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Angiotensin-(1-7): new perspectives in atherosclerosis treatment

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Abstract

Angiotensin (Ang)-(1-7) is recognized as a new bioactive peptide in renin-angiotensin system (RAS). Ang-(1-7) is a counter-regulatory mediator of Ang-II which appears to be protective against cardiovascular disease. Recent studies have found that Ang-(1-7) played an important role in reducing smooth muscle cell proliferation and migration, improving endothelial function and regulating lipid metabolism, leading to inhibition of atherosclerotic lesions and increase of plaque stability. Although clinical application of Ang-(1-7) is restricted due to its pharmacokinetic properties, identification of stabilized compounds, including more stable analogues and specific delivery compounds, has enabled clinical application of Ang-(1-7). In this review, we discussed recent findings concerning the biological role of Ang-(1-7) and related mechanism during atherosclerosis development. In addition, we highlighted the perspective to develop therapeutic strategies using Ang-(1-7) to treat atherosclerosis.

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1 Introduction

Angiotensin (Ang)-(1-7) is a new family member of the renin-angiotensin system and its appearance has challenged the traditional renin-angiotensin axis, angiotensin converting enzyme/angiotensin II/angiotensin II receptor type 1 (ACE/Ang-II/AT1) axis. In 1988, Santos, *et al*.[1] first found that Ang-(1-7), which was regarded as an inactive fragment, was the major product generated from Ang-I in the regions of the dog brainstem. Campagnole-Santos, *et al*.[2] then found that Ang-(1-7) was a bioactive peptide, which could cause hypotensive response accompanied with bradycardia after injection into the dorsal motor nucleus of the vagus. Furthermore, Benter, *et al*.[3] observed that Ang-(1-7) could counteract the effect of Ang-II on blood pressure. Recent studies have shown that Ang-(1-7) is a counter-regulatory mediator of Ang-II, which exerts protection against cardiovascular disease through the improvement of endothelial function, inhibition of vascular smooth muscle cell proliferation and migration, vasodilation and modulation of ventricular remodeling.[4,5] Atherosclerosis is characterized by endothelial dysfunction, smooth muscle cell proliferation and migration and disorder of lipid metabolism.[6] Recent studies have proved that Ang-(1-7) plays an important role in protecting against the development of atherosclerosis. In this review, we will discuss the recent findings concerning the biological role of Ang-(1-7) and related mechanism during atherosclerosis development, as illustrated in Figure 1.

2 The biosynthesis of Ang-(1-7)

In the traditional ACE/Ang-II/AT1 axis, Ang-I is cleaved by angiotensin converting enzyme (ACE) into Ang-II, which contains eight amino acids. Most of pathological effects of the renin-angiotensin system (RAS) are attributed to the overproduction of Ang-II. Activation of the Ang-II AT1 receptor results in vasoconstriction, elevation of blood pressure, cell proliferation and sodium retention.[7]

Recently, the appearance of new ACE2/Ang-(1-7)/MAS (G-coupled protein receptor of Ang-(1-7)) axis has challenged the existence of traditional ACE/Ang-II/AT1 axis. As shown in Figure 2, ACE2 cleaves one amino acid from Ang-I, generating Ang-(1-9). Ang-(1-9) is further cleaved by ACE into Ang-(1-7). In addition, Ang-(1-7) can be also directly generated from Ang-II through cleavage of ACE2.[8] ACE2 is distributed in various tissues, including kidney, heart, vessel and testis. Reduced concentrations of Ang-(1-7)

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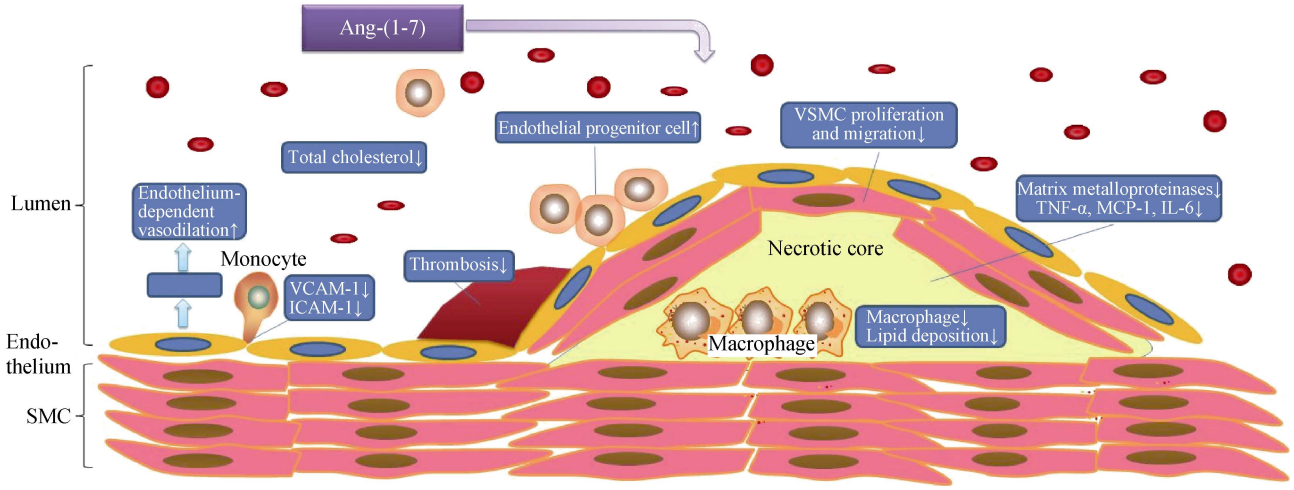

Figure 1. The role of Ang-(1–7) in atherosclerosis. Ang-(1–7) effectively suppresses expression of endothelial cell adhesion molecules, such as VCAM-1 and ICAM-1, resulting in reduction of monocyte adhesion into endothelial layer and subsequent decreased macrophage recruitment in atherosclerotic plaque. Ang-(1–7) increases eNOS expression, resulting in endothelium-dependent vasodilation. In addition, Ang-(1–7) stimulates proliferation of endothelial progenitor cell, regenerating the injured endothelial layer. Ang-(1–7) also causes a great reduction of thrombus formation. Vascular smooth muscle cell migration and proliferation can be also inhibited by Ang-(1–7). Ang-(1–7) effectively modulates lipid metabolism through reducing total cholesterol and triglycerides levels. Finally, Ang-(1–7) suppresses the formation of atherosclerotic lesion, and improves plaque stability by reducing proinflammatory cytokines levels, matrix metalloproteinases activities, macrophage contents and necrotic core in the plaque. Ang-(1-7): Angiotensin-(1-7); eNOS: endothelial nitric oxide synthase; ICAM-1: intercellular cell adhesion molecule 1; IL-6: Interleukin 6; MCP-1: monocyte chemotactic protein; SMC: smooth muscle cells; TNF-α: tumor necrosis factor α; VCAM-1: vascular cell adhesion molecule 1; VSMC: vascular smooth muscle cell.

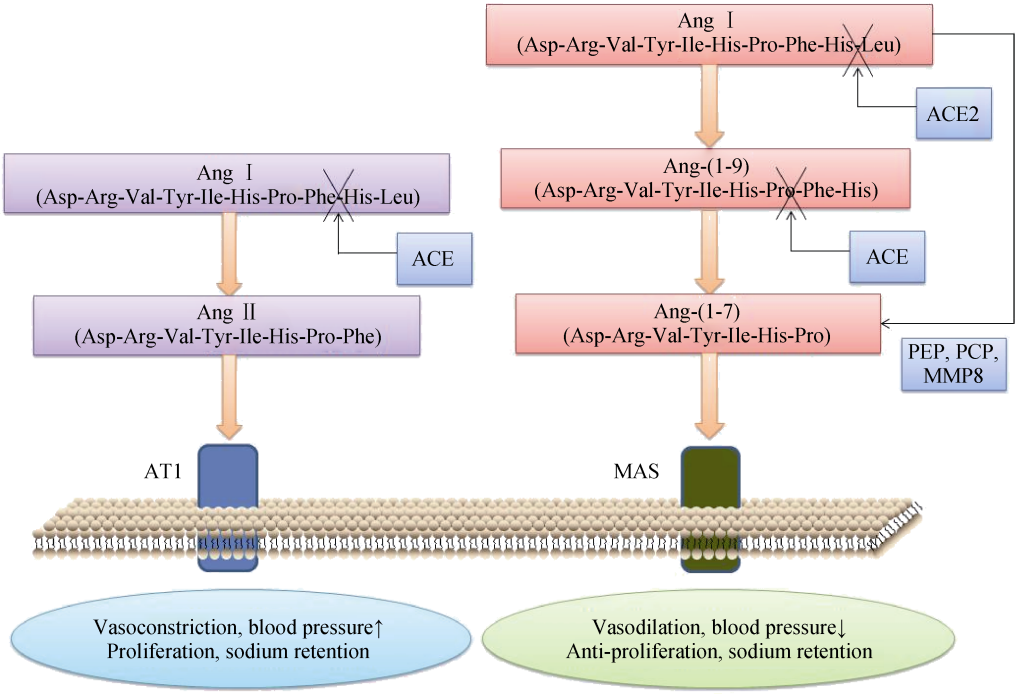

Figure 2. The comparison of ACE/Ang-II/AT1 axis and ACE2/Ang-(1–7)/MAS axis. Ang-I is cleaved by ACE into Ang-II. In contrast, Ang-I is cleaved by ACE2 and further cleaved by ACE, generating Ang-(1–7). Ang-(1–7) can counteract the effects of Ang-II, mediating vasodilation, blood pressure reduction, anti-proliferation and sodium secretion. ACE: angiotensin converting enzyme; Ang-II: angiotensin II; Ang-I: angiotensin I; Ang-(1-7): angiotensin-(1-7); AT1: angiotensin II receptor type 1; PEP: prolylendopeptidase; PCP: prolylcarboxypeptidase; MAS: G-coupled protein receptor of Ang-(1-7); MMP8: matrix metalloproteinase 8.

have been observed in renal cortical homogenates of ACE2 knockout mice.[9] Other enzymes can also generate Ang-(1-7) directly from Ang-I, including prolylendopeptidase (PEP),[10] prolylcarboxypeptidase (PCP),[11] neprilysin and matrix metalloproteinase 8 (MMP8).[12]

The Ang-(1-7) receptor was not elucidated until Santos, *et al*.[13] found that Ang-(1-7) acted through MAS receptors, which was a G protein-coupled, seven transmembrane protein. MAS deficient mice completely lacked the antidiuretic action of Ang-(1-7) after an acute water load, and also lost Ang-(1-7)-induced aortic vasodilation. Interestingly, Ang-(1-7) could also regulate the MAS receptor by inducing redistribution of MAS receptor from cell membrane to intracellular vesicles and subsequent endocytosis.[14]

3 Ang-(1-7) and vascular endothelial function

Ang-(1-7) has been demonstrated to protect against endothelial cell dysfunction, which is regarded as an early step in atherosclerotic plaque formation. Firstly, Ang-(1-7) negatively regulated Ang-II-induced intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) by suppressing P38 mitogen-activated protein kinase (MAPK) phosphorylation and attenuating nuclear translocation of NF-kappaB, suggesting that Ang-(1-7) could effectively modulate endothelial cell adhesion molecules.[15–17] Secondly, Ang-(1-7) has endothelium-dependent vasodilator properties. Ang-(1-7) treated mice showed reduced reactive oxygen species (ROS) production, decreased NADPH oxidase expression and increased endothelial nitric oxide synthase expression, resulting in renal endothelium-dependent vasorelaxation.[18] Similarly, Ang-(1-7) restored endothelium-dependent vasodilation in cerebral resistance arteries in rats fed with a high-salt diet.[19] Administration of Ang-(1-7) also restored vasodilation through reducing vascular oxidant stress in mesenteric arteries of salt-fed rats.[20] In addition, one study tested Ang-(1-7)-eluting stents, and found that Ang-(1-7) effectively improved aortic dilator function.[21] Thirdly, Ang-(1-7) stimulated proliferation of endothelial progenitor cells, regenerating the injured endothelial layer in the atherosclerotic vessel.[22] Seva, *et al*.[23] found that cyclic Ang-(1-7), a metabolically stable Ang-(1-7) analogue, increased circulating hematopoietic progenitor cell in mice with myocardial infarction. Fourthly, thrombus formation was associated with injury of vascular endothelial cells, and intravenous infusion of Ang-(1-7) into rats which developed venous thrombosis caused a great reduction of thrombus formation.[24] Interestingly, antithrombotic effects of captopril and losartan could be attenuated by Ang-(1-7) antagonist, indicating that Ang-(1-7) was involved in the antithrombotic process of angiotensin converting enzyme inhibitor and angiotensin receptor blocker (ARB) drugs.[24] Treatment of orally active formulation of Ang-(1-7) also promoted an antithrombotic effect in the abdominal vena cava of spontaneous hypertensive rats.[25] Moreover, Ang-(1-7) caused a decrease in endothelial cell tube formation and neovascularization.[16] The effect of Ang-(1-7) on the inhibition of angiogenesis was possible due to reduction in VEGF-A (vascular endothelial grow factor-A), a primary proangiogenic protein.[26]

4 Ang-(1-7) and vascular smooth muscle cell function

Vascular smooth muscle cell (SMC) proliferation and migration are important processes in the development of atherosclerosis. Ang-(1-7) can directly inhibit rat SMC proliferation.[27] However, Zhang, *et al*.[28] showed that Ang-(1-7) alone had no effect on mouse SMC growth, whereas Ang-(1-7) inhibited Ang-II induced mouse SMC proliferation. The reasons for these divergent effects of Ang-(1-7) might be due to differences in the repertoires of receptors, signaling molecules and cell cycling regulators in the different types of cells. Ang-(1-7) was also able to abrogate Ang-II induced SMC migration. Ang-(1-7) treatment attenuated neo-intimal formation in abdominal aorta after stent implantation and in balloon-injured carotid arteries in rats.[29,30] Ang-(1-7) treatment also resulted in a reduction of neo-intimal formation and collagen synthesis after angioplasty in rabbits.[31]

There are several mechanisms by which Ang-(1-7) regulates SMC migration. Firstly, Ang-(1-7) negatively modulated Ang-II-induced ERK1/2 activity in SMCs, which has been well established as a signaling pathway for SMC proliferation and migration.[28] Secondly, Ang-(1-7) was able to antagonize Ang-II-stimulated up-regulation of matrix metalloproteinases-9, which is an extracellular matrix protein-degrading enzyme known to facilitate cell migration.[32,33] Thirdly, Ang-(1-7) could down-regulate the Ang-II AT1 receptor in SMCs, resulting in reduced Ang-II binding to the receptor and subsequent decreased SMC migration.[34]

5 Ang-(1-7) and lipid metabolism

High blood cholesterol is a well-established contributor to atherosclerosis. Recent studies have demonstrated that Ang-(1-7) can effectively regulate lipid metabolism. Ang-(1-7) transgenic rats presented decreased cholesterol and triglycerides levels, as well as a reduction in abdominal fat mass.[35]

In the aspect of glucose metabolism, Ang-(1-7) transgenic rats showed enhanced glucose tolerance, insulin sensitivity and insulin-stimulated glucose uptake.[35] Furthermore, the study found that the improvement of lipid and glucose metabolism in Ang-(1-7) transgenic rats was possibly due to increase of adiponectin production in adipose tissue.[35] Oral formulation of Ang-(1-7) could effectively prevent high-fat diet-induced hepatic steatosis and decrease plasma total cholesterol with a reduction of inflammatory markers in the livers of mice.[36] In contrast, MAS deficient mice showed an increase of total cholesterol and triglycerides, as well as reduced glucose intolerance, insulin sensitivity and insulin-stimulated glucose uptake, resulting in 50% increase in abdominal fat mass.[37] Another study also showed that besides an increase of cholesterol and triglyceride, MAS/Apolipoprotein E (ApoE) double knockout mice were associated with increased hepatic lipid content and alanine aminotransferase with a change in hepatic protein content of mediators related to atherosclerotic inflammation, such as peroxisome proliferator-activated receptor-α and liver X receptor.[38] Moreover, recent studies have shown that statins, the most widely used lipid-lowering drugs, can exert additional "pleiotropic" actions partly through modulating Ang-(1-7) levels. Atorvastatin treatment attenuated the inhibitory effect of TNF-α on ACE2 and Ang-(1-7) production in SMC culture.[39] Another study showed that administration of rosuvastatin lead to increased Ang-(1-7) level and subsequent decreased vascular SMC proliferation and intimal thickening after vascular balloon injury in rats.[40] The effect of statins on Ang-(1-7) production is also demonstrated in clinical trials. Ang-(1-7) levels increased significantly after 30-day atorvastatin treatment in twelve hypercholesterolemic patients.[41] However, another clinical trial found no differences in Ang-(1-7) levels before and after atorvastatin treatment in healthy subjects, possibly because of the different study population and drug dosage.[42]

6 Ang-(1-7) and atherosclerotic plaque

Ang-(1-7) has been shown to inhibit the development of atherosclerotic plaque in many previous studies. Four-week Ang-(1-7) treatment suppressed the formation of atherosclerotic lesion in $ApoE^{-/-}$ mice, and increased the endothelium-dependent vaso-relaxation with decreased superoxide production and increased endothelial nitric oxide synthase.[37] Furthermore, Yang, *et al*.[43] showed that Ang-(1-7) enhanced plaque stability with high contents of collagen, and low contents of macrophages and lipids. In addition, Ang-(1-7) lowered the expression levels of pro-inflammatory cytokines and activities of matrix metalloproteinases in atherosclerotic lesions, stabilizing the plaques.[43] Another study also showed that treatment of Ang-(1-7) lead to decreased shear stress-induced carotid plaques, and reduced neutrophil and macrophage infiltration.[44] Interestingly, a study found that treatment of Ang-(1-7) and losartan were equivalent in the reduction of atherosclerotic plaque formation in $ApoE^{-/-}$ mice. Moreover, combination of Ang-(1-7) and losartan together enhanced the anti-atherosclerotic effects.[45]

Recently, the use of genetic modified animals has further demonstrated the role of Ang-(1-7) in atherosclerosis. ACE2 knockout mice showed an increase of atherosclerotic plaque formation. In the ACE2 knockout mice, the study observed more Ang-II and other inflammatory cytokines produced by macrophages, and adherence of more macrophages onto the layer of vascular endothelial cells, resulting in the development of atherosclerosis.[46] $ApoE^{-/-}$ mice that received ACE2 gene transfer showed a significant reduction of atherosclerotic plaque with decreased level of VCAM-1, monocyte chemotactic protein 1 (MCP-1) and interleukin 6 (IL-6).[47] Another study also showed that ACE2 over-expression in rabbits lead to stability of atherosclerotic plaque—fewer macrophages, less lipid deposition and more collagen contents with decreased Ang-II levels and increased Ang-(1-7) levels.[48] Further study showed that MAS knockout mice had an increased ratio of intima to media in the aorta in organ culture media after five weeks, suggesting the involvement of the MAS receptor in the suppression of intima proliferation.[49]

Furthermore, the utility of Ang-(1-7) agonist and antagonist also indicated the protective role of Ang-(1-7) in atherosclerosis. $ApoE^{-/-}$ mice that treated with Ang-(1-7) receptor agonist AVE0991 showed only half areas of atherosclerotic lesion compared to the control group.[50] Further study showed that NADPH oxidase expression, as well as macrophages and activated $CD4^+$ T cells, were decreased in AVE0991 treated mice.[51] In addition, inflammatory indicators such as MCP-1, IL-6 and IL-12 were diminished by AVE0991 in $ApoE^{-/-}$ mice.[52] AVE0991 also effectively attenuated Ang-II production, well known inducer of atherosclerosis, in the aortas of $ApoE^{-/-}$ mice compared to wild type animals.[53] Blocking of endogenous Ang-(1-7) with Ang-(1-7) antagonist, A779, decreased the plaque stability by increasing the lipid contents and reducing collagens in $ApoE^{-/-}$ mice, indicating that endogenous activated Ang-(1-7) was also involved in the protection against atherosclerosis.[54]

7 Ang-(1-7) and therapeutic potentials

Although most research concerning the role of Ang-(1-7)

in atherosclerosis remains at an animal experimental stage, more and more studies focused on human studies. Recent studies have found that Ang-(1-7) is involved in the neovascularization and inflammation in human. ACE2 and MAS receptor expressions reduced by 69% and 58% respectively in patients with aortic valve stenosis compared those with normal control valves. Interestingly, ACE2 was expressed in the macrophages and fibroblasts around neovessels in stenotic aortic valves.[55] Another study found that Ang-(1-7) may be associated with the protective effect of ARB drug. Serum levels of Ang-(1-7) were significantly increased, and Ang-II-induced vasoconstriction was reduced from 50% to 15% in the patients that had taken irbesartan for 30 days, suggesting the possible role of Ang-(1-7) in the protective mechanism of ARB drug.[42]

Although Ang-(1-7) has the ability to protect against atherosclerosis, the characteristics of metabolism of Ang-(1-7) have limited its oral pharmaceutical formulation in clinical utility. Ang-(1-7) can be rapidly cleaved by peptidase with half-life for just 10 s. In addition, Ang-(1-7) is easily degraded in gastrointestinal tract when orally administrated. Therefore, the clinical pharmacotherapy of Ang- (1-7) has encountered some problems. However, the recent research has found several possible ways to implement the clinical application of Ang-(1-7). Firstly, inclusion of Ang-(1-7) into the oligosaccharide hydroxypropyl-β-cyclodextrin (HPβCD) cavity can enhance stability, protect against digestive enzyme, and increase absorption of Ang-(1-7) across biological barriers. One study has found that oral intake of β-cyclodextrin/Ang-(1-7) effectively increased serum concentration of Ang-(1-7), and inhibited thrombosis formation in spontaneous hypertensive rats.[25] Marques, *et al*,[56] found that administration of β-cyclodextrin/Ang-(1-7) suppressed isoproterenol induced myocardial damage, remodeling and infarction in rats. Another study also showed that chronic treatment of β-cyclodextrin/Ang-(1-7) decreased blood pressure and heart rate, improved heart function, attenuated sympathetic modulation on heart and vessels, and enhanced parasympathetic modulation in spontaneously hypertensive rats, which were also observed in exercise-trained rats.[57] Secondly, Ang-(1-7) could be stabilized by introducing thioether bridges in plasmid-encoded prenisin-modification enzymes, which enhanced resistance to breakdown by protease and increased bioavailability of Ang-(1-7).[58] A recent study has shown that the thioether-bridged Ang-(1-7) increased relaxing activity on pre-contracted rat aorta rings by two times compared to natural counterpart of Ang-(1-7).[59] Thioether-bridged Ang-(1-7) also restored increased heart weight and myocyte size caused by myocardial infarction to sham levels. levels. Endothelial function was also improved after treatment of thioether-bridged Ang-(1-7) in rats with myocardial infarction.[60] Thirdly, Ang-(1-7), which is contained in liposome, has the ability to protect against degradation and maintain a sustained release.[61] The treatment of liposome-entrapped Ang-(1-7) resulted in attenuation of circadian variation of blood pressure and heart rate that lasted for several days.[62]

8 Conclusions

There is growing evidence that Ang-(1-7) plays an important role in protecting against atherosclerosis through regulating the function of smooth muscle cells and endothelial cells, and modulating lipid metabolism. The appearance of oral pharmaceutical formulation of Ang-(1-7) has brought great potential for atherosclerosis treatment. Ang-(1-7), the new member of RAS, is a new therapy option that merits further development and exploration.

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