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Niacin Suppresses Progression of Atherosclerosis by Inhibiting Vascular Inflammation and Apoptosis of Vascular Smooth Muscle Cells

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Data Interpretation D
Manuscript Preparation E
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Background: Niacin is a broad-spectrum lipid-regulating drug used for the clinical therapy of atherosclerosis; however, the mechanisms by which niacin ameliorates atherosclerosis are not clear.

Material/Methods: The effect of niacin on atherosclerosis was assessed by detection of atherosclerotic lesion area. Adhesion molecules in arterial endothelial cells were determined by using qRT-PCR and Western blot analysis. The levels of serum inflammatory cytokines in ApoE$^{-/-}$ mice were detected by using ELISA. We detected the expression levels of phosphorylated nuclear factors-κB (NF-κB) p65 in aortic endothelial cells of mice using Western blot analysis. Furthermore, we investigated the anti-inflammation effect and endothelium-protecting function of niacin and their regulatory mechanisms *in vitro*.

Results: Niacin inhibited the progress of atherosclerosis and decreased the levels of serum inflammatory cytokines and adhesion molecules in ApoE$^{-/-}$ mice. Niacin suppressed the activity of NF-κB and apoptosis of vascular smooth muscle cells (VSMCs). Furthermore, niacin induced phosphorylated focal adhesion kinase (FAK) and FAK inhibitor PF-573228 reduced the level of Bcl-2 and elevated the level of cleaved caspase-3 in VSMCs.

Conclusions: Niacin inhibits vascular inflammation and apoptosis of VSMCs via inhibiting the NF-κB signaling and the FAK signaling pathway, respectively, thus protecting ApoE$^{-/-}$ mice against atherosclerosis.

MeSH Keywords: Apoptosis • Atherosclerosis • Inflammation Mediators • Niacin

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Background

Atherosclerosis, the main cause of death in developed and some developing countries, is a systemic inflammatory disease characterized by the formation of atherosclerotic plaques [1]. Hyperlipidemia and obesity may increase the risk of atherosclerosis by triggering chronic inflammation of blood vessels [2]. The cell adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1), and oxLDL are involved in the initiation and development of atherosclerosis, and oxidized low density lipoprotein (oxLDL) can enhances hypoxia-reoxygenation-mediated apoptosis of human coronary artery endothelial cells (HCAECs) [3,4]. During the initiation of atherosclerosis, expression levels of cellular adhesion molecules are up-regulated by pro-inflammatory cytokines [5]. Monocytes adhere to vascular endothelial cells and accelerate the formation of plaque in atherosclerosis [6]. Nuclear factors-κB (NF-κB) has been proved to regulate expression of the inflammatory mediators in atherosclerosis and numerous negative regulators of NF-κB have been identified [7,8]. Caspase-3 is a dominant executioner caspase, which plays a central role in the execution-phase of cell apoptosis. Previous studies have shown that caspase-9 and cleaved caspase-3, but not caspase-8, play a key function in the ox-LDL-induced apoptotic signaling pathway in HCAECs [4]. Elucidating the molecular mechanisms of inflammation in atherosclerosis will contribute to identifying better therapeutic strategies.

Niacin (nicotinic acid, vitamin B3) can lower blood fat and prevent atherosclerosis, which is confirmed in large-scale clinical trials [9,10]. Peroxisome proliferators-activated receptor γ (PPARγ), a transcription factor for several genes, is involved in lipid metabolism. Niacin stimulates the ATP-binding cassette transporter A1 in monocytes and macrophages, up-regulates PPARγ, and ultimately results in reverse cholesterol transport [11]. Kamanna et al. provided direct evidence for anti-inflammatory properties of niacin in human aortic endothelial cells (HAECs) [9]. A study in guinea pig showed that niacin inhibited vascular inflammation by down-regulating the NF-κB signaling pathway [12]. However, how niacin participates in anti-inflammation and protecting endothelium in atherosclerosis remains largely unknown.

High concentrations of pro-inflammatory cytokines increase oxidative stress, down-regulate endothelial nitric oxide synthase (eNOS) bioactivity and induce endothelial cell apoptosis [13]. Vascular smooth muscle cells (VSMCs) are structural components of atherosclerotic plaque caps, and they play an important role in protection of plaque stability [14]. DNA damage has been identified in human VSMCs and alters plaque phenotype by inhibiting fibrous cap areas in advanced lesions [15,16]. Damaged smooth muscle cells will affect vascular tone and ultimately lead to plaque instability. Wang et al. found that VSMC apoptosis was an early trigger for hypothyroid atherosclerosis [17]. Oxidized LDL has been shown to promote VSMC apoptosis in culture media, in part by down-regulation of Bcl-2 and activation of caspase-3 [18]. Interleukin 1β (IL-1β) is a pro-inflammatory cytokine secreted by various cell types, including endothelial cells [19]. IL-1β acts on endothelial cells which line the arterial wall to up-regulate adhesion molecules during the initiation phase of atherosclerosis and drives smooth muscle cell expression and activation of proteases, such as matrix metalloproteinases, leading to plaque rupture and resulting occlusive thrombosis, in the progression and rupture of atherosclerotic plaques [20]. VSMC apoptosis promotes both thrombin generation and vascular calcification [21,22]. In this study, we used ApoE$^{-/-}$ mice with high fat diet to explore the protective mechanisms of niacin in atherosclerosis. In human aortic endothelial cells (HAECs), regulatory effect of niacin on the NF-κB signaling pathway was deeply investigated. In addition, the protective effect of niacin against VSMCs apoptosis was investigated.

Material and Methods

Animals and induction of atherosclerosis

Twelve 8-week-old male ApoE$^{-/-}$ mice with a C57BL/6J background (weighing 20–25 g) were purchased from Beijing Biocytogen (Beijing, China). Mice were kept in a temperature-controlled room with a 12-hour light/dark cycle and fed a high-fat diet composed of 20% fat, 20% sugar, and 1.25% cholesterol (Ssniff special diets, Soest, Germany) with free access to water for 10 weeks. At the same time, six of them were fed with niacin (100 mg/kg) (Sigma-Aldrich, St Louis, MO, USA) by oral gavage once daily for 10 weeks. The use of animals was in strict accordance with Guidance Suggestions for the Care of Laboratory Animals published by the Chinese Ministry of Science and Technology. The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Zhengzhou University. The number and suffering of animals was as minimized as much as possible.

Oil red O staining

The aortas were stored at –20°C for subsequent analysis. The aortic section was soak in 60% isopropanol (Aladdin, shanghai, China) for 25 s and then stained with oil red O (Sigma-Aldrich) for 8 min. Next, redundant dye was removed by soaking the slide in 60% isopropanol for10 s again. To measure the lesion area in the aortic root, sections of the aortic root were soaked in 60% isopropanol for 30 s and then in oil red O for 20 min. Rinsed sections were counterstained with hematoxylin (Sigma-Aldrich). The entire inner surface of aortic intima and sections of aortic roots were photographed, and positive

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lesions were analyzed using ImageJ software (NIH, Bethesda, MD, USA). Data were expressed as average lesion size in percentage of the total aortic surface or as the absolute lesion area in square micrometers.

Cell culture and treatment

HAECs (ATCC) were maintained in endothelial basal medium-2 and supplemented with 2% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA) in a 5% CO_2/95% air incubator at 37°C. The medium was changed every two days until the cells became confluent. To study the Anti-inflammatory effect of niacin on HAECs, HAECs (1×10^5/ml) were incubated with platelet (1.5×10^7/ml) or 25 mM D-glucose in the absence or presence of niacin (1 mM) for 24 h. The expression levels of TNF-α, IL-1β, and IL-6 were measured by using Western blot analysis. HAECs (1×10^5/ml) were incubated with high glucose (25 mM D-glucose) in the absence or presence of niacin (1 mM) for 24 h. The expression level of MCP-1 was determined using Western blot analysis.

Mouse aortic SMCs were isolated and maintained as described previously [23]. To study the effect of the FAK signaling on apoptosis of VSMCs, VSMCs were treated with 50 μM of focal adhesion kinase (FAK) inhibitor PF-573228 for 24 h. The levels of Bcl-2, pro-caspase-3, and cleaved caspase-3 were determined using Western blot analysis.

Preparation of oxLDL

Native LDL was obtained from Sigma. Native LDL (200 μg protein/ml) was oxidized by exposure to CuSO4 (5 μmol/l free Cu^{2+}) in phosphate-buffered saline at 37°C for 20 h. Control incubations were done in the presence of 200 μmol/l EDTA without CuSO4. Oxidation was terminated by refrigeration. Oxidation of LDL was identified using thiobarbituric acid-reactive substances (TBARS) with malonaldehyde bis (dimethyl acetal) as the standard. The levels of malondialdehyde (MDA) were detected by a spectrophotometric measurement of TBARS, depending on kit (Nanjing Jiancheng Bioengineering Institute, China). Protein concentration was measured by bicinchoninic acid (BCA) kit (Pierce Chemical, Rockford, IL, USA).

Western blot analysis

Protein samples in aortic endothelial cells of mice, HAECs, and VSMCs were extracted using RIPA Lysis buffer (Beyotime Institute of Biotechnology, Jiangsu, China). Proteins mixed with loading buffer were separated on SDS-PAGE and then transferred to a PVDF membrane (Millipore Inc., Billerica, MA, USA). Then, non-specific binding was blocked by incubating with 5% nonfat milk in TBST buffer at room temperature for 1 h. Immunodetection of ICAM-1, E-selectin, VCAM-1, p65, p-p65, TNF-α, IL-1β, IL-6, MCP-1, Bcl-2, Pro-caspase-3, cleaved caspase-3, FAK, p-FAK and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was done using monoclonal anti-ICAM-1 antibody (Santa Cruz, CA, USA), anti-E-selectin anti-body (Santa Cruz), anti-VCAM-1 anti-body (Santa Cruz), anti-p65 antibody (Abcam, Cambridge, UK), anti-p-p65 antibody (Abcam), anti-TNF-α antibody (Cell Signaling Technology, Boston, USA), anti-IL-1β antibody (Santa Cruz), anti-IL-6 antibody (Santa Cruz), anti-MCP-1 antibody (Santa Cruz), anti-Bcl-2 antibody (BD, CA, USA), anti-FAK antibody (Sigma), anti-p-FAK antibody (Sigma), anti-cleaved caspase-3 (Santa Cruz), polyclonal anti-caspase-3 (Santa Cruz) and anti-GAPDH (Sigma). After overnight incubation with the primary antibody, blots were incubated with corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies (Invitrogen) for an hour at room temperature. Protein concentration was determined using the BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). The blots were visualized by using the ECL chemiluminescence kit (CWBIO, Beijing, China) and exposed to film.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA were extracted from aortic endothelial cells of mice. 5 mg of the total RNA was reverse-transcribed into cDNA by M-MLV reverse transcriptase (Clontech). The following primers were used: ICAM-1: forward primer: 5'-CAGTGACCATCTACAGCTTTCCGG-3', reverse primer: 5'-GCTGCTACCACA GTGATGACAA-3'; E-selectin: forward primer: 5'-GGCAAATTCAACGGCACAGT-3', reverse primer: 5'-GGGTCTCGCTCCTGGAAGAT-3'; VCAM-1: forward primer: 5'-ACACTCTTACCTGTGCGCTGT-3', reverse primer: 5'-ATTTCCCGGTATCTTCAATGG-3'; GAPDH: forward primer: 5'-CCCATCTATGAGGGTTACGC-3', reverse primer: 5'-TTTAATGTCACGCACGAT TTC-3'. Real-time PCR was performed using a SYBR-green PCR master mix kit (TianGen Biotech, Beijing). The data obtained were calculated by $2^{-\Delta\Delta Ct}$ method and normalized against the housekeeping gene GAPDH. Each experiment was repeated three times.

Flow cytometry

According to the instructions, the apoptosis of VSMCs was detected by Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) double-staining assay (Sigma). VSMCs were treated with nLDL (150 μg/ml), oxLDL (150 μg/ml) or oxLDL (150 μg/ml)+niacin (1mM) for 24 h and then were centrifuged, washed twice with PBS, resuspended in 500 μL binding buffer, and incubated with 5 μL FITC-labeled Annexin V and 5μL PI for 10 min at room temperature in the dark. The scatter parameters of VSMCs were analyzed by FAC Scan flow cytometer (Beckman Coulter, Inc. CA, USA) and Cell Quest analysis software (Becton-Dickinson, CA, USA).

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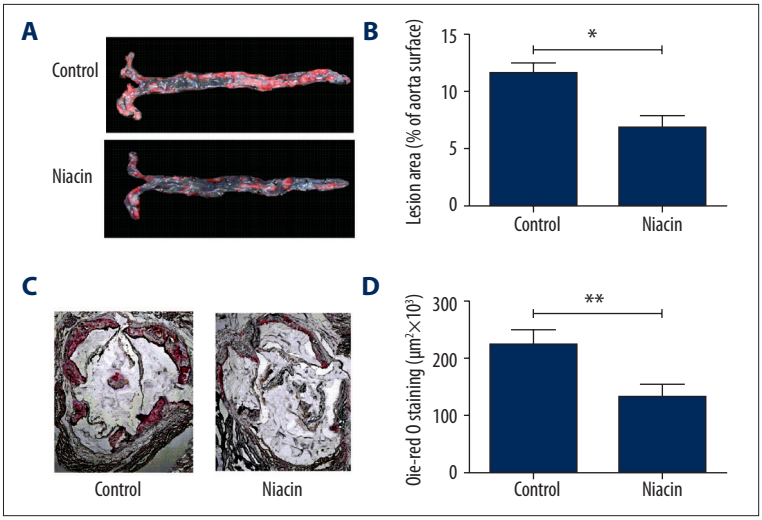

Figure 1. Niacin inhibits the progression of atherosclerosis in ApoE$^{-/-}$ mice. Representative oil red O-stained aortic arches (**A**) and cryosections of aortic roots (**C**) from untreated and niacin-treated ApoE$^{-/-}$ mice. The areas of atherosclerotic plaque were measured by image analysis. Data are presented as average lesion size in percentage of the total surface of the aorta (**B**) or as the absolute lesion area on the aortic root (D). Data are presented as mean ±SD (n=6).* P<0.05, ** P<0.01.

Measurement of inflammatory factors *in vivo*

According to the instructions of ELISA kit (R&D systems, Minneapolis, MN, USA), the serum levels of IL-6, TNF-α, MCP-1 and IL-1β which can be secreted during the process of inflammation were measured following the manufacturer's instructions. Results were calculated as pg/L.

Luciferase reporter assay

HAECs seeded in 24-well plates (2×10^5 per well) were co-transfected with NF-κB promoter-luciferase vector (Promega, Madison, WI, USA) for 14 h. Then, the transfected cells were incubated in presence of 2 ng/ml of TNF-α with or without 1 mM niacin for an additional 12 h. HAECs were washed with PBS and then lysed in a reporter lysis reagent (Promega). The luciferase activity in cell lysate was determined by the dual-luciferase reporter assay system (Promega).

Data analysis

Unpaired Student's *t*-test was used to analyze differences between two groups. ANOVA was used to compare the means of three or more groups. All the data are presented as the means ±SD. A value of P<0.05 was considered to indicate a statistically significant difference. All data analyses were performed using the SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

Results

Niacin suppressed atherosclerosis in ApoE$^{-/-}$ mice

To test whether niacin alleviates atherosclerosis, we measured the plaque area of aortic arches and staining area of aortic roots. Using oil red O staining, we found that aortic arches plaque area (%) (Figure 1A, 1B) and aortic root slice staining area (Figure 1C, 1D) of ApoE$^{-/-}$ mice fed niacin significantly decreased compared with the control group. The data suggest that niacin has an inhibitory effect on the initiation and progression of atherosclerosis in ApoE$^{-/-}$ mice.

Niacin restrained the expression of adhesion molecules in ApoE$^{-/-}$ mice

To explore the inhibition mechanism of niacin on the progression of atherosclerosis in mice, we determined the expression of adhesion molecules in ApoE$^{-/-}$ mice after treatment with niacin by using qRT-PCR and Western blot assay. Significantly decreased mRNA (Figure 2A) expression levels of ICAM-1, E-selectin and VCAM-1 were observed in niacin group compared with control group and the protein (Figure 2B) expression levels were obviously lower than that in control group.

Niacin decreased the levels of inflammatory cytokines *in vivo*

Previous studies have strongly suggested that inflammatory factors are involved in the initiation and progression of coronary atherosclerotic diseases. In this study, the concentrations of four major inflammatory cytokines (IL-1β, IL-6, TNF-α, and MCP-1) were determined by ELISA. As shown in Figure 3A–3D, the serum levels of TNF-α, IL-1β, IL-6 and MCP-1 in ApoE$^{-/-}$ mice treated with niacin decreased significantly versus the control group.

Inflammatory responses in vascular endothelial cells were inhibited by niacin

The activation of the NF-κB signaling pathway is known to result in the production of inflammatory cytokines and chemokines, which is constitutively activated in atherosclerosis [24]. To assess the role played by niacin in the NF-κB signaling, we

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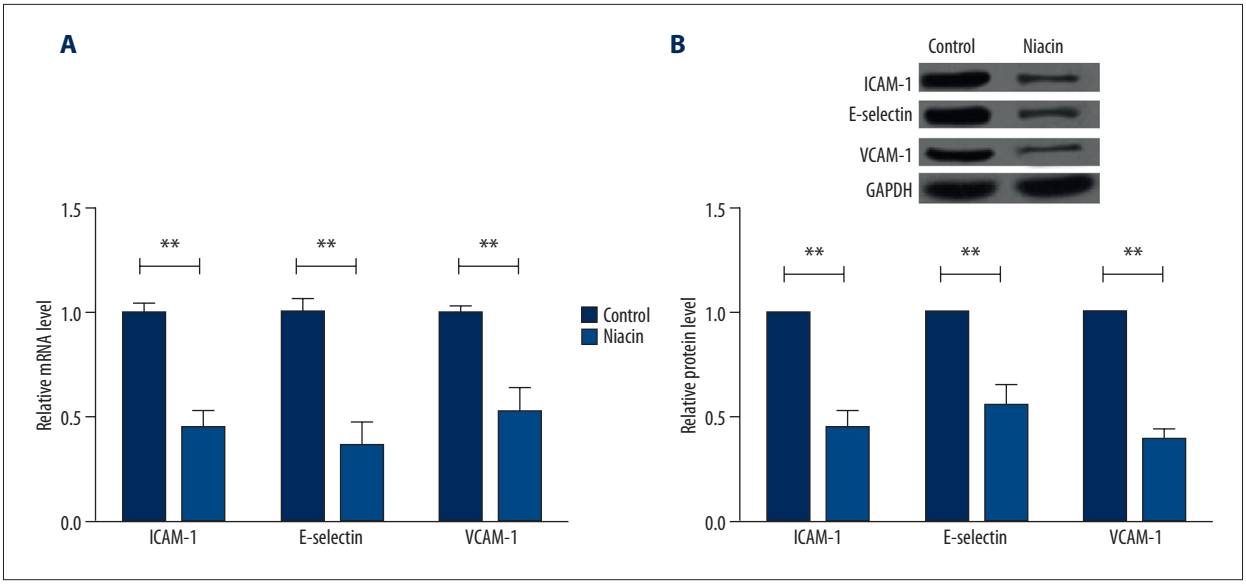

Figure 2. Niacin suppresses expression of adhesion molecules in ApoE$^{-/-}$ mice. qRT-PCR and Western blot analysis were used to analyze the mRNA and protein expression levels of adhesion molecules in different treatment groups of mice, respectively. The mRNA (**A**) and protein (**B**) level of ICAM-1, E-selectin and VCAM-1 in the mice vascular endothelial cells were obviously decreased after feeding niacin. Relative protein levels of ICAM-1, E-selectin and VCAM-1 were quantified using Image-Pro Plus 6.0 software and normalized to GAPDH. Data were expressed as mean ±SD from independent mice (n=6), ** P<0.01.

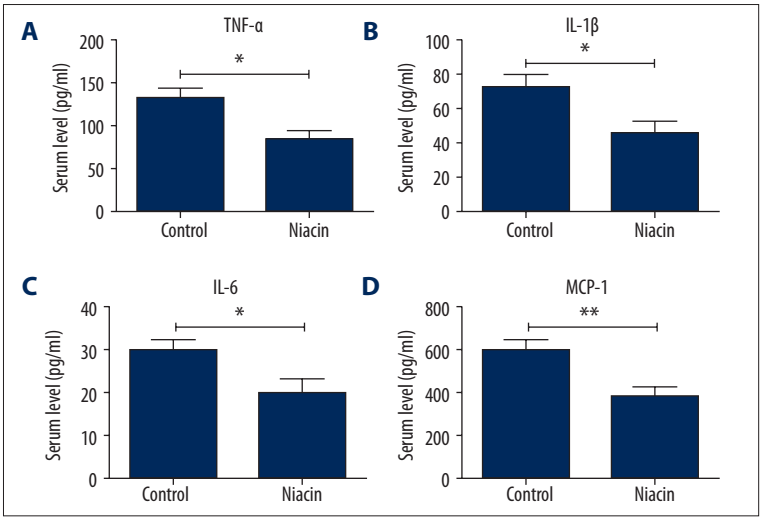

Figure 3. (**A–D**) Niacin decreases the levels of serum inflammatory cytokines in mouse models. The levels of IL-1β, IL-6, TNF-α, and MCP-1 in serum of ApoE$^{-/-}$ mice were determined by ELISA after treatment for 10 weeks. Compared with the control group, the levels of serum inflammatory cytokines were significantly lower in niacin group. Data presented as mean ±SD (n=6). * P<0.05; ** P<0.01.

evaluated the phosphorylation of NF-κB subunit p65 by Western blot analysis. In endothelial cells of ApoE$^{-/-}$ mice treated with niacin, we found that p65 protein phosphorylation was significantly decreased in contrast to the control group. However, there were no changes in p65protein expression (Figure 4A).

To further affirm the anti-inflammation effect of niacin, experiments in HAECs were conducted. In the platelet-stimulated HAECs, we found that the inflammatory cytokines including IL-1β, IL-6 and TNF-α increased; In contrast, HAECs co-cultured with niacin showed dramatically lower levels of inflammatory cytokines (Figure 4B). High glucose accelerates MCP-1 production [25–28]; In the presence of high glucose and niacin, the MCP-1 level of HAECs was lower than high glucose group (Figure 4C). Western blot results demonstrated that niacin significantly decreased p-p65 overexpression induced by platelet or high glucose (Figure 4D, 4E). To further verify the inhibitory effect of niacin on NF-κB, we examined the effect of niacin on the TNF-α-induced NF-κB activation in HAECs by luciferase reporter assay. As shown in Figure 4F, TNF-α-induced NF-κB transcriptional activity in HAECs was significantly inhibited by niacin. In summary, the above results indicated that niacin reduces the degree of inflammation in vascular endothelial cells via inhibiting NF-κB signaling pathway.

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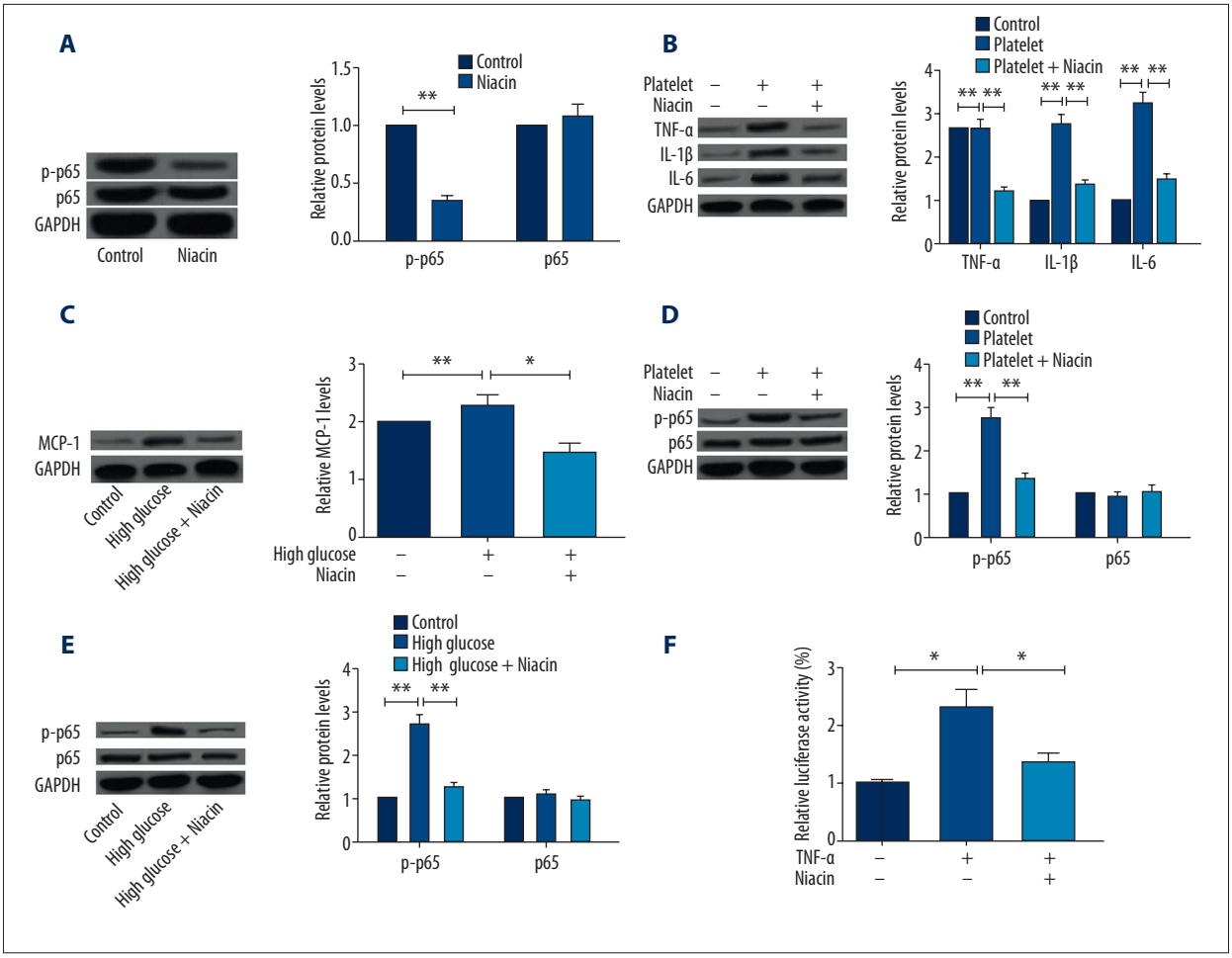

Figure 4. The molecular mechanism of niacin on NF-κB signaling pathway. (**A**) Western blot analysis was performed to detect the protein expression levels in aortic endothelial cells of ApoE$^{-/-}$ mice. Niacin significantly decreased the phosphorylation of p65. (**B, C**) Niacin reduced the expression levels of TNF-α, IL-1β, IL-6 and MCP-1. (**D, E**) Niacin suppressed the level of p-p65. (**F**) Luciferase reporter assay were used to detect the activity of NF-κB in HAECs. The relative luciferase activity was increased after treatment with TNF-α. However, niacin decreased the relative luciferase activity, showing that niacin inhibits the NF-κB signaling pathway. Data presented as mean ±SD (n=4). * P<0.05, ** P<0.01, *** P<0.001.

Niacin attenuated oxLDL-induced apoptosis of vascular smooth muscle cells

We determined the MDA levels in nLDL and oxLDL in order to guarantee the experimental rigor. Compared with that of the nLDL group, the level of MDA was significantly increased in oxLDL group (Figure 5A). When VSMCs were exposed to oxLDL, we observed a significant increase in the number of apoptotic cells and this increase was significantly reduced in the presence of niacin (Figure 5B). The expression level of Bcl-2, pro-caspase-3 and cleaved caspase-3 in each treatment group were detected. The result showed that oxLDL notably decreased the expression of Bcl-2 and elevated the expression level of cleaved caspase-3, but niacin reversed the effects of oxLDL. The expression level of pro-caspase-3 was not affected by nLDL, oxLDL and niacin (Figure 5C). To examined whether the FAK signaling participates in oxLDL-induced apoptosis of VSMCs and whether niacin inhibits apoptosis of VSMCs via blocking the FAK signaling, VSMCs were incubated with nLDL, oxLDL, oxLDL+0.25/0.50/1.00 mM niacin and the expression levels of p-FAK and FAK protein were detected. We observed a significant decrease of p-FAK after treatment of oxLDL compared with the control or nLDL group. Moreover, niacin increased the level of p-FAK in a concentration-dependent manner. However, no effects were observed on FAK expression levels in VSMCs treated under different conditions (Figure 5D). To further confirm the effect of the FAK signaling on apoptosis of VSMCs, we determined the expression of Bcl-2 and caspase-3 in VSMCs after treatment with FAK inhibitor PF-573228. The results exhibited that PF-573228 inhibited the expression of Bcl-2 and enhanced the expression of cleaved caspase-3 (Figure 5E), suggesting that blocking the FAK signaling pathway induces apoptosis of VSMCs. Taken together, these

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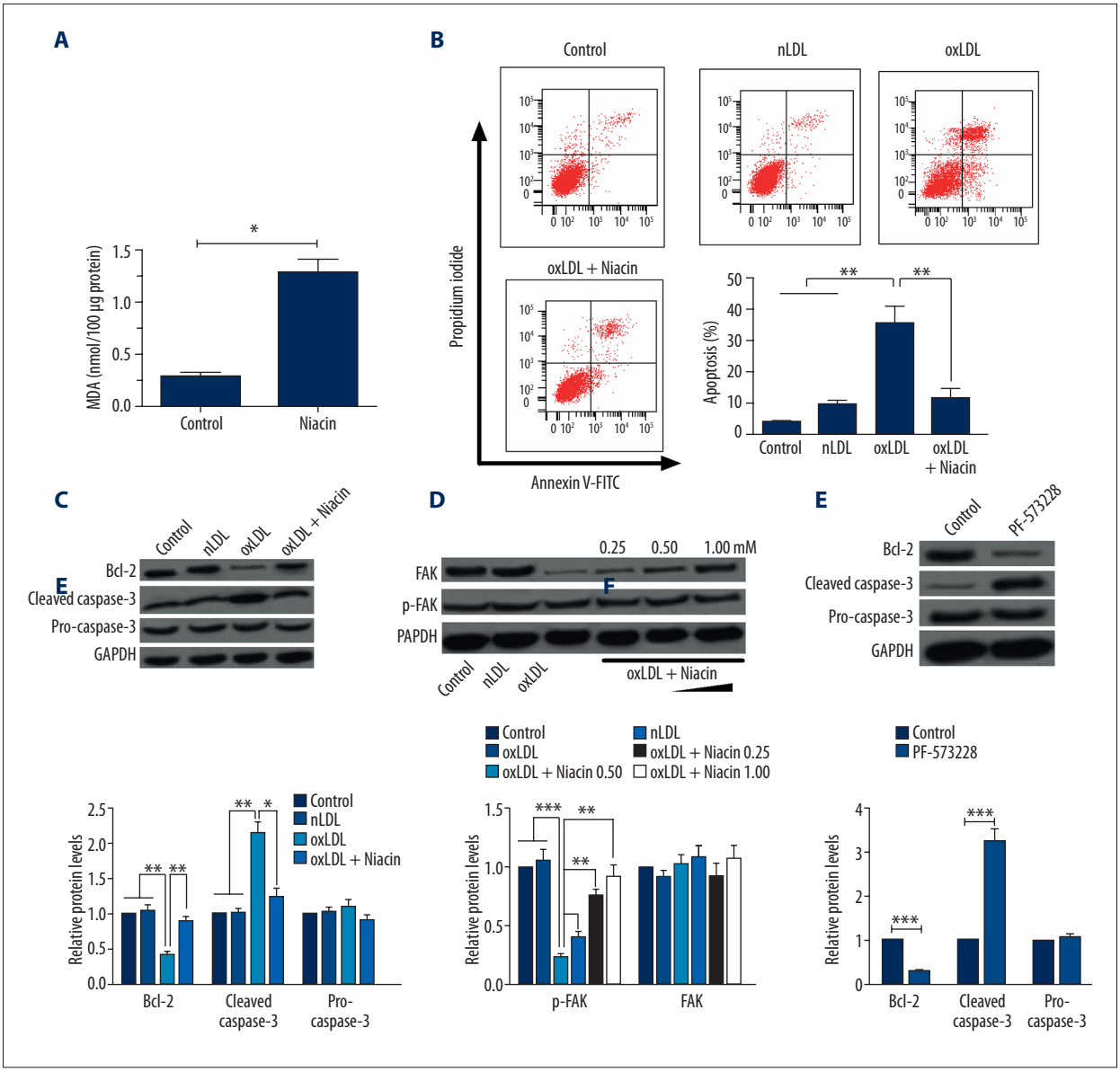

Figure 5. Niacin inhibits oxLDL-induced apoptosis of VSMCs through knocking the FAK signaling pathway. (**A**) MDA level increased in cells exposed to oxLDL. (**B**) The apoptosis rate was increased after treatment with oxLDL, but niacin resisted oxLDL-induced apoptosis of VSMCs. (**B**) Niacin reduced the apoptosis rate of VSMCs induced by oxLDL. (**C**) Niacin promoted the expression of Bcl-2 but repressed the expression of cleaved caspase-3. (**D**) Niacin induced the level of p-FAK in a concentration-dependent manner. (**E**) VSMCs were treated with 50 μM FAK inhibitor PF-573228 for 24 h. Blocking the FAK signaling decreased the expression level of Bcl-2 but increased the expression level of cleaved caspase-3. Data presented as mean ±SD (n=4). * P<0.05, ** P<0.01, *** P<0.001.

data revealed that niacin inhibited the apoptosis of VSMCs via inducing the FAK signaling pathway *in vitro*.

Discussion

Although many factors, such as aging, gender, smoking, diabetes, dyslipidemia, hypertension, physical and psychological stressors, and genetic factors are reported to have connection with atherosclerotic disease, the origination of atherosclerosis remains obscure [29]. A large number of researches have shown that atherosclerosis is a chronic inflammatory disease characterized by the presence of plaques and apoptosis of VSMCs [30]. Niacin is a broad-spectrum lipid drug, which has beneficial effects on plasma lipoproteins and has demonstrated clinical benefits in reducing cardiovascular events and

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atherosclerosis progression [31]. Niacin has been studied in 6 major clinical trials with cardiovascular endpoints and these clinical trials provide strong and consistent evidence that the use of niacin prevents atherosclerotic cardiovascular disease [32]. Numerous research studies have provided evidence that niacin has protective effects on cardiovascular disease through its vascular anti-inflammatory and antioxidant properties in addition to its function on regulating lipid metabolism. The ability of niacin to lower plasma levels of cholesterol was discovered 60 years ago by Rudolf Altschul [33]. The treatment with niacin, alone or in combination with other lipid-lowering agents, significantly reduces total mortality and coronary events and slows down the progression of coronary atherosclerosis [34]. Moreover, niacin attenuates obesity-induced adipose tissue inflammation and reduced pro-inflammatory cytokine expression in high fat diet-fed mice [35]. Besides its effects on lipid regulation, more and more researchers pay attention to the pharmacological potential of niacin. In the present study, the first part of results showed that niacin decreased the plaque area of atherosclerotic mice. In order to further explain the protection mechanism of niacin in the occurrence and development of atherosclerosis, we observed the effects of niacin on the expression of adhesion molecules and inflammatory cytokines in ApoE$^{-/-}$ mice. We provided evidence that niacin inhibited inflammatory responses and apoptosis of VSMCs through regulating the NF-κB and FAK signaling pathway, respectively.

NF-κB is a transcription factor that activates inflammation through regulating the gene expression of cytokines and adhesion molecules [36–38]. NF-κB causes early phenomenon in AS and the disruption of NF-κB activation has been shown to delay or prevent atherogenesis [39]. Oh et al. have shown that lipopolysaccharide-induced pro-inflammatory responses by *Buddleja officinalis* could be inhibited via negative regulation of the NF-κB and ERK1/2 signaling pathway. On the contrary, NF-κB activation promoted inflammation by TNF-α and IL-6 upregulation in serum [40]. So, we speculate that niacin suppresses inflammation via inhibiting the NF-κB signaling pathway. In human aortic endothelial cells *in vitro*, Ganji's team found that niacin inhibited reactive oxygen species (ROS) production, low density lipoprotein (LDL) oxidation, TNF-α-induced NF-κB activation, VCAM-1, MCP-1 secretion, and TNF-α-induced monocyte adhesion to HAECs in varying degrees [41], but it was unknown whether niacin had the same effects or not *in vivo*. Phosphorylation of several serine residues in p65 has been proved to be crucial for full activation of NF-κB [42]. To verify its function *in vivo*, ApoE$^{-/-}$ mice treated with niacin or not were studied. Phosphorylation level of p65 in aortic endothelial cells of mice was detected by immunoblot. We found that, as expected, niacin reduced the degree of inflammation via inhibiting the NF-κB signaling pathway.

A main function of VSMCs is to regulate the caliber of the blood vessels in the body. VSMCs compose the majority of atherosclerotic plaque caps, and they maintain the stability of plaque [14]. Apoptosis of VSMCs and macrophages were found in unstable plaques. To further confirm the protection of niacin on VSMCs, its effect on oxLDL-induced apoptosis of VSMCs was studied. OxLDL plays an important role in the development of atherosclerosis and under condition of high levels of ox-LDL, monocytes are converted to activated macrophages. As a non-receptor tyrosine kinase, FAK plays crucial roles in intracellular regulatory events, such as cell adhesion, proliferation, survival, angiogenesis and migration [43]. Peng et al. highlighted the *in vivo* functions of FAK in vascular endothelial cells [44]. Through complicated molecular mechanisms, FAK influences the cytoskeleton, structures of cell adhesion sites and membrane protrusions to regulate cell movement [45]. Blocking the FAK pathway is a key event in the inhibition of apoptosis induced by oxLDL. Our Western blot results indicated that niacin up-regulated the level of p-FAK in VSMCs. Si et al. found that niacin was able to inhibit vascular inflammation via down-regulating NF-κB signaling pathway in guinea pigs and human umbilical vein endothelial cells (HUVECs). Moreover, niacin attenuated oxLDL-induced apoptosis of HUVECs as well [12]. However, the protective mechanism of niacin against apoptosis of VSMCs remains unclear. In this paper, the regulation of niacin on FAK signaling pathway in apoptosis was studied. The data revealed that niacin induced phosphorylation of FAK in a concentration-dependent manner.

In summary, we verified that niacin alleviates atherosclerosis through restraining the expression of adhesion molecules and inflammatory cytokines secretion in serum. Niacin inhibits overexpression of TNF-α, IL-1β, IL-6, MCP-1 and p-p65 in HAECs treated with platelet or high glucose. The anti-inflammatory property of niacin is realized by down-regulating the NF-κB signaling pathway. Additionally, our data suggest that niacin attenuates oxLDL-induced apoptosis of VSMCs, accompanied by expression changes of Bcl-2, cleaved caspase-3 and p-FAK. However, the limitations of our study should be considered. The sample size of animals, a group of 6 mice only, is small. The molecular mechanisms by which niacin alleviates atherosclerosis should further explore. Together, these results indicated that niacin suppresses progression of atherosclerosis by inhibiting vascular inflammation and apoptosis of vascular smooth muscle cells.

Conclusions

Niacin inhibits vascular inflammation and apoptosis of VSMCs via inhibiting the NF-κB signaling and the FAK signaling pathway, respectively, thus protecting ApoE$^{-/-}$ mice against atherosclerosis.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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