Panax Notogingseng Saponins Suppress RAGE/MAPK Signaling and NF-kappaB Activation in Apolipoprotein-E-deficient Atherosclerosis-prone mice

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Key Words

Panax notoginseng saponins • Atherosclerosis • Nuclear factor kappa B • Receptor of advanced glycation end products • Adhesion molecules • ApoE-deficient mice

Abstract

Background: Panax notoginseng saponins (PNS) extracted from the roots of panax notoginseng are free radical-scavenger, with an antioxidant property, capable of inhibiting expression of adhesion molecules and chemokines. This study was designed to test the effects of PNS administration in apolipoprotein (apo)-E-deficient mice on the activation of JNK, p38^{MAPK}, ERK1/2 and NF-κB, and the expression of RAGE, adhesion molecules and chemokines in the atherosclerotic lesions. **Methods:** Wild type and apoE-null mice (male, 10-week-old) were treated with PNS for 4 weeks. Peripheral blood was collected for assessing the serum levels of glucose, lipids and MDA, and activities of SOD and GSH. The sizes of atherosclerotic lesions and numbers of macrophages in the branchiocephalic arteries, and the reactive oxygen species (ROS) production in the aortic root were analyzed. The levels of CD68, Galectin-3, RAGE, JNK, phosphor-JNK, p38^{MAPK}, phosphor-p38^{MAPK}, ERK1/2, phosphor-ERK1/2, I-κB,

phosphor-I-κB (Ser32), NF-κB, phosphor-NF-κB, MCP-1, VCAM-1 and ICAM-1 in the descending arteries were identified by Western blot. **Results:** The atherosclerotic lesion sizes, and macrophage numbers, but not the smooth muscle cell amounts and the collagen content, were decreased in apoE^{-/-} mice treated with PNS. After PNS administration for 4 weeks, the apoE^{-/-} mice displayed reduced level of serum MDA and enhanced activity of SOD and GSH, accompanied by impaired ROS generation in the aortic root. Moreover, PNS down-regulated the expression of VCAM-1, ICAM-1 and MCP-1, accompanied by reduced expression of RAGE and suppressed the activation of NF-κB, JNK, p38^{MAPK} and ERK1/2. **Conclusion:** PNS may inhibit progression of atherosclerotic lesions via their antioxidant/anti-inflammatory biological properties. PNS suppress the RAGE/MAPK signaling pathways, inactivate NF-κB, and reduce expression of pro-inflammatory factors, such as VCAM-1, ICAM-1 and MCP-1 in atherosclerotic lesions of apoE^{-/-} mice.

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Introduction

It has been suggested that endothelial dysfunction and inflammation is an early marker of atherosclerosis

[1, 2]. Normally, endothelium acts as a “barrier” to prevent interaction of vascular muscle layers with circulating inflammatory cells, mainly monocytes and lymphocytes. However, many atherogenic stresses or risk factors (e.g., dyslipidemia, hypertension, hyperglycemia, smoking and aging, etc) induce the excessive generation of reactive oxygen species (ROS), which may injury endothelial cells and cause inflammatory response [3]. The damaged or dysfunctional endothelial cells may synthesize and release pro-inflammatory factors, especially E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1), leading to attachment of the inflammatory cells to the dysfunctional endothelial cells [4-7]. It is considered that the pro-inflammatory factors play a major role in the formation of atherosclerotic plaques. Therefore, controlling inflammatory responses is an important therapeutic strategy for prevention of atherosclerosis initiation.

It is reported that the receptor of advanced glycation end products (RAGE) is a key regulator of adhesion molecules and chemokines, and it plays a pivotal role in amplifying inflammatory processes [8, 9]. Recently, the binding of RAGE to its ligands has been implicated in the plaque initiation and progression. Accumulating evidence has identified that RAGE, when binding to its ligands such as AGE, could trigger the activation of NADPH oxidase, mitogen-activated protein kinases (MAPK) and nuclear factor kappa B (NF- κ B), subsequently leading to excessive generation of ROS and up-regulation of VCAM-1, ICAM-1 and MCP-1 [10], which are major factors responsible for the infiltration of the inflammatory cells to the atheroma-prone sites [9, 11, 12]. RAGE, therefore, is considered as a linker between the release of pro-inflammatory cytokines and the initiation of atherosclerosis. Moreover, oxidative stress contributes to the injury of endothelial cells and expansion of vascular inflammation. Oxidative stress could directly activate the redox-sensitive transcription nuclear factor NF- κ B by several ways, leading to increased expression of pro-inflammatory cytokines and adhesion molecules [13]. Therefore, elevated production of ROS and sustained activation of the RAGE axis formed a vicious circle to perpetuate the injurious effects caused by chronic inflammation within the dysfunctional vascular wall. Thus, the suppression of ROS generation and RAGE expression may be useful as an anti-atherosclerotic target.

Panax notoginseng saponins (PNS) are effective free radical-scavenger, which have an antioxidant property

and are able to suppress adhesion molecules and chemokines. However, the precise mechanisms by which PNS reduce expression of adhesion molecules in atherosclerotic plaques have not been elucidated [14]. In the present study, we showed evidence that PNS suppress the progression of early lesions through their antioxidant/anti-inflammatory biological properties. PNS inhibit the expression of RAGE, MAPK signaling pathways and NF- κ B activation, leading to reduced expression of pro-inflammatory factors including VCAM-1, ICAM-1 and MCP-1 in the lesions of apoE^{-/-} mice.

Materials and Methods

Preparation of animals

Male apoE^{-/-} mice on a C57BL/6J background were provided from Peking University Health Science Center (purchased from Jackson Laboratory). 10-week-old apoE^{-/-} mice (n=20) were fed a high-fat, cholesterol-rich/atherogenic diet containing 21% fat, 19.5% casein, and 1.25% cholesterol in a temperature 20-24 °C and humidity (45-55%)-controlled environment with a 12-12 h light-dark cycle. apoE^{-/-} mice were divided into two groups: the PNS group (n=10) and the control group (n=10). PNS were purchased from Wu-Zhou pharmaceutical Group (WuZhou, China). They were extracted from the roots of panax notoginseng, which contained notoginsenoside R1 10%, ginsenoside Rg1 38%, ginsenoside Re 6%, ginsenoside Rb1 38% and ginsenoside Rd 5%. The chemical purity of PNS was about 97%. In the PNS group, PNS were dissolved in distilled water and administered daily by oral gavage at a dose of 60 mg/kg for 4 weeks, according to previous research [15, 16]. Blood samples were collected from the mice for measurement of glucose, lipids and malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) in serum. The hearts containing aortic root were embedded in the OCT compound and frozen immediately in the liquid nitrogen for further analysis. All the animal procedures were performed in accordance with the National Institutes of Health Animal Care and Use Guidelines. All the animal protocols were approved by the Animal Ethics Committee at the Beijing Institute of Geriatrics.

Measurement of serum glucose, lipids

The serum glucose levels were examined using glucose oxidase method (Beckman). The levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were measured using a kit from Sigma Diagnostics.

Determination of serum SOD and GSH activity, and MDA levels

The activity of SOD and GSH in serum was measured by a commercial assay kit (Cayman). The serum MDA level was measured using a TBARS assay kit (Cel Biolabs).

En face analysis of the endothelium of descending aortas

Six descending aortas of each group were used for en face lipid staining. The aortas were dissected from the left subclavian artery to the iliac bifurcation, then opened longitudinally and stained with Oil Red O to visualize the extent of the lipid deposition. Quantitative analysis of the lesion sizes was performed by first capturing aorta images with a Nikon D80. The data were analyzed with Image Pro-Plus-6 software.

Analysis of the lesions in the branchiocephalic artery and the aortic root

The hearts were sectioned throughout the aortic root with serial frozen sections taken every 10 μ m according to the identified methods.[17] The contiguous frozen sections were stained with Movat pentachrome stain and dihydroethidium (DHE) (Sigma). Other parts of branchiocephalic arteries (BCA) were dissected and fixed overnight in 4% polymerized formaldehyde, followed by paraffin embedded, and sectioned 5- μ m-thick. The sections were stained with a modified Movat pentachrome stain and Sirius Red stain to evaluate the areas and the content of collagen in the lesions separately. The atherosclerotic lesions were analyzed by Image Pro-Plus-6 software (Media Cybernetics).

In situ detection of ROS

To evaluate vascular ROS production in situ, the unfixed frozen cross-sections from the aortic root were stained with 10 μ mol/L DHE (Sigma) for 30 min in a dark humidified chamber at room temperature. The ROS generation was labeled with the red fluorescence, and visualized by a fluorescence microscopy (Carl Zeiss).

Immunohistochemistry

The sections from the BCA were used for immunohistochemistry staining to identify the macrophages, smooth muscle cells (SMC) by a method reported previously.[18] Briefly, the sections were incubated with the polyclonal antibodies at 37°C for 60 min or at 4°C overnight and then labeled with HRP-conjugated anti-rabbit IgG at 37°C for 60 min. Finally, the coverslips were mounted with the DABCO and analyzed by an upright microscope (Carl Zeiss). The antibodies against α -actin (1:100 dilution), CD68 (1:100 dilution), RAGE (1:60 dilution) were purchased from Santa Cruz and the antibody to MAC-3 (1:50 dilution) was purchased from BD Bioscience. MAC-3, a major surface receptor for galectin-3, indicates the amounts of macrophages. CD68 is a marker for foam cells. MAC-2/galectin-3 is a carbohydrate-binding protein expressed on the surface of inflammatory macrophages. Here we used three markers for macrophages such as CD68, MAC-3 and galectin-3 to analyze the amounts of macrophages, foam cells and inflammatory macrophages in the atherosclerotic lesions, respectively.

Western blot

The descending arteries were dissected and used to analyze the protein levels by Western blot. The lysates (10-30 μ g protein) were loaded onto 10% SDS-PAGE gels, blotted onto poly (vinylidene difluoride) membrane (Millipore), blocked with

8% nonfat dry milk for 60 min, and probed with the antibodies at 4°C overnight. The blots were incubated with HRP-conjugated anti-IgG for 1h at 37°C, followed by detection using enhanced chemiluminescence (Millipore). The antibodies against I- κ B (1:1000 dilution), phosphor-I- κ B (Ser32) (1:1000 dilution), MCP-1 (1:1000 dilution), Galectin-3 (1:1000 dilution), VCAM-1 (1:1000 dilution) and ICAM-1 (1:1000 dilution) were purchased from Santa Cruz. The antibody to RAGE (1:500 dilution) was purchased from Sigma Aldrich. The antibodies to phosphor-JNK (Thr¹⁸³ / Tyr¹⁸⁵) (1:1000 dilution), JNK (1:1000 dilution), phosphor-p38 (Thr¹⁸⁰/Tyr¹⁸²) (1:1000 dilution), p38 (1:1000 dilution), phosphor-ERK1/2 (Thr202/Tyr204) (1:1000 dilution), ERK1/2 (1:1000 dilution), NF- κ B p65 (1:1000 dilution) and phosphor-NF- κ B (Ser536) (1:1000 dilution) were obtained from Cell Signaling and the antibody to CD68 (1:1000 dilution) was bought from AbD serotec.

Statistical analysis

All values are represented as means $\pm$ SE of the indicated number of measurements. One-way ANOVA test was used to determine significance, requiring $P < 0.05$ for statistical significance.

Results

PNS treatment enhances activities of SOD and GSH, lowers the levels of MDA in blood, and diminishes generation of ROS in the aortic root

To evaluate the antioxidant ability of PNS, the apoE^{-/-} mice fed a high-fat, cholesterol-rich/atherogenic diet containing 21% fat, 19.5% casein, and 1.25% cholesterol were administered with PNS at a dose of 60mg/kg for 4 weeks. As shown in Fig.1, there was no statistic difference in the body weight and the levels of serum GLU (8.51 $\pm$ 0.69mmol/L vs 8.31 $\pm$ 0.27mmol/L, $p > 0.05$), TC (23.04 $\pm$ 0.52mmol/L vs 24.45 $\pm$ 0.62mmol/L, $p > 0.05$), TG (1.32 $\pm$ 0.18mmol/L vs 1.37 $\pm$ 0.19mmol/L, $p > 0.05$), LDL-C (13.67 $\pm$ 1.67mmol/L vs 12.94 $\pm$ 1.59mmol/L, $p > 0.05$) and HDL-C (0.23 $\pm$ 0.04mmol/L vs 0.29 $\pm$ 0.07mmol/L, $p > 0.05$) between the PNS group and the controls (Fig.1A, B, C). However, as expected, the apoE^{-/-} mice administered with PNS displayed increased activity of serum SOD (120.16 $\pm$ 4.20U/ml vs 56.72 $\pm$ 7.62U/ml, $p < 0.01$) and GSH (6.09 $\pm$ 0.84 μ m vs 3.67 $\pm$ 0.13 μ m, $p < 0.01$), and decreased MDA level (3.91 $\pm$ 1.11 μ m vs 12.13 $\pm$ 1.65 μ m, $p < 0.01$) (Fig.1D, E, F). Moreover, the DHE staining was used to detect the level of superoxide anion to evaluate the superoxide clearance efficiency by PNS in situ. Fig.1G showed that PNS impaired the ROS generation in the aortic root (159.08 $\pm$ 23.29 vs 237.68 $\pm$ 26.01, $p < 0.05$). Our results suggest that PNS may suppress oxidative stress under hyperlipidemic conditions.

Fig. 2. PNS reduce the atherosclerotic lesion area of branchiocephalic arteries (BCA) in apoE^{-/-} mice. The percentage of aortic lesion area (lesion area compared to total arch area) was measured by quantitative histomorphology of Oil Red O stained en face specimens (A). The lesion sizes of BCA and aortic root were analyzed in the PNS group and the control group, as shown by Movat staining (B and C). Data represent the means $\pm$ SE (n=6). * P<0.05 versus control.

PNS treatment reduces atherosclerosis in apoE^{-/-} mice

We examined the effect of PNS on the atherosclerotic lesion sizes in apoE^{-/-} mice. The percentage of aortic lesion area was determined by quantitative histomorphology of Oil Red O stained en face specimens. There was a decreased tendency of the

percentage of aortic area (lesion area compared to total arch area) after PNS treatment ($9.40 \pm 0.83\%$ vs $11.39 \pm 1.43\%$, $p > 0.05$) (Fig.2A). Similarly, there is a statistic decreased lesion area of BCA in apoE^{-/-} mice treated with PNS ($2.64 \pm 0.35 \times 10^4 \mu\text{m}^2$ vs $6.42 \pm 0.44 \times 10^4 \mu\text{m}^2$, $p < 0.05$) (Fig.2B). However, no significant difference in the aortic root areas was found between

Fig. 3. PNS decrease accumulation of macrophages in the lesions of BCA. The amounts of macrophages (A), smooth muscle cells (SMC) (C) and content of collagen (B) in the lesions from BCA were determined by immunohistochemistry and Sirius Red staining. Data represent the means $\pm$ SE (n=6). ** P<0.01 *versus* control.

Fig. 4. PNS inhibit expression of adhesion molecules by inhibition of nuclear factor kappa B (NF- κ B) activation. The expression of macrophage markers (galectin-3/MAC-2 and CD-68) in the aortic root and the descending arteries of apoE^{-/-} mice was detected by immunofluorescent staining and Western blot (A). The activation of NF- κ B in the descending arteries was analyzed by measurement of NF- κ B, phosphor-NF- κ B, I- κ B and phosphor-I- κ B levels (B). The expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1(MCP-1) (C) in the descending arteries was detected by Western blot. Data represent the means $\pm$ SE (n=4). * P<0.05 *versus* control; ** P<0.01 *versus* control.

the PNS group and the control group, as shown by Movat staining ($10.03 \pm 1.70 \times 10^4 \mu m^2$ vs $13.62 \pm 1.71 \times 10^4 \mu m^2$, $p > 0.05$) (Fig.2C). The results indicate that PNS have the ability to suppress the progression of lesions in the BCA, but not the aortic root.

PNS treatment reduces accumulation of macrophages in branchiocephalic arteries

The characters of early stage of atherosclerotic lesions are the accumulation or the infiltration of inflammatory cells, mostly the monocytes-derived macrophages, and less SMCs and collagen in the lesions. We, therefore, evaluated the plaque composition in apo E^{-/-} mice. The immunohistochemistry using the antibody against MAC-3, a marker of macrophages, indicated a

decrease of macrophage amounts in the atherosclerotic lesions ($2.39 \pm 0.07 \times 10^4 \mu m^2$ vs $5.78 \pm 0.22 \times 10^4 \mu m^2$, $p < 0.01$) (Fig.3A). However, no significant difference in the content of collagen ($3.22 \pm 0.91\%$ vs $5.29 \pm 2.79\%$, $p > 0.05$) (Fig.3B) and the amount of SMC ($5.15 \pm 2.28\%$ vs $4.25 \pm 1.33\%$, $p > 0.05$) (Fig.3C) in the plaques was found between the PNS group and the control group.

PNS treatment inactivates NF- κ B and inhibits expression of adhesion molecules

It has been found that PNS have an anti-inflammatory activity beyond its antioxidant property [14, 15, 19, 20]. The pro-inflammatory factors including VCAM-1, ICAM-1 and MCP-1 are responsible for the accumulation of macrophages into the atheroma-prone area. PNS may

Fig. 5. PNS suppress the expression of advanced glycation end products (RAGE) and the activation of ERK, JNK and p38^{MAPK}. The activation of ERK, JNK and p38^{MAPK} were determined by analysis of ERK, phosphor-ERK, JNK, phosphor-JNK, and p38^{MAPK}, phosphor- p38^{MAPK} (A). PNS suppressed the expression of RAGE in the descending arteries and the BCA, as showed by Western blot and immuno-histochemistry (B). Data represent the means $\pm$ SE (n =4). * P<0.05 *versus* control; ** P<0.01 *versus* control.

manifest through its ability to inhibit the expression of a variety of pro-inflammatory cytokines and adhesion molecules. The expression of CD68 and VCAM-1, ICAM-1, MCP-1 and the activation of NF- κ B in the descending arteries and the sections of aortic root were analyzed by Western blot and immunofluorescent staining. As shown in Fig.4, PNS administration led to reduced expression of CD68 (0.14 ± 0.05 vs 0.54 ± 0.13 , $p < 0.05$), MAC-2 (0.49 ± 0.12 vs 1.28 ± 0.10 , $p < 0.05$) (Fig.4A), and VCAM-1 (0.27 ± 0.03 vs 0.42 ± 0.02 , $p < 0.01$), ICAM-1 (0.13 ± 0.01 vs 0.29 ± 0.04 , $p < 0.01$) and MCP-1 (0.20 ± 0.01 vs 0.30 ± 0.04 , $p < 0.05$) (Fig.4C), displaying the anti-inflammatory activity of PNS. We also evaluated the activation of NF- κ B. The inactive NF- κ B is restricted to the cytoplasm by binding to its major inhibitor I κ B as a complex. The phosphorylation of I κ B results in the ubiquitination and degradation of itself, allowing NF- κ B to translocation to the nucleus. The results show decreased level of p-I- κ B/ I- κ B (0.49 ± 0.11 vs 1.17 ± 0.15 , $p < 0.05$) and p-NF- κ B/ NF- κ B (0.31 ± 0.04 vs 0.61 ± 0.11 , $p < 0.05$) in the descending aorta of apoE^{-/-} mice administered with PNS (Fig.4B). The results indicate that PNS suppressed the activation of NF- κ B, leading to reduced expression of adhesion molecules.

PNS treatment inhibits expression of RAGE and the activation of JNK and p38^{MAPK}

MAPK are the important downstream signaling molecules involved in RAGE signal pathway. Previous studies identified that JNK and p38^{MAPK} are mainly responsible for the expression of VCAM-1, ICAM-1 and MCP-1 [10] when RAGE signal pathway are activated *in vivo* and *in vitro* [9, 12, 21]. The phosphorylation of JNK, p38^{MAPK} and ERK1/2 leads to the activation of

themselves. Here the phosphorylation of MAPK molecules including JNK, p38^{MAPK} and ERK1/2 was analyzed by Western blot. PNS suppressed the phosphorylation of JNK (0.43 ± 0.07 vs 0.76 ± 0.06 , $p < 0.05$), p38^{MAPK} (0.28 ± 0.01 vs 0.88 ± 0.07 , $p < 0.01$) and ERK1/2 (0.31 ± 0.12 vs 0.86 ± 0.07 , $p < 0.01$) (Fig.5A). Moreover, the expression of RAGE in the descending arteries and the BCA of apoE^{-/-} mice was measured by Western blot and immunohistochemistry respectively. The RAGE expression was down-regulated in the descending arteries and the BCA of apoE^{-/-} mice (0.61 ± 0.09 vs 1.30 ± 0.20 , $p < 0.05$). (Fig.5B). Taken together, these results suggest that PNS might suppress expression of RAGE and activation of JNK, p38^{MAPK} and ERK1/2, which might lead to inhibit the progression of early lesions in apoE^{-/-} mice.

Discussion

In this study, we showed experimental evidence that PNS suppress the progression of atherosclerotic lesions in apoE^{-/-} mice. In particular, our findings have demonstrated (i) the inhibitory effects of PNS on activation of NF- κ B; (ii) the association of suppression of RAGE, VCAM-1, ICAM-1 and MCP-1 by PNS with atherosclerotic plaque initiation; (iii) the PNS suppression of oxidative stress under hyperlipidaemic conditions, which may inhibit atherosclerosis.

To evaluate the effect of PNS on atherosclerosis, apoE^{-/-} mice were fed atherogenic diet (1.25% cholesterol contained in the diet) for 4 weeks leading to the formation of early plaques in the BCA and the aortic root. In this study, we evaluated the composition of atherosclerotic plaques in apoE^{-/-} mice administered with PNS. The results

show that PNS reduced the accumulation of macrophages in the BCA, although there was not an effect of PNS on the content of collagen and the amount of SMC in the plaques. PNS also decreased the sizes of lesions accompanied by the reduction of macrophages infiltration. Moreover, the initiation of atherosclerosis is driven by inflammatory cytokines, in particular, the selectins, adhesion molecules and chemokines, which are all controlled by NF- κ B [4, 7]. Because there are more activated NF- κ B in the endothelial cells of atherogenic prone regions [22], NF- κ B is crucial for the initiation and the progression of atherosclerosis. The activated NF- κ B regulates the expression of many atherogenic genes, making a local inflammatory condition and inducing chemotactic factors and adhesion molecules on the surface of endothelial cells, which leads to the attraction of monocytes to the site which is prone to develop atheroma. In the present study, we found decreased activation of NF- κ B in the atherosclerotic plaques of apoE^{-/-} mice administered with PNS. In our previous studies we have addressed the role of PNS in the progression of atherosclerosis and to explore the possible relevant molecular mechanisms. The results showed that PNS treatment significantly decreased the mRNA expression levels of MCP-1 and NF- κ B/p65 in the aorta wall after 8 weeks of treatment, indicating that PNS attenuates atherogenesis through an anti-inflammatory action [23]. In addition, the observations in lipopolysaccharide (LPS)-stimulated mouse macrophage cells, RAW264.7 cells suggested that PNS have a strong anti-inflammatory property to suppress the expression of the inflammation-associated genes including inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor- α and interleukin-1 β by inhibiting the NF- κ B activation, resulting in its anti-inflammatory effects [24].

Oxidative stress has been implicated in the progression of atherosclerosis and vascular inflammation. It has been suggested that the excessive ROS generation up-regulated the expression of pro-inflammatory cytokines and adhesion molecules, consequently resulted in lesion initiation [25]. Based on its antioxidant property, PNS are proposed to suppress the progression of plaques by reducing oxidative stress. In the present study, we found reduced serum MDA level and elevated SOD and GSH activity, accompanied by impaired ROS generation in the aortic root in apoE^{-/-} mice administered with PNS. Our results suggest that PNS inhibit the progression of early lesion, at least in part, by suppression of oxidative stress under hyperlipidemic conditions.

To date, there has been accumulating evidence showing that elevated RAGE contributes to atherosclerosis through activation of NF- κ B, generation of adhesion molecules, and oxidative stress in the dysfunctional endothelium. Almost all the atherogenic stresses, such as hypercholesterolemia, diabetes, hypertension, smoking and aging, stimulate the production of ROS. Firstly, oxidative stress induces the expression of RAGE and contributes to increased generation of advanced oxidation protein products (AOPP), AGEs and AGE-modified ox-LDL, which are the major ligands for RAGE in the atherosclerotic plaques [21, 26]. Secondly, ROS could activate NF- κ B directly or indirectly, which is responsible for the expression of RAGE [27]. It has been reported that panax notoginseng can suppress the phosphorylation of JNK, ERK1/2 and p38^{MAPK} and the translocation of NF- κ B p65 subunit into nucleus [23, 24]. Thereby, we proposed that PNS impair the generation of ROS and subsequently suppress the activation of NF- κ B, in turn inhibit the expression of RAGE. Previous studies have identified that the binding/ interaction of RAGE with its ligands activated the NADPH oxidase, the major source of ROS in the endothelial cells, and subsequently elevated the generation of ROS, followed by the activation of JNK, P38^{MAPK} and NF- κ B [9, 11, 21], leading to the expression of pro-inflammatory genes and RAGE itself which involved in the initiation and the progression of atherosclerosis. Thus, it is considered that ROS facilitate the accumulation of RAGE ligands in the lesions. The interaction of RAGE with its ligands triggers the activation of intracellular signaling pathways including NADPH oxidase, MAPK and NF- κ B, leading to vascular perturbation and inflammation. MAPK, the serine/threonine kinases, are the important downstream signaling molecules for RAGE signal pathway and are activated by various stimuli including ROS. Moreover, previous studies identified that JNK and p38^{MAPK} are mainly responsible for the expression of VCAM-1, ICAM-1 and MCP-1 [10] when RAGE signal pathway is activated *in vivo* and *in vitro* [9, 12, 21]. Although our results cannot exclude the possibility that PNS directly suppress the activation of NF- κ B and the expression of pro-inflammatory factors via its anti-oxidative effect, PNS inhibit the progression of atherosclerosis in apoE^{-/-} mice, at least partly, by suppressing the activation RAGE axis and subsequently decreasing adhesion molecule-induced monocyte/macrophage infiltration in the damaged endothelial cells.

In conclusion, this study provides the experimental data that support the concept that PNS may serve as

anti-atherosclerotic agent via their anti-oxidant/anti-inflammatory biological properties. PNS suppress the expression of RAGE, MAPK signaling pathways and NF- κ B activation, leading to reduced expression of pro-inflammatory factors including VCAM-1, ICAM-1 and MCP-1 in the lesions of apoE^{-/-} mice.

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