Int J Clin Exp Pathol 2016;9(7):6896-6904
www.ijcep.com /ISSN:1936-2625/IJCEP0020114

Original Article

Fucoidan attenuates atherosclerosis in LDLR$^{-/-}$ mice through inhibition of inflammation and oxidative stress

Xin Wang[1], Lili Pei[2], Haibo Liu[1], Kai Qv[2], Weiwei Xian[2], Jia Liu[2], Guomin Zhang[2]

[1]*Department of Emergency, The First Hospital of Jilin University, Changchun 130021, China;* [2]*The Department of Cardiology, The First Affiliated Hospital of Qiqihar Medical University, Qiqihar, Heilongjiang, China*

Received November 19, 2015; Accepted May 26, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: Background and purpose: Fucoidan is a major bioactive polysaccharide which contains substantial percentages of L-fucose and sulfate ester groups, mainly isolated from brown seaweed. For the past decade fucoidans have been extensively studied due to their varied biological activities, including anti-inflammatory, blood lipids reducing, antioxidant and anticomplementary properties. However, it is not known whether fucoidan can prevent experimental atherosclerosis. The present study was designed to investigate the protective effects of fucoidan on atherosclerosis and its molecular mechanisms of action. Methods: Low Density Lipoprotein Receptor-deficient (LDLR$^{-/-}$) mice, fed an atherogenic diet, were dosed daily with Fucoidan (50, 100 mg/kg day) by oral gavage. *In vitro* studies were carried out in oxidized LDL (oxLDL)-stimulated RAW264.7 cells treated with or without Fucoidan. Results: Fucoidan significantly attenuated atherosclerotic plaque formation and enhanced plaque stability in LDLR$^{-/-}$ mice by decreasing the serum lipids and inhibiting the macrophage infiltration, as well as inhibiting reactive oxygen species (ROS) generation. Fucoidan treatment significantly decreased the expression of LOX-1 and the levels of pro-inflammatory mediators in vivo. *In vitro*, fucoidan decreased oxLDL-induced LOX-1, pro-inflammatory mediators as well as adhesion molecules (ICAM-1 and VCAM-1). Furthermore, fucoidan inhibited NADPH oxidase subunit 4 (NOX4)-mediated ROS generation. Conclusion: Fucoidan was shown to have anti-atherosclerotic activity, which was mediated through inhibition of the inflammation and oxidative stress. This suggests that Fucoidan is a vasculoprotective drug that has potential therapeutic value for the clinical treatment of atherosclerotic cardiovascular diseases.

Keywords: Fucoidan, atherosclerosis, inflammation, oxidative stress

Introduction

Atherosclerotic cardiovascular disease, driven and regulated by lipid retention (in the artery wall), lipid oxidation, persistent inflammation and immune response disturbances, is the leading cause of premature death in developed and developing countries [1, 2]. One critical event in the initiation of atherosclerosis is the adhesion of leukocytes to activated endothelium and their subsequent migration into the vessel wall. These cellular processes are mediated by the up-regulation of adhesion molecules in endothelial cells (ECs) and an increased expression of leukocyte chemotactic factors in the vascular wall [3]. One major determinant of this alteration could be oxidative stress. Modified LDL, in particular oxidized LDL (oxLDL; the pathological form of oxidatively modified LDL), exerts several pro-atherogenic effects to facilitate atherosclerosis [4]. The plasma oxLDL level is transiently increased before the development of atherosclerotic lesions in apolipoprotein E-deficient (LDLR$^{-/-}$) mice [5], suggesting that oxLDL may play a crucial role in the early stages in the formation of atherosclerotic lesions. Previous studies have shown that lectin-like oxLDL receptor-1 (LOX-1), a primary scavenger receptor expressed in ECs [6], is up-regulated in atherosclerotic plaques of experimental animals and humans [7]. LOX-1 facilitates the uptake of oxLDL by ECs and macrophages, thus mediating several of its biological effects [8-11]: oxLDL induces (i) apoptosis of ECs and phagocytosis of aged and apoptotic cells; (ii) the adhesion of monocytes to activated endothelium; and (iii) macrophage-derived foam cell formation. More recently, it has been demonstrated that LOX-1 is the main culprit that transduces the signal of the adverse effects of dys-

Table 1. Effects of fucoidan on serum blood lipids in LDLR$^{-/-}$ mice

Group	TG (mg/dl)	TC (mg/dl)	LDL-C(mg/dl)	HDL-C (mg/dl)
Control	127.24±20.76	403.76±41.09	292.98±36.70	11.78±2.13
Fucoidan (50 mg/kg)	107.13±13.20*	354.61±31.67	206.34±26.47*	19.36±3.17*
Fucoidan (100 mg/kg)	86.32±11.09**	256.71±21.37**	168.00±20.31**	26.08±2.84**

TG: triglyceride, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol. *p<0.05, **p<0.0.1.

functional high-density lipoprotein (HDL; such as oxidized HDL and HDL from patients with coronary artery disease) [12]. *In vitro*, LOX-1 expression is up-regulated by various pro-atherogenic stimuli, including oxLDL, TNF-α, angiotensin II, shear stress, homocysteine and high glucose [11]. Therefore, LOX-1 has recently been suggested as an attractive therapeutic target for atherosclerosis [11].

The brown seaweed, Laminaria japonica, is common seafood in China and many other countries, and documented as a drug in traditional Chinese medicine for over a 1000 years. Fucoidan is a complex sulfated polysaccharide, derived from marine brown seaweed. Fucoidan has been reported to possess diverse biological activity of potential medicinal value, such as antioxidant, anticoagulant, antitumor, antiviral and anti-inflammatory activity [13, 14]. Notably, its anti-inflammatory has drawn much attention in recent years [15, 16]. However, there is no report on the potential anti-atherosclerotic effects of fucoidan *in vivo*.

Therefore, in the present study, we investigated the potential effects of fucoidan on atherosclerotic plaque development in LDLR$^{-/-}$ mice kept on a high cholesterol diet (HCD). Additionally, we examined the modulation of LOX-1 expression by CTS in HUVECs stimulated with oxLDL.

Materials and methods

Materials

The test compound, fucoidan, was prepared from L. japonica cultured in Yantai, China and was identified by Prof. Benming Xu, School of Pharmacy, Yantai University. A voucher specimen was deposited in the herbarium of the school of pharmacy, Yantai University. Fucoidan was isolated as described previously [17].

Animal experiment

Experimental protocols were approved by the Animal Care and Use Committee of Jilin University (Jilin, China). All animal experimental procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [18, 19]. Male LDLR$^{-/-}$ mice on C57BL/6J background were purchased from Peking University Experimental Animal Center (Beijing, China). The mice were housed under a 12-h light/dark cycle in specific pathogen-free facility at Institutional Experimental Animal Center. Starting from 6 weeks, the mice were fed with a HCD (10% fat, 1.25% cholesterol, 0% cholic acid) for 16 weeks. All LDLR$^{-/-}$ mice were dosed daily via intragastric gavage with 50 or 100 mg/kg Fucoidan dissolved in 0.5% carboxymethyl-cellulose sodium (CMC-Na) or administered 0.5% CMC-Na alone (control) (n = 5 per group). All animals received food and water *ad libitum*. Body weight and food intake were monitored during the study.

Detection of serum lipid profile

For measurement of the lipids, blood samples were collected at baseline (6 week) and the end of the diet treatment period (22 week), by retro-orbital venous plexus bleeding, from animals that had been fasted overnight. Serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured by colorimetric assays as previously described [20].

Morphometric analysis of atherosclerotic lesions

The *en face* and aortic sinus cryosection techniques [20] were used to quantify atherosclerosis development throughout the aorta as well as at the vessel origin. Oil Red O (ORO) staining was used to measure lesion area in *en face* aorta and aortic sinus. For quantitative analysis of the total lesion area in aortic sinus, eight separate cryosections (spacing 50 μm apart) from each mouse were manually analysed with the Leica Qwin PLUS Software (Leica Micro-

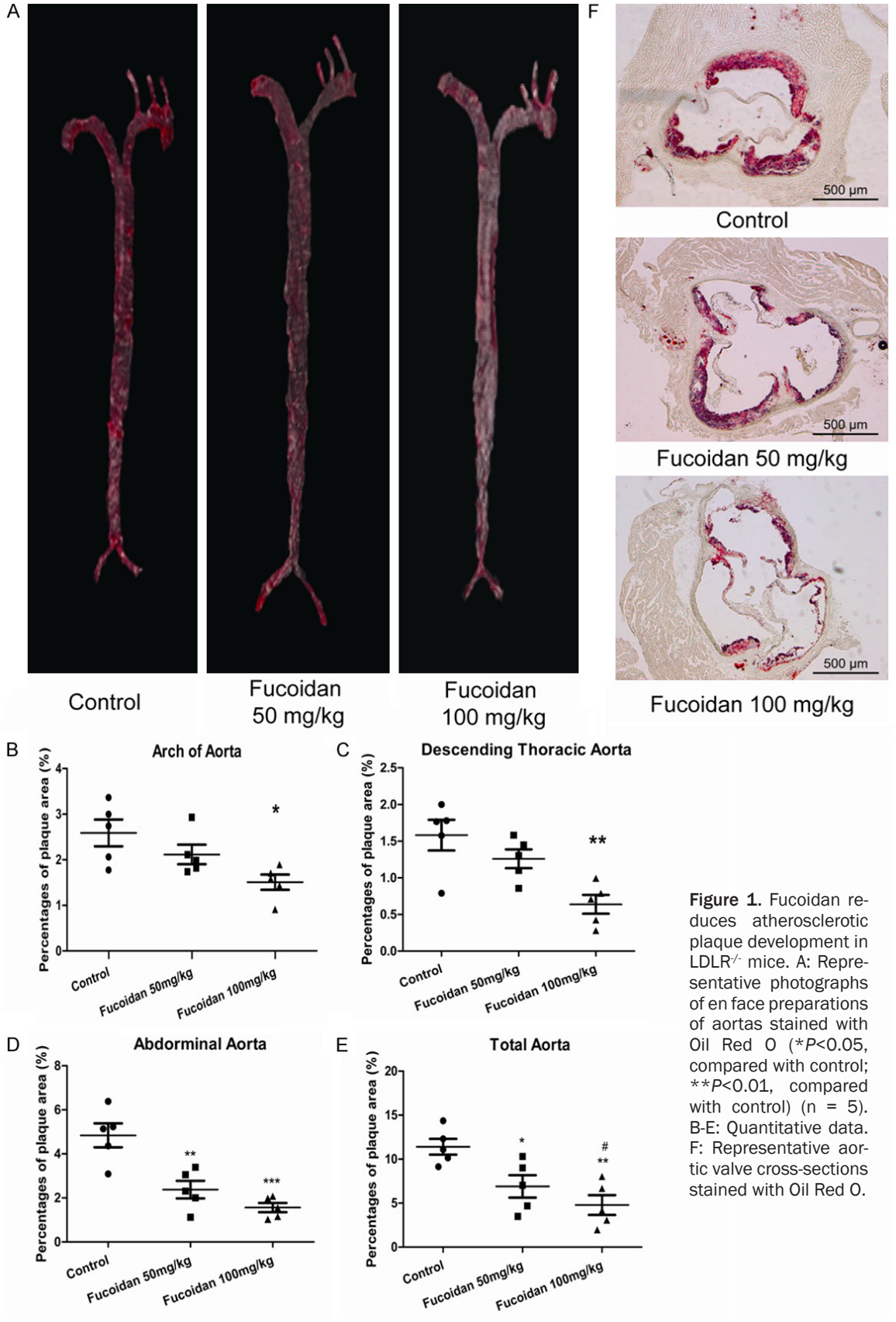

Figure 1. Fucoidan reduces atherosclerotic plaque development in LDLR$^{-/-}$ mice. A: Representative photographs of en face preparations of aortas stained with Oil Red O (*P<0.05, compared with control; **P<0.01, compared with control) (n = 5). B-E: Quantitative data. F: Representative aortic valve cross-sections stained with Oil Red O.

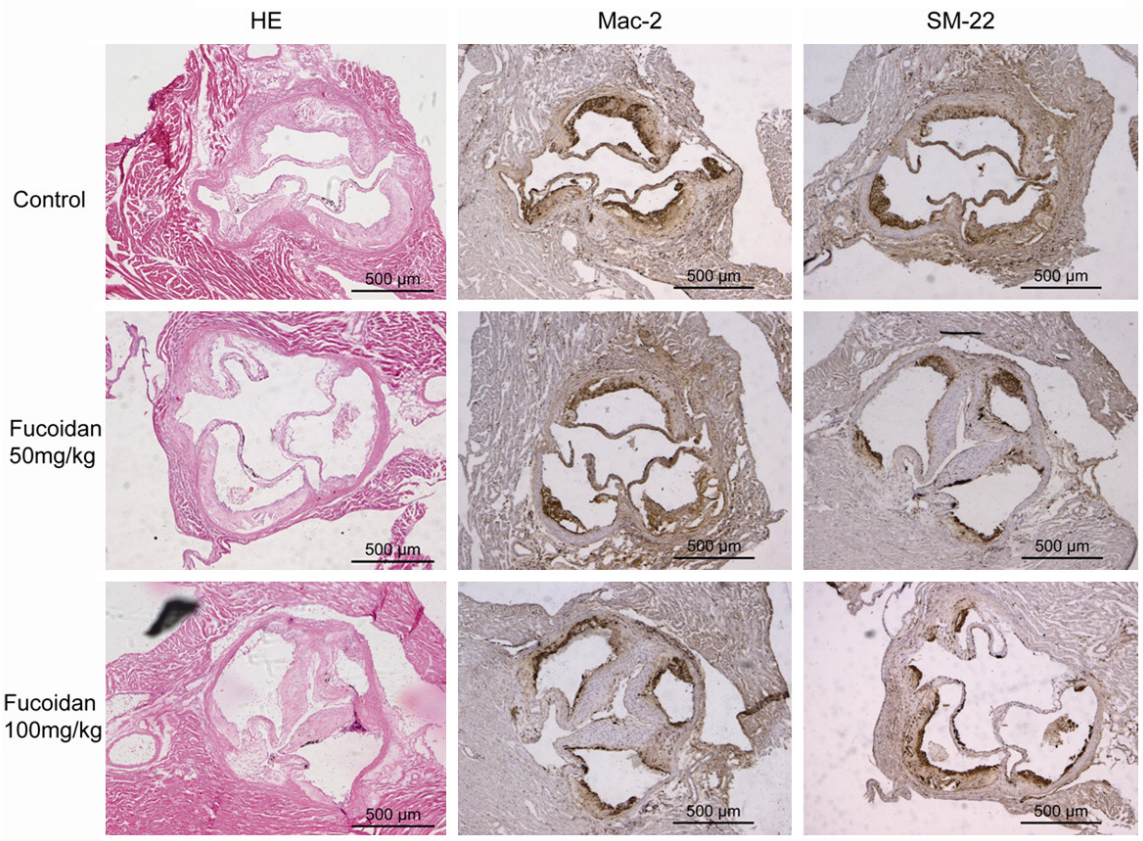

Figure 2. Fucoidan reduces Mac-2 and SM-22 expression in aorta atherosclerosis plaque of LDLR$^{-/-}$ mice. Immunohistochemistry result of Mac-2 and SM-22 in aorta atherosclerosis plaque of different groups.

systems, Heidelberg, Germany). For plaque area in whole aorta, the percentage of ORO-positive stained area in relation to total luminal surface area was quantified using computer-assisted morphometry with NIH ImageJ software (http://imagej.nih.gov). Lesion size in the aortic sinus and *en face* arterial tree was measured by two observers blinded to experimental groups.

Histology and immunohistochemistry analysis

Aortic sinus morphometric and immunohistochemical analysis was performed as described previously in detail [20]. Sections of 8 μm thickness were used for immunohistochemical staining with Mac-2 (Boster, Wuhan, China) and SM-22 antibodies (Abcam, Cambridge, MA, USA). Colour reaction was developed with diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA). The remainders of the sections were utilized for hematoxylineosin (H&E) staining to examine basic lesion morphology.

Reactive oxygen species (ROS) production in situ

The production of ROS in aortic root cryosections was assessed *in situ* by fluorescence microscopy of dihydroethidium (DHE)-stained sections as described, in detail, previously [20]. The fluorescence was quantified using NIH ImageJ software.

Cell culture

RAW264.7 murine macrophage cells (American Type Culture Collection, Rockville, MD, USA) were cultured in DMEM supplemented with 10% FBS and 1% penicillin and streptomycin at 37°C in the presence of 5% CO_2. RAW 264.7 cells were treated with 100 ng/mL LPS with or without different concentrations of fucoidan (50 and 100 μg/mL) for 24 h. Cells treated with LPS alone were utilised as the control. After 24 h of treatment, the cell culture supernatants were collected for cytokine and nitrite assays.

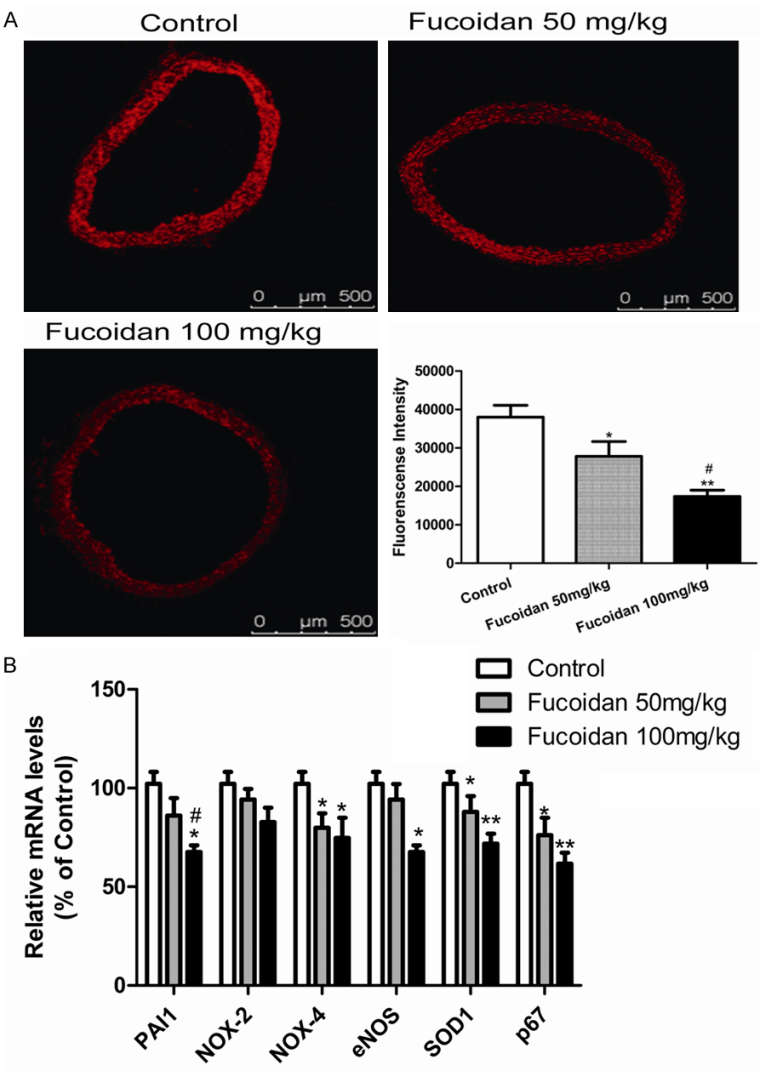

Figure 3. Fucoidan abolished the ROS and mRNA expression of ROS related protein in AS aorta of LDLR$^{-/-}$ mice. A. Micrographs and quantitative data of sequential sections of aorta from AS mice were obtained after staining with dihydroethidium (DHE). B. Relative mRNA level of ROS-generation related protein was measured by realtime PCR. (*P<0.05, compared with control; **P<0.01, compared with control; #P<0.05, compared with fucoidan 50 mg/kg) (n = 5).

The cell lysates were collected for reverse transcriptase-polymerase chain reaction (RT-PCR) assays.

Realtime PCR

Real-time PCR was conducted as previously described [20]. Sequences for the oligonucleotide primers used were designed with Primer 5.0 software (Premier Biosoft, Palo Alto, CA, USA) and were custom synthesized by Invitrogen. mRNA levels of PAI1, NOX-2, p67, LOX-1, IL-1b, IL-6, TNF-α, intracellular adhesion molecule 1 (ICAM-1), vascular cellular adhesion molecule 1 (VCAM-1), eNOS, SOD1 and NOX-4 were measured by qRT-PCR and quantified with respect to the GAPDH housekeeping gene. The fold increase/decrease versus control cells presented was calculated by the $2^{-\Delta\Delta Ct}$ method [21]. Statistical differences between the treatment group and control group were subjected to Student's unpaired *t*-tests.

Detection of reactive oxygen species in RAW264.7 cells

The level of superoxide production was determinedby staining with dichlorodihydrofluorescein diacetate (DCFH), according to the manufacturer's protocol. Briefly, following treatment as mentioned in the section "Anoxia/reoxygenation injury model", above, the cells were then washed once with phenolred-free medium, and incubated in 200 mL of a working solution of DCFH-DA (10 mmol/L) at 37°C for 30 min. The cells were observed under a fluorescence microscope (Olympus, Japan). The fluorescence of DCFH was monitored at the excitation and emission wavelengths of 485 nm and 530 nm.

Statistical analysis

Fifteen LDLR$^{-/-}$ mice were randomly allocated to groups and equal group sizes were obtained (n = 5 per group). Data are presented as mean ± SEM unless specified otherwise. Images shown are representative of five or more independent experiments. Statistical significance of differences was calculated using one-way ANOVA with Bonferroni *post hoc* for multiple group comparison or Student's unpaired *t*-test for two-group comparison where appropriate. The analyses were performed using GraphPad Prism Software version 5.02 (GraphPad Inc., La

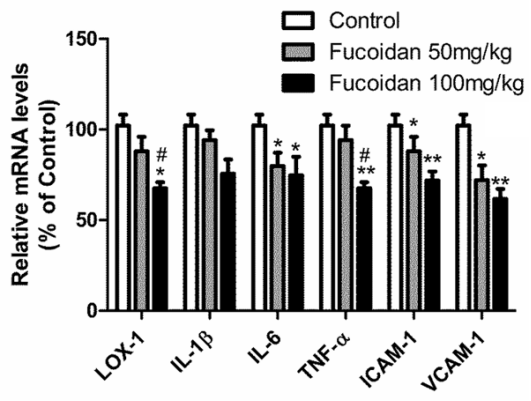

Figure 4. Fucoidan reduces the LOX-1 and pro-inflammatory mediators expression in AS aorta of LDLR$^{-/-}$ mice. The mRNA level of LOX-1, IL-1b, IL-6, TNF-α, ICAM-1 and VCAM-1 in AS aorta of LDLR$^{-/-}$ mice were measured by RT-PCR (*P<0.05, compared with control; **P<0.01, compared with control; #P<0.05, compared with fucoidan 50 mg/kg) (n = 5).

Jolla, CA, USA). A P value<0.05 was considered to be statistically significant.

Results

Fucoidan decreases the serum lipid level in LDLR$^{-/-}$ mice

Similar to previous study, fucoidan notably reduced the concentration of serum total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) of hyperlipidemic mice and increased the concentration of high-density lipoprotein cholesterol (HDL-C) (**Table 1**) [22].

Fucoidan reduces atherosclerotic plaque development in LDLR$^{-/-}$ mice

The efficacy of fucoidan in diet-induced atherosclerosis in LDLR$^{-/-}$ mice was examined. Notably, compared with vehicle control group, fucoidan treatment (50 mg/kg, 100 mg/kg) significantly attenuated atherosclerotic lesion formation in the *en face* and prepared aorta and cross-sections aortic valve (**Figure 1A**, **1F**) of LDLR$^{-/-}$ mice fed a HCD for 16 weeks. These data suggest that fucoidan has potent anti-atherosclerotic effects in experimental atherosclerosis.

Fucoidan reduces macrophage infiltration and smooth muscle cell proliferation in atherosclerotic plaque development in LDLR$^{-/-}$ mice

Macrophage infiltration and smooth muscle cell proliferation are the key initiating and primary process in atherosclerosis. Macrophage infiltration and smooth muscle cells proliferation in atherosclerosis plaque of different groups were measured by immunohistochemistry of Mac-2 and SM-22 (**Figure 2**). Compared with control group, fucoidan reduces both the macrophage infiltration and smooth muscle cell proliferation.

Fucoidan inhibits ROS generation in LDLR$^{-/-}$ mice aorta

ROS in the plaque could modify LDL, react with endothelial-derived nitric oxide subsequently forming peroxynitrite, and amplify the expression of various genes important for leukocyte recruitment within the arterial wall and leading to oxidant injury [23]. As compared to vehicle control, treatment with fucoidan abolished superoxide production as detected by dihydroethidium (DHE) fluorescence (**Figure 3A**). The following realtime PCR results of related proteins (PAI1, NOX-2, NOX-4, eNOS, SOD1, p67) revealed that 100 mg/kg fucoidan could inhibit the expression of ROS-generation related protein significantly.

Fucoidan inhibits LOX-1 expression and pro-inflammatory mediators expression in LDLR$^{-/-}$ mice

To investigate the impact of fucoidan on LOX-1 expression and pro-inflammatory mediators expression, the mRNA level of LOX-1, IL-1b, IL-6, TNF-α, ICAM-1 and VCAM-1 were measured by RT-PCR (**Figure 4**). The results showed that 100 mg/kg fucoidan could reduce LOX-1 and pro-inflammatory mediators expression in LDLR$^{-/-}$ significantly.

Fucoidan inhibits pro-inflammatory mediators expression and ROS generation in RAW264.7 cells

We further examined the anti-ROS generation and anti-inflammation effects of fucoidan on RAW264.7 cells, to confirm the in-vivo findings. The results indicated that fucoidan could abolish the expression of LOX-1 and pro-inflammatory mediators significantly. DCFH staining and realtime PCR showed that fucoidan could reduce ROS generation as well as the expression of ROS-generation related protein (**Figure 5**).

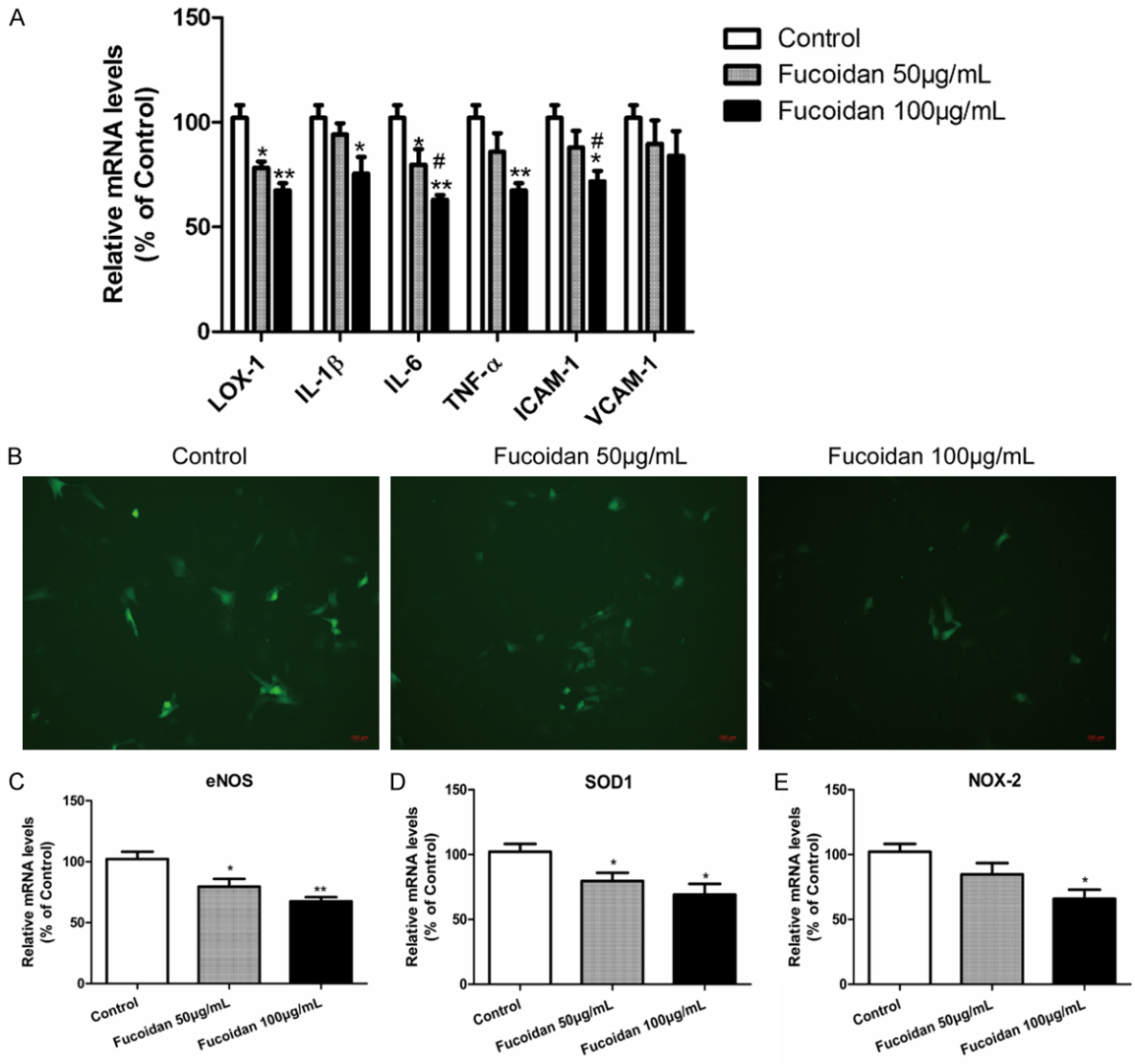

Figure 5. Fucoidan inhibit LOX-1, pro-inflammatory mediators, ROS and ROS-generation related protein expression in vitro. (A) Fucoidan inhibit the mRNA level of LOX-1, IL-1b, IL-6, TNF-α, ICAM-1 and VCAM-1 on RAW264.7 cells. (B) Fluorescence microscopy image of RAW264.7 stained with DCFH after treated with fucoidan at different concentration. Fucoidan inhibit the mRNA level of eNOS (C), SOD1 (D) and NOX-4 (E) on RAW264.7 cells.

Discussion

Previous study has indicated fucoidan may reduce the concentration of serum total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) of heperlipidemic rats and increase the concentration of high-density lipoprotein cholesterol (HDL-C) [22]. Our study reproduced similar results in HCD-fed LDLR$^{-/-}$ mice. Previous studies also found that fucoidan could inhibit the proliferation of arterial smooth muscle cells (SMC) both in animal models and in vitro. Hyperlipidemia and the proliferation of arterial SMC are both major risk factors for the development of atherosclerosis. The regulation effects of fucoidan on lipid level and arterial SMC indicated that fucoidan may be atheroportective.

So we investigated the effect of fucoidan on the development of atherosclerosis in HCD-fed LDLR$^{-/-}$ mice, a well established model for studying atherosclerosis. Our data suggested that high dose of fucoidan (100 mg/kg) resulted in a significant reduction in atherosclerosis plaque area. Meanwhile the expression of MAC-2 in plaque lesion was suppressed in the high dose fucoidan group, indicating that fucoidan

could reduce the local inflammation. Furthermore, we found that the expression of SM-22, a SMC marker, was significantly attenuated by fucoidan, suggesting that the anti-atherosclerosis effects of fucoidan may associated with the reduction of SMC proliferation. Moreover, the inhibitory effects of fucoidan on atherosclerosis may be involved in with its antioxidant activity [24], reflected by the decrease of ROS level in the aorta of high dose fucoidan group. Previous studies have demonstrated the pleiotropic effects of fucoidan on cardiovascular disease. Our results showed that the benefits effects of fucoidan on cardiovascular disease may be a combined results of various action.

To gain further insight into the molecular mechanism by which fucoidan inhibits atherosclerosis progress, we examined the expression of LOX-1, pro-inflammatory mediators (IL-1β, IL-6, TNF-α, ICAM-1, VACM-1) in LDLR$^{-/-}$ mice. LOX-1, a versatile scavenger receptor that is ubiquitously expressed in vascular cells is critical for the initiation and progression of atherosclerosis [25]. Our study, for the first time, showed that fucoidan could attenuate the expression of LOX-1 with in plaque lesions. Further in-vitro experiment confirmed our in-vivo finding. Fucoidan reduces the mRNA expression level of LOX-1 and pro-inflammatory mediators on RAW264.7 cells. Simultaneously, fucoidan abolished the ROS generation as well as the ROS-generation related protein expression.

Taken together, our present results have indentified a novel atheroprotective effect of fucoidan. Fucoidan was shown to suppress the ROS related pathway and reduce the expression of LOX-1 and the diminishing the inflammation response. These observation indicate that fucoidan could be exploited as an innovative cardiovascular drug to prevent or retard the pathogenesis of atherosclerotic cardiovascular diseases.

Acknowledgements

This study was supported by Qiqihar science and technology commission fundation (SPZD-2013123).

Disclosure of conflict of interest

None.

Abbreviations

LDLR$^{-/-}$, Low Density Lipoprotein Receptor-deficient; CD36, cluster of differentiation 36; HCD, high cholesterol diet; ICAM-1, intracellular adhesion molecule 1; NOX4, NADPH oxidase subunit 4; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; SMCs, smooth muscle cells; SR-A, scavenger receptor-A; VCAM-1, vascular cellular adhesion molecule 1.

Address correspondence to: Xin Wang, Department of Emergency, The First Hospital of Jilin University, Changchun 130021, China. E-mail: dr_xwang@outlook.com

References

[1] Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340: 115-126.

[2] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. Circulation 2013; 127: e6-e245.

[3] Hansson GK and Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 2006; 6: 508-519.

[4] Li AC and Glass CK. The macrophage foam cell as a target for therapeutic intervention. Nat Med 2002; 8: 1235-1242.

[5] Kato R, Mori C, Kitazato K, Arata S, Obama T, Mori M, Takahashi K, Aiuchi T, Takano T and Itabe H. Transient increase in plasma oxidized LDL during the progression of atherosclerosis in apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 2009; 29: 33-39.

[6] Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T and Masaki T. An endothelial receptor for oxidized low-density lipoprotein. Nature 1997; 386: 73-77.

[7] Kataoka H, Kume N, Miyamoto S, Minami M, Moriwaki H, Murase T, Sawamura T, Masaki T, Hashimoto N and Kita T. Expression of lectin-like oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. Circulation 1999; 99: 3110-3117.

[8] Li D, Liu L, Chen H, Sawamura T, Ranganathan S and Mehta JL. LOX-1 mediates oxidized low-density lipoprotein-induced expression of matrix metalloproteinases in human coronary artery endothelial cells. Circulation 2003; 107: 612-617.

[9] Li D and Mehta JL. Intracellular signaling of LOX-1 in endothelial cell apoptosis. Circ Res 2009; 104: 566-568.

[10] Xu S, Liu Z, Huang Y, Le K, Tang F, Huang H, Ogura S, Little PJ, Shen X and Liu P. Tanshinone II-A inhibits oxidized LDL-induced LOX-1 expression in macrophages by reducing intracellular superoxide radical generation and NF-kappaB activation. Transl Res 2012; 160: 114-124.

[11] Xu S, Ogura S, Chen J, Little PJ, Moss J and Liu P. LOX-1 in atherosclerosis: biological functions and pharmacological modifiers. Cell Mol Life Sci 2013; 70: 2859-2872.

[12] Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, Chroni A, Yonekawa K, Stein S, Schaefer N, Mueller M, Akhmedov A, Daniil G, Manes C, Templin C, Wyss C, Maier W, Tanner FC, Matter CM, Corti R, Furlong C, Lusis AJ, von Eckardstein A, Fogelman AM, Luscher TF and Landmesser U. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest 2011; 121: 2693-2708.

[13] Feldman SC, Reynaldi S, Stortz CA, Cerezo AS and Damont EB. Antiviral properties of fucoidan fractions from Leathesia difformis. Phytomedicine 1999; 6: 335-340.

[14] Wang J, Zhang Q, Zhang Z, Song H and Li P. Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from Laminaria japonica. Int J Biol Macromol 2010; 46: 6-12.

[15] Bojakowski K, Abramczyk P, Bojakowska M, Zwolinska A, Przybylski J and Gaciong Z. Fucoidan improves the renal blood flow in the early stage of renal ischemia/reperfusion injury in the rat. J Physiol Pharmacol 2001; 52: 137-143.

[16] Li N, Zhang Q and Song J. Toxicological evaluation of fucoidan extracted from Laminaria japonica in Wistar rats. Food Chem Toxicol 2005; 43: 421-426.

[17] Zhang Q, Li Z, Xu Z, Niu X and Zhang H. Effects of fucoidan on chronic renal failure in rats. Planta Med 2003; 69: 537-541.

[18] Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG; National Centre for the Replacement, Refinement and Reduction of Amimals in Research. Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 2010; 160: 1577-1579.

[19] McGrath JC, Drummond GB, McLachlan EM, Kilkenny C and Wainwright CL. Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 2010; 160: 1573-1576.

[20] Xu S, Little PJ, Lan T, Huang Y, Le K, Wu X, Shen X, Huang H, Cai Y, Tang F, Wang H and Liu P. Tanshinone II-A attenuates and stabilizes atherosclerotic plaques in apolipoprotein-E knockout mice fed a high cholesterol diet. Arch Biochem Biophys 2011; 515: 72-79.

[21] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.

[22] Huang L, Wen K, Gao X and Liu Y. Hypolipidemic effect of fucoidan from Laminaria japonica in hyperlipidemic rats. Pharm Biol 2010; 48: 422-426.

[23] Mugge A. The role of reactive oxygen species in atherosclerosis. Z Kardiol 1998; 87: 851-864.

[24] Religa P, Kazi M, Thyberg J, Gaciong Z, Swedenborg J and Hedin U. Fucoidan Inhibits Smooth Muscle Cell Proliferation and Reduces Mitogen-activated Protein Kinase Activity. Eur J Vasc Endovasc Surg 2000; 20: 419-426.

[25] Chen M, Masaki T and Sawamura T. LOX-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: implications in endothelial dysfunction and atherosclerosis. Pharmacol Ther 2002; 95: 89-100.