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ARTICLE

Panax notoginseng Reduces Atherosclerotic Lesions in ApoE-Deficient Mice and Inhibits TNF-α-Induced Endothelial Adhesion Molecule Expression and Monocyte Adhesion

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It is widely recognized that atherogenesis is associated with vascular inflammation. *Panax notoginseng*, a commonly used herb in China, has been shown to possess anti-inflammatory activity. In the present study, the antiatherogenic effect of *P. notoginseng* saponins (PNS) was examined in apolipoprotein E (apoE)-deficient mice. The molecular mechanisms responsible for the antivascular inflammatory effect of PNS on human coronary artery endothelial cells (HCAECs) were also investigated in vitro. PNS, dissolved in drinking water, was administered orally to two treatment groups at dosages of 4.0 and 12.0 mg/day/mouse, respectively. After 8 weeks, atherosclerosis in the entire aortic area was assessed using an en face method. Compared with the control group, both low- and high-dose PNS-treated groups showed a significant decrease in extent of atherosclerotic lesions by 61.4 and 66.2%, respectively ($P < 0.01$). PNS also notably reduced serum lipid levels. Serum levels of IL-6 and TNF-α in all groups of apoE-deficient mice were below the detection limit. In vitro studies showed that PNS dose-dependently inhibited monocyte adhesion on activated endothelium, as well as the expression of TNF-α-induced endothelial adhesion molecules, such as ICAM-1 and VCAM-1. In conclusion, PNS has antiatherogenic activity through, at least in part, its lipid-lowering and antivascular inflammatory mechanisms.

KEYWORDS: *Panax notoginseng*; atherosclerosis; inflammation; apoE-deficient mice; adhesion molecules

INTRODUCTION

Atherosclerosis underlies most cardiovascular disease and causes more death and disability worldwide than any other pathology except infection (*1*). In the past decade, many lines of evidence have indicated that vascular inflammation mechanisms are associated with the initiation and progression of atherosclerotic lesions (*2*, *3*). Therefore, reducing inflammation in atherosclerotic plaques may help to reduce the risk of atherosclerosis.

Panax notoginseng is one of the most commonly used herbs for the prevention and treatment of cardiovascular diseases, either alone or in combination (*4*, *5*). *P. notoginseng* belongs to the same genus as Asian ginseng (*Panax ginseng* C. A. Mey) and American ginseng (*Panax quinquefolius* L.), and their main components are very similar (*6*). Modern pharmacological studies have demonstrated that *P. notoginseng* and its components possess several beneficial effects on the cardiovascular system (*7*), including protection against cardiac ischemia and ischemia/reperfusion injury, calcium channel antagonism, reduction in lipid peroxidation, and inhibition of platelet aggregation. Dammarane triterpene saponins are generally considered to be the major bioactive ingredients in *P. notoginseng* (*7*). In vitro (*8*) and in vivo (*9*) studies have demonstrated that *P. notoginseng* and its saponins (PNS) have anti-inflammatory effects, suggesting that this herb may possess antiatherosclerotic properties. Several previous animal studies using rabbits or wild-type rats/mice models have shown the antiatherosclerotic activity of *P. notoginseng* and its ingredients (*10*−*13*). However, the methods used to evaluate the extent of atherosclerosis were limited to the cross section of the artery (*11*) and measurement of several atherosclerotic or inflammatory markers, such as blood lipids, C reactive protein, and superoxide dismutase (SOD) (*10*, *12*), which could not accurately and directly evaluate the overall extent of the atherosclerotic lesions. Recently, it has been reported that PNS inhibits zymosan A-induced inflammation and atherosclerosis in rats (*13*). However, modeling with the chemical or reagent might affect the variability of action. For providing reliable and convincing evidence, the methodology used is critical. The effects of *P. notoginseng* remain to be explored by using well-qualified approaches or models. The apoE knockout mouse is a well-characterized model that spontaneously

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develops atherosclerotic lesions with high consistency when fed a Western-type diet (*14*). The en face lesion evaluation method allowed us to observe the entire surface of the endothelium with a clear focused image and, thus, to quantify the extent of atherosclerotic lesions (*15*−*17*).

In the present study, the potential therapeutic effect of total saponins from *P. notoginseng* on atherosclerotic development was examined in apolipoprotein E (apoE)-deficient mice by using en face evaluation method. In addition, the possible molecular mechanisms underlying the antivascular inflammatory effects of PNS were investigated in vitro on human coronary artery endothelial cells (HCAECs).

MATERIALS AND METHODS

Animals and Treatment. Double-homozygosis apoE-deficient female mice (C57BL/6 L genetic background) were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were maintained on 12 h dark/12 h light cycles in air-conditioned rooms (22.5 ± 0.5 °C, 50 ± 5% humidity) with access to diet and water ad libitum. At the age of 6 weeks, mice (weight = 16.0 ± 2.0 g) in the control group were fed a Western-type diet (consisting of 21% fat, 0.15% cholesterol, made by Research Diets Inc., New Brunswick, NJ) for 8 weeks. Low-dose group and high-dose group animals received the same diet and an aqueous solution of PNS at doses of 4.0 and 12.0 mg/day/mouse, respectively. Compliance was confirmed by measuring daily consumption of PNS-supplemented drinking water. This study was conducted according to protocols approved by the center for comparative medicine, Massachusetts General Hospital, in agreement with NIH guidelines.

Quality Control of PNS. PNS was purchased from International Laboratory, and its labeled purity was >95%. The chemical characteristics of PNS were determined using HPLC to ensure the consistency of efficacy. An Aglient 1100 series HPLC apparatus (Palo Alto, CA) was used under optimized conditions (*6*, *18*). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany), and deionized water was purified by a Milli-Q purification system (Millipore, Bedford, MA).

Assessment of Atherosclerotic Lesions. At the end of the experiment, mice were sacrificed by isoflurane inhalation (Abbott Laboratories, Abbott Park, IL). Blood was immediately collected by cardiac puncture from the right ventricle. After blood clotting for 30 min, serum was separated by centrifugation at 1000*g* for 10 min and stored at −80 °C for lipid analysis and cytokine assay. After perfusion with PBS (pH 7.4), the heart and entire aorta with its main branches were rapidly dissected entirely to the iliac bifurcation for histopathological analyses of aortic atherosclerotic lesions.

The extent of atherosclerosis was determined using an en face method (*15*−*17*). The dissected entire aorta was placed in PBS, and the fat and connective tissue adhering to the adventitia were carefully cleaned from the aorta as much as possible under a dissecting microscope. Vessels were removed and fixed with formal sucrose (4% paraformaldehyde, 5% sucrose, 20 μmol/L butylated hydroxytoluene, 2 μmol/L EDTA, pH 7.4) at 4 °C overnight. The aorta was then opened longitudinally and pinned onto black wax plates using micrometereedles (Fine Science Tools, Foster City, CA). Lipid-rich intraluminal lesions were stained with Sudan IV (Sigma-Aldrich, St. Louis, MO). Images were recorded using a Coolpix 990 digital camera (Nikon Corp., Tokyo, Japan) and saved as TIF files. Image analysis was done using Image-Pro Plus software (version 5.1, Media Cybernetics, Bethesda, MD), and the amount of aortic lesion formation in each animal was measured as percent lesion area per total area of the aorta.

Serum Lipid Analysis and Inflammatory Cytokines Assay. Serum concentrations of total cholesterol (TC) and triacylglycerol (TG) were assayed enzymatically using commercial kits (Merck). Levels of HDL and LDL were determined by precipitation with phosphotungstic acid/magnesium chloride and heparin/sodium citrate, respectively, using reagents supplied by Merck.

The levels of TNF-α and IL-6 in the serum samples derived from apoE-deficient mice were determined using commercial ELISA kits (BD Biosciences, San Jose, CA) following the manufacturer's protocols.

Cell Culture and Treatment. Human coronary artery endothelial cells (HCAECs; Cambrex Corp., East Rutherford, NJ) were cultured in EGM-2 MV medium supplemented with endothelial cell growth supplement (SingleQuots kit, including hydrocortisone, hFGF, VEGF, IGF-1, ascorbic acid, hEGF, R^3-IGF-1, and gentamicin/amphotericin-B; Cambrex) and 5% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. Cells with 85−90% confluence from passages 3 to 6 were used.

PNS was dissolved in Mill-Q water, and the solution was filtered through a 0.22 μm Econofilter (Agilent Technologies). PNS was added to cultured cells at final concentrations of 30, 100, and 300 μg/mL and incubated for 24 h. To initiate an inflammatory response, 10 ng/mL recombinant human tumor necrosis factor-α (TNF-α; expressed in *Escherichia coli*, Sigma) was added to culture medium and incubated with endothelial cells for 4 h. Pyrrolidine dithiocarbamate (PDTC, >99.0% from Sigma), an inhibitor of nuclear factor kappa B (NF-κB), was the positive control and incubated for 2 h.

Cell Adhesion Assay. Human acute monocytic leukemia THP-1 cells were obtained from American Type Culture Collection (ATCC; Rockville, MD) and cultured in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS). Before the adhesion test, THP-1 cells were starved for 24 h in RPMI medium containing 1% FBS, then stimulated with 20 ng/mL phorbol ester (PMA) for 3 h, and washed with RPMI medium. THP-1 cells were labeled with the fluorescent dye calcein-AM for quantitative adhesion assay. After loading of calcein-AM, cells were washed three times with PBS to remove excess dye. Cells were resuspended in culture medium at a density of 1×10^5 cells/mL.

HCAECs (5×10^3 cells/well) grown to confluence in a 96-well plate were pretreated with various concentrations of PNS at 37 °C for 24 h and then stimulated with 10 ng/mL TNF-α for 4 h before the adhesion assay. Calcein-AM-labeled THP-1 cells and HCAECs were co-incubated for 30 min at 37 °C in 5% CO_2. Culture medium alone was used as a blank. The total fluorescence intensity of each well (F_t) and blank (F_{b1}) was measured using a fluorescence multiwell plate reader (Wallac 1420, Freiburg, Germany) at excitation and emission wavelengths of 485 and 530 nm, respectively. Subsequently, the plate was washed with PBS three times to remove excess calcein-AM-labeled THP-1 cells and refilled with culture medium. The remaining fluorescence of each well (F_r) and the blank (F_{b2}) was measured. The adhesion ratio (AR) of HCAECs was calculated using the following equation:

$$\mathrm{AR}\ (\%) = \frac{(F_r - F_{b2})}{(F_t - F_{b1})} \times 100\%$$

Immunostaining Assay. After fixation with 80% ethanol, HCAECs were incubated with PBS containing 0.5% Triton X-100 and then washed again three times with PBS. Cells were incubated with mouse monoclonal antibody (mAb) against intercellular adhesion molecule-1 (ICAM-1; 1:100) or vascular cell adhesion molecule-1 (VCAM-1; 1:100) for 1 h at room temperature. Subsequently, cells were washed sufficiently with cold PBS and incubated for 30 min with anti-mouse IgG Alexa 488 antibody (1:100) and then incubated for 10 min with propidium iodide solution (1:1000) (Molecular Probes, Eugene, OR) for nuclear staining at room temperature. Finally, after 3 washings with PBS, cells were examined and photographed using a fluorescence microscope at a magnification of ×400.

Cellular ELISA Assay. Cellular ELISA, modified from the method of Rothlein (*19*), was used for measuring the expression of ICAM-1 and VCAM-1 on the surface of endothelial cells. Briefly, HCAECs were pretreated with or without PNS at desired concentrations for 24 h followed by stimulation with 10 ng/mL TNF-α in 0.5% FBS medium for 4 h before the ELISA assay. After fixation and blocking, cells were incubated with anti-ICAM-1 (1:500) or anti-VCAM-1 (1:300) mAb for 1 h and then with horseradish peroxidase-conjugated goat anti-mouse IgG at VCAM-1 (1:200) or ICAM-1 (1:400), respectively. Cells were exposed to the peroxidase substrate, and absorbance was read at 490 nm using a fluorescence multiwell plate reader.

Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Analysis. Total RNA was extracted from endothelial cells using the RNeasy mini Kit (Qiagen, Valencia, CA). The same amount of mRNA (0.7 μg) was reverse-transcribed and amplified into cDNA using the SuperScript III first-strand synthesis system for real-time RT-PCR

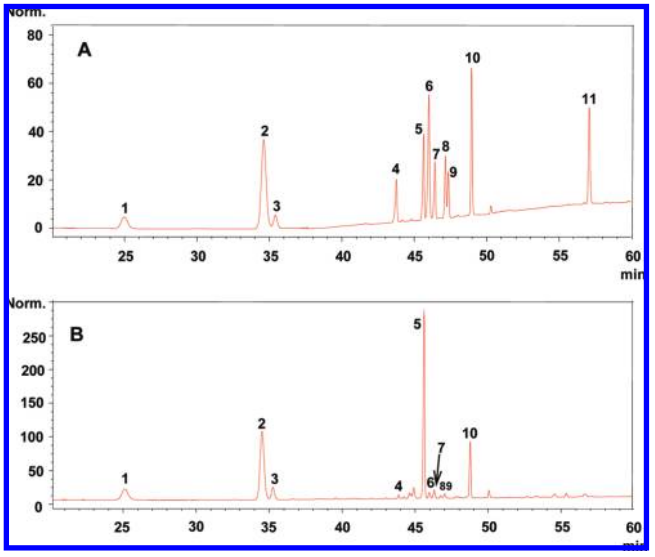

Figure 1. HPLC-DAD chromatograms of (**A**) mixed standards and (**B**) PNS tested. Chromatographic conditions: Zorbax SB-C18 (250 × 4.6 mm i.d., 5 μm) and Zorbax SB-C18 guard columns (12.5 × 4.6 mm i.d., 5 μm) were used at a column temperature of 40 °C. The mobile phase consisted of water (A) and acetonitrile (B) using the following gradient program: 0−30 min, 18−19% B; 30−40 min, 19−31% B; 40−60 min, 31−70% B. Flow rate was kept at 1.5 mL/min, the detection wavelength was set at 203 nm, and the sample injection volume was 10 μL. Peaks: 1, notoginsenoside R1; 2−11, ginsenosides Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3, Rd, and Rg3, respectively.

(Invitrogen Corp., Carlsbad, CA). Oligonucleotide primers and TaqMan probes for human GAPDH (assay 4326317E), VCAM-1 (Hs00174239_m1), and ICAM-1 (Hs00164932_m1) were purchased from Applied Biosystems. TaqMan universal PCR master mix was used for quantitative assay. The RT-PCR was carried out with an ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA). Samples was assayed in triplicate and normalized according to GAPDH content.

Statistical Analysis. The value from in vivo study was expressed as mean ± SD, and the data from in vitro study were expressed by normalization of the responses of the TNF-α group. Student's *t* test was used for comparison between the two groups. $P < 0.05$ was considered to be statistically significant.

RESULTS

Chemical Characteristics of PNS. PNS is a mixture of several compounds from the herb, and the chemical composition of the herb may vary depending on origin and processing. It was therefore necessary to determine the chemical characteristics of PNS to ensure quality consistency and standardization of the sample tested. Under the optimized chromatographic conditions, the peaks corresponding to 11 chemical standards were well separated in 60 min (**Figure 1A**). From the chromatographic profile of PNS (**Figure 1B**), the five compounds (notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, and Rd) were clearly identified as the major components, and their total content was >90.2% of PNS; ginsenosides Rf, Rg2, Rc, Rb2, and Rb3 were the minor components, and their content was <5% of PNS.

Effect of PNS on Atherosclerotic Lesions. After 8 weeks of feeding with the Western-type diet, apoE-deficient mice in the control group developed severe atherosclerotic lesions as measured by an en face method (**Figure 2A**). Sudan IV stains of whole aortas from the mice confirmed the presence of prominent, lipid-rich atheromas. Total lesion areas were dramatically reduced

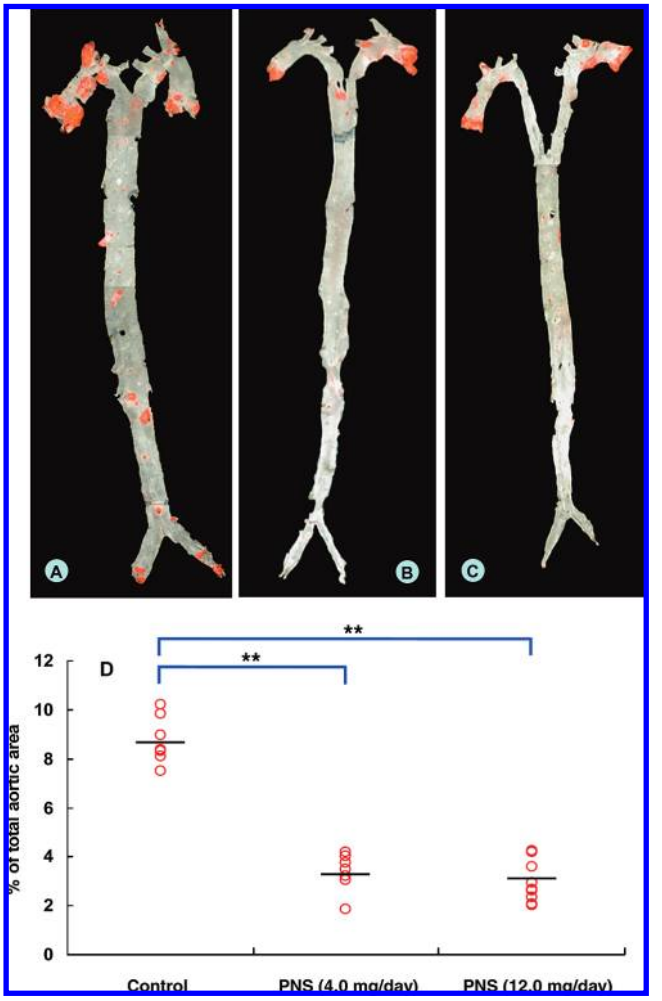

Figure 2. Atherosclerotic lesions in untreated (control) and PNS-treated apoE-deficient mice. Representative Sudan IV-stained, longitudinally opened aortas from (**A**) control, (**B**) PNS-treated (4.0 mg/day), and (**C**) PNS-treated (12.0 mg/day) apoE-deficient mice. Luminal side facing up, displaying lipid-rich (red) atherosclerotic lesions. (**D**) Individual values (○) for lipid lesion area of the entire aorta (percentage) in control and PNS-treated apoE-deficient mice ($n = 6-8$ per group); (—) indicates the median value for each study group. **, $P < 0.01$ versus control; NS, no significant difference.

in PNS-treated mice (**Figure 2B,C**). The percentage of the aorta stained with Sudan IV was significantly lower in the PNS-treated groups (3.37 ± 0.96% for the low-dose group; 2.90 ± 0.86% for the high-dose group, $P < 0.01$) compared to the control group (8.74 ± 0.96%) (**Figure 2D**).

Levels of Serum Lipids and Inflammatory Cytokines. To test whether PNS had lipid-lowering properties, several lipid parameters in the serum of mice from all groups were determined (**Table 1**). Both low-dose and high-dose PNS-treated groups significantly decreased the serum lipids, including total cholesterol, triglycerides, HDL, and LDL, in apoE-deficient mice. The ratio of HDL to LDL in PNS-treated group mice was not significantly different from that in control group, although the average values were mildly elevated.

PNS Inhibits Adhesion of Monocytes to the Endothelial Monolayer. To determine the effect of PNS on endothelial−monocyte interaction, HCAECs were incubated with or without various concentrations of PNS for 24 h before induction with TNF-α (10 ng/mL) for 4 h. There was a low adherence of monocytes on unstimulated HCAECs, and adherence increased significantly

Table 1. Effect of PNS on Blood Lipids of apoE-Deficient Mice[a]

group	cholesterol (mg/dL)	triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	HDL/LDL
control	1051.5 ± 77.6	103.2 ± 18.5	308.8 ± 31.7	726.8 ± 70.3	0.43 ± 0.07
PNS (4.0 mg/day)	891.8 ± 53.4**	86.2 ± 5.9*	249.8 ± 34.6*	609.7 ± 36.0**	0.45 ± 0.10
PNS (12.0 mg/day)	861.8 ± 100.1**	75.3 ± 17.4*	263.1 ± 29.5*	577.0 ± 75.1**	0.47 ± 0.08

[a] Data are presented as mean ± SD for 6−8 mice per group. *, $P < 0.05$, and **, $P < 0.01$, as compared with control group.

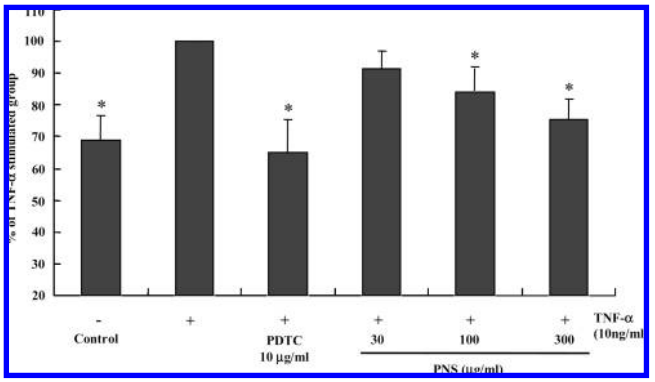

Figure 3. Effects of PNS on the adhesion of THP-1 cells to TNF-α-stimulated HCAECs. Data are expressed by normalization of the responses of TNF-α group and represent means ± SD of three separate assays ($n = 3$). ∗, $P < 0.05$, and ∗∗, $P < 0.01$, versus TNF-α group.

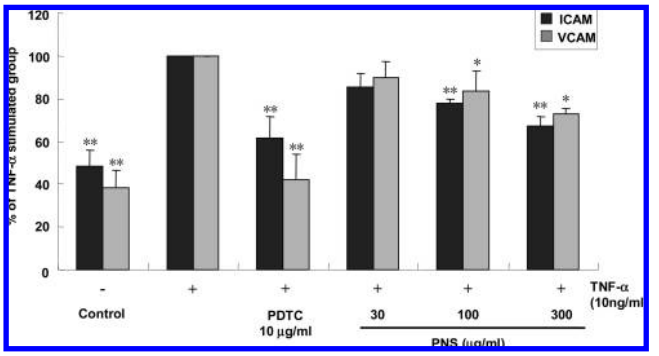

Figure 4. Effects of PNS on TNF-α-induced ICAM-1 and VCAM-1 expressions on the surface of HCAECs determined by cellular ELISA. Data shown are expressed by normalization of the responses of TNF-α group and represent means ± SD of three separate assays ($n = 3$). ∗, $P < 0.05$, and ∗∗, $P < 0.01$, versus TNF-α group.

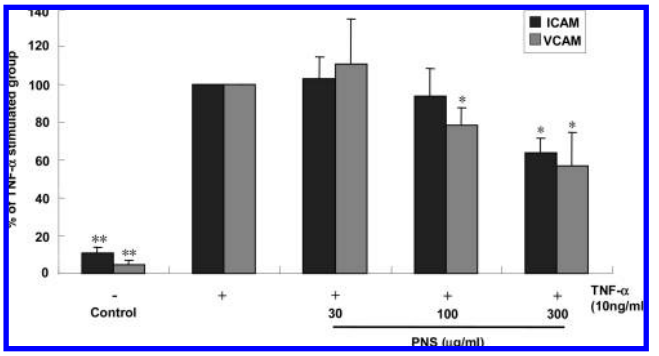

Figure 5. Effect of PNS on mRNA levels of ICAM-1 and VCAM-1 in HCAECs using real-time RT-PCR. Data shown are expressed by normalization of the responses of TNF-α group and represented means ± SD of three separate assays ($n = 3$). *$P < 0.05$, **$P < 0.01$ versus TNF-α group.

(>30%) upon stimulation with TNF-α (**Figure 3**). Pretreatment of endothelial cells with PNS inhibited the TNF-α-induced adhesion of monocytes to the endothelial cells in a concentration-dependent manner, with ~25% inhibition at a concentration of 300 μg/mL. PDTC (10 μg/mL), a NF-κB inhibitor, could greatly inhibit the adhesiveness of THP-1.

PNS Inhibits the Expression of TNF-α-Induced Endothelial Adhesion Molecules. To assess whether the PNS modulate expression of TNF-α-induced adhesion molecules, we examined the effect of PNS on TNF-α-induced surface expression of ICAM-1 and VCAM-1 by immunostaining assay and cellular ELISA. As detected qualitatively by the immunostaining assay of ICAM-1, there was almost no staining in the controls, whereas a strong ICAM-1 staining in TNF-α-treated cells was observed, indicating increased expression of ICAM-1 protein at the single-cell level. PDTC greatly inhibited the expression of ICAM-1 as the positive control. PNS-treated groups showed a tendency to inhibit ICAM-1 expression on the surface of HCAECs (Figure 1S of the Supporting Information). The same results occurred in the immunostaining assay of VCAM-1 expression (data not shown). Cellular ELISA was utilized to quantify expression of ICAM-1 and VCAM-1 on the surface of HCAECs (**Figure 4**). ICAM-1 and VCAM-1 were expressed at low levels on unstimulated HCAECs, and there was a >2-fold increase in their expression after stimulation with TNF-α; this increase was inhibited dose-dependently by PNS.

PNS Reduces the mRNA Levels of Adhesion Molecules. The experiments described above demonstrated that PNS inhibits ICAM-1 and VCAM-1 expression on the surface of HCAECs stimulated with TNF-α. It is possible that PNS inhibited expression of these adhesion molecules by modulating the mRNA levels of ICAM-1 and VCAM-1. To investigate this possibility, the total RNAs were isolated from HCAECs and analyzed by real-time RT-PCR (**Figure 5**). Pretreatment of HCAECs with PNS dose-dependently decreased the TNF-α-induced mRNA production of ICAM-1 and VCAM-1 in HCAECs. The level of inhibition of mRNA appears consistent with the level of inhibition of surface expression.

DISCUSSION

In the present study, we demonstrated that PNS, administered orally, notably decreased the formation of atherosclerotic plaques and the level of serum lipids in the apoE-deficient mice fed a Western-type diet for 8 weeks. In addition, PNS inhibited monocyte adhesion to activated endothelial cell and the expression of TNF-α-induced endothelial adhesion molecules such as ICAM-1 and VCAM-1 on HCAECs.

ApoE is a 34-kDa glycoprotein that circulates in plasma as a component of several lipoproteins, such as HDL, chylomicrometer remnants, intermediate-density lipoprotein (IDL), very-low-density lipoprotein (VLDL), and β-migrating VLDL, except LDL (*20*). Genetic deficiency of apoE in humans and murine has shown accumulation of plasma remnant lipoproteins and development of atherosclerosis. The apoE-deficient mice, generated in 1992 (*21*), are perhaps the most popular model for atherosclerotic studies because of their propensity to spontaneously develop atherosclerotic lesions, similar to those found in humans on a standard diet (*22*). To the best of our knowledge, this study is the first to use this model to examine the

antiatherosclerotic effect of *P. notoginseng*. The result showed that at a dose as low as 4.0 mg/day/mouse of PNS, the main ingredient of *P. notoginseng*, could decrease lesion formation by 61.4%, as evidenced by an en face method, indicating a potent antiatherogenic effect of PNS.

Various cytokines play a significant role in the development of an acute or chronic inflammatory response. Endothelial cells increase expression and secretion of these cytokines, including IL-6 and TNF-α, in response to inflammatory stimulation (*23*). In our study, serum levels of IL-6 and TNF-α in all groups of apoE-deficient mice were below the limits of detection, as noted in previous studies (*24*, *25*). We presumed that an inflammatory reaction occurred in the local artery during atherosclerosis, which could not induce the increase of inflammatory cytokines in serum.

An antivascular inflammatory mechanism is now considered to be a new target for the treatment of atherosclerosis. The focus of our study was on the antivascular inflammation of *P. notoginseng*, which has not been well studied previously. HCAECs are recently commercially available and have become very popular for studies of atherogenesis, because they are more relevant to atherosclerosis and coronary heart disease than any other cell type/origin. To the best of our knowledge, our study is the first to utilize HCAECs to explore the effect of *P. notoginseng* on vascular inflammation and atherosclerosis.

The vascular endothelium serves as an important "gatekeeper", regulating the movement of blood-borne molecules and leukocytes into the tissues. For circulating leukocytes to enter inflamed tissue, the cells must adhere to and pass between the endothelial cells lining the walls of blood vessels (*26*). Endothelial cells increase expression of leukocyte-specific cell-adhesion molecules (CAMs), such as ICAM-1 and VCAM-1, during an inflammatory response (*2*, *3*). Circulating lymphocytes, monocytes, and granulocytes bear receptors that bind to CAMs on the vascular endothelium, enabling these cells to migrate into the tissues. Endothelial-leukocyte adhesion is governed largely by the interaction of complementary adhesion molecules on endothelia and leukocytes. Up-regulation of these adhesion molecules in endothelia is important in the initial stages of inflammatory response in atherosclerosis. The present study clearly demonstrates that PNS has a potent inhibitory effect on TNF-α-induced adhesion of monocytes to HCAECs due to its ability to inhibit TNF-α-induced surface expression and mRNA levels of the cell adhesion molecules ICAM-1 and VCAM-1. These inhibitory mechanisms are related to antiatherosclerotic action of PNS.

Atherosclerosis is increasingly appreciated as a complex and multifaceted disease that can be caused by elevated and modified LDL, free radicals, hypertension, diabetes mellitus, genetic alterations, elevated concentrations of plasma homocysteine, infectious microorganisms, and combinations of these or other factors (*3*), and atherosclerosis is a series of multiple pathological processes (*2*, *3*). Therefore, drugs that can act on multiple targets should be more effective than those having a narrow spectrum of action. It has been reported that *P. notoginseng* and its saponins could regulate lipid metabolism (*5*), inhibit the proliferation of vascular smooth muscle cells (*27*), scavenge free radicals, inhibit platelet aggregation, and improve the blood hypercoagulable state (*28*). Our results confirmed the lipid-lowering property of PNS and also showed that PNS possess antivascular inflammatory activity, which is a new target for the treatment of atherosclerosis. Given the multiple beneficial effects, *P. notoginseng* has the potential to be an effective antiatherogenic agent.

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Supporting Information Available: Representative immunofluorescence staining of ICAM-1 on the surface of HCAECs. This material is available free of charge via the Internet at http://pubs.acs.org.

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