergistically increased the gene expressions of ABCG5 and ABCG8, which are responsible for cholesterol lowering. ABCG5 and ABCG8 are half-transporters, that is, their genes only encode half of the structural motifs that are necessary to produce a functional transporter, and the two half-transporters form a functional heterodimer (27, 28). In mice, ABCG5/ABCG8 deficiency increases plasma sitosterol and decreases bile cholesterol (29), whereas ABCG5/ABCG8 over-expression increases biliary cholesterol secretion and decreases dietary cholesterol absorption (30). It has been reported that fecal cholesterol excretion, in terms of both the concentration and the daily excretion, increased significantly in rats fed sesamin isomers (a mixture of sesamin and episesamin) with a cholesterol-enriched diet (12). This report might partially reflect our results that the hypocholesterolemic action was due to up-regulation of the genes for cholesterol efflux transporter, ABCG5 and ABCG8. The other cholesterol efflux transporters were not considered to affect the cholesterol lowering in the blood because co-ingestion of sesamin and α-tocopherol did not increase the gene expression of these transporters. The close head-to-head juxtaposition of the ABCG5 and ABCG8 genes suggests that they share a bidirectional promoter, which like other similarly arranged genes may provide a means for coordinately regulating their expression (31 – 33). Thus, it seemed reasonable that the expression patterns between ABCG5 and ABCG8 were similar in our experiment. Several potential regulatory elements were found for the ABCG5 and ABCG8 genes, and the intergenic region was found to act as a bidirectional promoter (34). Transcriptional control of ABCG5 and ABCG8 genes can be attributed to the nuclear hormone receptor family, including HNF4α, LRH1, and LXRα (34 – 36), but the co-ingestion of sesamin and α-tocopherol did not

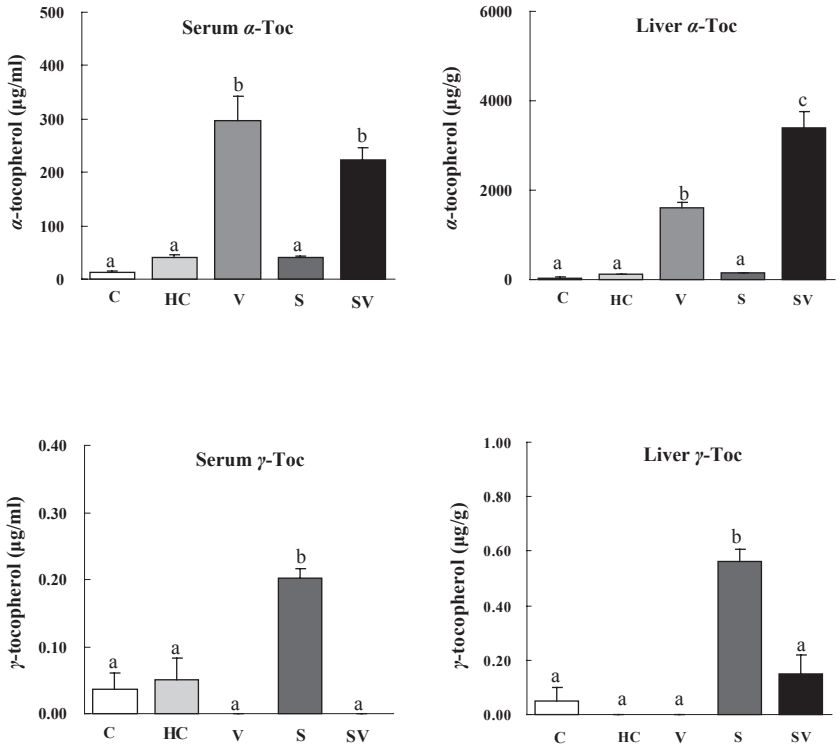

Fig. 7. Effects of sesamin and α-tocopherol on the α- and γ-tocopherol concentrations in liver and blood. Rats were fed the experimental diets for 10 days. Concentrations of α- and γ-tocopherol in liver and serum were measured. Data are expressed as the mean ± S.E.M. (n = 6). Labeled means without a common letter are significantly different by Tukey's multiple comparison test ($P < 0.05$).

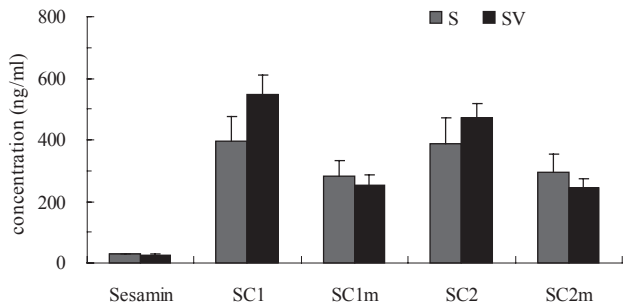

Fig. 8. Sesamin metabolism in rats. Plasma concentrations of sesamin and sesamin metabolites in rats fed a 0.2% sesamin-containing diet were measured. Data are expressed as the mean ± S.E.M. (n = 6).

increase the gene expression of these nuclear receptors in the present study. It seemed that the change in these gene expressions might not participate in the up-regulation of ABCG5 and ABCG8 genes.

Secondly, we analyzed the gene expression of proteins involved in hepatic cholesterol circulation. As co-expression of ApoB and ApoA4 modified with the carboxyl-terminal endoplasmic reticulum retention signal reduced the secretion of ApoB in COS cells, ApoA4 may physically interact with ApoB in the secretory pathway (37). This report suggests that down-regulation of ApoA4 gene expression reduced ApoB secretion. Therefore, it was supposed that the co-ingestion of sesamin and α-tocopherol generated the down-regulation of ApoA4, which caused LDL-C–lowering in the blood attributed to ApoB. A study on male patients with hypercholesterolemia has been reported. The sesamin-treated group received 32.4 mg of sesamin isomers and 162 mg of vitamin E per day for 4 weeks, followed by 64.8 mg of sesamin isomers and 324 mg of vitamin E per day for 4 weeks, and the placebo-treated group received 162 and 324 mg of vitamin E for 4 weeks each. LDL-C and ApoB were significantly lower in the sesamin-treatment group than in the placebo group (14). This effect might be ascribed to the reduction of ApoB secretion as a result of the down-regulation of ApoA4 expression by sesamin and α-tocopherol ingestion. Additionally, as co-ingestion of sesamin and α-tocopherol maintained the low gene expression of LDLR, which was decreased in the rats fed with a high-cholesterol diet, LDLR seemed not to be responsible for the LDL-C reduction in the blood.

Finally, we analyzed the gene expression involved in hepatic cholesterol synthesis. Our results revealed that the gene expression of HMGCR in rats fed diets containing cholesterol became nearly less than one-third of that in the animals fed a control diet. There were no significant differences in the values among the HC, V, S, and SV groups owing to the potent effect of the high-cholesterol diet on lowering this gene expression. Although the gene-expression profiles of the regulators involved in cholesterol synthesis, except for INSIG1, were consistent

with the inhibition of cholesterol synthesis, they were not specific to the ingestion of sesamin and α-tocopherol. Therefore, it was considered that this inhibition was attributable not to sesamin and α-tocopherol but rather to the high-cholesterol diet. Thus, the ingestion of sesamin together with α-tocopherol was not considered to affect the gene expression associated with cholesterol synthesis.

In the course of the study on the mechanism involved, we observed that the concentrations of α-tocopherol in serum and liver were clearly higher in the V group than in the S group. In contrast, the concentrations of γ-tocopherol in serum and liver were clearly higher in the S group than in the V group. In spite of these results, the effect on cholesterol lowering was similar between the V and S groups. Therefore, α- or γ-tocopherol concentration in the serum or liver seemed not to affect the cholesterol reduction in the blood. It is known that dietary sesamin isomers elevate γ-tocopherol concentrations in liver and serum by the inhibition of CYP3A- or CYP4F2-dependent metabolism of γ-tocopherol (38, 39). In this study, we showed that the concentrations of γ-tocopherol in serum and liver were clearly lower in the V and SV groups than in the S group. Therefore, it was supposed that α-tocopherol decreased the concentrations of γ-tocopherol in liver and serum as a result of the up-regulation of the CYP3A2 gene. CYP4F2 and α-TTP were not considered to affect the concentrations of γ- and α-tocopherol in the blood and liver because sesamin and α-tocopherol did not increase the expression of these genes. As the concentrations of sesamin and sesamin metabolites in plasma were comparable between the S group and the SV group, they also seemed not to affect the cholesterol reduction in the blood.

Patients with hypercholesterolemia can be treated with a change in diet, statins, fibrates, cholesterol absorption inhibitors, and bile acid sequestrants (40, 41). Statins are the most commonly used and effective forms of medication for the treatment of high cholesterol. They lower cholesterol by inhibiting the enzyme HMGCR, which is the rate-limiting enzyme of cholesterol synthesis. Fibrates induce hepatic lipid metabolism through a mechanism involving the peroxisome proliferator-activated receptor. Bile acid sequestrants disrupt the enterohepatic circulation of bile acids by sequestering them and preventing their reabsorption from the gut. Cholesterol absorption inhibitors localize at the brush border of the small intestine, where they inhibit the absorption of cholesterol from the diet. Fibrates, bile acid sequestrants, and cholesterol absorption inhibitors may be used together with statins when cholesterol levels cannot be controlled with statins alone. In this study, we suggested that the mechanism underlying the hypocholesterolemic action by the co-ingestion of sesamin and α-tocopherol was up-regulation of ABCG5 and ABCG8 and down-regulation of ApoA4. This novel mechanism might be applicable for hypercholesterolemia treatment.

In summary, we postulate a mechanism for the combined hypocholesterolemic action of sesamin and α-tocopherol: ingestion effectively increases biliary excretion of cholesterol by up-regulation of the gene expression for cholesterol efflux transporter, ABCG5 and ABCG8, and reduces ApoB secretion into the bloodstream by down-regulation of ApoA4 gene expression related to secretion of ApoB.

References

1 Shimizu S, Akimoto K, Shinmen Y, Kawashima H, Sugan M, Yamada H. Sesamin is a potent and specific inhibitor of delta 5 desaturase in polyunsaturated fatty acid biosynthesis. Lipids. 1991;26:512–516.

2 Shimizu S, Akimoto K, Shinmen Y, Sugano M, Yamada H. Production of dihomo-γ-linolenic acid by Mortierella alpina 1S-4. J Am Oil Chem Soc. 1989;66:237–241.

3 Fukuda Y, Nagata T, Osawa T, Namiki M. Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil. J Am Oil Chem Soc. 1986;63:1027–1031.

4 Ikeda S, Kagaya M, Kobayashi K, Tohyama T, Kiso Y, Higuchi N, et al. Dietary sesame lignans decrease lipid peroxidation in rats fed docosahexaenoic acid. J Nutr Sci Vitaminol. 2003;49: 270–276.

5 Hirose N, Doi F, Ueki T, Akazawa K, Chijima K, Sugano M, et al. Suppressive effect of sesamin against 7,12-dimethylbenz-[a]anthracene-induced rat mammary carcinogenesis. Anticancer Res. 1992;12:1259–1265.

6 Matsumura Y, Kita S, Morimoto S, Akimoto K, Furuya M, Oka N, et al. Antihypertensive effect of sesamin. I. Protection against deoxycorticosterone acetate-salt-induced hypertension and cardiovascular hypertrophy. Biol Pharm Bull. 1995;18:1016–1019.

7 Kita S, Matsumura Y, Morimoto S, Akimoto K, Furuya M, Oka N, et al. Antihypertensive effect of sesamin. II. Protection against two-kidney, one-clip renal hypertension and cardiovascular hypertrophy. Biol Pharm Bull. 1995;18:1283–1285

8 Matsumura Y, Kita S, Tanide Y, Taguchi Y, Morimoto S, Akimoto K, et al. Antihypertensive effect of sesamin. III. Protection against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats. Biol Pharm Bull. 1998; 21:469–473.

9 Nakano D, Itoh C, Ishii F, Kawanishi H, Takaoka M, Kiso Y, et al. Effects of sesamin on aortic oxidative stress and endothelial dysfunction in deoxycorticosterone acetate-salt hypertensive rats. Biol Pharm Bull. 2003;26:1701–1705.

10 Nakano D, Kurumazuka D, Nagai Y, Nishiyama A, Kiso Y, Matsumura Y. Dietary sesamin suppresses aortic NADPH oxidase in DOCA salt hypertensive rats. Clin Exp Pharmacol Physiol. 2008;35:324–326.

11 Miyawaki T, Aono H, Toyoda-Ono Y, Maeda H, Kiso Y, Moriyama K. Antihypertensive effects of sesamin in humans. J Nutr Sci Vitaminol. 2009;55:87–91.

12 Hirose N, Inoue T, Nishihara K, Sugano M, Akimoto K, Shimizu

S, et al. Inhibition of cholesterol absorption and synthesis in rats by sesamin. J Lipid Res. 1991;32:629–638.
13 Ashakumary L, Rouyer I, Takahashi Y, Ide T, Fukuda N, Aoyama T, et al. Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. Metabolism. 1999;48:1303–1313.
14 Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Nakamura H. Hypocholesterolemic effect of sesamin lignan in humans. Atherosclerosis. 1996;122:135–136.
15 Nakabayashi A, Kitagawa Y, Suwa Y, Akimoto K, Asami S, Shimizu S, et al. alpha-Tocopherol enhances the hypocholesterolemic action of sesamin in rats. Int J Vitam Nutr Res. 1995; 65:162–168.
16 Yamashita K, Nohara Y, Katayama K, Namiki M. Sesamin seed lignans and gamma-tochopherol act synergistically to produce vitamin E activity in rats. J Nutr. 1992;122:2440–2446.
17 Yamashita K, Iizuka Y, Imai T, Namiki M. Sesame seed and its lignans produce marked enhancement of vitamin E activity in rats fed a low alpha-tocopherol diet. Lipids. 1995;30:1019–1028.
18 Tsuruoka N, Kidokoro A, Matsumoto I, Abe K, Kiso Y. Modulating effect of sesamin, a functional lignan in sesame seeds, on the transcription levels of lipid- and alcohol-metabolizing enzymes in rat liver: a DNA microarray study. Biosci Biotechnol Biochem. 2005;69:179–188.
19 Noguchi T, Ikeda K, Sasaki Y, Yamamoto J, Seki J, Yamagata K, et al. Effects of vitamin E and sesamin on hypertension and cerebral thrombogenesis in stroke-prone spontaneously hypertensive rats. Hypertens Res. 2001;24:735–742.
20 Kong X, Yang JR, Guo LQ, Xiong Y, Wu XQ, Huang K, et al. Sesamin improves endothelial dysfunction in renovascular hypertensive rats fed with a high-fat, high-sucrose diet. Eur J Pharmacol. 2009;620:84–89.
21 Khan MM, Ishrat T, Ahmad A, Hoda MN, Khan MB, Khuwaja G, et al. Sesamin attenuates behavioral, biochemical and histological alterations induced by reversible middle cerebral artery occlusion in the rats. Chem Biol Interact. 2010;183:255–263.
22 Ide T, Nakashima Y, Iida H, Yasumoto S, Katsuta M. Lipid metabolism and nutrigenomics – impact of sesame lignans on gene expression profiles and fatty acid oxidation in rat liver. Forum Nutr. 2009;61:10–24.
23 Ide T, Lim JS, Odbayar TO, Nakashima Y. Comparative study of sesame lignans (sesamin, episesamin and sesamolin) affecting gene expression profile and fatty acid oxidation in rat liver. J Nutr Sci Vitaminol. 2009;55:31–43.
24 Akimoto K, Asami S, Shimizu S, Sugano M, Yamada H. Antioxidant activity of sesamin on NADPH-dependent peroxidation in liver microsomes. In: Kumpulainen JT, Salonen JT, editors. Natural antioxidants and food quality in atherosclerosis and cancer prevention. London: The Royal Society of Chemistry; 1996. p. 241–246.
25 Nakai M, Harada M, Nakahara K, Akimoto K, Shibata H, Miki W, et al. Novel antioxidative metabolites in rat liver with ingested sesamin. J Agric Food Chem. 2003;51:1666–1670.
26 Akimoto K, Kigawa Y, Akamatsu T, Hirose N, Sugano M, Shimizu S, et al. Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. Ann Nutr Metab. 1993;37:218–224.
27 Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science. 2000;290: 1771–1775.
28 Lu K, Lee MH, Hazard S, Brooks-Wilson A, Hidaka H, Kojima H, et al. Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. Am J Hum Genet. 2001;69:278–290.
29 Yu L, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, et al. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. Proc Natl Acad Sci U S A. 2002;99:16237–16242.
30 Yu L, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, et al. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J Clin Invest. 2002;110:671–680.
31 Wright KL, White LC, Kelly A, Beck S, Trowsdale J, Ting JP. Coordinate regulation of the human TAP1 and LMP2 genes from a shared bidirectional promoter. J Exp Med. 1995;181: 1459–1471.
32 Pollner R, Schmidt C, Fischer G, Kühn K, Pöschl E. Cooperative and competitive interactions of regulatory elements are involved in the control of divergent transcription of human Col4A1 and Col4A2 genes. FEBS Lett. 1997;405:31–36.
33 Ryan MT, Herd SM, Sberna G, Samuel MM, Hoogenraad NJ, Hoj PB. The genes encoding mammalian chaperonin 60 and chaperonin 10 are linked head-to-head and share a bidirectional promoter. Gene. 1997;196:9–17.
34 Remaley AT, Bark S, Walts AD, Freeman L, Shulenin S, Annilo T, et al. Comparative genome analysis of potential regulatory elements in the ABCG5-ABCG8 gene cluster. Biochem Biophys Res Commun. 2002;295:276–282.
35 Sumi K, Tanaka T, Uchida A, Magoori K, Urashima Y, Ohashi R, et al. Cooperative interaction between hepatocyte nuclear factor 4 alpha and GATA transcription factors regulates ATP-binding cassette sterol transporters ABCG5 and ABCG8. Mol Cell Biol. 2007;27:4248–4260.
36 Freeman LA, Kennedy A, Wu J, Bark S, Remaley AT, Santamarina-Fojo S, et al. The orphan nuclear receptor LRH-1 activates the ABCG5/ABCG8 intergenic promoter. J Lipid Res. 2004;45:1197–1206.
37 Gallagher JW, Weinberg RB, Shelness GS. apoA-IV tagged with the ER retention signal KDEL perturbs the intracellular trafficking and secretion of apoB. J Lipid Res. 2004;45:1826–1834.
38 Ikeda S, Tohyama T, Yamashita K. Dietary sesame seed and its lignans inhibit 2,7,8-trimethyl- 2(2′-carboxyethyl)-6-hydroxychroman excretion into urine of rats fed gamma-tocopherol. J Nutr. 2002;132:961–966.
39 Sontag TJ, Parker RS. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. J Biol Chem. 2002;277:25290–25296.
40 Pignone M. Primary prevention: dyslipidaemia. Clin Evid. 2005; 14:142–150.
41 Gami A. Secondary prevention of ischaemic cardiac events. Clin Evid. 2006;15:195–228.