Angiotensin-(1-7) Attenuates Angiotensin II-Induced ICAM-1, VCAM-1, and MCP-1 Expression via the MAS Receptor Through Suppression of P38 and NF-κB Pathways in HUVECs

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Key Words
Atherosclerosis • Angiotensin-(1-7) • Angiotensin II • ICAM-1 • VCAM-1 • MCP-1

Abstract
Background/Aims: Atherosclerosis is a chronic inflammatory disease. Intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1) play important roles in inflammatory processes. P38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB signaling regulate ICAM-1, VCAM-1, and MCP-1 expression. Angiotensin (Ang) II upregulates ICAM-1, VCAM-1, and MCP-1 expression through the P38 MAPK and NF-κB pathways. Ang-(1-7) may oppose the actions of Ang II. We investigated whether Ang-(1-7) prevents Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression in human umbilical vein endothelial cells (HUVECs). Methods: ICAM-1, VCAM-1, and MCP-1 expression was estimated by real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA); P38, NF-κB, and p-IκB-α expression was estimated by western blotting. Results: Ang-(1-7) inhibited Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression and secretion in HUVECs. Ang II sharply increased P38 MAPK phosphorylation, which was inhibited by pretreatment with Ang-(1-7). Moreover, Ang-(1-7) significantly inhibited Ang II-induced IκB-α phosphorylation and NF-κB P65 nuclear translocation. The MAS receptor antagonist A-779 abolished the suppressive effects of Ang-(1-7). Conclusion: Ang-(1-7) attenuates Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression via the MAS receptor by suppressing the P38 and NF-κB pathways in HUVECs. Ang-(1-7) might delay the progression of inflammatory diseases, including atherosclerosis.

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Introduction

Atherosclerotic cardiovascular diseases such as coronary heart disease (CHD), peripheral artery disease, and carotid artery stenosis are leading causes of morbidity and mortality. Atherosclerosis is a chronic inflammatory disease [1]. Monocytes adhere to vascular endothelial cells and transform into macrophages during atherosclerosis prophase [2]. Intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1) play important roles in this process. ICAM-1 and VCAM-1, which are adhesion molecules, facilitate firm adhesion of leukocytes to endothelial cells [3]. MCP-1 is involved in leukocyte rolling and cross-endothelium migration [4]. ICAM-1, VCAM-1, and MCP-1 have been used as noninvasive markers for the evaluation of endothelial dysfunction and modification in patients with CHD [5]. Thus, downregulating the expression of ICAM-1, VCAM-1, and MCP-1 may retard the development of atherosclerosis.

P38 mitogen-activated protein kinase (MAPK) is involved in directing cellular responses to various stimuli and in the regulation of cellular processes such as inflammation, proliferation, and cell survival [6, 7]. The transcription factor, nuclear factor kappa B (NF-κB), is also involved in cellular responses to various stimuli, including inflammatory diseases such as atherosclerosis [8]. P38 MAPK and NF-κB promote the expression of genes encoding cytokines, the adhesion molecules ICAM-1, VCAM-1, and the chemoattractant protein MCP-1 [9, 10].

Angiotensin (Ang) II is an important biologically active peptide of the rennin-angiotensin system (RAS). Ang II induces numerous biological effects such as vasoconstriction, increased myocardial contractility, cell proliferation, and water-sodium retention [11, 12]. Recent studies have shown that Ang II increases ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells (HUVECs) and MCP-1 expression in vascular smooth muscle cells through the P38 MAPK and NF-κB pathways [13-16].

Ang-(1-7) is a novel component of RAS that is synthesized from Ang I and Ang II by the catalytic action of enzymes. Ang-(1-7) exerts its biological effects through the autospecific acceptor MAS. Recent studies have demonstrated that Ang-(1-7) can antagonize the above-mentioned physiological effects of Ang II [17, 18]. Accordingly, in contrast to Ang II, Ang-(1-7) induces relaxation of blood vessels, and it inhibits smooth muscle cell growth. Moreover, Ang-(1-7) has anti-atherosclerotic and antiproliferative effects [19-21]. Wang et al. [22] recently reported that Ang-(1-7) prevents Ang II-induced VCAM-1 and MCP-1 expression by inhibiting lectin-like oxidized low-density lipoprotein receptor (LOX)-1 in HUVECs. However, to the best of our knowledge, whether Ang-(1-7) prevents the Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 through the P38 MAPK and NF-κB pathways in HUVECs has not been investigated.

In this study, we investigated the effects of Ang-(1-7) on Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression in HUVECs and its possible molecular relationship with the P38 MAPK and NF-κB pathways. Our results revealed that Ang (1–7) attenuates Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression via the MAS receptor through suppression of the P38 MAPK and NF-κB pathways in HUVECs. These results might provide the basis for the treatment of clinical cardiovascular diseases.

Materials and Methods

Reagents

Ang II, Ang-(1-7), SB203580, and pyrrolidine dithiocarbamate (PDTC) were purchased from Sigma Aldrich (St. Louis, MO, USA). A-779 was purchased from the American Peptide Company (Sunnyvale, CA, USA). ICAM-1, VCAM-1, and MCP-1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from the DA KE WEI company (Shenzhen, China). Antibodies against, P38 MAPK, phospho-P38, NF-κB p65, IκB-α, phosphor-IκB-α, proliferating cell nuclear antigen (PCNA), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from Abcam (Cambridge, UK).
Cells culture and identification

A fresh umbilical cord was obtained from a healthy neonate under aseptic conditions in the Department of Gynaecology and Obstetrics at the Shanxi Medical University, and HUVECs were isolated. Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen Corporation, Carlsbad, California) was added to the culture fluid. The HUVECs were allowed to grow to the deuto-confluence phase, and then they were digested with 0.25% trypsin (Invitrogen Corporation, Carlsbad, California). Subsequently, cell passage was performed. The cells were used for experiments at passages two to four (cell number, $1 \times 10^6$). Endothelial cells were identified by morphology.

RNA isolation and real-time polymerase chain reaction (PCR)

Total RNA was extracted using TRIzol® reagent according to the manufacturer’s protocol. The primer sequences for the real-time PCR assays are listed in Table 1. Real-time PCR, using SYBR® Green detection chemistry, was performed on an ABI QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems; Carlsbad, CA, USA) under the following conditions: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s and 60°C for 30 s. Quantitative measurements for real-time PCR were determined using the $\Delta\Delta C_T$ method, and $\beta$-actin expression was used as the internal control.

ELISA

The levels of MCP-1, VCAM-1, and ICAM-1 in the culture supernatant were estimated by ELISA according to the manufacturer’s protocol. Briefly, 100 µL of standard cytokine or culture supernatant was added to each well and incubated for 30 min at 37 °C. The cells were then incubated for 60 min with 100 µL of 1× biotin and for 60 min with 100 µL of 1× horseradish peroxidase (HRP). After every incubation, each well was washed five times with wash buffer. Finally, 100 µL of stop solution was added to each well to terminate the reaction, and the plates were incubated in the dark for 30 min with 100 µL of 3,3′,5,5′-tetramethylbenzidine at 37 °C. The optical density of each well was determined using a microplate reader at 450 nm within 30 min. A standard curve for each cytokine was generated. On the basis of this standard curve, linear regression analysis was performed using the Microcal Origin 6.0 software.

Western blot analyses

Briefly, after treatment, $1 \times 10^6$ HUVECs were harvested, and protein extracts were prepared. For western blot analyses, 50 µg of protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes (Millipore Inc., Billerica, MA, USA). The membranes were then blocked for 1 h in 3% non-fat dry milk, and incubated at 4 °C overnight with anti-mouse monoclonal PCNA, GAPDH (1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), p38, phospho-p38, NF-kB p65, phosphor-IκB-α antibodies (1:1,000 dilution; Cell Signaling, Boston, MA, USA). The membranes were then incubated with anti-mouse or anti-rabbit secondary antibody (1:10,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h. The proteins were visualized using a chemiluminescence detection system (Amersham, Poole, UK), and the signal intensities were analyzed using a Gel Imaging System (Bio-Rad). The relative protein expression level was determined by normalizing the values obtained to that of GAPDH.

Statistical analysis

All values are expressed as means ± standard deviations (SD). Student’s t-test was used to analyze differences between two groups. Analysis of variance (ANOVA) was used to compare the means of three or
more groups, and post-hoc multiple comparisons were used to compare the means of any two groups. All data analyses were performed using the SPSS v16.0 software. Statistical significance was assumed when the p-values were less than 0.05.

Results

Isolation and identification of HUVECs

Immunofluorescence staining of Von Willebrand factor (vWF), which is specifically expressed in blood vessel endothelial cells (HUVECs), was performed to identify HUVECs. The results revealed that nearly 100% of the isolated cells were vWF-positive (Fig. 1).

Ang-(1-7) attenuates Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 in HUVECs

To evaluate effects of Ang-(1-7) on Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 in HUVECs, the cells were pre-treated with 1000 nM Ang-(1-7) for 30 min and then stimulated with 100 nM Ang II for 6 h. Culture supernatants were collected, and the levels of ICAM-1, VCAM-1, and MCP-1 were determined by ELISA. Meanwhile, the HUVECs were harvested, and the mRNA levels of ICAM-1, VCAM-1, and MCP-1 were estimated by real-time PCR. Ang II treatment resulted in marked increase in ICAM-1, VCAM-1, and MCP-1 mRNA expression and secretion, compared with the control. However, pretreatment with Ang-(1-7) inhibited ICAM-1, VCAM-1, and MCP-1 mRNA expression and secretion, compared with Ang II treatment alone. Pretreatment with A779 blocked the Ang-(1–7)-mediated inhibition of Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 in HUVECs (Fig. 2).

Ang-(1-7) inhibits Ang II-induced P38 MAPK phosphorylation in HUVECs

The P38 MAPK signaling pathway plays an important role in the regulation of inflammation. Therefore, we investigated the effect of Ang-(1-7) on the activation of phospho-P38. The phosphorylation level of P38 MAPK increased significantly upon treatment with Ang II. The phosphorylation level decreased upon pretreatment with Ang-(1-7). However, treatment with A779 significantly inhibited Ang-(1-7)-induced reduction in the level of phospho-P38 (Fig. 3).

Ang-(1-7) inhibits Ang II-induced IκB-α phosphorylation and NF-κB translocation in HUVECs

The NF-κB pathway is a key mediator of inflammation. Therefore, we estimated the effect of Ang-(1-7) on the activation of the Ang II-induced NF-κB pathway. As shown in Fig
Fig. 2. Ang-(1-7) attenuates angiotensin (Ang) II-induced intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1) expression in HUVECs. HUVECs were incubated with 100 nM Ang II alone for 6 h. Furthermore, HUVECs were pretreated with 1000 nM Ang-(1-7) or 1000 nM A-779 (MAS receptor antagonist) plus 1000 nM Ang-(1-7) for 30 min before being incubated with 100 nM Ang II for another 6 h. A, C, and E. mRNA expression of ICAM-1, VCAM-1, and MCP-1 was measured by real-time polymerase chain reaction (PCR). B, D, and F. Culture supernatants were collected, and the secretion levels of ICAM-1, VCAM-1, and MCP-1 were determined by enzyme-linked immunosorbent assay (ELISA). Data are presented as the means ± standard deviation (SD) of three independent experiments. *P < 0.05 compared with the control group; #P < 0.05 compared with the Ang II group; &P < 0.05 compared with the Ang II + Ang-(1-7) group.

4. Ang II sharply increased the translocation of NF-κB p65 from the cytosol to the nucleus, and this increase was inhibited by pretreatment with Ang-(1-7). Translocation of NF-κB to the nucleus depends on the phosphorylation and degradation of IκB-α. Therefore, we also examined the protein levels of phosphorylated IκB-α by western blot analysis. Ang-(1-7) inhibited the Ang II-induced phosphorylation of IκB-α (Fig. 4). These results indicated that Ang-(1-7) inhibited Ang II-induced inflammatory responses through inhibition of Ang II-induced IκB-α phosphorylation and NF-κB translocation in HUVECs. The MAS receptor antagonist A-779 completely abolished the effects of Ang-(1-7).

Effects of SB203580 and PDTC on Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 in HUVECs

A recent study demonstrated that Ang II increases ICAM-1 and VCAM-1 expression in HUVECs and MCP-1 expression in vascular smooth muscle cells through the P38 MAPK and...
NF-κB pathways. To investigate whether the Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 is induced by the P38 MAPK and NF-κB pathways in HUVECs, the cells were pretreated with SB203580 (an inhibitor of P38 MAPK) and PDTC (an inhibitor of NF-κB). As shown in Fig. 5, treatment with Ang II caused a significant increase of ICAM-1, VCAM-1, and MCP-1 expression.
MCP-1 expression, compared with the control. SB203580 inhibited the effects of Ang II on the expression of ICAM-1, VCAM-1, and MCP-1. PDTC also abolished the effect of Ang II on HUVECs (Fig. 6). These results indicate that P38 MAPK, as well as NF-κB, may be essential for Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression in HUVECs.

Discussion

In recent years, it has been generally accepted that atherosclerosis is a chronic hyperplasia inflammatory process. ICAM-1 and VCAM-1 are members of the immunoglobulin superfamily, and they are mainly expressed in vascular endothelial cells, lymph cells, and monocytes. MCP-1 is involved in leukocyte rolling and cross-endothelium migration. In recent years, several studies have suggested that the expression of ICAM-1, VCAM-1, and MCP-1 is correlated with the development of atherosclerosis. In addition, P38 MAPK and NF-κB signaling have been shown to be involved in inflammatory process, especially in the regulation of ICAM-1, VCAM-1 and MCP-1 expression.

Ang II is an important biologically active peptide of RAS. Previous in vivo and in vitro studies have suggested that Ang II upregulates the expression of ICAM-1, VCAM-1, and MCP-1 [23, 24]. Recently, it was reported that Ang II increases the expression of ICAM-1 and VCAM-1 through P38 MAPK and NF-κB signaling in HUVECs. Moreover, Ang II-mediated
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Fig. 6. Effects of pyrrolidinedithiocarbamate (PDTC) on Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 in HUVECs. HUVECs were incubated with 100 nM Ang II alone for 6 h. Furthermore, HUVECs were pretreated with 10 mM PDTC for 30 min before being incubated with 100 nM Ang II for another 6 h. A, C, and E. mRNA levels of ICAM-1, VCAM-1, and MCP-1 were estimated by real-time PCR. B, D, and F. Culture supernatants were collected and the secretion levels of ICAM-1, VCAM-1, and MCP-1 were estimated by ELISA. Data are presented as the means ± SD of three independent experiments. *P < 0.05 compared with the control group; #P < 0.05 compared with the Ang II group.

activation of the P38 MAPK pathway is not involved in the activation of NF-κB. In addition, Ang II upregulates the expression of MCP-1 through P38 MAPK and NF-κB signaling in vascular smooth muscle cells. In this study, we incubated HUVECs with 100 nM Ang II, and the expression levels of ICAM-1, VCAM-1, and MCP-1 increased significantly compared with the control (P < 0.05). When the HUVECs were pretreated with SB203580 or PDTC, the effect of Ang II on the expression of ICAM-1, VCAM-1, and MCP-1 was inhibited. Together, these results suggest that Ang II upregulates the expression of ICAM-1 and VCAM-1 through P38 MAPK and NF-κB signaling in HUVECs. This finding is consistent with the results of some previous reports [13-16]. Our findings also suggest that Ang II increases MCP-1 expression through P38 MAPK and NF-κB signaling in HUVECs. To the best of our knowledge, this effect of Ang II has not been reported previously.

The heptapeptide Ang-(1-7) is an independent member of RAS, and it is produced directly from Ang II by the action of the recently discovered carboxypeptidase angiotensin-converting enzyme 2. Alternatively, Ang I can be converted by a neutral endopeptidase to Ang-(1-7). Initially, it was believed that Ang-(1-7) was the non-active metabolite of RAS. Subsequently, many studies have demonstrated that Ang-(1-7) can antagonize the biological effects of Ang II. Zhang et al. [25] demonstrated that Ang-(1-7) inhibits Ang II-induced SMC proliferation and migration through negative modulation of Ang II-induced ERK1/2 activity. Yang et al. [26] reported that Ang-(1-7) inhibited atherosclerotic lesion formation by targeting vascular smooth muscle cells and that a large dose of Ang-(1-7) may stabilize mature plaque by targeting macrophages in ApoE(-/-) mice. Recently, our laboratory demonstrated that Ang-(1-7) improves reverse cholesterol transport by improving the expression of adenosine...
triphosphate-binding cassette transporter A1 and adenosine triphosphate-binding cassette transporter G1, which protect against atherosclerosis [27, 28]. However, whether Ang-(1-7) attenuates Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression through P38 MAPK and NF-κB pathways in HUVECs was unclear. In this study, we first examined the effect of Ang-(1-7) on ICAM-1, VCAM-1, and MCP-1 expression. We found that Ang-(1-7) inhibited Ang II-induced mRNA and secretion of ICAM-1, VCAM-1, and MCP-1 in HUVECs, which suggests a possible beneficial effect of Ang-(1-7) via the attenuation of HUVECs activation and inflammation. Therefore, Ang-(1-7) might delay the progression of inflammatory diseases.

In order to further elucidate the mechanisms underlying the inhibitory effect of Ang-(1-7) on the expression of ICAM-1, VCAM-1, and MCP-1 in HUVECs, we investigated the effect of Ang-(1-7) on the activation (phosphorylation) of P38 MAPK induced by Ang II in HUVECs. The results revealed that Ang II sharply increases the phosphorylation of P38 MAPK, which was, in turn, inhibited by pretreatment with Ang-(1-7). Our results suggested that the Ang-(1-7)-mediated attenuation of inflammation is associated with the downregulation of P38 MAPK phosphorylation.

NF-κB is clearly one of the most important regulators of inflammation. The expression of ICAM-1, VCAM-1, and MCP-1 is mediated by NF-κB. NF-κB dimers are rendered inactive in the cytosol by inhibitory proteins of the IκB family in unstimulated cells [29]. The crucial step in NF-κB activation is IκB phosphorylation, which occurs because of activation of the IκB kinase complex. The phosphorylation of inhibitory IκB proteins triggers their ubiquitination and subsequent proteasomal degradation, followed by the release and nuclear translocation of active NF-κB [30-32]. We examined whether Ang-(1-7)-mediated signaling pathways modulate NF-κB signaling to study the molecular mechanisms underlying the anti-inflammatory effect of Ang-(1-7). Incubation of HUVECs with Ang II caused marked cytosolic phosphorylation of IκB-α and NF-κB p65 translocation into the nucleus, but pretreatment with Ang-(1-7) significantly inhibited IκB-α phosphorylation and NF-κB p65 nuclear translocation. These results indicated that Ang-(1-7) suppressed Ang II-induced inflammatory responses by inhibiting Ang II-induced IκB-α phosphorylation and NF-κB p65 nuclear translocation in HUVECs.

Many of the cardiovascular effects of Ang-(1-7) are completely blocked by the selective Ang-(1-7) antagonist, A-779, suggesting that the effects of Ang-(1-7) are mediated by a specific receptor sensitive to A-779. Recently, Santos et al. [33] reported that Ang-(1-7) is an endogenous ligand for the G-protein-coupled receptor, MAS. Specific binding of $^{125}$I-Ang-(1-7) in mouse kidney slices was abolished by genetic deletion of MAS, while binding of $^{125}$I-Ang II and $^{125}$I-Ang IV was fully preserved. Furthermore, $^{125}$I-Ang-(1-7) bound with high affinity to MAS-transfected COS cells. In order to investigate whether Ang-(1-7) attenuates Ang II-induced ICAM-1, VCAM-1, and MCP-1 expression via the MAS receptor in HUVECs, we incubated HUVECs with the Ang-(1-7) antagonist, A-779, for 30 min. Treatment with A779 almost completely reversed the effects of Ang-(1-7) on ICAM-1, VCAM-1, and MCP-1 expression, and P38 phosphorylation and NF-κB activation, suggesting that the actions of Ang-(1-7) are mediated via the MAS receptor.

In conclusion, our data suggest that Ang-(1-7) inhibits Ang II-induced expression of ICAM-1, VCAM-1, and MCP-1 through P38 MAPK- and NF-κB-dependent signaling in HUVECs. Moreover, the beneficial effects of Ang-(1-7) are reversed by A779. Thus, these results suggest that the Ang-(1-7)/MAS receptor axis is an important mechanism that reverses the inflammatory cascades involved in atherosclerosis, and activation of this pathway may be a novel therapeutic approach for the treatment of atherosclerosis.

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Disclosure Statement

The authors declare no conflict of interest.

References

1. Ross R: Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115-126.
2. Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. Circulation 2002;105:1135-1143.
3. Blankenberg S, Barbaux S, Tirié L: Adhesion molecules and atherosclerosis. Atherosclerosis 2003;170:191-203.
4. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rolls BJ: Absence of monocyte chemotactant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998;2:275-281.
5. Hillis GS, Flapan AD: Cell adhesion molecules in cardiovascular disease: a clinical perspective. Heart 1998;79:429-431.
6. Han J, Lee JD, Bibbs L, Ulevitch RJ: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science 1994;265:808-811.
7. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH: Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 2001;22:153-183.
8. Monaco C, Andreoulos E, Kiriakidis S, Mauri C, Bicknell C, Foxwell B, Cheshire N, Paleolog E, Feldmann M: Canonical pathway of nuclear factor kappa B activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. Proc Natl Acad Sci U S A 2004;101:5634-5639.
9. Dou L, Lu Y, Shen T, Huang X, Man Y, Wang S, Li J: Panax notoginseng saponins suppress RAGE/MAPK signaling and NF-kappaB activation in apolipoprotein-E-deficient atherosclerosis-prone mice. Cell Physiol Biochem 2012; 9:875-882.
10. Lu Y, Zhu X, Liang GX, Cui RR, Liu Y, Wu SS, Liang QH, Liu QY, Liu GY, Zhang Y, Wang S, Li J: Panax notoginseng saponins suppress RAGE/MAPK signaling and NF-kappaB activation in apolipoprotein-E-deficient atherosclerosis-prone mice. Cell Physiol Biochem 2012; 9:875-882.
11. Chae YJ, Kim CH, Ha TS, Hescheler J, Ahn HY, Sachinidis A: Epigallocatechin-3-O-gallate inhibits the angiotensin II-induced expression of human umbilical vein endothelial cell via inhibition of MAPK pathways. Cell Physiol Biochem 2007;20:859-866.
12. Costanzo A, Moretti F, Burgio VL, Bravi C, Guido F, Levero M, Puri PL: Endothelial activation by angiotensin II through NFkappaB and p38 pathways: Involvement of NFkappaB-inducible kinase (NIK), free oxygen radicals, and selective inhibition by aspirin. J Cell Physiol 2003;195:402-410.
13. Takahashi M, Suzuki K, Takeda R, Oba S, Nishimatsu H, Kimura K, Nagano T, Nagai R, Hirata Y: Angiotensin II and tumor necrosis factor-alpha synergistically promote monocyte chemotactant protein-1 expression: roles of NF-kappaB, p38, and reactive oxygen species. Am J Physiol Heart Circ Physiol 2008;294:H2879-2888.
14. Suzuki K, Satonaka H, Nishimatsu H, Oba S, Takeda R, Omata M, Fujita T, Nagai R, Hirata Y: Myocyte enhancer factor 2 mediates vascular inflammation via the p38-dependent pathway. Circ Res 2004;95:42-49.
15. Jiang B, Yang J, Zhang Y, Hong M, Wang S, Zhang Q, Liu FF, Zhang K, Zhang C: Angiotensin-converting enzyme 2 and angiotensin 1-7: novel therapeutic targets. Nat Rev Cardiol 2014;11:413-426.
16. Santos RA: Angiotensin-(1-7). Hypertension 2014;63:1138-1147.
17. Freeman EJ, Chisolm GM, Ferrario CM, Tallant EA: Angiotensin-(1-7) inhibits vascular smooth muscle cell growth. Hypertension 1996;28:104-108.
18. Loo AE, Rosk RA, Henning RH, Tio RA, Suurmeijer A, Boomsma F, van Gilst WH: Angiotensin-(1-7) attenuates the development of heart failure after myocardial infarction in rats. Circulation 2002;105:1548-1550.
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21 Tallant EA, Clark MA: Molecular mechanisms of inhibition of vascular growth by angiotensin-(1-7). Hypertension 2003;42:574-579.
22 Wang L, Hu X, Zhang W, Tian F: Angiotensin (1-7) ameliorates angiotensin II-induced inflammation by inhibiting LOX-1 expression. Inflamm Res 2013;62:219-228.
23 Chen XL, Tummala PE, Olbrych MT, Alexander RW, Medford RM: Angiotensin II induces monocyte chemotactant protein-1 gene expression in rat vascular smooth muscle cells. Circ Res 1998;83:952-959.
24 Pastore L, Tessitore A, Martinotti S, Tonato E, Alesse E, Bravi MC, Ferri C, Desideri G, Gulino A, Santucci A: Angiotensin II stimulates intercellular adhesion molecule-1 (ICAM-1) expression by human vascular endothelial cells and increases soluble ICAM-1 release in vivo. Circulation 1999;100:1646-1652.
25 Zhang F, Hu Y, Xu Q, Ye S. Different effects of angiotensin II and angiotensin-(1-7) on vascular smooth muscle cell proliferation and migration. PLoS One 2010;5:e12323.
26 Yang J, Dong M, Meng X, Zhao Y, Yang Y, Liu X, Hao P, Li J, Wang X, Zhang K, Gao F, Zhao X, Zhang M, Zhang Y, Zhang C. Angiotensin-(1-7) dose-dependently inhibits atherosclerotic lesion formation and enhances plaque stability by targeting vascular cells. Arterioscler Thromb Vasc Biol 2013;33:1978-85.
27 Liang B, Wang X, Bian Y, Yang H, Liu M, Bai R, Yang Z, Xiao C: Angiotensin-(1-7) upregulates expression of ABCA1 and ABCG1 through the Mas receptor via the liver X receptor alpha signaling pathway in THP-1 macrophages treated with Angiotensin II. Clin Exp Pharmacol Physiol 2014;41:1023-1030.
28 Liang B, Wang X, Yan F, Bian Y, Liu M, Bai R, Yang HY, Zhang NN, Yang ZM, Xiao CS: Angiotensin-(1-7) upregulates (ATP-binding cassette transporter A1) ABCA1 expression through cyclic AMP signaling pathway in RAW 264.7 macrophages. Eur Rev Med Pharmacol Sci 2014;18:985-991.
29 Torres L, Serna E, Bosch A, Zaragoza R, Garcia C, Miralles V, Sandoval J, Vina JR, Garcia-Trevijano ER: NF-kB as node for signal amplification during weaning. Cell Physiol Biochem 2011;28:833-846.
30 Ghosh S, Hayden MS: New regulators of NF-kappaB in inflammation. Nat Rev Immunol 2008;8:837-848.
31 Perkins ND: Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol 2007;8:49-62.
32 Xie S, Liu B, Fu S, Wang W, Yin Y, Li N, Chen W, Liu J, Liu D: GLP-2 suppresses LPS-induced inflammation in macrophages by inhibiting ERK phosphorylation and NF-kappaB activation. Cell Physiol Biochem 2014;34:590-602.
33 Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RF, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M et al: Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A 2003;100:8258-8263.