Phoenixin 20 Prevents ox-LDL-Induced Attachment of Monocytes to Human Aortic Endothelial cells (HAECs): A Protective Implication in Atherosclerosis

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Research

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Abstract

Background: The cause of atherosclerosis is not known, and therefore the current treatment options are limited. In the present study, we aimed to investigate the effects of Phoenixin 20 and its receptor G protein-coupled receptor 173 (GPR173) against ox-LDL- induced endothelial dysfunction.

Materials and Methods: Human aortic endothelial cells (HAECs) were treated with 10 μg/ml ox-LDL in the presence or absence of phoenixin 20. Gene expression of GPR173, ICAM-1, VCAM-1, IL-1β, IL-8, MCP-1, and NOX-4 were measured by real time PCR. Protein expression was assayed by western blot analysis. Secretions of pro-inflammatory cytokines were measured by ELISA. The attachment of THP-1 monocytes to HAECs was detected using calcein-AM staining. Transcriptional activity of NF-κB was measured using dual-luciferase reporter assay.

Results: Our findings indicate that ox-LDL significantly lowered the expression of GPR173 in HAECs and triggered an increase in ROS, NOX-4, and proinflammatory cytokine expression. Importantly, we demonstrate that agonism of GPR173 using phoenixin 20 significantly ameliorated these harmful effects of ox-LDL. We also show that agonism of GPR173 can prevent the attachment of monocytes to endothelial cells, which is an important therapeutic approach to prevent atherogenesis.

Conclusion: Here, for the first time to our knowledge, we provide a basis for future research on the role of GPR173 as a new potential treatment against atherosclerosis.

1. Introduction

During the development of atherosclerosis, monocytes attach to the arterial walls and form a plaque buildup. This process, as the main event of atherogenesis, can cause the cessation of arterial blood flow and the formation of blood clots in the later stages. However, little about this process is understood. It is estimated that by 2020 over 50 million people will suffer from cardiovascular diseases such as atherosclerosis. By some estimates, almost all adults already suffer from atherosclerosis to some degree [1]. Thus, it is of great importance to research potential treatments for atherosclerosis and similar diseases. The pleiotropic peptide phoenixin 20 is part of the C4orf52 C-terminus family and is found in a diverse range of species, with few variations. Currently, phoenixin 20 is recognized as having a possible link to a range of beneficial effects, such as reduced anxiety and improved memory, as well as a role in the gut-brain axis [2]. Through its wide-ranging effects and impressive potential to ameliorate various aspects of disease, we identified this protein as a possible treatment for atherosclerosis. In order to test the potential antiatherosclerotic properties of phoenixin 20, we used oxidized low-density lipoprotein (ox-LDL) as a catalyst to simulate atherogenesis in an *in vitro* model using human aortic endothelial cells (HAECs). Ox-LDL has been shown to induce atherogenesis by triggering the release of proinflammatory cytokines and chemokines, inducing oxidative stress, and initiating crosstalk between various cellular signaling pathways via specific receptors [3].

G protein-coupled receptors (GPCRs) have recently emerged as a promising class of specific targets to modulate disease activity. In this study, we demonstrate that G protein-coupled receptor 173 (GPR173), which had previously only been linked to the the activity of the hypothalamus, is expressed in HAECs (Stein et al., 2016). We also found that GPR173 expression was diminished upon exposure to ox-LDL, thereby indicating a potential role of GPR173 in ox-LDL-mediated atherogenesis. To confirm this connection, we employed the specific GPR173 agonist phoenixin 20. We studied the effects of GPR173 agonism on the rate of attachment of monocytes to HAECs induced by ox-LDL and found that phoenixin 20 did, in fact, greatly inhibit this process. A 2016 study suggested that ox-LDL levels are significantly induced by NADPH oxidases [4]. Therefore, we also investigated the role of GPR173 in the production of NADPH oxidase 4 (NOX-4) and found that ox-LDL increased NOX-4 production. These findings together suggest a possible positive feedback loop between the two factors. Taking into consideration the role of increased levels of ROS in the production of proinflammatory cytokines, we investigated the involvement of GPR173 in ox-LDL-induced oxidative stress. Numerous studies have focused on the role of oxidative stress in atherogenesis [5, 6], but there is little research regarding the role of specific GPCRs in specific cell types in terms of oxidative stress. Here, we found that the introduction of phoenixin 20 reduced the production of ROS in HAECs. As oxidative stress upregulates cytokine production, which initiates a cycle of chronic inflammation, the next focus of our study was the effect of phoenixin 20 on the overproduction of ROS, proinflammatory cytokines, and chemokines induced by ox-LDL. We found that IL-1β, IL-8, and MCP-1 levels were all increased upon exposure to ox-LDL, but diminished under the influence of phoenixin 20. Previous studies have confirmed this process and have found high levels of these and other cytokines in atherosclerotic plaque [7], as well as in mouse model experiments [8]. We, therefore, believe that phoenixin 20 may have significant treatment potential against the development of atherosclerosis.

2. Materials And Methods

2.1. *Cell culture, treatment*

HAECs were from ATCC (USA) and maintained with EGM-2 BulletKit (Lonza, Switzerland) supplemented with 5% FBS (Gibco, USA) and an antibiotic mixture. Cells were maintained in a Humidified incubator with 5% CO_2 at 37 °C. Phoenixin 20 was dissolved in dimethyl sulfoxide (DMSO). HAECs were stimulated with 10 μg/ml ox-LDL (Yiyuan Biotechnologies, China) with or without phoenixin 20 (10, 20 nM) for 24 h.

2.2. *Quantitative real-time PCR*

Total RNAs in HAECs were extracted using Qiazol reagent (Qiagen, Germany). About 1 μg of total isolated RNA was used to generate cDNA with a cDNA Reverse Transcription Kit (Bio-Rad, USA) through reverse transcription PCR (RT-PCR). Synthesized cDNA was used for real-time PCR to measure the expression of the target genes using iQTM SYBR Green Supermix (Bio-Rad, USA). The primer sequences are shown in Table 1.

2.3. *Western blot analysis*

After the necessary stimulation, cell lysates from HAECs were prepared with RIPA lysis (Thermo Fisher Scientific, USA) buffer containing protease inhibitor cocktail (Sigma-Aldrich, USA). For determination of nuclear levels of NF-κB p65, nuclear fractions of HAECs were extracted using a Nuclear/Cytosol Fractionation kit (Thermo Fisher Scientific, USA). Then, 20 μg of extracted proteins were loaded onto 10% SDS-PAGE and electronically transferred onto PVDF membranes (Bio-Rad, USA). A blocking buffer containing 5% non-fat milk was used to eliminate non-specific binding sites on the membranes. The membranes were then probed with primary antibodies and HRP-conjugated secondary antibody. Immuno-bands were visualized using an ECL kit (Thermo Fisher Scientific, USA). The following antibodies were used in this study: anti-GPR173 (1:500, #ab136255, Abcam, USA); anti-β-acitn (1:5000, #sc-47778, Santa Cruz Biotechnology, USA); anti-NOX4 (1:1500, #NB110-58849, Novus Biologicals, USA); anti-NF-κB p65 (1:2000, #sc-8008, Santa Cruz Biotechnology, USA); anti-lamin B1 (1:5000, #ab16048, Abcam, USA).

2.4. *Attachment of THP-1 monocytes to endothelial cells*

HAECs were cultured with 10 μg/ml ox-LDL with or without phoenixin 20 (10, 20 nM) for 24 h. THP-1 monocytes were stained with 2 μM calcein-AM (Sigma-Aldrich, USA) for 30 min at 37 °C. Stained THP-1 monocytes at a density of 5×10^6 cells/ml were added to the HAEC culture monolayer and maintained for 2 h. Unattached THP-1 cells were then washed away. Green fluorescence was detected using a fluorescence microscope.

2.5. *ELISA*

After the necessary stimulation, cells were lysed. The cell lysate was used for ELISA analysis to examine the expression of ICAM-1 and VCAM-1. Meanwhile, the cell supernatant was collected for the ELISA analysis of secretions of IL-1β, IL-8, and MCP-1. ELISA kits were obtained from R&D Systems, USA.

2.6. *Dihydroethidium (DHE) staining*

The level of ROS in HAECs was assessed by dihydroethidium (DHE) staining. After the necessary treatment, cells were washed 3 times with PBS and loaded with DHE (Thermo Fisher Scientific, USA) (10 μM) for 30 min in an incubator at 37 °C in darkness. After carefully washing 3 times, red fluoresce was observed under a fluorescence microscope. Five random fields were selected in each well to calculate the fluorescent density of DHE.

2.7. *Dual-luciferase reporter assay*

Cells were co-transfected with 400 ng NF-kB luciferase reporter (Clontech, USA) and 5 ng pRL-TK renilla luciferase (Clontech, USA) using Lipofectamine 2000 (Invitrogen, USA). At 24 h post transfection, cells were stimulated with 10 μg/ml ox-LDL with or without phoenixin 20 (10, 20 nM) for 24 h. Cells were then lysed, and the luciferase activity was measured with a Dual-Luciferase Assay Kit (Promega, USA).

2.8 Immunostaining of GPR173

SH-SY5Y cells and HAECs were washed and fixed with 4% paraformaldehyde for 15 minutes on ice. Cells were then blocked by culturing with 5% goat serum for 1 h at room temperature, and sequentially

incubated with the primary GPR173 antibody overnight and Alaxa-488 secondary antibody (Invitrogen, USA) for 1 h in the darkness. The cells were then counterstained with DAPI. Fluorescent signals were visualized with a fluorescent microscope.

2.9. *Statistical analysis*

Experimental data are shown as the mean ± S.E.M. Statistical analysis was performed with ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1 GPR173 is strongly expressed in HAECs and is reduced by ox-LDL

We theorized that GPR173 might play a regulatory role in HAECs, so we tested for the presence of this receptor in those same cells using SH-SY5Y cells as a positive control. We found that HAECs have moderate expression of GPR173 at both the mRNA (Fig. 1A) and protein levels (Fig. 1B by western blot analysis, Fig. 1C by immunostaining). Next, we went on to explore the effect of ox-LDL on this receptor by testing the levels of GPR173 expression in two regards—in its mRNA expression, via real-time PCR analysis, and in its protein expression, via western blot analysis. We found that when ox-LDL was introduced, GPR173 expression was significantly reduced in both tested aspects. For GPR173 mRNA, we found that GPR173 expression was reduced to 67%, 46%, and 32% by the three doses of ox-LDL, respectively (Fig. 2). At the protein level, GPR173 expression was reduced to 68%, 51%, and 36%, respectively (Fig. 2).

3.2 Phoenixin 20 reduces ox-LDL-induced monocyte attachment to endothelial cells

In order to examine the eligibility of the specific GPR173 agonist phoenixin 20 as a potential treatment for atherosclerosis, we investigated the potential regulatory effect of GPR173 activation on various molecular events. The effects of phoenixin 20 on cell viability are shown in supplementary Fig. 1. First, we determined the effect of GPR173 on the attachment of monocytes to HAECs. The number of attached THP-1 monocytes increased to approximately 4.5-fold when the cells were only introduced to ox-LDL 10 μg/ml (Fig. 3) alone, an increase of approximately 2.5-fold when introduced to ox-LDL 10 μg/ml and 10 nM phoenixin 20 (Fig. 3), and an increase of approximately 2-fold when introduced to ox-LDL 10 μg/ml and phoenixin 20 20 nM (Fig. 3). Therefore, phoenixin 20 was shown to significantly decrease ox-LDL-induced THP-1 monocyte adherence.

To clarify the mechanism behind this beneficial effect of phoenixin 20, we next investigated the involvement of the two major cellular adhesion molecules, ICAM-1 and VCAM-1. We determined that indeed the increase in THP-1 attachment happened alongside an increase in the levels of ICAM-1 and

VCAM-1 triggered by ox-LDL. Expression of ICAM-1 and VCAM-1 was measured at the mRNA level with data collected via real-time PCR analysis, and at the protein level with data collected via ELISA analysis. The mRNA expression of ICAM-1 increased to approximately 8-fold in the presence of ox-LDL, but with the addition of phoenixin 20, this increase was cut in half by a dose of 10 nM (Fig. 4) and, significantly, reduced to only 2-fold by the 20 nM dose (Fig. 4). The mRNA expression of VCAM-1 increased approximately 7.5-fold by the presence of ox-LDL, while with the addition of the two doses of phoenixin 20 suppressed this increase to only 3.5-fold and 2-fold, respectively (Fig. 4). Consistently, western blot analysis revealed that ox-LDL treatment significantly increased the expression of ICAM-1 and VCAM-1, which was blocked by phoenixin 20 in a dose dependent manner (Fig. 4).

3.3 Phoenixin 20 ameliorates ox-LDL-induced expression of proinflammatory cytokines

Overproduction of cytokines and chemokines is linked to endothelial dysfunction and inflammatory issues play a causal role in atherosclerosis. Thus, we found it significant to study the effects of GPR173 agonism on ox-LDL-induced expression of the pro-inflammatory cytokines IL-1β, IL-8, and MCP-1. We used real-time PCR and ELISA analyses to assess the change in expression of these cytokines. The mRNA results were as follows: IL-1β levels increased approximately 5-fold when HAECs were incubated with ox-LDL alone, but the two doses of phoenixin 20 reduced this increase to approximately 3-fold and 1.5-fold, respectively (Fig. 5). IL-8 levels increased by approximately 6-fold when HAECs were incubated with ox-LDL alone, while the introduction of the two doses of phoenixin 20 limited IL-8 expression to only approximately 3-fold and 1.5-fold, respectively (Fig. 5). MCP-1 levels increased approximately 7-fold when HAECs were incubated with ox-LDL alone, with the two doses of phoenixin 20 reducing this increase to approximately 3.5-fold and 2-fold, respectively (Fig. 5). Next, the protein level determined by ELISA analysis were as follows: IL-1β levels increased by approximately 4-fold when HAECs were incubated with ox-LDL alone, with the two doses of phoenixin 20 reducing these numbers to approximately 2-fold and one 1.5-fold (Fig. 5), respectively. Levels of IL-8 increased approximately 4.5-fold when HAECs were incubated with ox-LDL alone and were reduced to approximately 2.5-fold and 2-fold by the two doses of phoenixin 20, respectively (Fig. 5). Finally, MCP-1 levels increased approximately 6-fold when HAECs were incubated with ox-LDL alone, while the two doses of phoenixin 20 reduced the increase to only approximately 3-fold and 2-fold, respectively (Fig. 5).

3.4 Phoenixin 20 ameliorates ox-LDL-induced oxidative stress

Although the production of ROS plays a role in normal cell function and proliferation, excessive ROS production can shatter the oxidant/antioxidant balance, thereby leading to endothelial dysfunction and oxidative stress. We decided to study the effects of phoenixin 20 on ox-LDL-induced production of ROS to determine whether agonism of GPR173 can alleviate oxidative stress. DHE staining was used to determine the levels of ROS. When HAECs were incubated with ox-LDL alone, the level of ROS increased

approximately 4-fold, while the two doses of phoenixin 20 reduced this increase in ROS to only approximately 2-fold and 1.5-fold, respectively (Fig. 6).

NOX-4 plays a significant role in oxidative stress and the pathogenesis of atherosclerosis. We tested the effects of GPR173 agonism on the expression of NOX-4. At the mRNA level, NOX-4 expression increased approximately 4-fold in the presence of ox-LDL alone, while with the addition of the two doses of phoenixin 20, this increase was reduced to only approximately 2.5-fold and 1.5-fold, respectively (Fig. 7). At the protein level, NOX-4 expression increased approximately 3.5-fold when incubated with ox-LDL alone, while the two doses of phoenixin 20 reduced this increase to only approximately 2-fold and 1.5-fold, respectively (Fig. 7).

3.5 The effects of phoenixin 20 are mediated through the NF-κB signaling pathway

The increase of activity of NF-κB is known to cause a cascade of deleterious effects including overproduction of proinflammatory cytokines. Thus, we assessed the effects of GPR173 agonism by phoenixin 20 on ox-LDL-induced nuclear translocation of NF-κB p65 and luciferase activity of NF-κB. When incubated with ox-LDL alone, nuclear levels of NF-κB p65 increased approximately 4-fold, while the addition of the two doses of phoenixin 20 reduced this increase to only approximately 2-fold and 1.5-fold, respectively (Fig. 8). When incubated with ox-LDL alone, the luciferase activity of NF-κB increased approximately 87-fold, which was reduced to only approximately 37-fold and 15-fold by the two doses of phoenixin 20, respectively (Fig. 8).

4. Discussion

Although the treatment of atherosclerosis remains challenging due to the seeming ambiguity of its causes, new studies on various treatment potentials contribute to a growing understanding of what aspects induce the development of this disease. Many treatment potentials for atherosclerosis are currently being researched with varying results. A recent study used lixisenatide on the GLP-1 receptor as a treatment for atherosclerosis and type 2 diabetes and found that this activation aided in reducing proinflammatory cell absorption and neutralizing plaque buildup [9]. We established in our research that GPR173 plays a role in the effects of ox-LDL on HAECs, which is a new finding as in the past it was linked to the reproductive effect of phoenixin [10]. As we found GPR173 to be expressed in HAECs and downregulated in response to ox-LDL, we theorized that it might play a regulatory role in atherogenesis, making it a significant player as a potential treatment for atherosclerosis. The specific GPR173 agonist phoenixin 20 was found to play a significant role in reducing THP-1 monocyte attachment to HAECs, which we demonstrate to likely be due to the reduced expression of the key adhesion molecules VCAM-1 and ICAM-1. These adhesion molecules have been found in previous research to be a major cause of atherogenesis, and inhibiting their expression has been shown to greatly reduce the formation of aortic plaque buildup [11, 12].

Monocyte attachment has been presented by previous researchers as playing a significant role in atherosclerosis and has been proposed as a primary treatment target [13]. In light of our results showing that phoenixin 20 significantly reduced the rate of attachment of monocytes to endothelial cells, we believe that GPR173 has great promise as a therapeutic candidate in this regard. Proinflammatory cytokines are another significant causative factor in the progression of many diseases, including atherosclerosis. In the present study, we focused on the effect of GPR173 agonism on the expression of IL-8, IL-1β, and MCP-1 induced by ox-LDL. IL-8 has been found to be an important predictor of mortality rates in another related disease, acute coronary syndrome [14]. Furthermore, MCP-1 has been previously identified as playing a significant role in atherosclerosis by recruiting immune cells to invade the arterial wall, while the presence of IL-1β has been identified to significantly promote atherogenesis by triggering the inflammatory response as well as acting as a signaling molecule [15, 16]. In our research, we found that the addition of phoenixin 20 significantly alleviated the increase in the levels of IL-8, IL-1β, and MCP-1 induced by ox-LDL. Consistently, a recent study demonstrates that the activation of GPR173 by phoenixin-20 exerts a robust anti-inflammatory effect in dental pulp cells by reducing the expression of proinflammatory cytokines, cell adhesion molecules, MMP-2, and MMP-9 [17]. NOX-4, which is present in mitochondria and localizes to membranes, has been shown to be a major source of ROS in endothelial cells. NOX-4 plays a direct role in modulating the production of mitochondrial ROS within the mitochondrial membrane by affecting the respiratory chain or influencing other ROS-producing enzymes. Additionally, NOX-4 could regulate cytosolic ROS, thereby leading to increased mitochondrial ROS production by opening the mitochondrial permeability transition pore. In the current study, we found that phoenixin 20 could reduce the expression of NOX-4 and intracellular levels of ROS, suggesting that mitochondria might play an important role in mediating the protective effects of phoenixin 20 in HAECs.

The activation of GPR173 by its natural ligand Phoenixin-20 exhibits robust anti-inflammatory effects in dental pulp cells Oxidative stress is another aspect of atherosclerosis which has been previously noted for its integral role in promoting endothelial cell dysfunction. We found that ox-LDL-induced production of ROS was significantly reduced in the presence of phoenixin 20, and previous studies have also come to similar conclusions [18, 19]. Similarly, NOX-4 levels are recognized to contribute to the complications of atherosclerosis and a reduction in its expression has been noted as a potential treatment [20, 21]. Due to the role of NF-κB role as a critical inflammatory signaling complex that drives the creation of inflammatory cytokines, among other functions, it is of great value to understand the effect of GPR173 agonism by phoenixin 20 on its action. We found that ox-LDL-induced overexpression of NF-κB was reduced under the influence of phoenixin 20. While ox-LDL has been shown to activate NF-κB, research has shown that may occur through the ERK-RSK pathway [22]. It has also been widely explored as a potential treatment target against atherosclerosis [23, 24]. In the present study, we demonstrated the treatment potential of the specific GPR173 agonist phoenixin 20 by investigating its role in mediating the expression of various molecules involved in ox-LDL-induced atherogenesis. Our findings show that phoenixin 20 has the ability to regulate several important factors which are proven or thought to contribute to atherosclerosis, such as overproduction of proinflammatory cytokines, increased production

of ROS, and activation of NF-κB in HAECs. We also found that perhaps most importantly of all, phoenixin 20 significantly ameliorated monocyte attachment to artery walls.

A variety of risk factors have been associated with the pathogenesis of atherosclerosis, including genetics, ageing, and obesity [25]. In addition to ox-LDL, several other toxins, such as pro-inflammatory cytokines and reactive oxygen species (ROS), are reported to play a critical role in the pathophysiology of atherosclerosis [26]. The main limitation of the current study is that we only investigated the beneficial effects of phoenixin 20 in an ox-LDL-stimulated endothelial cell culture model. It should be noted that the pathological mechanism of atherosclerosis is complicated and needs to be elucidated. Therefore, further research with animal models or possible clinical trials will help to verify the atheroprotective effects of phoenixin 20 and the physiological function of GPR173 in cardiovascular diseases.

Conclusion

Our results suggest that the activation of GPR173 using its natural ligand Phoenixin-20 might have a beneficial effect against ox-LDL- induced endothelial dysfunction. We provide a basis for future research on the role of GPR173 as a new potential therapeutic target against atherosclerosis.

Abbreviations

human aortic endothelial cells (HAECs); G protein-coupled receptor 173 (GPR173); oxidized low-density lipoprotein (ox-LDL); NADPH oxidase 4 (NOX-4); dimethyl sulfoxide (DMSO); dihydroethidium (DHE); polyvinylidene Fluoride (PVDF); Radioimmunoprecipitation assay (RIPA); horseradish peroxidase (HRP); standard error of mean(S.E.M.); 4',6-Diamidino-2-Phenylindole, dihydrochloride (DAPI); reactive oxygen species (ROS); intercellular cell adhesion molecule-1 (ICAM-1); vascular cell adhesion molecule-1 (VCAM-1); interleukin-1β (IL-1β); interleukin-8 (IL-8); monocyte chemotactic protein 1 (MCP-1); enzyme linked immunosorbent assay (ELISA).

Declarations

Conflict interest statement: All authors declared they have no conflict interest need to be disclosed.

Ethics statement

This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of Fujian Medical University (FJMU-E-20170023).

Consent for publication: Not applicable

Availability of data and material: Experimental data are available from the corresponding author by request.

Author contribution statement:

Hua-Song Lin: Conceptualization, Methodology, Software, Investigation;

Xiao-Lan Wei: Conceptualization, Methodology, Software, Investigation;

Bi-Rong Zheng: Methodology, Software, Visualization; resources

Ruo-Ting Lin: Methodology, Software, Visualization;

Ya-li Huang: Investigation, Software; Resources

Mi-Mi Li: Investigation, Software;

Ya-Fang Chen: Investigation, Resources;

Jin-Ying Zhang: Resource, Software;

Wei-wei Li: Supervision, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing.

Li-Chao Ye: Supervision, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing.

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References

1. Aronow WS, Fleg JL, Rich MW, editors. Tresch and Aronow's Cardiovascular Disease in the Elderly. CRC Press. 2013 Sep 4.
2. Schalla M, Stengel A. Phoenixin—A Pleiotropic Gut-Brain Peptide. Int J Mol Sci. 2018;19(6):1726.
3. Preising MN, Forster H, Gonser M, Lorenz B. Screening of TYR, OCA2, GPR143, and MC1R in patients with congenital nystagmus, macular hypoplasia, and fundus hypopigmentation indicating albinism. Molecular vision. 2011;17:939.
4. Cross S. Identification of oxLDL-induced oxidative stress sources in cardiovascular disease. 2016, http://hdl.handle.net/10092/12505.
5. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. Histology histopathology. 2008;23(3):381–90.
6. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. The American journal of cardiology. 2003;91(3):7–11.
7. Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis. 1996;127(2):263–71.
8. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiological reviews. 2006;86(2):515–81.

9. Vinué Á, Navarro J, Herrero-Cervera A, García-Cubas M, Andrés-Blasco I, Martínez-Hervás S, Real JT, Ascaso JF, González-Navarro H. The GLP-1 analogue lixisenatide decreases atherosclerosis in insulin-resistant mice by modulating macrophage phenotype. Diabetologia. 2017;60(9):1801–12.
10. Stein LM, Tullock CW, Mathews SK, Garcia-Galiano D, Elias CF, Samson WK, Yosten GL. Hypothalamic action of phoenixin to control reproductive hormone secretion in females: importance of the orphan G protein-coupled receptor Gpr173. Am J Physiol Regul Integr Comp Physiol. 2016;311(3):R489-96.
11. Vogel ME, Idelman G, Konaniah ES, Zucker SD. Bilirubin prevents atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice by inhibiting endothelial VCAM-1 and ICAM-1 signaling. Journal of the American Heart Association. 2017;6(4):e004820.
12. Vogel ME, Idelman G, Zucker SD. Bilirubin Prevents Atherosclerotic Plaque Formation in LDLR-/-Mice by Inhibiting Monocyte Migration Through the Disruption of Endothelial Vascular Cell Adhesion Molecule 1 (VCAM-1) and Intercellular Adhesion Molecule 1 (ICAM-1) Signaling. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016;36(suppl_1):A661-.
13. Hilgendorf I, Swirski FK, Robbins CS. Monocyte fate in atherosclerosis. Arterioscler Thromb Vasc Biol. 2015;35(2):272–9.
14. Cavusoglu E, Marmur JD, Yanamadala S, Chopra V, Hegde S, Nazli A, Singh KP, Zhang M, Eng C. Elevated baseline plasma IL-8 levels are an independent predictor of long-term all-cause mortality in patients with acute coronary syndrome. Atherosclerosis. 2015;242(2):589–94.
15. Husain K, Hernandez W, Ansari RA, Ferder L. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. World journal of biological chemistry. 2015;6(3):209.
16. Lombardi M, Mantione ME, Baccellieri D, Ferrara D, Castellano R, Chiesa R, Alfieri O, Foglieni C. P2 × 7 receptor antagonism modulates IL-1β and MMP9 in human atherosclerotic vessels. Scientific reports. 2017;7(1):4872.
17. Sun G, Ren Q, Bai L, Zhang L. Phoenixin-20 suppresses lipopolysaccharide-induced inflammation in dental pulp cells. Chem Biol Interact. 2020;318:108971.
18. Yang X, Li Y, Li Y, Ren X, Zhang X, Hu D, Gao Y, Xing Y, Shang H. Oxidative stress-mediated atherosclerosis: mechanisms and therapies. Frontiers in physiology. 2017;8:600.
19. Kattoor AJ, Pothineni NV, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. Curr Atheroscler Rep. 2017;19(11):42.
20. Vendrov AE, Lozhkin A, Madamanchi NR, Runge MS. Nox4 Deletion Attenuates Age-Associated Vascular Inflammation and Atherosclerosis Burden in Hyperlipidemic Mice by Modulating Macrophage Phenotype. Circulation. 2018;138(Suppl_1):A17163.
21. Koulis C, Watson AM, Gray SP, Jandeleit-Dahm KA. Linking RAGE and Nox in diabetic micro-and macrovascular complications. Diabetes Metab. 2015;41(4):272–81.
22. Yurdagul A, Sulzmaier FJ, Chen XL, Pattillo CB, Schlaepfer DD, Orr AW. Oxidized LDL induces FAK-dependent RSK signaling to drive NF-κB activation and VCAM-1 expression. J Cell Sci. 2016;129(8):1580–91.

23. Gargiulo S, Gamba P, Testa G, Rossin D, Biasi F, Poli G, Leonarduzzi G. Relation between TLR4/NF-κB signaling pathway activation by 27-hydroxycholesterol and 4-hydroxynonenal, and atherosclerotic plaque instability. Aging cell. 2015;14(4):569–81.
24. Bhaskar S, Sudhakaran PR, Helen A. Quercetin attenuates atherosclerotic inflammation and adhesion molecule expression by modulating TLR-NF-κB signaling pathway. Cellular immunology. 2016;310:131–40.
25. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med. 2014;5(8):927–46.
26. Zmysłowski A, Szterk A. Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols. Lipids Health Dis. 2017;16(1):188.

Figures

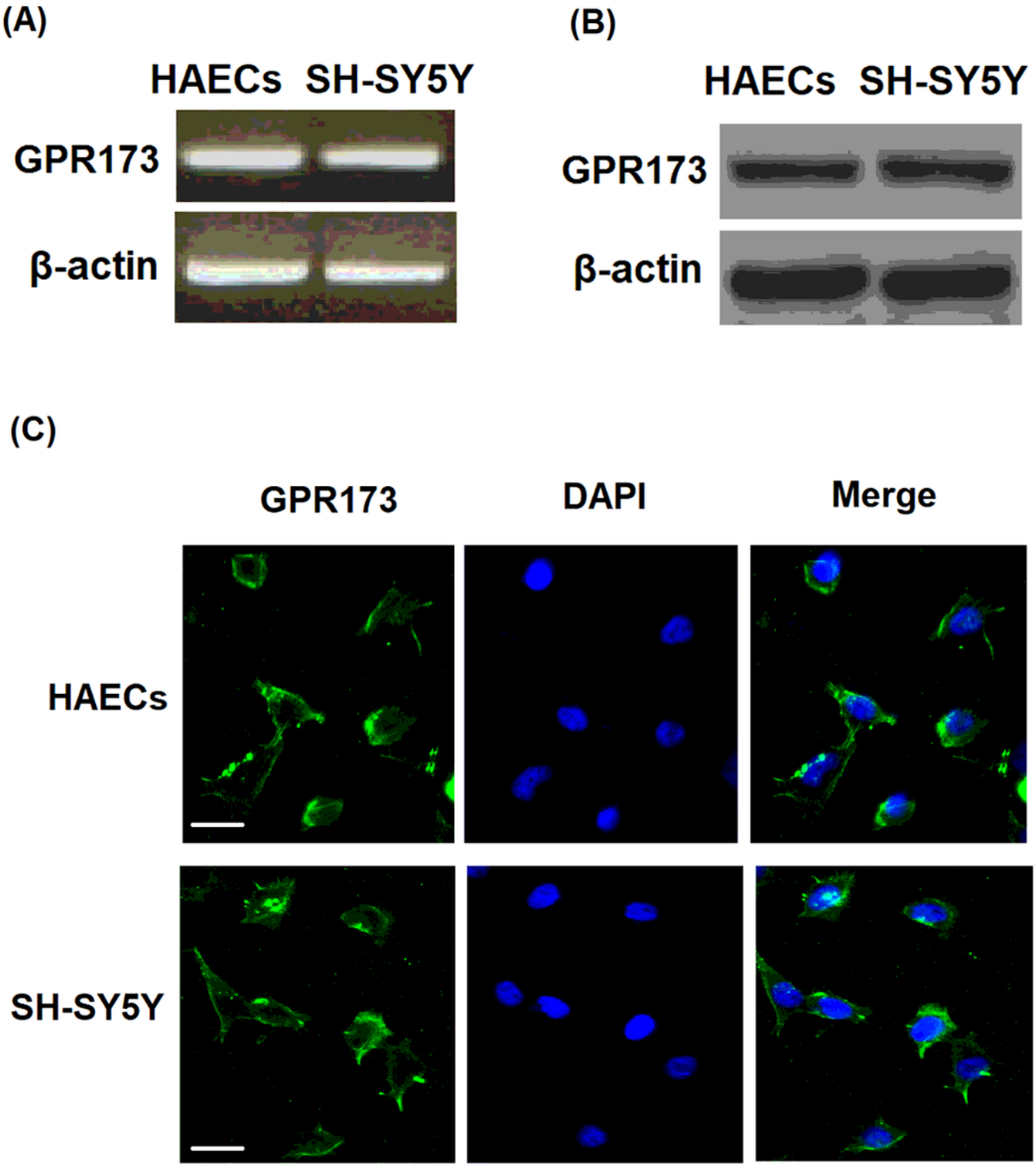

Figure 1

GPR173 is expressed in HAECs. Human SH-SY5Y cells were used as a positive control. (A). Reverse transcription PCR (RT-PCR) analysis of GPR173; (B). Western blot analysis of GPR173; (C). Immunostaining of GPR173; Scale bar, 50 μm.

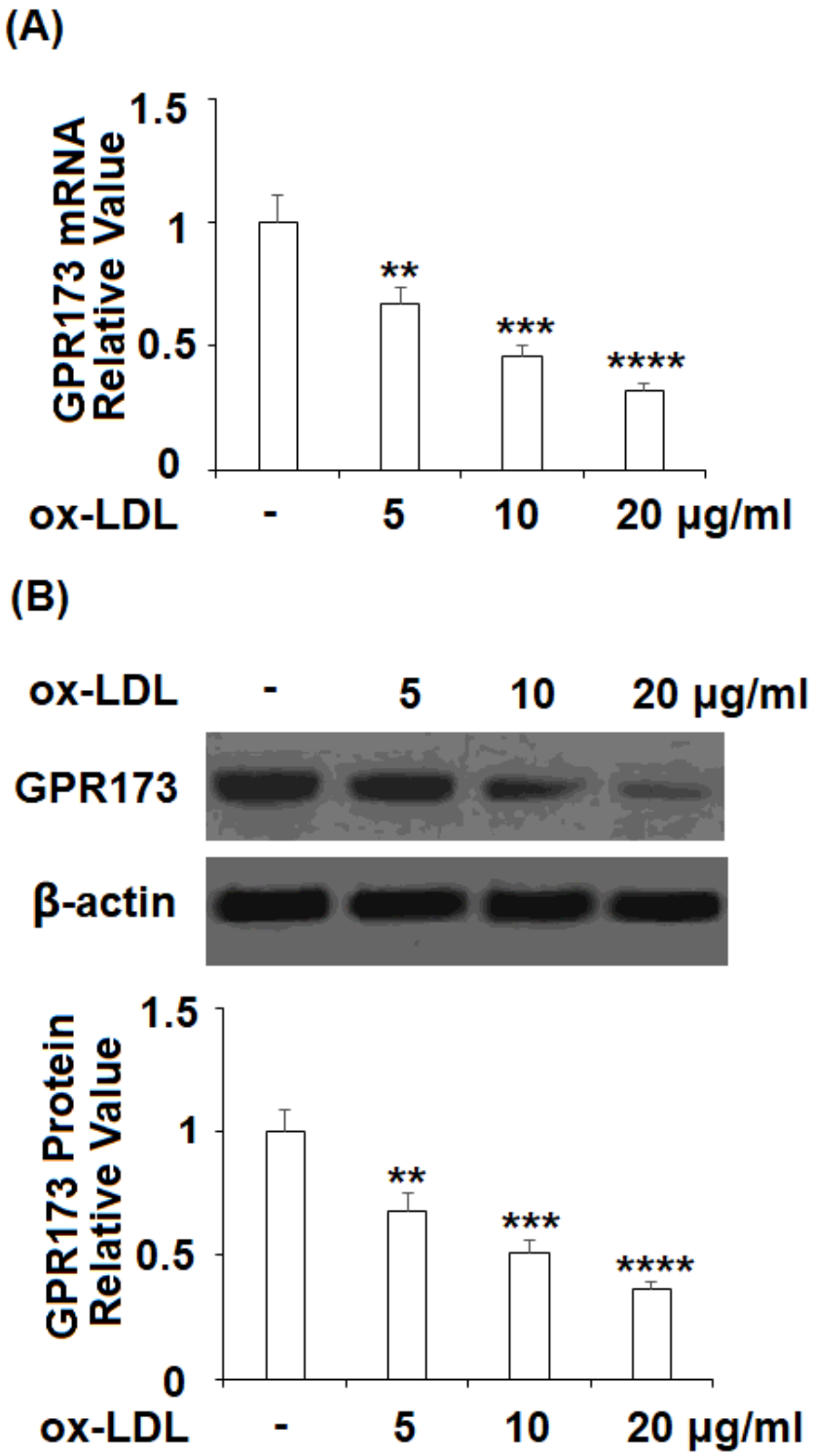

Figure 2

Ox-LDL reduced the expression of GPR173 in HAECs. Cells were treated with ox-LDL at the concentrations of 5, 10, 20 μg/ml. (A). mRNA expression of GPR173; (B). Protein levels of GPR173. Control, DMSO group (**, ***, ****, P<0.01, 0.001, 0.0001 vs control group).

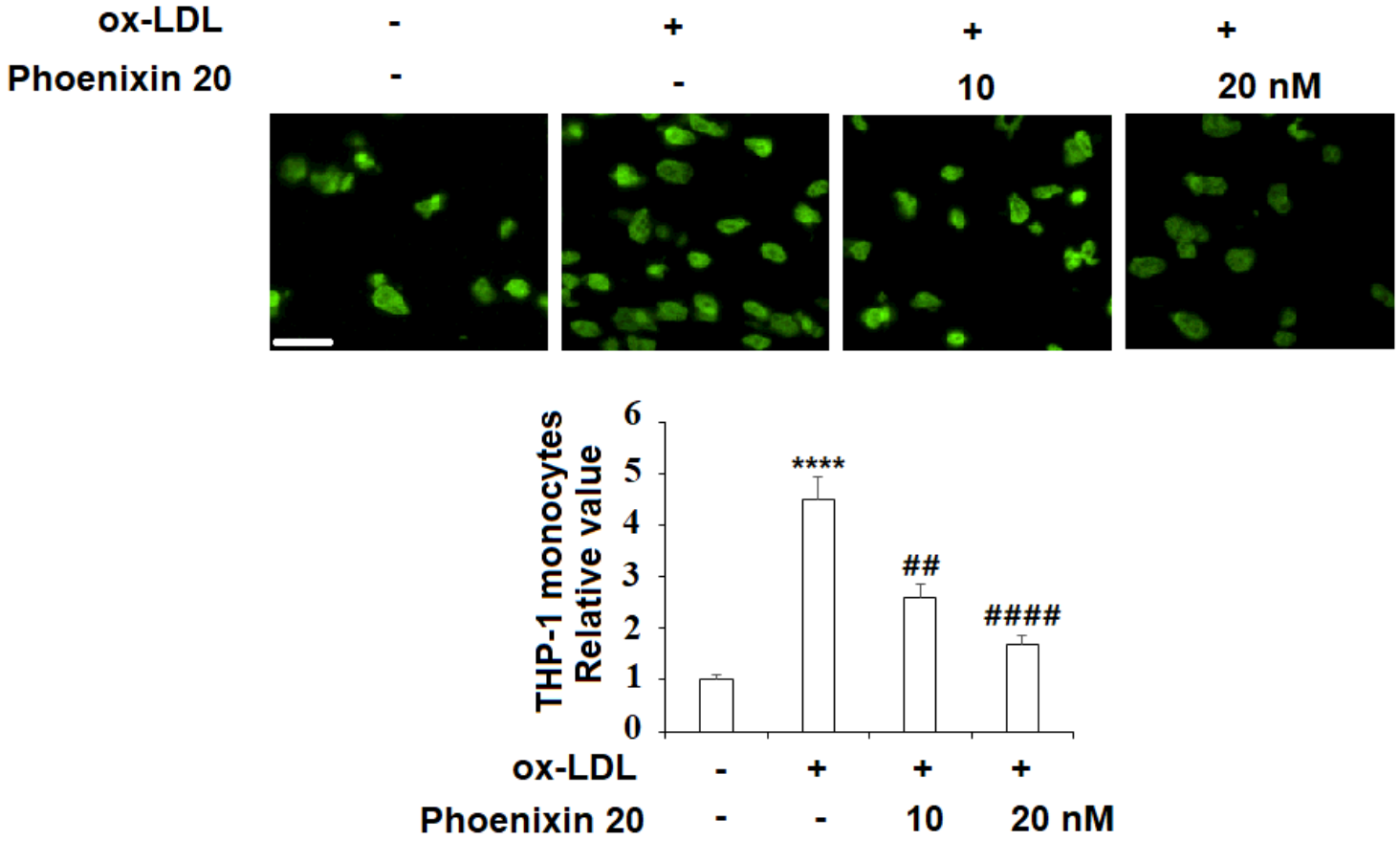

Figure 3

Activation of GPR173 with Phoenixin 20 attenuated ox-LDL-induced attachment of THP-1 monocytes to HAECs. HAECs were stimulated with 10 μg/ml ox-LDL with or without Phoenixin 20 (10, 20 nM) for 24 h. The attachment of THP-1 monocytes to HAECs was determined. Scale bar, 100 μm (****, P<0.0001 vs. vehicle group; ##, ####, P<0.01, 0.0001 vs. ox-LDL treatment group).

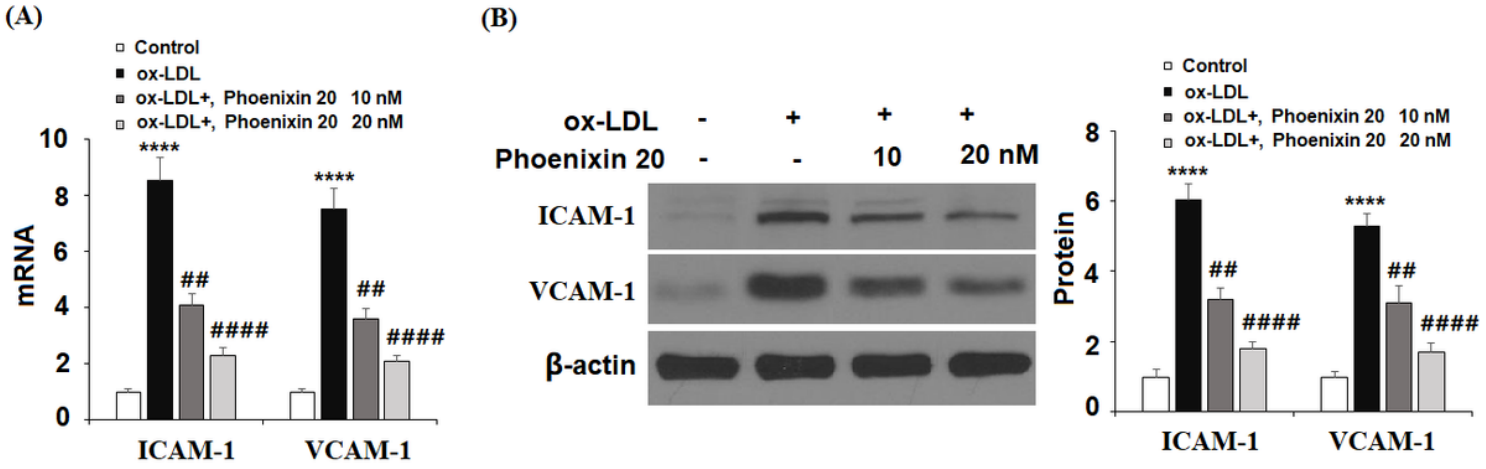

Figure 4

Phoenixin 20 reduced ox-LDL- induced expression of ICAM-1 and VCAM-1 in HAECs. HAECs were stimulated with 10 μg/ml ox-LDL with or without Phoenixin 20 (10, 20 nM) for 24 h. (A). Real time PCR

analysis of ICAM-1 and VCAM-1; (B). Western blot analysis of ICAM-1 and VCAM-1 (****, P<0.0001 vs. vehicle group; ##, ####, P<0.01, 0.0001 vs. ox-LDL treatment group).

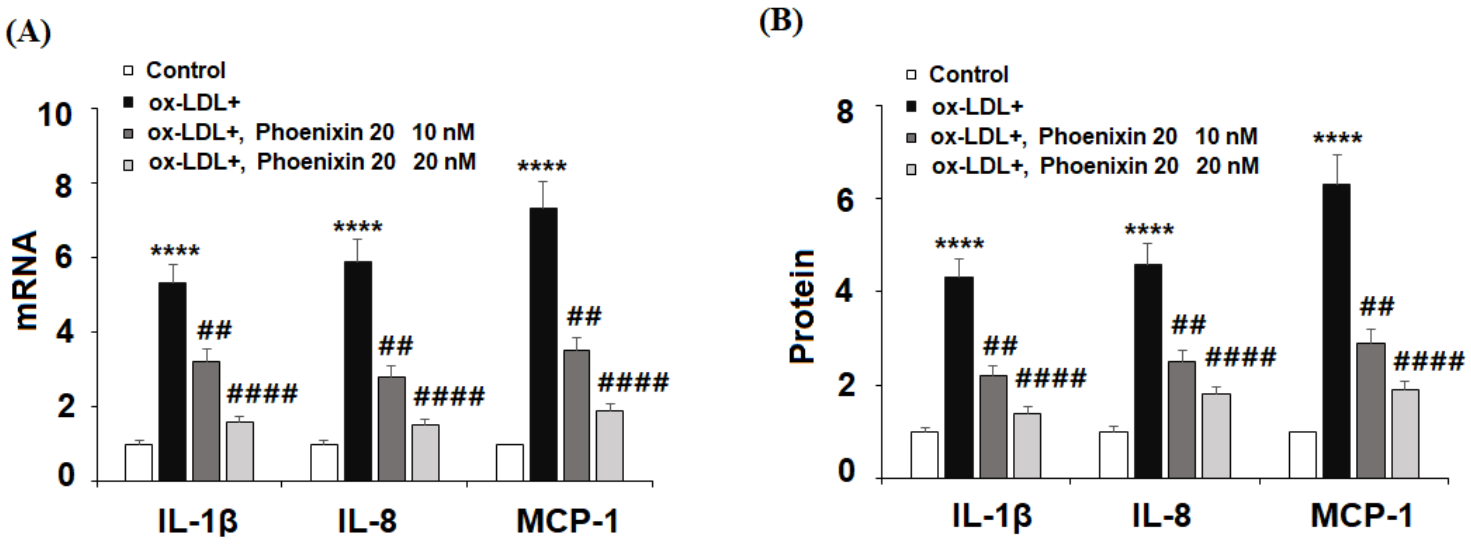

Figure 5

Phoenixin 20 suppressed ox-LDL- induced expression and secretions of IL-1β, IL-8, MCP-1 in HAECs. HAECs were stimulated with 10 μg/ml ox-LDL with or without Phoenixin 20 (10, 20 nM) for 24 h. (A). Real time PCR analysis of IL-1β, IL-8, and MCP-1; (B). Elisa analysis of IL-1β, IL-8, and MCP-1 (****, P<0.0001 vs. vehicle group; ##, ####, P<0.01, 0.0001 vs. ox-LDL treatment group).

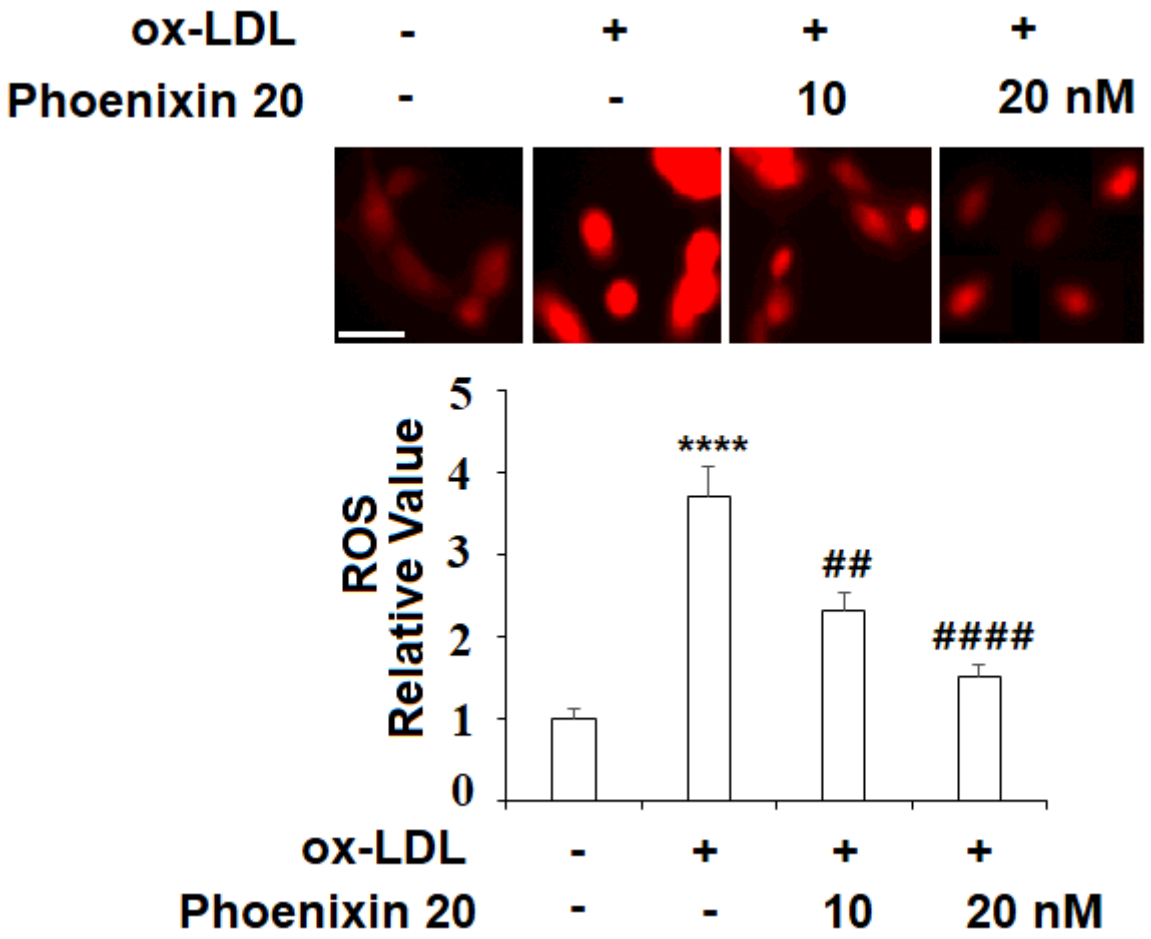

Figure 6

Phoenixin 20 suppressed ox-LDL- induced generation of intracellular ROS in HAECs. HAECs were stimulated with 10 μg/ml ox-LDL with or without Phoenixin 20 (10, 20 nM) for 24 h. ROS was determined by Dihydroethidium (DHE) staining. Scale bar, 100 μm (****, P<0.0001 vs. vehicle group; ##, ####, P<0.01, 0.0001 vs. ox-LDL treatment group).

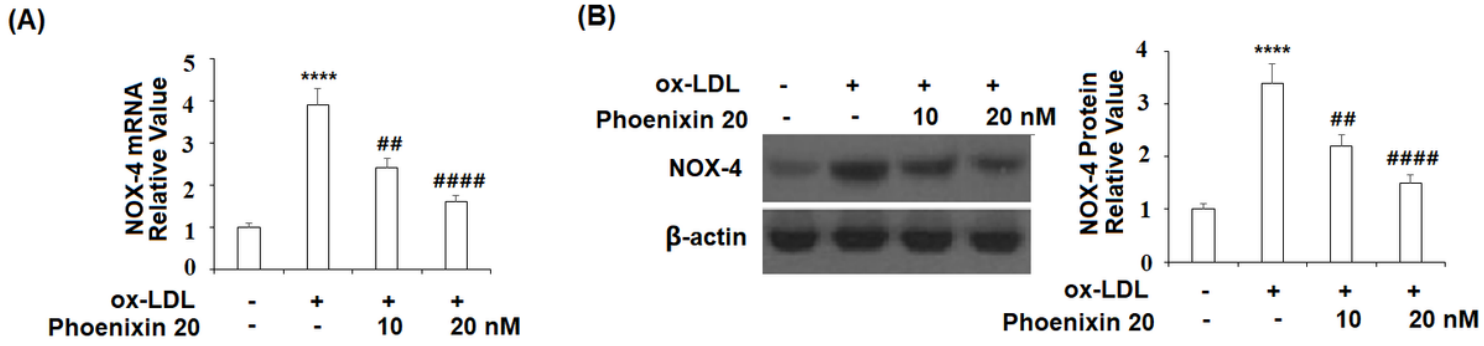

Figure 7

Phoenixin 20 suppressed ox-LDL- induced expression of NOX-4 in HAECs. HAECs were stimulated with 10 μg/ml ox-LDL with or without Phoenixin 20 (10, 20 nM) for 24 h. (A). Real time PCR analysis of NOX-4;

(B). Western blot analysis of NOX-4 (****, P<0.0001 vs. vehicle group; ##, ####, P<0.01, 0.0001 vs. ox-LDL treatment group).

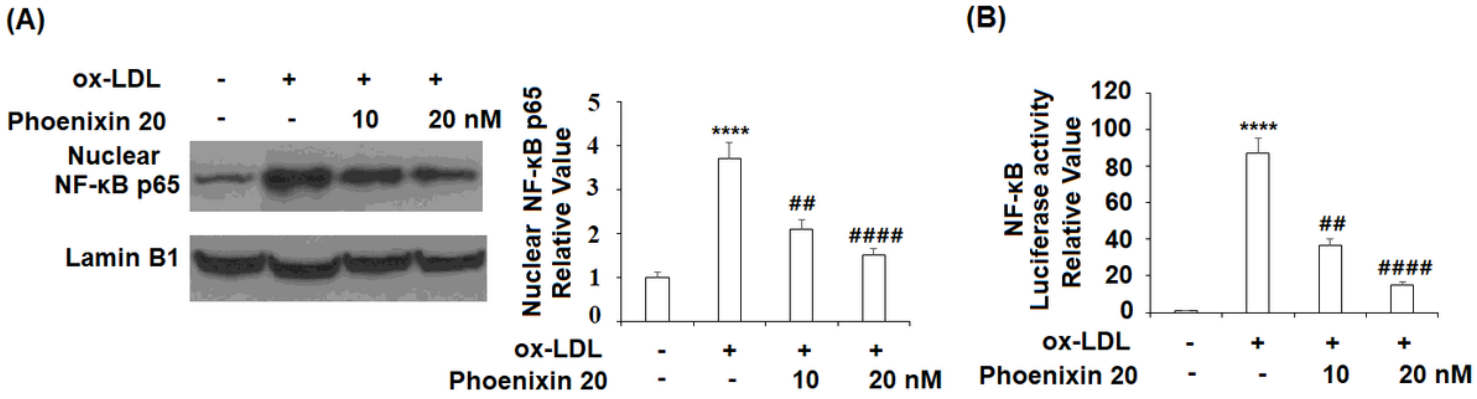

Figure 8

Phoenixin 20 suppressed ox-LDL- induced activation of NF-κB in HAECs. HAECs were stimulated with 10 μg/ml ox-LDL with or without Phoenixin 20 (10, 20 nM) for 24 h. (A). Nuclear levels of NF-κB p65; (B). Luciferase activity of NF-κB (****, P<0.0001 vs. vehicle group; ##, ####, P<0.01, 0.0001 vs. ox-LDL treatment group).

Supplementary Files

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- supplementaryfig.1.tif