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Chinese red yeast rice attenuates the development of angiotensin II-induced abdominal aortic aneurysm and atherosclerosis☆

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Abstract

Objective: Abdominal aortic aneurysm (AAA) is a chronic vascular disease characterized by medial degradation and inflammation. No medical approaches have been validated for treating AAA, and therapeutic options are limited to regular surveillance leading to surgical intervention. This study aimed to investigate whether administration of Chinese red yeast rice (*Monascus purpureus*; RYR) suppressed angiotensin II (AngII)-induced AAA and atherosclerosis.
Methods and Results: Apolipoprotein E-deficient male mice fed a normal diet were administered either RYR extract (200 mg/kg/day) or vehicle by gavage for 1 week before initiating AngII infusion (1000 ng/kg/min) via subcutaneous osmotic pumps for 28 days. Red yeast rice extract administration significantly suppressed AngII-induced expansion of suprarenal diameter and area ($P<.05$). Furthermore, RYR extract significantly reduced atherosclerotic lesion areas in both the intima of aortic arches and cross sections of aortic roots ($P<.05$). These effects were associated with reductions of serum total cholesterol, intercellular adhesion molecule 1, vascular cell adhesion molecule 1, matrix metalloproteinase (MMP) 2 and increases of serum macrophage migration inhibitory factor, but no changes in serum interleukin (IL) 1α, IL-6, monocyte chemoattractant protein 1, MMP-9 and expression of MMP-2 and MMP-9 in aortic walls.
Conclusions: This study demonstrated that RYR extract administration suppressed AngII-induced AAA and atherosclerosis associated with regulating inflammation responses independent of lipid-lowering effects. Red yeast rice may have preventive potential for patients with AAA.

Keywords: Abdominal aortic aneurysm; Angiotensin II; Chinese red yeast rice; Atherosclerosis

1. Introduction

Abdominal aortic aneurysm (AAA) is a common health problem in the Western world, with a lower prevalence in Chinese population [1]. There is an increased focus on the physiopathology and therapeutic strategy of this disease. Current therapeutic options are limited to surgery or endovascular stenting for AAA patients with a maximum diameter exceeding 5.5 cm. Patients with smaller AAA must undergo regular ultrasound surveillance before surgical intervention. Based on the current understanding of molecular mechanisms of AAA, potential medical treatment options include inhibitors of matrix metalloproteinase (MMP) [2], serine proteases [3] and cysteine proteases [4]. No medical therapies have been proven to be beneficial in humans, although studies have demonstrated the potential for attenuated aneurysm expansion by macrolides administration [5].

Red yeast rice (RYR) is a traditional Chinese cuisine and medicinal agent prepared by using *Monascus purpureus* fermented with rice, which has been recorded in ancient Chinese pharmacopoeias since the Ming Dynasty (1368–1644). Red yeast rice extracts contain a mixture of starch, phytocholesterols, isoflavones, monounsaturated fatty acids and polyketides called monacolins, one of which, monacolin K, is identical to lovastatin [6,7]. Several randomized clinical trials have indicated beneficial effects of RYR extract including *Xuezhikang* and *Zhibituo* in the treatment of hyperlipidemic patients [8,9]. *Xuezhikang* has been in clinical use as a Chinese proprietary medicine in China and demonstrated to prevent

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cardiovascular events and mortality in patients with coronary heart disease [10–12]. Therefore, *Xuezhikang* is commonly prescribed to patients with ischemic cardiovascular disease and its risk equivalent as a supplementary therapy.

Infusion of angiotensin II (AngII) into hypercholesterolemic mice induces both AAA formation and augments atherosclerosis [13,14]. Angiotensin II changes the expression of many molecules that affect inflammation based on cardiovascular disease. This includes increased expression of cyclooxygenase 2, interleukin 6 (IL-6), IL-1α, intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), MMP-2, MMP-13, and MMP-14 [15,16]. The rationale of this study is based on the reports that RYR extract has shown to protect vascular endothelial function by decreasing inflammatory mediators, such as IL-6 and VCAM-1 [17,18].

Here, we hypothesized that RYR may suppress development of AngII-induced AAA and atherosclerosis. To determine whether RYR exerted a protective effect, we used apolipoprotein E-deficient (apoE−/−) male mice infused with AngII to promote development of AAAs and atherosclerosis. Red yeast rice extract (*Xuezhikang*) was administered 1 week before and during AngII infusion. Abdominal aortic aneurysms were evaluated as well as atherosclerotic lesions. Inflammatory markers and MMP involved were also investigated to interpret the potential mechanisms.

The purpose of this study was to define whether a substance used in traditional Chinese cuisine and as a medicinal agent, RYR, contributes to the lower prevalence of AAAs in Chinese population. The present study provides the potential interpretation that this traditional Chinese food herb may be used as a preventive treatment of AAAs.

2. Materials and methods

2.1. Animals

Apolipoprotein E-deficient (ApoE−/−) mice on C57BL/6 background (n=34) were originally purchased from the Beijing Vital River Laboratory Animal Technology Corporation and bred in-house. Mice were maintained under specific pathogen-free conditions and fed normal laboratory diet. All studies were performed with the approval of Zhejiang University Institutional Animal Care and Use Committee.

2.2. Study design

Male apoE−/− mice (8–16 weeks old) were randomly divided into two groups, administered with RYR extract (*Xuezhikang* kindly provided by Beijing Peking University WBL Biotech) or vehicle 7 days before AngII infusion and throughout the whole study. Mice were inspected daily and weighed weekly. Red yeast rice extract was dissolved in water at concentrations that gave an approximate dose of 200 mg/kg/day when delivered by daily gavage. Osmotic minipumps (Alzet Model 2004; Durect) were implanted subcutaneously and delivered AngII (1000 ng/kg/min; Bachem catalog no. H1705) for 28 days [13,14]. Systolic blood pressure was measured in conscious mice using a computerized tail cuff (CODA 6+; Kent Scientific, Torrington, CT) [19]. All mice were acclimated to the system for 1 week before the start of the study. Individual mice were kept at a constant temperature (28°C–32°C on tail). Ten pressure cycles were used to obtain daily mean systolic blood pressures after acclimation as described previously [2]. Maximal dimensions and areas of suprarenal lumen were monitored at selected intervals (Day 0 and 28) by a high-frequency ultrasound imaging system (Visualsonics; Toronto, Ontario, Canada) as described previously [20]. Abdominal aortic aneurysm was defined as an increase of 50% or greater in the maximal suprarenal diameter compared with the baseline.

2.3. Abdominal aortic aneurysm and atherosclerosis analyses

Mice were terminated after 28 days of AngII infusion, with blood harvested from the left ventricle and aortic arch immersion fixed with 10% neutral buffer formalin. Aortic roots were embedded in OCT and frozen at −20°C. Atherosclerosis was assessed both in the intimas of aortic arches by an en face technique and also using cross sections (10 μm thick) of aortic roots as described previously [21]. Oil red O staining was used to assist in visualization of lesions but not in the quantification of lesion size. Quantitative analysis of atherosclerosis was performed using Image-Pro software (Media Cybernetics) as described previously [13,14]. Suprarenal aortas were dissected where abdominal aortas gave off branches from superior mesenteric artery to right renal artery and removed adventitial fat tissue. Cellular components of suprarenal aortas (n=3 in every group) were detected by immunostaining with rabbit antisera against mouse macrophage (catalog no. AIAD31240; Accurate Chemical Company), rabbit polyclonal to α-smooth muscle actin (α-SMA; catalog no. ab5694; Abcam), rat monoclonal to mouse CD19 (catalog no. 553783; BD Pharmingen) and rat monoclonal to mouse CD90.2 (catalog no. 553009; BD Pharmingen). Frozen aortas (n=8–10 in each group) were pulverized for protein extraction in protease inhibitor cocktail and protein concentration was determined with a bicinchoninic acid (BCA; Pierce) assay kit. Concentrations of total MMP-2 and total MMP-9 were measured in the supernatant of aortic lysates by ELISA kit (R&D Systems). Aortic lysates (30 μg protein) were resolved by 10% SDS-PAGE containing 2% gelatin. Gelatinolytic activity was visualized as negative staining with Coomassie Brilliant Blue (Sigma) as described previously [22]. All the data were quantified by two observers that were blinded to the study design.

2.4. Serum measurements

Serum total cholesterol concentrations were determined using enzymatic assay kits (catalog no. 294-65801; Wako Chemical, Japan). Serum alanine aminotransferase and glucose were measured with the Olympus commercial kits OSR6107 and OSR6121, respectively (Olympus Diagnostica, Hamburg, Germany). Serum cytokines and enzymes were measured with ELISA kits according to manufacturer's recommendation, including IL-1α, IL-6, monocyte chemoattractant protein 1 (MCP-1), macrophage migration inhibitory factor (MIF), soluble ICAM-1 (CD54), soluble VCAM-1, total MMP-2 and total MMP-9 (all purchased from R&D Systems, except MCP-1, which was purchased from Bender MedSystems).

2.5. Statistical analyses

Mean and S.E.M. were calculated for each parameter. Data were tested for use of parametric or nonparametric post hoc analysis and then analyzed by Student's t test or Mann–Whitney Rank Sum test using SigmaStat version 3.5 software (Systat Software, Chicago, IL). P<.05 was considered to be statistically significant.

3. Results

3.1. Red yeast rice extract suppressed AngII-induced AAAs

During the 28 days of AngII infusion, there was no statistical significance of mortalities between the RYR (2/17, 12%) and control groups (4/17, 24%; P>.05 by Fisher's Exact Test). Death due to aortic rupture incidence of AAA was 25% (1/4) in the control group. No mice died from AAA rupture in the group administered RYR extract. The mortality in the RYR group was not significantly different from the control group by Fisher's Exact Test.

Administration of RYR extract significantly decreased both suprarenal aortic diameter and area compared with mice administered with vehicle (Fig. 1, P<.05). Abdominal aortic aneurysm incidence was decreased significantly in RYR extract-administered mice (27%, 4/15) compared with vehicle-administered mice (77%, 10/13; P<.05 by Fisher's Exact Test). Consistently, administration of RYR extract significant reduced the percent differences in suprarenal aortic diameter and area compared with mice with vehicle (Fig. 1, P<.05).

3.2. Red yeast rice extract attenuated AngII-induced atherosclerotic lesions

We determined the effects of RYR extract in apoE−/− mice infused with AngII on atherosclerotic lesion areas in aortic roots (from aortic sinuses to ascending aorta) and percent of lesion area on the intimas of aortic arches. Administration of RYR extract significantly decreased lesion areas in aortic roots compared with mice with vehicle. Consistently, administration of RYR extract also significantly decreased percent lesion areas on the intimas of aortic arches compared with mice administered vehicle (Fig. 2, P<.05, Supplemental Fig. 1). Atherosclerotic lesion areas were reduced by 48% in aortic

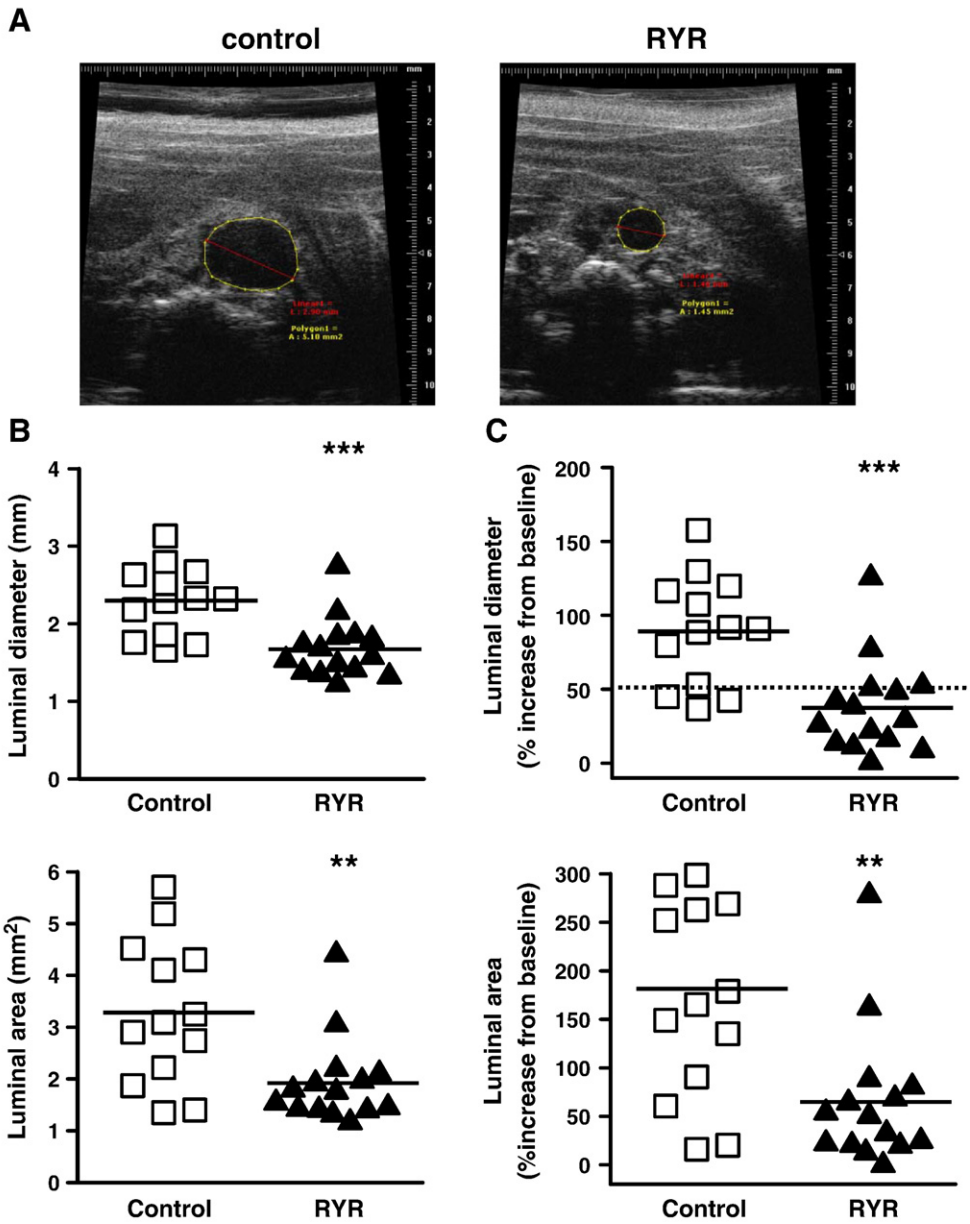

Fig. 1. Red yeast rice extract attenuated AngII-induced AAAs. Abdominal aortic aneurysms were assessed by *in vivo* ultrasound imaging (A) to determine the maximal diameter and area of suprarenal aortas (B) in apoE−/− mice infused with AngII for 28 days; and the percent increase in luminal diameter and area of suprarenal aortas (C) before and after AngII infusion. The images shown are representative aortas after 28-day infusion of AngII. Magnifications presented in Supplemental Fig. 1. Each symbol represents an individual mouse; bars represent means. ***$P<.001$; **$P<.01$.

roots and 36% in aortic arch intima, respectively, by administration of RYR extract.

3.3. Red yeast rice extract lowered serum total cholesterol concentrations

Serum total cholesterol concentrations were measured by enzymatic assay kits. Administration of RYR extract significantly decreased serum total cholesterol concentrations by 19% (Table 1, $P<.05$). Red yeast rice extract administration had no effect on body weight or serum concentrations of glucose and alanine transaminase (as shown in Table 1). Consistent with our previous reports, AngII infusion led to a 26±2-mmHg increase of systolic blood pressure in apoE−/− male mice, which was not affected by administration of RYR extract (165±3 vs. 170±2 mmHg at Day 28).

3.4. Red yeast rice extract influenced serum inflammatory markers and MMPs

To investigate the potential mechanisms for RYR extract reducing AAAs and atherosclerosis, we measured serum concentrations of IL-1α, IL-6, MCP-1, MIF, ICAM-1 and VCAM-1 as an index of circulatory inflammatory markers. Administration of RYR extract did not alter serum concentrations of IL-1α, IL-6, or MCP-1 (Fig. 3A, B and D). Serum concentrations of ICAM-1 and VCAM-1 were significantly reduced by administration of RYR extract (Fig. 3E and F, $P<.05$). In contrast, serum MIF concentrations were significantly increased by administration of RYR extract (Fig. 3C, $P<.01$).

We further determined serum concentrations of total MMP-2 and total MMP-9, which are thought to be associated with the formation and expansion of AAAs. Serum concentrations of MMP-2 were

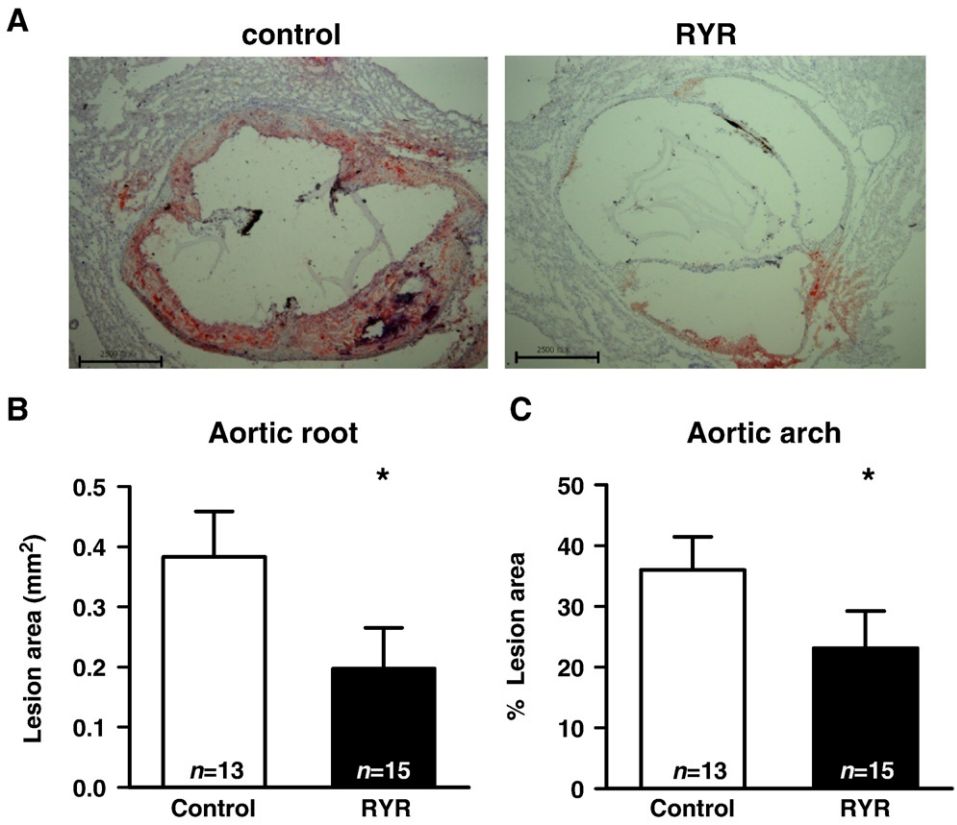

Fig. 2. Red yeast rice extract reduced AngII-induced atherosclerosis. Measurement of atherosclerotic lesion size both in the intimas of aortic arches (B) and cross sections of aortic roots (A) in apoE−/− mice infused with AngII for 28 days. The images shown are representative atherosclerotic lesions in aortic root visualized with oil red O staining after a 28-day duration of AngII infusion. Values shown are means±S.E.M. *P<.05.

approximately 100-fold higher than MMP-9 in mice infused with AngII for 28 days (Fig. 3G and H). Red yeast rice extract administration did not affect serum MMP-9 concentrations, however, serum MMP-2 concentrations was significant decreased by RYR extract administration (Fig. 3G, P<.05).

3.5. Red yeast rice extract did not change MMP-2 and MMP-9 abundance in aortas but attenuated inflammatory cells accumulation

To investigate further potential mechanisms of RYR extract on reducing AAAs, MMP-2 and MMP-9 concentrations were determined by ELISA kits in tissue extract of aortic wall, as well as gelatin zymography for MMP activity. Compared with the bands of MMP-2 activity on zymography, MMP-9 bands were not detected (data not shown). Neither protein concentrations of MMP-2 and MMP-9 (Fig. 4A and B) nor the gelatinase activities of MMP-2 and MMP-9 in aortic lysates were changed by administration of RYR extract (data not shown).

Table 1
Serum panels and other parameters in AngII-infused ApoE−/− male mice treated with vehicle or RYR extract

	Vehicle (n=17)	RYR (n=17)	P*
Total cholesterol (mg/dL)	602±46	489±28	.048
Blood glucose (mg/dL)	104±10	121±12	NS
Alanine transaminase (U/l)	32±7	27±5	NS
Body weight (g)			
Baseline	24.9±0.9	24.3±0.8	NS
End	25.3±0.9	23.6±0.8	NS

* P value was obtained using Student's t test for the statistical comparison between the control and RYR group. NS indicates no statistically significant difference.

Histological staining showed that aortas extracted from mice administered with vehicle had prominent proliferated adventitia with hemorrhage, ruptured media and abundant accumulation of macrophages and lymphocytes in the media and adventitia (Fig. 4C and Supplemental Fig. 2). However, administration of RYR extract dramatically decreased the accumulation of macrophages and lymphocytes and increased atherosclerotic lesions content of smooth muscle cells (Fig. 4C).

4. Discussion

In this study, we demonstrated that administration of RYR extract significantly suppressed development of AngII-induced AAA and atherosclerosis. Reductions in vascular pathologies during RYR extract administration were associated with decreased serum ICAM-1 and VCAM-1 concentrations and increased serum MIF concentrations. Red yeast rice extract administration also decreased serum MMP-2 concentrations but not serum MMP-9 concentrations. As a dietary supplement and Chinese medicinal herb, RYR extract administration had significant potential to reduce serum total cholesterol concentrations.

There is substantial evidence that the incidence of AAA in the Chinese population is lower in comparison to Western countries [23,24]. Considering the risk factors involved in the development of AAA, it is possible that changes of lifestyle and dietary habits are benefit for prevention of AAA formation. Red yeast rice has a long history in China and is in common used as a natural pigment for traditional cuisine and food additives. It is prepared by using *Monascus purpureus* fermented with rice. The lower prevalence of AAA in Chinese population may be attributed to the widely application of RYR in unconscious conditions.

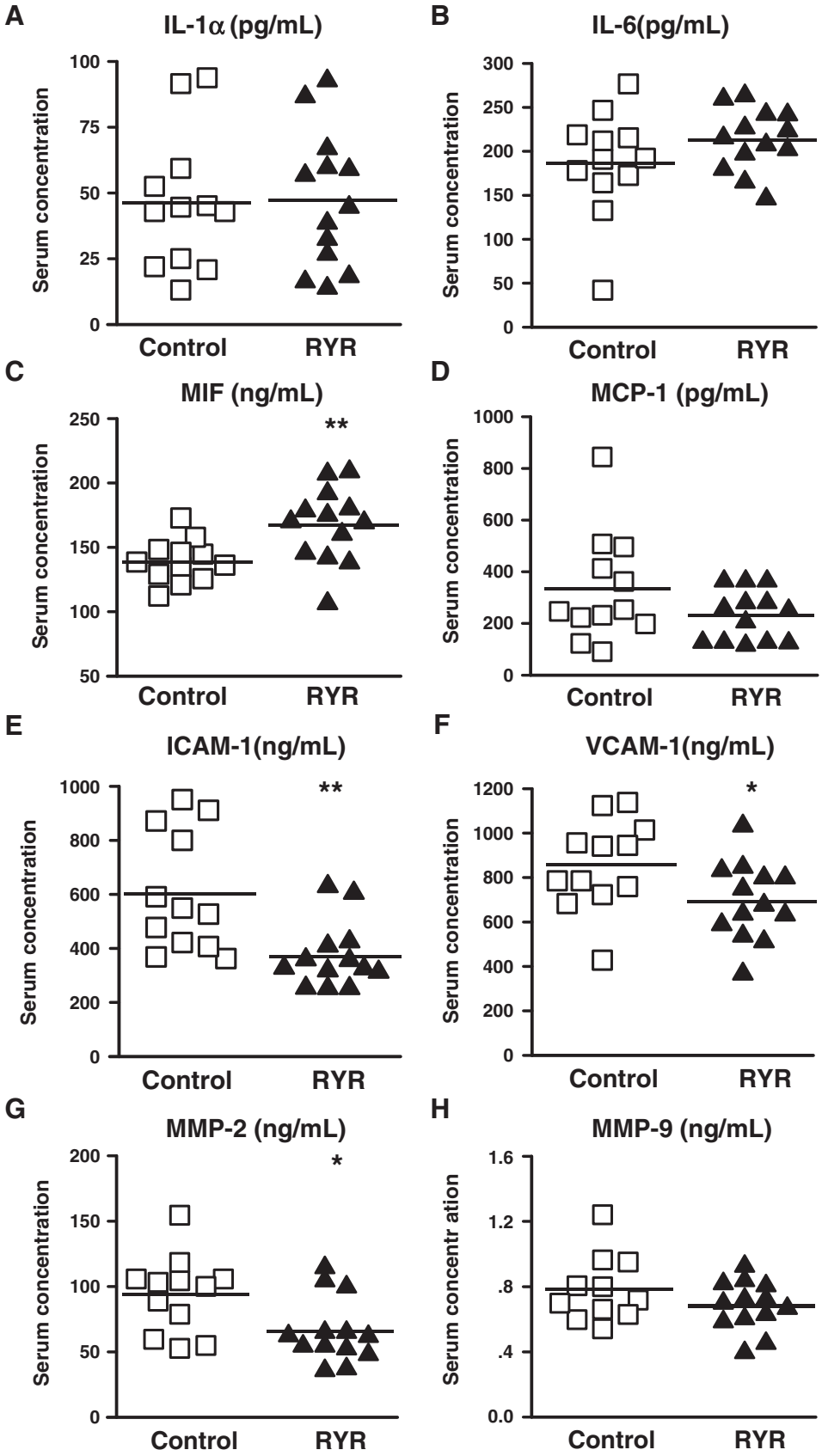

Fig. 3. Red yeast rice extract had an effect on serum concentrations of inflammatory cytokines and MMPs. Serum concentrations of IL-1α (A), IL-6 (B), MIF (C), MCP-1 (D), ICAM-1 (E), VCAM-1 (F), MMP-2 (G) and MMP-9 (H) in apoE−/− mice infused with AngII for 28 days. Each symbol represents an individual mouse; bars represent means. $^{**}P<.01$; $^{*}P<.05$.

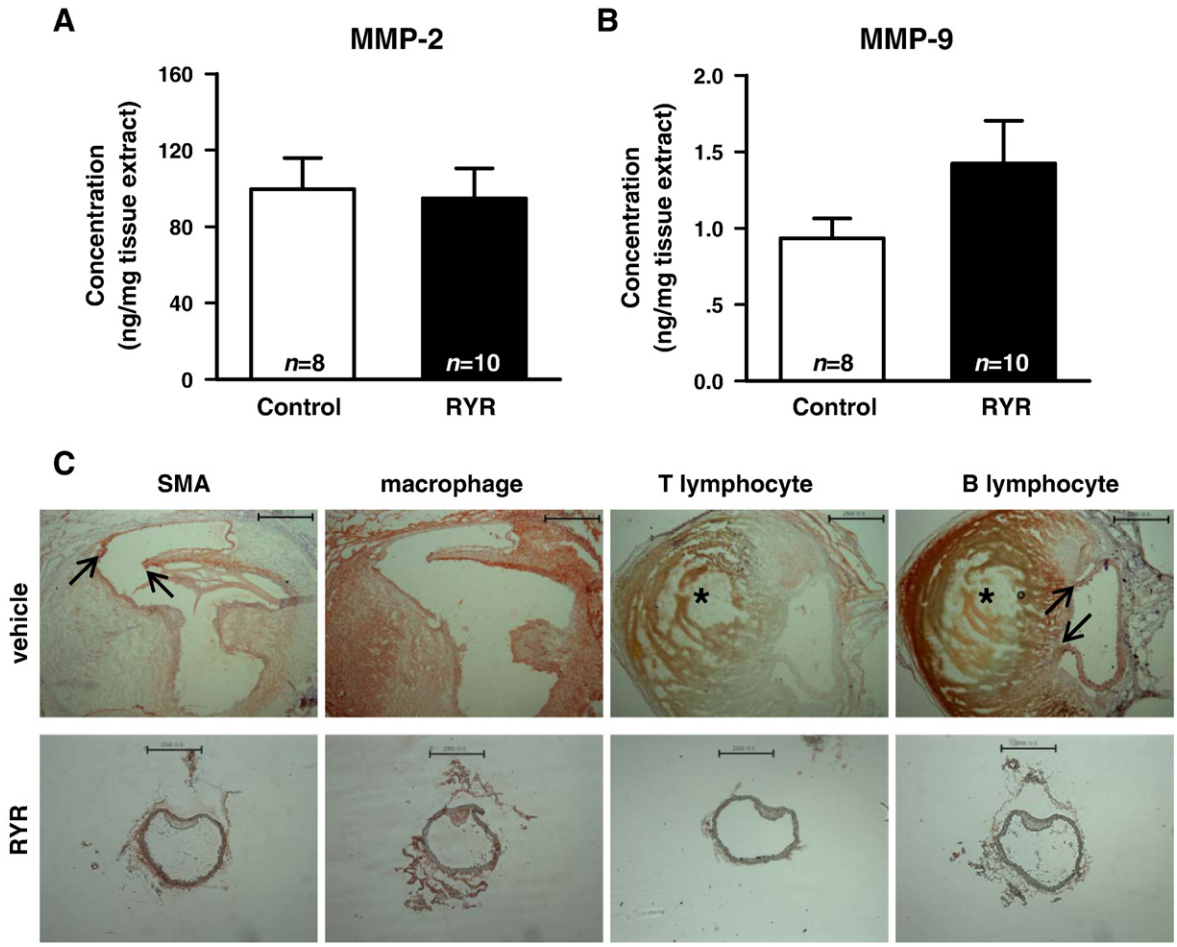

Fig. 4. Red yeast rice extract did not change MMP-2 and MMP-9 abundance in aortic wall but attenuated infiltrations of macrophage and lymphocyte to aortic wall. MMP-2 (A) and MMP-9 (B) concentrations in tissue extract of aortic walls in apoE−/− mice infused with AngII for 28 days. Values shown are means±S.E.M. The images (C) shown are representative aneurysm sections of immunostaining with SMA, macrophage, T lymphocyte (CD90.2) and B lymphocyte (CD19) in suprarenal aortas from AngII-infused apoE−/− mice administrated with vehicle or RYR extract. Medial rupture seen at arrows and hemorrhage at asterisks. Bars=2500 μm. Magnifications presented in Supplemental Fig. 2.

Red yeast rice has been recently regarded as a native statin due to its lipid-regulating effect [8]. The present study showed a 200-mg/kg/day dose of RYR extract for 28 days reduced serum total cholesterol concentrations by 19% in apoE−/− mice. It is comparable to a previous report that 400 or 1350 mg/kg/day of RYR extract administration reduced serum total cholesterol concentrations by 25% and 40%, respectively, in rabbits on high-fat diet [25]. Furthermore, we found atherosclerotic lesion areas were 48% lower in aortic roots and 36% lower in the intimas of aortic arches, respectively, by administration of RYR extract. It was consistent that RYR extract administration reduced atherosclerotic lesions by 63% in the intimas of aortic arches of hypercholesterolemic rabbits [25]. The effect of RYR extract on lipid regulation and atherosclerotic protection is associated with the components of RYR, monacolin K, which has been marketed under the names of mevinolin or lovastatin. Although the total monacolins content of RYR extract is 0.4%, there are 13 different monacolins identified in RYR, and monacolin K represents about half of the total monacolin yield [5]. In a previous study, administration of lovastatin at 2.36 mg/kg/day achieved the comparative reduction of serum total cholesterol concentrations and atherosclerotic lesions. In addition, RYR extract also contains monounsaturated fatty acids, palmitic acid, linoleic acid, oleic acid, stearic acid, some of which may benefit for serum lipid regulation.

Although the pathogenesis in the development of AAA and atherosclerosis differs, chronic inflammatory responses are involved in these two vascular diseases. We demonstrated that chronic infusion of AngII in apoE−/− mice up-regulated gene expressions of IL-6, MMP-2, MMP-13, MMP-14, cyclooxygenase 2 in suprarenal aortas [15]. Other reports showed that AngII infusion elevated the mRNA aortic abundance of MCP-1, ICAM-1, VCAM-1, cyclooxygenase 2, macrophage colony-stimulating factor [26]. Angiotensin II infusion has been demonstrated to increase serum concentrations of proinflammatory cytokines such as IL-1α and IL-6 [16]. To interpret the effect of RYR extract administration on inflammatory responses, some key mediators were investigated both in serum samples and suprarenal aortas by protein measurements. Red yeast rice extract led to reductions of serum concentrations of ICAM-1 and VCAM-1, which may attenuate transmigration of leukocytes across vascular endothelial and recruitment of inflammatory immune cells into the lesions. Migration inhibitory factor is a multifunctional protein that expressed abundantly by many cells types and exhibits a role in the regulation of inflammation and immune response [27]. Red yeast rice extract exerted a protective effect on atherosclerosis with elevation of serum MIF concentrations, which might be supported by the evidence that MIF has a cardioprotective role in the postischemic heart by reducing oxidative stress [28]. Furthermore, recent studies have demonstrated beneficial effects of MIF on alleviating myocardial ischemia and preserving cardiac contractile function by AMP-activated protein kinase signaling pathway

[29,30]. Abdominal aortic aneurysm formation is also a process of vascular remodeling, including media rupture, adventitial proliferation and neoangiogenesis. The role of MIF on the pathological process of AAA is controversial. It has demonstrated previously that circulatory MIF concentrations are associated with human AAA expansion [31]. However, recent studies showed that deficiency of MIF delayed the healing of medial collateral [32]. The reduction of AngII-induced AAA by RYR extract administration may be attributed to the facilitation of vascular healing and remodeling process by elevated serum MIF concentrations.

Matrix metalloproteinases have been implicated in the initiation, progression and rupture of AAA, with greatest emphasis on MMP-2 and MMP-9. Deficiency of MMP-2 or MMP-9 had been demonstrated to suppress the development of experimental AAAs [33,34]. In AngII-induced AAA models, MMP-2 expression has been upregulated that is consistent with a role, in AAA development [14]. Currently there are no publications demonstrating the response of deficiency of specific MMPs on the development of AngII-induced AAAs. Specific MMPs degrade collagen and elastin, which are assumed to be critical to AAA development. Red yeast rice extract administration might reduce MMP-2 expression to keep the integrity of aortic walls and protect from the early formation of AngII-induced AAA.

Recent studies have claimed that statins have cardiovascular protection by complex effects in addition to lowering plasma cholesterol concentrations. While statins administration is considered a standard of care for patients with coronary heart disease, it is still controversial whether statins can suppress AAA expansion [35]. The beneficial role of RYR extract may not be attributed to the monacolin K component. Another component of RYR extract is isoflavones, including diadzein, glycitein and saponin, with a content of 0.05% [5]. Similar in the chemical structure to estrogen, isoflavones may have an effect similar to estrogens, which had been identified to prevent the development of AngII-induced AAA [36].

In summary, the present study showed that RYR extract administration suppressed development of AngII-induced AAA and atherosclerosis associated with a reduction of serum concentrations of ICAM-1, VCAM-1 and MMP-2 and an increase of serum MIF concentrations, thus attenuating the infiltration of inflammatory cells. These data suggest that RYR may have preventive potential for patients suffered with AAA.

Supplementary materials related to this article can be found online at doi:10.1016/j.jnutbio.2011.02.011.

References

[1] Salem MK, Rayt HS, Hussey G, Rafelt S, Nelson CP, Sayers RD, et al. Should Asian men be included in abdominal aortic aneurysm screening programmes. Eur J Vasc Endovasc Surg 2009;38:748–9 [PubMed: 19666232].

[2] Manning MW, Cassis LA, Daugherty A. Differential effects of doxycycline, a broad-spectrum matrix metalloproteinase inhibitor, on angiotensin II-induced atherosclerosis and abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 2003;23:483–8 [PubMed: 12615694].

[3] Pagano MB, Bartoli MA, Ennis TL, Mao D, Simmons PM, Thompson RW, et al. Critical role of dipeptidyl peptidase I in neutrophil recruitment during the development of experimental abdominal aortic aneurysms. Proc Natl Acad Sci U S A 2007;104:2855–60 [PubMed: 17301245].

[4] Sukhova GK, Wang B, Libby P, Pan JH, Zhang Y, Grubb A, et al. Cystatin C deficiency increases elastic lamina degradation and aortic dilatation in apolipoprotein E-null mice. Circ Res 2005;96:368–75 [PubMed: 15653570].

[5] Mosorin M, Juvonen J, Biancari F, Satta J, Surcel HM, Leinonen M, et al. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: a randomized, double-blind, placebo-controlled pilot study. J Vasc Surg 2001;34:606–10 [PubMed: 11668312].

[6] Ma J, Li Y, Ye Q, Li J, Hua Y, Ju D, et al. Constituents of red yeast rice, a traditional Chinese food and medicine. J Agric Food Chem 2000;48:5220–5 [PubMed: 11087463].

[7] Heber D, Lembertas A, Lu QY, Bowerman S, Go VL. An analysis of nine proprietary Chinese red yeast rice dietary supplements: implications of variability in chemical profile and contents. J Altern Complement Med 2001;7:133–9 [PubMed: 11327519].

[8] Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff RM, Go VL. Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. Am J Clin Nutr 1999;69:231–6 [PubMed: 9989685].

[9] Liu J, Zhang J, Shi Y, Grimsgaard S, Alraek T, Fonnebo V. Chinese red yeast rice (*Monascus purpureus*) for primary hyperlipidemia: a meta-analysis of randomized controlled trials. Chin Med 2006;1:4 [PubMed: 17302963].

[10] Li JJ, Lu ZL, Kou WR, Chen Z, Wu YF, Yu XH, et al. Impact of Xuezhikang on coronary events in hypertensive patients with previous myocardial infarction from the China Coronary Secondary Prevention Study (CCSPS). Ann Med 2010;42:231–40 [PubMed: 20350253].

[11] Lu Z, Kou W, Du B, Wu Y, Zhao S, Brusco OA, et al. Effect of Xuezhikang, an extract from red yeast Chinese rice, on coronary events in a Chinese population with previous myocardial infarction. Am J Cardiol 2008;101:1689–93 [PubMed: 18549841].

[12] Zhao SP, Liu L, Cheng YC, Shishehbor MH, Liu MH, Peng DQ, et al. Xuezhikang, an extract of cholestin, protects endothelial function through antiinflammatory and lipid-lowering mechanisms in patients with coronary heart disease. Circulation 2004;110:915–20 [PubMed: 15313947].

[13] Daugherty A, Cassis L. Chronic angiotensin II infusion promotes atherogenesis in low density lipoprotein receptor −/− mice. Ann N Y Acad Sci 1999;892:108–18 [PubMed: 10842656].

[14] Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. J Clin Invest 2000;105:1605–12 [PubMed: 10841519].

[15] Zack M, Boyanovsky BB, Shridas P, Bailey W, Forrest K, Howatt DA, et al. Group X secretory phospholipase A(2) augments angiotensin II-induced inflammatory responses and abdominal aortic aneurysm formation in apoE-deficient mice. Atherosclerosis 2011;214:58–64 [PubMed: 20833395].

[16] Zhang LN, Vincelette J, Cheng Y, Mehra U, Chen D, Anandan SK, et al. Inhibition of soluble epoxide hydrolase attenuated atherosclerosis, abdominal aortic aneurysm formation, and dyslipidemia. Arterioscler Thromb Vasc Biol 2009;29:1265–70 [PubMed: 19667112].

[17] Li JJ, Wang Y, Nie SP, Li Q, Li YS, Huang Y, et al. Xuezhikang, an extract of cholestin, decreases plasma inflammatory markers and endothelin-1, improve exercise-induced ischemia and subjective feelings in patients with cardiac syndrome X. Int J Cardiol 2007;122:82–4 [PubMed: 17196275].

[18] Lin CP, Chen YH, Chen JW, Leu HB, Liu TZ, Liu PL, et al. Cholestin (*Monascus purpureus* rice) inhibits homocysteine-induced reactive oxygen species generation, nuclear factor-kappaB activation, and vascular cell adhesion molecule-1 expression in human aortic endothelial cells. J Biomed Sci 2008;15:183–96 [PubMed: 17906965].

[19] Daugherty A, Manning MW, Cassis LA. Antagonism of AT2 receptors augments angiotensin II-induced abdominal aortic aneurysms and atherosclerosis. Br J Pharmacol 2001;134:865–70 [PubMed: 11606327].

[20] Barisione C, Charnigo R, Howatt DA, Moorleghen JJ, Rateri DL, Daugherty A. Rapid dilation of the abdominal aorta during infusion of angiotensin II detected by noninvasive high-frequency ultrasonography. J Vasc Surg 2006;44:372–6 [PubMed: 16890871].

[21] Daugherty A, Whitman SC. Quantification of atherosclerosis in mice. Methods Mol Biol 2003;209:293–309 [PubMed: 12357958].

[22] Haas TL, Davis SJ, Madri JA. Three-dimensional type I collagen lattices induce coordinate expression of matrix metalloproteinases MT1-MMP and MMP-2 in microvascular endothelial cells. J Biol Chem 1998;273:3604–10 [PubMed: 9452488].

[23] Hobbs SD, Sam RC, Bhatti A, Rehman A, Wilmink AB, Adam DJ, et al. The low incidence of surgery for non-cardiac vascular disease in UK Asians may be explained by a low prevalence of disease. Eur J Vasc Endovasc Surg 2006;494–99:32 [PubMed: 16679039].

[24] Wang LX, Fu WG, Guo DQ, Xu X, Chen B, Jiang JH, et al. Analysis of contrast medium induced pancreatitis. Zhonghua Yi Xue Za Zhi 2010;90:1159–61 [PubMed: 20646559].

[25] Wei W, Li C, Wang Y, Su H, Zhu J, Kritchevsky D. Hypolipidemic and antiatherogenic effects of long-term Cholestin (*Monascus purpureus*-fermented rice, red yeast rice) in cholesterol fed rabbits. J Nutr Biochem 2003;14:314–8 [PubMed: 12873712].

[26] Tham DM, Martin-McNulty B, Wang YX, Wilson DW, Vergona R, Sullivan ME, et al. Angiotensin II is associated with activation of NF-kappaB-mediated genes and downregulation of PPARs. Physiol Genomics 2002;11:21–30 [PubMed: 12361987].

[27] Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 2003;3:791–800 [PubMed: 14502271].

[28] Koga K, Kenessey A, Powell S, Sison CP, Miller EJ, Ojamaa K. Macrophage migration inhibitory factor provides cardioprotection during ischemia/reperfusion by reducing oxidative stress. Antioxid Redox Signal 2011;14:1191–202 [PubMed: 20831446].

[29] Ma H, Wang J, Thomas DP, Tong C, Leng L, Wang W, et al. Impaired macrophage migration inhibitory factor-AMP-activated protein kinase activation and ischemic recovery in the senescent heart. Circulation 2010;122:282–92 [PubMed: 20606117].

[30] Tong C, Morrison A, Yan X, Zhao P, Yeung ED, Wang J, et al. Macrophage migration inhibitory factor deficiency augments cardiac dysfunction in

Type 1 diabetic murine cardiomyocytes. J Diabetes 2010;2:267–74 [PubMed: 20923497].
[31] Pan JH, Lindholt JS, Sukhova GK, Baugh JA, Henneberg EW, Bucala R, et al. Macrophage migration inhibitory factor is associated with aneurysmal expansion. J Vasc Surg 2003;37:628–35 [PubMed: 12618703].
[32] Kitayama S, Onodera S, Kondo E, Kobayashi T, Miyatake S, Kitamura N, et al. Deficiency of macrophage migration inhibitory factor gene delays healing of the medial collateral ligament: a biomechanical and biological study. J Biomech 2011;44:494–500 [PubMed: 20950810].
[33] Pyo R, Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. J Clin Invest 2000;105:1641–9 [PubMed: 10841523].
[34] Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110:625–32 [PubMed: 12208863].
[35] Baxter BT, Terrin MC, Dalman RL. Medical management of small abdominal aortic aneurysms. Circulation 2008;117:1883–9 [PubMed: 18391122].
[36] Martin-McNulty B, Tham DM, da CV, Ho JJ, Wilson DW, Rutledge JC, et al. 17 Beta-estradiol attenuates development of angiotensin II-induced aortic abdominal aneurysm in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2003;23:1627–32 [PubMed: 12855485].