Full paper

Fucoidan improves serum lipid levels and atherosclerosis through hepatic SREBP-2-mediated regulation

Jinhee Park a, 1, Mijung Yeom a, 1, Dae-Hyun Hahm a, b, *

a Acupuncture and Meridian Science Research Center, College of Korean Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea
b Department of Science in Korean Medicine, College of Korean Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea

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ABSTRACT

Hyperlipidemia is associated with increased risk of the development of cardiovascular diseases. Although a great deal of attention has been paid to the hypolipidemic activity of fucoidan, complex polysaccharides from brown seaweeds, the underlying mechanism is still unclear. This study was performed to investigate whether and how fucoidan has lipid-lowering potential in poloxamer-407 (P407)-induced hyperlipidemic mice. Fucoidan treatment 2 h after acute administration of P407 in these mice significantly reduced serum total cholesterol, triglycerides, and LDL cholesterol levels, but increased the levels of HDL cholesterol. In HepG2 hepatocytes and the liver, fucoidan decreased the expression of FAS and ACC mRNA with no or only a moderate inhibitory effect on SREBP-1c mRNA expression. Furthermore, fucoidan attenuated the hepatic expression of mature SREBP-2 protein with a subsequent decrease in hepatic HMG-CoA reductase mRNA expression and an increase in hepatic LDL receptor mRNA expression. In addition, atherosclerotic lesions in the aorta of chronically P407-treated mice were also reduced by fucoidan. These findings indicate that fucoidan improves serum lipid levels by regulating the expression of key enzymes of cholesterol and triglyceride synthesis in the liver through modulation of SREBP-2.

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1. Introduction

Hyperlipidemia, in which the levels of any or all lipids and/or lipoproteins are abnormally elevated in the blood, contributes to the development of atherosclerosis and related cardiovascular diseases, which are the primary leading causes of death worldwide (1). For example, the recent study indicated that one of the most strongly predictive cardiovascular risk factors for myocardial infarction is dyslipidemia characterized by elevated concentrations of serum triglyceride (TG) as well as increased levels of low-density lipoprotein cholesterol (LDL-c) and decreased levels of high-density lipoprotein cholesterol (HDL-c) (2,3). Therefore, any rational strategies for prevention and treatment of atherosclerosis as well as reduction of the incidence of related cardiovascular diseases should be closely associated with targeting hyperlipidemia by drugs and/or dietary intervention.

The liver plays a central role in the maintenance of systemic lipid homeostasis through regulating several lipid metabolic pathways, such as lipogenesis and lipoprotein uptake and secretion in response to nutritional and hormonal signals (4). However, dysregulation of lipid metabolism in the liver results in hyperlipidemia, characterized by an excess of lipids, such as total cholesterol (TC), TG, and oxidized LDL, and a lack of HDL in the bloodstream. Recently, sterol regulatory element-binding proteins (SREBPs) have attracted a great deal of attention as important transcription factors involved in the regulation of lipid metabolism and homeostasis in the liver, inducing many genes involved in lipid synthesis and the uptake of lipoproteins (5). There are three SREBP isoforms, SREBP-1a, SREBP-1c, and SREBP-2, encoded by two genes, SREBP1 and SREBP2, which have different roles in lipid metabolism (6); SREBP-1c plays an essential role in TG and fatty acid synthases by regulating the expression of lipogenic genes, including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (7), whereas SREBP-2 is relatively specific to cholesterol synthesis by regulating the expression of genes involved in cholesterol synthesis and uptake, such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCr) and LDL receptor (LDLR) (7,8). When excess...
levels of cholesterol are sensed by SREBP cleavage activating protein (SCAP), which contains a cholesterol-sensing domain. SCAP strongly interacts with a retention protein (Insig-1 or -2), tethering the SREBP–SCAP complex in the endoplasmic reticulum. When cholesterol levels are low, the SREBP–SCAP complex is transported to the Golgi where it is processed by two proteases (site-1 protease and site-2 protease) into the mature form (6). Therefore, release of SREBP–SCAP complex from the ER is the critical point in feedback regulation of cholesterol metabolism.

Marine natural products, especially polysaccharides from marine resources, have attracted increasing attention due to their various biological activities (9). Fucoidan, a sulfated polysaccharide from marine resources, have attracted increasing attention due to their anti-inflammatory effects (15). The potential lipid-lowering property of fucoidan was reported previously in several studies. Administration of fucoidan from *Laminaria japonica* (family Laminariaceae) decreased serum TC, TG, and LDL-c concentrations, while increasing HDL-c levels, in a high-fat emulsion-fed hyperlipidemic rat model (16). Fucoidan from *Cladosiphon okamuranus* (family Chordariaceae) also reduced the isoproterenol-induced increase in concentrations of TC, TG, and LDL-c but increased that of HDL-c in a rat model of myocardial infarction (17). These benefits of fucoidan may have been mediated by inhibition of lipid accumulation and stimulation of lipolysis (14). However, it remains unclear whether and how fucoidan exerts hypolipidemic effects.

The present study was performed to investigate whether and how fucoidan affects lipid metabolism in P407-induced hyperlipidemic mice and human hepatoma HepG2 cells. In addition, the hypolipidemic effect of fucoidan and its hepatic regulation of lipid synthesis were investigated. The antiatherogenic effect of fucoidan was also examined in the atherosclerotic mouse aorta.

2. Materials and methods

2.1. Chemicals and reagents

Fucoidan from *Fucus vesiculosus* (family Fucaceae) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Poloxamer-407 was obtained from BASF Co. (Parsippany, NJ, USA). Minimum essential medium (MEM), fetal bovine serum (FBS), penicillin/streptomycin, and phosphate-buffered saline (PBS) were purchased from WelGENE (Seoul, Korea). An EZ-Cytox cell viability assay kit was purchased from Daeil Lab (Seoul, Korea). TRizol reagent was purchased from Life Technologies (Carlsbad, CA, USA). Reverse transcriptase and Taq polymerase were purchased from Takara Bio (Shiga, Japan). The nucleic acid dye GelRed was purchased from Biotium Inc. (Hayward, CA, USA). Goat anti-human HMGGCR, goat anti-mouse LDLR and rabbit anti-human SREBP-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-human β-actin was purchased from Sigma–Aldrich (St. Louis, MO, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit goat anti-mouse, and donkey anti-goat secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Oligonucleotides used as primers for PCR were synthesized by COSMO Genetech (Seoul, Korea).

Commercially available diagnostic kits for cholesterol, TG and HDL-c were purchased from Bio Clinical System Co. (Anyang, Korea). All other chemicals and reagents not specifically cited here were purchased from Sigma–Aldrich.

2.2. Cell culture

HepG2, a human hepatoma cell line, was obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were grown in MEM supplemented with 10% heat-inactivated FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) mixture, and 25 mM HEPES at 37 °C in a humidified incubator with 5% CO2. To upregulate the mevalonate pathway, the cells were incubated in medium containing LPDS (18). For the experiments, HepG2 cells were plated in 10% FBS-DMEM and incubated for 24 h. Thereafter, all cells were treated with 10% LPDS-MEM with or without the indicated concentrations of fucoidan for 24 h.

2.3. Animals

Adult male C57BL/6NTacSam mice (7 weeks old) were obtained from Samtako (Yongin, Korea). The animals were acclimatized to the laboratory environment for 1 week before starting the experiments. All mice were maintained on a 12-h light–dark cycle with free access to food and water. The experimental procedures were approved by the Kyung Hee University Institutional Animal Care and Use Committee.

2.4. Cytotoxicity assay

Cytotoxicity was assessed by WST assay using the EZ-Cytox cell viability assay kit (Daeil Lab) according to the manufacturer’s instructions. Briefly, HepG2 cells were treated with various concentrations of fucoidan (up to 500 µg/ml). Twenty-four hours later, 10 µl kit reagent was added into each well. After 2 h, the plates were read at 450 nm using a microplate reader ( Molecular Devices, Sunnyvale, CA, USA). The results are expressed as percentages of untreated controls.

2.5. Schedules for poloxamer-407-induced hyperlipidemia, fucoidan treatment, and sampling

Aqueous P407 solutions were prepared according to the cold method (19). Briefly, P407 was mixed with cold saline and stored overnight at 4 °C to facilitate dissolution of P407. As shown in Fig. 1A, for the acute studies, mice were fasted overnight prior to P407 injection and then injected intraperitoneally with a single 250 mg/kg dose of P407 followed by another 6-h fast. Control animals received the same volume of cold sterile saline as vehicle. Animals were randomly divided into six experimental groups (n = 6 each): NOR, normal mice treated with saline; P407, P407-induced hyperlipidemic mice treated with saline; FCN10, P407-induced hyperlipidemic mice treated with 10 mg/kg fucoidan; FCN30, P407-induced hyperlipidemic mice treated with 30 mg/kg fucoidan; FCN50, P407-induced hyperlipidemic mice treated with 50 mg/kg fucoidan; STA, P407-induced hyperlipidemic mice treated with 10 mg/kg atorvastatin. Fucoidan was dissolved in normal saline just before use and administered intraperitoneally to mice 2 h after P407 administration. As a positive control, atorvastatin was used instead of fucoidan (STA group). Blood samples were collected 24 h after P407 injection. To examine the effects of fucoidan on P407-induced atherogenesis, mice were injected intraperitoneally with 400 mg/kg P407 every third day for 16 weeks (Fig. 1B). Fucoidan (50 mg/kg) and atorvastatin (10 mg/kg) were only administered intraperitoneally 2 h after each P407 injection. Aortas were collected 24 h following the final injection of P407.

2.6. Determination of serum lipid levels and the atherogenic index

Twenty-four hours after P407 injection, blood was collected through cardiac puncture, and the serum was separated. Before blood sampling, mice were fasted for 8 h. Serum levels of TC, TG, and HDL-c were measured using commercial kits according to the manufacturer’s instructions. LDL-c was calculated using the equation LDL = TC/
1.19 + TG/1.9–HDL/1.1–38, as reported by Ahmadi et al. (20). The atherogenic index (AI) was calculated as \((TC – HDL-c)/HDL-c\) (21).

2.7. Quantification of aortic intima-media thickness

Formalin-fixed, paraffin-embedded sections of thoracic aortas were stained with hematoxylin and eosin (H–E). Quantification of the maximal intimal thickness was then accomplished by determining the maximal intima-media thickness defined as the maximal measurement from the lesion luminal surface to the outer surface of the vessel wall using DP2-BSW software (Olympus Ltd., Tokyo, Japan) at \( \times 400 \) magnification. Three measurements of intima-media thickness were taken at the site of the greatest thickness and expressed as arbitrary units (pixels). The results are presented as the mean lesion size ± SEM for each group.

2.8. Total RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from mouse livers and HepG2 cells using TRIzol reagent in accordance with the manufacturer’s instructions. First-strand cDNA was reverse transcribed from 2 \( \mu \)g total RNA using reverse transcriptase (Takara Bio) with random hexamers. PCR was performed with specific primers using Taq polymerase (Takara Bio) in a thermal cycler (MJ Research, Waltham, MA, USA). The sequences of the primers are shown in Table 1. PCR products were confirmed by electrophoresis on agarose gels. The primer sequences are shown in Table 1.

### Table 1

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<th>Gene Name</th>
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<th>Annealing temp. (°C)</th>
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<td></td>
<td>Reverse: GTACTAGTTGCTGCTCAGGCA</td>
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<tr>
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SREBP-1c, sterol regulatory element-binding protein-1c; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
were electrophoresed on 1.2% agarose gels and visualized under UV after GelRed staining (Biotium Inc.). Quantification of band intensities was performed using Image Master Total Lab (Amersham Pharmacia, Piscataway, NJ, USA). GAPDH was used as an endogenous control for normalization. Data are expressed relative to untreated controls.

2.9. Western blot analysis

Liver tissue was homogenized in RIPA buffer containing protease and phosphatase inhibitor cocktail (Sigma–Aldrich). Protein samples (50 μg) were separated on a 7.5% polyacrylamide gel (SREBP-2, HMGR) and transferred onto PVDF membranes. After blocking nonspecific binding using 5% skim milk for 1 h, the blots were incubated with a primary antibody against SREBP-2 (1:250; Santa Cruz), HMGR (1:500; Abcam), LDLR (1:200; Santa Cruz) or β-actin (1:2000; Sigma–Aldrich) at 4 °C overnight, followed by appropriate secondary antibodies conjugated to horseradish peroxidase (HRP; 1:2000–5000; Santa Cruz) for 1 h. The signals were detected using ECL or ECL plus reagents (Thermo Fisher Scientific Inc., Waltham, MA, USA). β-actin was used as a loading control. The band intensity was quantified using image-analysis software (Image Master Total Lab; Amersham Pharmacia Biotech) and normalized to that of β-actin. Data are expressed relative to untreated controls.

2.10. Statistical analysis

The data are presented as the means ± SEM. The experimental data were analyzed using one-way ANOVA with Tukey’s post hoc test (SPSS 13.0; SPSS, Chicago, IL, USA). In all analyses, P < 0.05 represented statistical significance.

3. Results

3.1. Fucoidan improves serum lipid parameters in P407-induced hyperlipidemic mice

To verify whether fucoidan has lipid-lowering activity, acute hyperlipidemia was elicited by a single intraperitoneal injection of P407 into mice, and the antihyperlipidemic effect of intraperitoneally administered fucoidan was investigated in this mouse model. The serum lipid levels were measured 24 h after P407 injection (Fig. 2). Atorvastatin, an inhibitor of HMGR with beneficial effects, was used as a positive control. Before fucoidan treatment, a single injection of P407 resulted in highly significant increases in serum TC, TG, and LDL-c levels in the mice by 216%, 1522%, and 1409%, respectively, and a marked decrease in serum HDL-c level by 51% compared with those in the NOR group. Serum TC, TG, and LDL-c concentrations were lower in the fucoidan-treated mice by 31%, 41%, and 32%, respectively, compared with those in P407-induced hyperlipidemic mice in a dose-dependent manner. On the other hand, fucoidan significantly increased the HDL-c level by 92% in a dose-dependent manner. To support the lipid-lowering potential of fucoidan, the AI, a possible indicator of predisposition to heart disease, was also evaluated. Consistent with the above observations, fucoidan treatment significantly decreased the AI value in a dose-dependent manner in comparison with that in the non-treated P407 group. The restorative effect of fucoidan and the AI in the FCN50 group (50 mg/kg) were compatible with those in the STA group (10 mg/kg atorvastatin).

3.2. Fucoidan regulates mRNA expression of genes involved in lipogenesis regulation in HepG2 cells

Elevated fatty acid synthesis in the liver and subsequent TG synthesis predominantly contribute to the accumulation of lipids in the liver. Our study was conducted to determine whether fucoidan affects the lipid accumulation in the liver and subsequently regulate the expression of lipogenesis-related genes in HepG2 cells. Fucoidan significantly decreases the expression of fatty acid synthase (FAS) and sterol regulatory element-binding protein-1c (SREBP-1c) in a dose-dependent manner. In addition, the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), which is known to play a key role in lipid metabolism, was also decreased in a dose-dependent manner. These results suggest that fucoidan may play a role in regulating lipid metabolism at the transcriptional level.

Fig. 2. Effects of fucoidan on serum lipids and atherogenic index in P407-induced hyperlipidemic mice. Fucoidan was injected intraperitoneally 2 h after acute P407 (250 mg/kg) administration in mice. The serum samples were collected 24 h after administration of P407. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) levels and the atherogenic index (AI) were evaluated. Data are presented as means ± SEM (n = 6 each). NOR, normal mice treated with saline; P407, P407-induced hyperlipidemic mice treated with saline; FU10, P407-induced hyperlipidemic mice treated with 10 mg/kg fucoidan; FU30, P407-induced hyperlipidemic mice treated with 30 mg/kg fucoidan; FU50, P407-induced hyperlipidemic mice treated with 50 mg/kg fucoidan; STA, P407-induced hyperlipidemic mice treated with 10 mg/kg atorvastatin. *P < 0.05, **P < 0.01, and ***P < 0.001 determined by one-way ANOVA compared with the P407 group.
the liver and serum. Therefore, the effects of fucoidan on the mRNA expression levels of genes involved in control of lipogenesis (FAS and ACC) were analyzed in human hepatoma HepG2 cells using RT-PCR. Compared with untreated HepG2 cells, fucoidan treatment significantly decreased the levels of FAS and ACC mRNA by 87% and 89%, respectively, in a concentration-dependent manner (Fig. 3A). Fatty acid synthesis mediated by ACC and FAS is transcriptionally regulated by SREBP-1c (7). To determine whether SREBP-1c is involved in the suppressive effect of fucoidan on lipogenesis, the mRNA level of the SREBP-1c gene itself was examined in fucoidan-treated HepG2 cells. However, in contrast to our expectations, fucoidan did not significantly reduce the SREBP-1c mRNA level in fucoidan-treated cells (Fig. 3B).

To examine whether the inhibitory effects of fucoidan on mRNA levels of SREBP-1c and its target genes in HepG2 cells were due to cytotoxicity by fucoidan itself, the cell viability was also examined by WST assay 24 h after fucoidan treatment. The concentrations of fucoidan used in this study (up to 100 μg/ml) did not lead to any significant cytotoxicity (data not shown).

3.3. Fucoidan regulates expression of genes involved in cholesterol metabolism in HepG2 cells

Next, we investigated the effects of fucoidan on the expression of genes involved in cholesterol metabolism (HMGCR and LDLR) in HepG2 cells. RT-PCR analysis revealed that both HMGCR and LDLR mRNA levels were significantly reduced by 86% and 55%, respectively, in HepG2 cells following treatment with fucoidan (Fig. 4A). To determine the molecular mechanism underlying the transcriptional inhibition of the HMGCR and LDLR genes by fucoidan, the effects of fucoidan on SREBP-2 mRNA expression were also evaluated by RT-PCR, because SREBP-2 plays a critical role in hepatic cholesterol homeostasis by regulating lipogenic genes, such as HMGCR and LDLR. As shown in Fig. 4B, fucoidan treatment resulted

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**Fig. 3. Effects of fucoidan on the mRNA levels of genes involved in fatty acid metabolism in HepG2 cells.** HepG2 cells were treated with the indicated concentrations of fucoidan in MEM containing 10% lipoprotein-deficient serum (LPDS) for 24 h. The mRNA levels of lipogenic genes (A) and of SREBP-1c (B) were analyzed by RT-PCR. GAPDH was used as an internal control. Results were normalized to GAPDH expression and expressed as percentages of the untreated control. Each bar represents the mean ± SEM of three independent experiments performed in triplicate. *P < 0.05, **P < 0.01, and ***P < 0.001 determined by one-way ANOVA compared to the untreated controls.

**Fig. 4. Effects of fucoidan on mRNA levels of genes involved in cholesterol metabolism in HepG2 cells.** HepG2 cells were treated with the indicated concentrations of fucoidan in MEM medium containing 10% lipoprotein-deficient serum (LPDS) for 24 h. The mRNA expression levels of lipogenic genes (A) and of SREBP-2 (B) were analyzed by RT-PCR and Western blot, respectively. Results were normalized to GAPDH or β-actin expression and expressed as percentages of the untreated control. Each bar represents the mean ± SEM of three independent experiments performed in triplicate. **P < 0.01 and ***P < 0.001 determined by one-way ANOVA compared with the untreated controls.
in a marked decrease in the level of SREBP-2 mRNA expression in a concentration-dependent manner.

3.4. Fucoidan regulates the expression of genes controlling fatty acid metabolism in P407-induced hyperlipidemic mice

The liver is one of the most important organs for maintaining the homeostasis of fatty acids and cholesterol in the body. To further confirm the in vitro effects of fucoidan on SREBP-1c and its target genes, changes in the mRNA expression of genes involved in the control of fatty acid metabolism by fucoidan treatment in the liver of P407-i-induced hyperlipidemic mice were determined by RT-PCR. Acute administration of P407 to the mice increased the hepatic expression of FAS and ACC mRNA by 30% and 60%, respectively, in comparison with the NOR group. However, as expected, fucoidan inhibited FAS by 70% and ACC by 36% in the mouse liver in a dose-dependent manner in comparison with the P407 group, as also observed in HepG2 cells (Fig. 5A). P407 administration did not alter the SREBP-1c mRNA level. Although fucoidan caused a slight decrease in the level of SREBP-1c mRNA, the change was not significant (Fig. 5B). These results were also consistent with the findings in hyperlipidemic rats treated with simvastatin (STA group).

3.5. Fucoidan decreases the mRNA and protein levels of genes controlling cholesterol metabolism in the livers of P407-induced hyperlipidemic mice

We also analyzed the effects of fucoidan on the expression of SREBP-2 and its target genes in the livers of P407-induced hyperlipidemic mice to confirm the in vitro effects of fucoidan on the expression of cholesterogenic genes. As shown in Fig. 6A, the acute
administration of P407 resulted in an increase in hepatic expression of HMGCR mRNA by 294% but a decrease in that of LDLR mRNA by 60% in comparison with the NOR group. Fucoidan treatment significantly lowered HMGCR mRNA expression by 51% in a dose-dependent manner in comparison with the P407 group, while it markedly increased the level of LDLR mRNA expression by 160% also in a dose-dependent manner. Similar to their mRNA levels, fucoidan-treated mice showed a markedly decreased HMGCR protein level and a significantly increased LDLR protein level (Fig. 7A). The acute administration of P407 resulted in an increase in hepatic expression of SREBP-2 mRNA by 221% in comparison with the NOR group. Fucoidan treatment significantly lowered SREBP-2 mRNA expression by 60% in a dose-dependent manner in comparison with the P407 group (Fig. 6B). Since the transcription factor activity of SREBP is largely regulated by the maturation process of precursor proteins (22), Western blotting was performed to determine the protein level of mature SREBP (mSREBP). The changes in SREBP-2 protein level paralleled those of the mRNA level (Fig. 7B).

3.6. Fucoidan decreases atherosclerosis development in P407-induced atherogenic mice

Elevated fatty acid synthesis in the liver and subsequent TG synthesis predominantly contributes to the accumulation of cholesterol within the intima of arterial blood vessels, which is a prominent feature of atherosclerosis. The effects of fucoidan on the formation of atherosclerotic lesions were explored in the P407-induced atherogenic mouse model. As indicated in Fig. 8A, the aortas of P407-induced atherogenic mice showed pathological lesions that were thickened. The arterial thickening (atherosclerotic

![Image](image1.png)

**Fig. 7. Effects of fucoidan on the expression of proteins involved in cholesterol metabolism in the liver of P407-induced hyperlipidemic mice.** Fucoidan was injected intraperitoneally 2 h after acute P407 (250 mg/kg) administration. Liver samples were collected 24 h after administration of P407. The protein levels of cholesterogenic genes (A) and mSREBP-2 (B) were analyzed by Western blot analysis. NOR, normal mice treated with saline; P407, P407-induced hyperlipidemic mice treated with saline; FU10, P407-induced hyperlipidemic mice treated with 10 mg/kg fucoidan; FU30, P407-induced hyperlipidemic mice treated with 30 mg/kg fucoidan; FU50, P407-induced hyperlipidemic mice treated with 50 mg/kg fucoidan; STA, P407-induced hyperlipidemic mice treated with 10 mg/kg atorvastatin. *P < 0.05, **P < 0.01, and ***P < 0.001 determined by one-way ANOVA compared with the P407 group.

![Image](image2.png)

**Fig. 8. Effects of fucoidan on P407-induced atherogenesis in mice.** C57BL/6 mice were injected intraperitoneally with 400 mg/kg P407 every third day for 16 weeks. Fucoidan and atorvastatin were only administered intraperitoneally 2 h after every P407 injection. Aortas were collected 24 h following the last P407 injection. Representative images of H&E-stained aortas (A) were obtained from untreated normal mice (NOR), P407-induced atherogenic mice (P407), P407-induced atherogenic mice treated with 50 mg/kg fucoidan (FU50), and P407-induced atherogenic mice treated with 10 mg/kg atorvastatin (STA). Original magnification ×400 (inset, ×40). Intima-media thicknesses (B) are expressed as the means ± SEM (n = 6 each). *P < 0.05, **P < 0.01, and ***P < 0.001 determined by one-way ANOVA compared with the P407 group.
plaque islands) occurred throughout the entire surface of the vessels. The tunica intima of the aorta in the fucoidan- or atorvastatin-treated atherogenic mice was significantly lower than that in untreated P407-induced atherogenic mice (Fig. 8A and B). The above data indicate that fucoidan effectively inhibits atherosclerosis lesion formation.

4. Discussion

Here, we demonstrated that fucoidan improves abnormal serum lipid levels and reduces atherogenesis in P407-induced hyperlipidemic mice. The molecular mechanism underlying this effect may be related to inhibition of hepatic expression of lipogenic enzymes (FAS and ACC), in particular, cholesterogenic genes (HMGCR and LDLR) by SREBP-2.

In the present study, P407 (also known as Pluronic® F-127) was used to induce the development of acute hyperlipidemia and chronic atherosclerosis in mice. As a nongenetically altered, nondiet-induced mouse model of hyperlipidemia and atherosclerosis, these mice have many advantages in that they have much more controllable hyperlipidemia levels and atherosclerotic lesions than the classic high-fat diet-induced mouse model, being closer to the human condition than knock-out mouse models, and less toxic than other chemically induced mouse models using biphensyl, nicotine, isoretinoin, carbon disulfide, etc. (23). Since it has a structure of synthetic block copolymers consisting of alternating units of hydrophobic propylene oxide and hydrophilic ethylene oxide, P407 may physically inhibit the passage of small-sized lipoproteins (<100–200 nm in diameter) from the bloodstream into the liver sinusoidal endothelial cells, prior to receptor-mediated uptake, which results in increased levels of lipoproteins in the blood and consequently causes massive hyperlipidemia and atherosclerosis in rodent models (24).

The beneficial effect of fucoidan on lipid metabolism was demonstrated by significant decreases in TC, TG, and LDL-c levels and an increase in HDL-c. These results confirmed those of several previous studies indicating that fucoidan plays a role in lowering serum lipid levels in hyperlipidemia or helping to prevent atherosclerosis (16,17,25). These beneficial effects of fucoidan seem to be mediated by inducing LPL secretion from adipocytes (26) or improving an antioxidant defense system (17). In addition, we attempted to elucidate another mechanism by which fucoidan improves lipid metabolism in hyperlipidemic mice by investigating the effects of fucoidan on the expression of genes involved in fatty acid biosynthesis and cholesterol metabolism.

In human hepatoma HepG2 cells and liver tissues, fucoidan treatment resulted in marked decreases in the expression of genes affecting fatty acid synthesis, e.g., FAS and ACC, a significant reduction in HMGCR, the rate-limiting enzyme in the biosynthesis of cholesterol, and a marked increase in LDLR, a hepatic cholesterol uptake-related protein. The regulatory effect of fucoidan on the expression of these genes in the liver is likely to be responsible for its lipid-lowering activities in the blood of hyperlipidemic mice.

SREBP1s are important transcription factors that regulate the biosynthesis of fatty acids and cholesterol (27); SREBP-1c enhances transcription of genes involved in fatty acid biosynthesis (28), whereas SREBP-2 preferentially regulates genes involved in cholesterol biosynthesis (8). The patterns of mature SREBP-1c expression and its activity were closely associated with the changes in mRNA levels of the lipogenic genes, whereas the expression pattern of mature SREBP-2 protein paralleled the changes in mRNA levels of genes related to cholesterol metabolism (29). However, although fucoidan significantly reduced mRNA levels of both lipogenic enzymes of FAS and ACC in vivo as well as in vitro in the present study, the expression of SREBP-1c mRNA seemed to be unaffected. SREBP-1c is a lipogenic transcriptional factor that regulates the transcription of lipogenic enzymes (7). These observations suggest that fucoidan may decrease hepatic lipogenesis through the SREBP-1c-dependent mechanism. However, contrary to our expectations, fucoidan did not affect the mRNA level of SREBP-1c, suggesting that inhibition of these genes by fucoidan may be SREBP-1c-independent. Although we focused on the involvement of SREBP-2 on the beneficial activity of fucoidan in dyslipidemia, we cannot exclude the possibility that SREBP-1c is involved in the fucoidan-elicited changes in expression of hepatic lipogenic genes because the mRNA level of SREBP-1c does not always correspond with the level of the mature SREBP-1c protein located in the nucleus (30,31). Accordingly, it is necessary to determine the protein level of the mature form of SREBP-1c in order to verify the role of this transcription factor in the inhibitory effect of fucoidan on expression of hepatic lipogenic genes.

Acute administration of P407 to the mice increased the levels of mature SREBP-2 mRNA and protein as well as the expression of HMGCR mRNA and protein, but it decreased the expression of LDLR mRNA and protein at the same time in the liver. Although this was explained by the significant reductions of serum TC and LDL-c levels associated with the inhibition of HMGCR and the elevation of LDLR through the inhibition of SREBP-2, it was also notable that the expression of LDLR was attenuated in the liver of P407-induced hyperlipidemic mice because activation of SRPBP-2 generally leads to transcriptional activation of LDLR. However, hepatic LDLRs may also be regulated post-transcriptionally by proprotein convertase subtilisin/kexin type 9a (PCSK9). As SREBP-2 also activates PCSK9 in the liver, the increase in PCSK9 expression through SREBP-2 activation can attenuate hepatic LDLR protein expression and subsequently reduce serum cholesterol levels (32). Therefore, we could not exclude the possibility that fucoidan can elevate LDLR through the PCSK9-mediated pathway.

In addition to its hypolipidemic effect, the protective effect of fucoidan against atherogenesis was also observed in this study. Increased levels of TC, TG, and LDL-c and decreased levels of HDL-c are associated with an increased incidence of cardiovascular disease in the general population. AI based on serum lipid profile is a reliable indicator of predisposition to cardiovascular disease. In addition, a low level of HDL-c is also considered an important risk factor for cardiovascular diseases due to the strong association between lower HDL-c level and the incidence of coronary heart disease (33,34). In this study, fucoidan significantly reduced AI and markedly increased serum HDL-c level as compared with P407-induced hyperlipidemic mice. These effects may partly explain its beneficial protective effect against the development of atherosclerosis, suggesting that fucoidan may confer significant protection against cardiovascular disease and mortality. The other evidence, however, shows fucoidan also exerts anti-inflammatory and antioxidative effects (10), which may contribute to the attenuation of atherosclerosis as well as hypolipidemic effect. It can also raise the possibility that fucoidan cannot only improve serum lipid profiles, but protect against the development of atherosclerosis via its anti-inflammatory and antioxidative effects.

Taken together, the results of the present study indicated that fucoidan inhibits hyperlipidemia and aortic lesion development in P407-induced hyperlipidemic mice and may affect hepatic lipid metabolism. To our knowledge, this is the first study to reveal a novel mechanism of action for fucoidan in which it lowers serum lipid levels by suppressing SREBP-2. In conclusion, the hypolipidemic effects of fucoidan could account for decreased HMGCR via suppression of SREBP-2. Therefore, fucoidan may be beneficial against hyperlipidemia and atherosclerosis and, therefore, be cardioprotective and reduce the risk of CHD.
Conflict of interest statement

No conflict of interest was declared.

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