Title Page:

Targeting the Protective Arm of the Renin-Angiotensin System: Focused on Angiotensin-(1-7)

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Running Title Page:

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  Introduction: 833

Abbreviations
ACCA: cis-3-(Aminomethyl)cyclobutanecarboxylic acid
ACE: Angiotensin converting enzyme
ACEI: Angiotensin converting enzyme inhibitor
ARB: Angiotensin receptor blocker
AT1R: Angiotensin II type 1 receptor
AT2R: Angiotensin II type 2 receptor
BAL: Broncho alveolar lavage
β-CD: Beta-cyclodextrin
CTB: Cholera non-toxic B subunit
LP: Lactobacillus paracasei
MI: Myocardial infarction
MrgD: Mas related G-protein–coupled receptor
PMAM-OH: Hydroxyl-terminated poly(amidoamine)
PRR: (Pro)renin receptor
RAS: Renin-Angiotensin System
RVLM: Rostral ventrolateral medulla

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Abstract

The in vivo application and efficacy of many therapeutic peptides is limited due to their instability and proteolytic degradation. Novel strategies for developing therapeutic peptides with higher stability toward proteolytic degradation would be extremely valuable. Such approaches could improve systemic bioavailability and enhance therapeutic effects. The renin-angiotensin system (RAS) is a hormonal system within the body essential for the regulation of blood pressure and fluid balance. The RAS is composed of two opposing classical and protective arms. The balance between these two arms is critical for the homeostasis of the body's physiological function. Activation of the RAS results in the suppression of its protective arm that has been reported in inflammatory and pathological conditions such as arthritis, cardiovascular diseases, diabetes, and cancer. Clinical application of the Angiotensin-(1-7) (Ang-(1-7)), a RAS critical regulatory peptide, augments the protective arm and restores balance hampered by its enzymatic and chemical instability. Several attempts to increase the half-life and efficacy of this heptapeptide using more stable analogs and different drug delivery approaches have been made. This review article provides an overview of efforts targeting the RAS protective arm. It provides a critical analysis of Ang-(1-7) or its homologs' novel drug delivery systems using different administration routes, their pharmacological characterization, and therapeutic potential in various clinical settings.

Significance Statement: Ang-(1-7) is a unique peptide component of the RAS system with vast potential for clinical applications that modulate various inflammatory diseases. Novel Ang-(1-7) peptide drug delivery could compensate its lack of stability for effective clinical application.
1 Introduction

The renin-angiotensin (Ang) system (RAS) regulates multiple tissue and organ functions by maintaining blood pressure, body fluid, and electrolyte balance homeostasis (Carey and Padia, 2018). The RAS is a significant contributor to vascular, cardiac, renal, liver, gastrointestinal (GI), reproductive, respiratory, and musculoskeletal system physiology. Its pathophysiological effects include inflammation and fibrosis that link the RAS to the initiation, development, and progression of several diseases (Ko and Bakris, 2018).

The RAS has two opposing arms: the classical arm composed of ACE (angiotensin-converting enzyme) /Ang II /AT1R (Ang II type 1 receptor) and the protective arm composed of ACE2 /Ang-(1-7) /MasR (Mas receptor) (Figure 1). As one of the components of the RAS, renin is released primarily by the kidneys and breaks down angiotensinogen to form Ang I (Wu et al., 2019), constituting the harmful, proinflammatory actions of Ang II mediated by AT1R. In healthy individuals, the two arms maintain a dynamic balance (South et al., 2019). However, in the activated RAS, the balance shifts toward the potentiation of the classical arm (Santos et al., 2019).

The RAS major vasoactive effector peptide, Ang II, is produced through enzymatic reactions of peptidases, mainly ACE from Ang I in plasma and various tissues (Turner and Hooper, 2002). Vasoconstriction, cardiac hypertrophy and remodeling, inflammation, and fibrosis may result from this conversion either directly through action on the AT1R or indirectly through aldosterone stimulation (Hahn et al., 1993). Ang II is additionally associated with the development, proliferation, and metastasis of several cancers (Penafuerte et al., 2016; Pei et al., 2017; Ekambaram et al., 2018). The pathophysiological mechanisms of the RAS are attributed to an Ang II-dependent increase of nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase activity via the AT1R in endothelial and vascular smooth muscle cells (VSMCs) (Zafari et al., 1998), stimulating reactive oxygen (ROS) and nitrogen species (RNS) formation (Lacolley et al.,
Many signaling pathways (e.g., superoxide and H$_2$O$_2$) may be activated by ROS products and subsequently trigger mitogen-activated protein kinases (MAPK), tyrosine kinases, phosphatases, calcium channels, and redox-sensitive transcription factors (Montezano and Touyz, 2014), which result in cell growth and proinflammatory gene expression.

Ang II can also be generated from non-ACE-related enzymes (Wei et al., 2002) through the chymase pathway (Figure 1). This pathway forms Ang-(1-12) from angiotensinogen using the chymase enzyme. Extremely efficient, this enzyme’s protease action has been implicated in many human diseases. Previously considered a mast cell product, chymase is produced and distributed in many organs, including the heart (Dell’Italia et al., 2018).

The generation of Ang II from Ang I has long been considered the final product of the RAS biological cascade. However, recent studies have demonstrated that there are other Ang peptides in the RAS that may either contribute to or oppose the actions of Ang II, giving this system greater flexibility than initially thought (Arendse et al., 2019).

Scientists recently discovered another arm of the RAS. Composed of ACE2/Ang-(1-7)/MasR, the protective arm regulates many body functions, including the cardiovascular (CV), central nervous system (CNS), GI, musculoskeletal, and immune systems.

ACE2, a homolog of ACE, was discovered two decades ago (Tipnis et al., 2000; Douglas et al., 2004). ACE2 functions as a carboxypeptidase, a major enzyme involved in the conversion of Ang II to Ang-(1-7). In marked contrast to ACE, ACE2 does not convert Ang I to Ang II, and its enzyme activity is not blocked by ACE inhibitors. Thus, ACE2 effectively inhibits Ang II formation by stimulating alternate pathways for Ang I and, particularly, Ang II degradation (Dell’Italia et al., 2018).

MasR, a specific receptor of Ang-(1-7), is a member of the G protein-coupled receptors (GPCRs) family (Solinski et al., 2014). As the research evolves (Jackson et al., 1988), scientists have determined that Ang II is not a MasR ligand (Dong et al., 2001) and that the Mas proto-
oncogene, which codifies MasR, has less tumorigenic properties than initially believed (Kostenis et al., 2005).

Like the RAS’s protective arm, the classical arm can also have beneficial effects through the action of Ang II on AT_2R. When stimulated, AT_2R, a functional antagonist of AT_1R, can promote vasodilation, anti-proliferation, anti-inflammatory, and anti-fibrotic. Although some controversy exists regarding its beneficial role, new studies suggest a protective role for AT_2R activation, especially in renal diseases and injuries (Hashimoto et al., 2004; Hakam and Hussain, 2005). An interesting therapeutic target, the RAS has been studied for several decades. Examining its classical arm has resulted in Ang converting enzyme inhibitors (ACEIs) and Ang II receptor blockers (ARBs) discoveries. The accumulating evidence proves that Ang-(1-7), the main component of the protective arm, counteracts the actions of Ang II (Santos et al., 2000). Due to Ang-(1-7’s) rapid systemic clearance, its short half-life (3-15min) restricts its potential therapeutic benefits. Structural modifications or appropriate drug delivery systems that extend its circulation half-life would enhance the therapeutic application of peptide drugs in general and Ang-(1-7) in specific (Chappell et al., 1998). This review updates the RAS conceptual development and discusses different Ang-(1-7) structural modifications and delivery approaches.

2 Targeting the RAS Classical Arm

The balance between the RAS’s two arms is critical to the body’s homeostasis with imbalance resulting in complications such as CV, renal (Fraune et al., 2012), and pulmonary disease, diabetes, neuropathic pain (Smith and Muralidharan, 2015), Alzheimer’s disease (Ohrui et al., 2004), and cancer (Guo et al., 2015). The ability to suppress the classical RAS response is a significant therapeutic advancement. Targeting ACE with ACEIs is an effective way to inhibit the conversion of Ang I to Ang II and inhibit the downstream effects of Ang II on AT_1R. ACEIs are used for different indications such as hypertension, acute coronary syndrome, aldosteronism,
and Raynaud's phenomenon (Antman et al., 2004). Another means of blocking Ang II from acting on AT1R is the use of ARBs (Li et al., 2010). Originally used to control hypertension, both ACEIs and ARBs can modulate other complications such as diabetic nephropathy and decrease rates of renal disease, heart failure, and cognitive decline (Ohrui et al., 2004; Rozzini et al., 2006). An alternative approach for targeting and attenuating the activated classical RAS is suppressing AT1R gene expression. Guo et al. used miR-410, a microRNA, to silence the gene and suppress AT1R expression. This mitigated the receptor's downstream cascade events, resulting in inhibition of pancreatic cancer cell growth and invasion in both in vitro and in vivo studies (Guo et al., 2015).

Renal injury through AT1R, CV remodeling, tumor progression, and cancer proliferation are all undesirable Ang II outcomes (Cambados et al., 2017; Pei et al., 2017). However, beneficial results should not be overlooked. Ang II is a ligand for both AT1R and AT2R, and its binding to these receptors results in opposing effects (AbdAlla et al., 2001; Padia and Carey, 2013). Ang II activity is not the focus of this review article. Gasparo (2000) and Miura (2010) provide excellent detail on Ang II receptors activation (De Gasparo et al., 2000; Miura et al., 2010). Also, there are several peptides and non-peptide compounds that are under development for targeting AT2R and have been discussed previously (Unger et al., 2015).

3 Targeting the RAS Protective Arm

Ang-(1-7) is a biologically active heptapeptide that is mostly formed through the direct action of ACE2 on Ang II (Crackower et al., 2002). In 2000, Santos (Santos et al., 2000) reported that Ang-(1-7) interacts with putative receptors such as the losartan-sensitive receptor, PD sensitive receptor (PD123319, an AT2R antagonist), and AT1-R. Santos further concluded that AT1R is not a target receptor for this peptide. In 2003, the same group reported using A-779 (D-Ala7-Ang-(1-7), an Ang-(1-7) antagonist, to identify the G protein-coupled receptor Mas as a specific receptor (Santos et al., 2003).
The physiological repercussions of MasR can be illustrated by experiments with MasR-deficient mice. Completely lacking the antidiuretic action of Ang-(1-7), the animals' aortas lost the Ang-(1-7)-induced relaxation response following an acute water load (Santos et al., 2003). Activation of MasR induces the phosphatidylinositol 3-kinase/Akt pathway and this, in turn, activates the endothelial Nitric oxide (NO) synthase and the consequent NO release causes vasodilation (Sampaio et al., 2007). Ang-(1-7) (Ferreira et al., 2007; Santos and Ferreira, 2007) exerts its action by counteracting the effects of Ang II (Schiavone et al., 1988; Santos et al., 2000). Evidence suggests that Ang-(1-7) is also involved in blocking and directly interacting with AT1R (Garcia and Garvin, 1994), diminishing its functional regulatory effects (Raizada and Ferreira, 2007).

When synthesized in the kidney, Ang-(1-7), through MasR, increases the glomerular filtration rate (GFR), inhibits Na/K/ATPase, and leads to vasorelaxation, natriuresis, diuresis, and downregulation of AT1R, opposing the action of Ang II on this receptor (Zimmerman and Burns, 2012). Many studies have investigated the effects of Ang-(1-7) on heart conditions and cardiac tissue localization (Ferreira and Santos, 2005). Ang-(1-7) contrasts vasoconstrictive, proliferative, hypertrophic, and proinflammatory actions of Ang II in the CV system (Passos-Silva et al., 2015; e Silva and Teixeira, 2016; Machado-Silva et al., 2016; Villalobos et al., 2016). The benefits of Ang-(1-7), especially in the endocrine and musculoskeletal systems, kidneys, and lungs, are well established. Santos et al. provided a detailed review of Ang-(1-7)'s benefits to the body (Santos et al., 2018).

An effective approach for reversing the imbalance between the classical and protective arms of the activated RAS is to increase the Ang-(1-7) peptide concentration. Wysocki et al. reported targeting the protective arm using recombinant ACE2 (rACE2). This led to increased systemic ACE2 activity that consequently lowered plasma levels of its substrate, Ang II, by enhancing its biotransformation to Ang-(1-7) (Wysocki et al., 2010).
The heptapeptide's short half-life makes its delivery impractical for the effective augmentation to reestablish RAS balance levels. Feasible approaches to address this issue include using Ang-(1-7) precursors and/or protecting the peptide bonds prone to degradation. Several studies have focused on the development of peptide and non-peptide agonists to simulate the protective actions of Ang-(1-7) through MasR activation. The following sections discuss Ang-(1-7) analogs and their clinical use.

3.1.1 Ang-(1-7) analog, A-1317

Different groups have worked to identify Ang-(1-7) analogs and MasR agonists that are less prone to degradation and hold the possibility of therapeutic application. Barbosa et al., for example, tested an analog of Ang-(1-7) in a diabetic mouse model to determine if the analog showed similar benefits as Ang-(1-7). This compound was engineered by adding L-arginine amino acid to the Ang-(1-7) sequence and administering it orally to hypertensive rats to determine its effect on liver glucose metabolism (Barbosa et al., 2020). The results indicated that an A-1317 oral treatment had similar efficacy to reducing hypertension as Ang-(1-7); however, it was more efficient at improving β-cell functionality and reducing body mass gain and liver damage. Additional data on A-1317 pharmacokinetics and characteristics are required before a comparison to the parent Ang-(1-7) can be made.

3.1.2 MasR agonist, AVE 0991

AVE 0991 (Figure 2) is an orally active non-peptide analog of the Ang-(1-7) peptide and MasR agonist that mimics Ang-(1-7) effects in different organs (Wiemer et al., 2002). This compound has different in vitro and in vivo effects and can resist proteolytic enzymes found in the GI tract. Similar to Ang-(1-7), AVE 0991 results in up to 5 times more NO release than Ang-(1-7); however, it reduced superoxide production in different cell lines and kidney slices (Santos and Ferreira, 2006). The effect of this compound can be selectively blocked by A-779 (Santos et al., 1994). Tested primarily on the heart and CV system, Ferreira et al. demonstrated that it attenuates MI-
induced heart failure (Ferreira et al., 2007). In mouse models, AVE 0991 produces antidiuretic
effects that could prevent end-organ damage and morphological changes to the heart, kidney,
and mesenteric vessels. In rats, it could improve endothelial function through MasR and NO
synthesis (Santos and Ferreira, 2006). Ample studies suggest that compounds capable of
mimicking the Ang-(1-7) peptide can exert their actions through the ACE2/Ang-(1-7)/MasR axis
and may constitute a new class of drugs for the treatment of CV and related diseases. A likely
candidate, AVE 0991, is the first synthetic physiologically safe and effective compound.

3.1.3 MrgD agonist, Alamandine

Etelvino et al. recently introduced alamandine [Ala–Arg–Val–Tyr–Ile–His–Pro], a RAS
component that forms through either of two pathways including Ang A hydrolysis by ACE2 at the
C-terminal or decarboxylation of Aspartate at Ang-(1-7's) N-terminal (Etelvino et al., 2014).
Based on functional data, alamandine interacts with the MrgD (Mas-related G protein-coupled
receptor D) (Figure 3) and exerts CV protective effects (Li et al., 2018; Liu et al., 2018; Santos
et al., 2019) through various mechanisms such as blocking the p38 MAPK signaling pathway
(Yang et al., 2020) and attenuating cardiac dysfunction and fibrosis induced by chronic
hypertension (Wang et al., 2019). Alamandine is effective in reversing hyperhomocysteinemia-
induced vascular dysfunction (Qaradakhi et al., 2017). Alamandine's action resembles Ang-(1-7)
in the rostral and caudal ventrolateral medulla, and hypothalamus (Shen et al., 2018). Similar to
Ang-(1-7) and Ang II actions through the MasR and AT2R, these observations suggest that
alamandine may act as a neuronal excitatory molecule in the brain (Marins et al., 2014).
Activation of MrgD by almandine leads to NO release using a different mechanism than Ang-(1-
7) by targeting AMP-activated protein kinase (AMPK), primarily to induce NO formation (Figure
3) (Jesus et al., 2018).
4 Ang-(1-7) novel drug delivery

The key points on various approaches to targeting the RAS protective arm are summarized in Table 1 and elaborated in detail in the following sections.

4.1 Application of Ang-(1-7) complex formulation for various diseases

4.1.1 Cardiovascular disorders and diabetes

Myocardial infarction generates cellular events that begin with an inflammatory response and are followed by fibrogenic pathway activation needed for cardiac tissue remodeling (Sutton and Sharpe, 2000). Ang-(1-7) has the potential to act as a cardioprotective agent due to its anti-inflammatory and anti-fibrotic properties in cardiac ischemia (Rodrigues Prestes et al., 2017). Tijsma EJ et al. introduced an Ang-(1-7) eluting polymer-coated medical stent to reduce restenosis and improve endothelial cell function. Using different concentrations of peptide-to-polymer ratios, these vascular stents have improved vascular endothelial cell function by successfully inhibiting smooth muscle cell proliferation and restenosis at the vascular injury site. This patented invention is an open-ended cylindrical insert that has both the outer and inner surfaces coated with a controlled-release matrix comprised of an amphiphilic copolymer and an Ang-(1-7) receptor agonist. The surfaces are engineered to release the Ang-(1-7) receptor agonist as the stent is inserted into mammalian tissue. The first-order release of Ang-(1-7) agonist inhibits post-stent implantation restenosis and improves vascular endothelial functioning (Tijsma et al., 2007).

In another study, liposomal Ang-(1-7) (LAng) was evaluated for its sustained release potential in the rostral ventrolateral medulla (RVLM), an area of the brain that controls blood pressure. Ang-(1-7) was selected for both its CV system effects and pathophysiological role as a neuromodulator. Small unilamellar vesicles (SUVs) were fabricated with and without Ang-(1-7) and were injected into rats' RVLM. Although the authors claimed that LAng had a long-lasting effect, outcome data were not reported, nor was LAng compared to the pure peptide. However,
the effects of LAng on blood pressure (MAP) and heart rate were evaluated, indicating a significant pressor impact during the day (5 days duration) and bradycardia (3 days duration) during the night. These benefits were not observed following vehicle microinjection. The data proves that this novel technique can be utilized in chronic conditions and reveals a new physiological role (i.e., modulation of the circadian rhythms of mean arterial pressure (MAP) and heart rate) for Ang-(1-7) at the RVLM (Silva-Barcellos et al., 2001).

In an innovative delivery approach, Lula et al. formed and characterized an inclusion of Ang-(1-7) with β-cyclodextrin (βCD/Ang-(1-7)) using a freeze-drying method (Lula et al., 2007). Santos et al. tested the oral administration of βCD/Ang-(1-7) on type 2 diabetes mellitus. The data suggested that βCD/Ang-(1-7) prevented and modulated hyperglycemia, making it a novel therapeutic option for the treatment of type 2 diabetes. This observation was attributed to the modulation of insulin signaling and better glucose uptake, especially in tissues with insulin signaling targets such as adipose tissue and skeletal muscle (Santos et al., 2014).

Furthermore, Ang-(1-7) enclosed in hydroxypropyl β-cyclodextrin (HPβCD/Ang-(1-7)) also has been tested in an experimental myocardial infarction (MI) rat model as a mean of reversing cardiac tissue dysregulation after oral administration (Marques et al., 2011). HPβCD/Ang-(1-7) treatment improved the rats' post-infarction condition by triggering an intense anti-inflammatory response and modulating proteins linked with inflammation and mitochondrial dysfunction. This study was the first to demonstrate that Ang-(1-7) treatment following experimental MI significantly downregulated the C-X-C chemokine receptor type 4 (CXCR4) (Marques et al., 2012). In a similar study, using an oral HPβCD/Ang-(1-7), the researchers confirmed the findings of the previous group (Gómez-Mendoza et al., 2019). However, none of these investigators addressed the peptide half-life alteration following oral administration.

4.1.2 Musculoskeletal disease

A new strategy for degenerative diseases and damaged bone tissue include autografts, allografts, xenografts, and artificial materials (metals and bioceramics). Fabricating scaffolds for
meeting the needs of specific repair sites is challenging due to bone tissue's complexity and property variability. Macedo et al. combined beta-tricalcium phosphate-hydroxyapatite bioceramic, polycaprolactone and Ang-(1-7) composite to form a porous 3-D biodegradable scaffold using "solvent casting and particulate leaching" methods (SC/PL). This innovative combination joins a mechanical anchor for osteoblastic cells and a more favorable surface for cell attachment (BCP) with a semi-crystalline, bioresorbable polymer and is well known for its slow degradation rate (PCL) with the Ang-(1-7) composite. This promising method provides a porous structure suitable for bone structure. Incorporating Ang-(1-7) in the bone structure may counteract Ang II and eventually decrease interleukin-6 (IL-6) levels that act as a bone-resorbing factor and also induce osteoclast formation that stimulates bone resorption. Viability, in vitro, demonstrates that the scaffolds may hold promise as a drug delivery system (Macedo et al., 2012).

A non-cytotoxic hydroxyl-terminated poly(amidoamine) (PAMAM-OH) dendrimer has been used as an Ang-(1-7) carrier, (Ang-(1-7)/PAMAM-OH), by Marquez-Miranda et al. (Márquez-Miranda et al., 2017). Molecular dynamics simulation data suggests this dendrimer could protect Ang-(1-7) and form a stable complex due to retarding peptide mobility. When administered intraperitoneally, the Ang-(1-7) dendrimer but not plain Ang-(1-7) could demonstrate anti-atrophic properties in skeletal muscle tissue. In vivo toxicity studies showed no significant toxic effect on male mice. The authors attribute the improvement of anti-atrophic effects to the advancement of the half-life or kinetic release of Ang-(1-7) peptide from the PAMAM-OH dendrimer complex.

In line with the discussed Ang-(1-7) advanced drug delivery studies, our group has developed a novel bone-targeting Ang-(1-7) conjugate (Ang Conj.), which presents with a more than 10-fold longer half-life. Upon administration, Ang Conj. loads on the bone and releases the active peptide in a sustained manner. We have studied the pharmacodynamic effects of Ang Conj. on
different cell lines and animal models of cancer and osteoarthritis. A patent application has been submitted, and a detailed manuscript is being reviewed for publication (Habashi et al., 2020).

4.1.3 Pulmonary disease

Investigations showed Ang-(1-7) ’s role in the prevention of chronic allergic lung inflammation. Resolving eosinophilic inflammation in an asthmatic model characterized by inflammation, pulmonary remodeling, and bronchial hyperresponsiveness were achieved after the inclusion of Ang-(1-7) in hydroxypropyl β-cyclodextrin (HPβ-CD) administered by inhalation. Pulmonary remodeling in a murine model of ovalbumin (OVA)-induced chronic allergic lung inflammation demonstrated that Ang-(1-7) could reduce eosinophils in the lung. Treating OVA-sensitized mice with Ang-(1-7) by inhalation reversed pulmonary remodeling by reducing collagen, Matrix metallopeptidase (MMP)-9, MMP-12, and alpha-smooth Muscle Actin (α-SMA). The inflammatory response and the deposition of fibrotic factors induced by the OVA-challenge were attenuated by the Ang-(1-7) inhaled formulation (Magalhães et al., 2018).

Using oral HPβCD/Ang-(1-7) combination, Bastos et al. studied the systemic and pulmonary effect of orally administered Ang-(1-7) on pulmonary emphysema. Their data suggest a significant improvement in alveolar vascularity, cytokine modulation, and alveolar space (Bastos et al., 2020).

4.2 Structurally modified homologs of Ang-(1-7)

Some researchers have studied structurally modified Ang-(1-7) in different disease states to determine whether these structural changes could alter the peptide's pharmacokinetics and pharmacodynamics.

The beneficial effects of Ang-(1-7) as a tissue-protective peptide has been shown in different studies (Santos et al., 2018). Recent scientific research indicates that the progression of many neurodegenerative diseases results from vascular influences that contribute to cognitive impairment and dementia (VCID). Alzheimer's disease and related dementia result from
decreased brain blood flow, increased production of reactive oxygen species (ROS), and proinflammatory mechanisms (Pavol et al., 2018). Hay et al. (Hay et al., 2019) tested their novel glycosylated Ang-(1-7) peptide Ang-1-6-O-Ser-Glc-NH2 (PNA5) as a therapy to treat VCID on a mouse model of VCID and heart failure (HF) (VCID/HF). This compound is the analog of an Ang-(1-7) peptide where proline is substituted with serine and a β-D-glucose bound to serine side methyl. PNA5 showed greater brain penetration and higher stability and bioavailability when compared with the native Ang-(1-7) peptide. Improved spatial memory and ROS inhibition were also observed due to its preserved MasR activation activity. Decreased VCID/HF-induced activation of brain microglia/macrophages could dramatically reduce circulating tumor necrosis factor α (TNF-α), interleukin (IL)-7, and granulocyte cell-stimulating factor serum levels (Jiang et al., 2014; Hay et al., 2017).

Native Ang-(1-7) has multiple therapeutic effects but is susceptible to degradation by ACE and other peptidases. This susceptibility is primarily reduced by lanthionine-stabilized Ang-(1-7) (cAng-(1-7)) (Figure 4), which has been shown to be fully resistant to ACE as well as other peptidases (Figure 4) (Kluskens et al., 2009). The interaction of Ang-(1-7) with ACE is thought to occur in the C domain, which contains a C-terminal proline residue (Patchett and Cordes, 1985). In cAng-(1-7), this residue was replaced by a thioether-bridged amino acid, which reduced its affinity for the binding site by cyclization.

Kuipers A et al. tested cAng-(1-7) in the streptozotocin-induced mice model of diabetes and compared it to the vehicle (saline). In the type 1 diabetes model, cAng-(1-7) increased the insulin level, and in the type 2 diabetes model, it generated a 55% increase in the insulin level in week eight and reduced glycated hemoglobin levels. cAng-(1-7) reduced blood glucose levels in both type 1 and 2 diabetes and after an oral glucose tolerance test in type 2 diabetes. These findings are consistent with cAng-(1-7) therapeutic potentials for both type 1 and 2 diabetes (Kuipers et al., 2019).
In another study, de Vries et al. investigated cyclized thioether bridge Ang-(1-7) compound delivery through both oral and pulmonary administration. Stability data shows that the bridge was stable at pH 2.0 and demonstrated increased resistance to breakdown by pancreatic proteases at pH 7.4. Additionally, it was resistant to liver protease breakdown at the lysosomal pH 5.0. Their findings show that the thioether stabilized Ang-(1-7) can be directly delivered orally and via the pulmonary route. Systemic drug absorption, on the other hand, was maximized with subcutaneous administration. All three delivery methods generated therapeutic plasma concentrations. This observation indicates that including a thioether bridge in the peptide structures can open up a new delivery method for medically important and promising peptides (de Vries et al., 2010).

Cassis P et al. investigated the impact of cAng-(1-7) in BTBR ob/ob mice suffering from type 2 diabetic nephropathy. The BTBR ob/ob mice strain received either the cAng-(1-7) vehicle or the ACE inhibitor, lisinopril. cAng-(1-7) limited albuminuria progression and also reduced podocyte dysfunction (this result was similar for lisinopril). But unlike lisinopril, cAng-(1-7) reduced glomerular fibrosis and inflammation and improved glomerular capillary rarefaction. Furthermore, the combination of cAng-(1-7) with lisinopril produced a superior anti-proteinuric effect compared to lisinopril alone due to better preservation of podocyte proteins and capillary density amelioration. Adding cAng-(1-7) to ACEI therapy could benefit diabetics who have responded unsatisfactorily to ACEI therapy (Cassis et al., 2019).

Ma X. et al. used an alternative Ang-(1-7) analog peptide with N- and C-terminals protected by acetylation and amination (Ang-AA). Evaluated in mice, Ang-AA pharmacokinetics and toxicity results suggest that amination and acetylation significantly reduced Ang-(1-7) hydrolysis in vitro and in vivo. Ang-(1-7) half-life in rats increased from 2.4 ± 0.6 min to 238.7 ± 61.3 min. The specific binding of Ang-AA to the MasR was well preserved; Ang-AA had greater inhibitory effects on proliferation, migration, and invasion in the A549 cell line than Ang-(1-7). Acetylation
and amination appear as a simple and effective method to produce a bioactive peptide of Ang-(1-7) (Ma et al., 2018).

Wester et al. reported three novel Ang-(1-7) analogs assembled by substitution of a cyclic non-natural δ-amino acid, cis-3(aminomethyl) cyclobutane carboxylic acid (ACCA), at the cleavage site of ACE and dipeptidyl peptidase 3 (DPP 3) enzymes. ACCA substitution used solid-phase peptide synthesis on Ile5, His6, and Val3 positions, and named ACCA 1, 2, and 3, respectively. ACCA 1 and 2 showed complete resistance to ACE, and ACCA analog 1 was resistant to DPP 3 hydrolysis. All analogs had preserved activity against breast cancer and fibrosarcoma cells (Figure 3) (Wester et al., 2017).

Diabetes is a disorder that can delay wound repair, which may result in colonized chronic wounds (Rodgers et al., 2011). Diabetic patients may experience a 25% incidence of foot ulcers (DFUs) during their lifetime, which increases the risk of morbidity, osteomyelitis, and amputations. DFUs are treated via strict offloading, bandaging, and debridement treatments that dramatically increase healthcare costs. The only approved product to treat diabetic ulcers, Regranex® (0.01% becaplermin gel), has limited efficacy and severe side effects, and is not widely prescribed. DSC127, a relatively new topical treatment that hastens to heal and may increase the proportion of fully healed DFUs, is in Phase III clinical trials (NCT01830348 and NCT01849965) (Balingit et al., 2012). Aclerastide (AKADSC127) with the active ingredient NorLeu3-Ang-(1-7) is an analog of Ang-(1-7) that induces proliferation, accelerates vascularization, collagen deposition, and re-epithelialization. Preclinical and clinical research indicates DSC127 is highly effective in treating diabetic wounds and generates better results than commercially available treatments for DFU (Rodgers et al., 2003; Rodgers et al., 2005; Rodgers et al., 2015).

4.3 Bio expressing Ang-(1-7)

Recently, Carter et al. studied the effect of multiple doses of an oral formulation of modified Ang-(1-7)-expressing probiotic bacteria Lactobacillus paracasei (LP) (LP-A) on rat gut-brain axis
physiologic parameters. The method was also compared with the subcutaneous delivery of synthetic Ang-(1-7) peptide on increasing circulating Ang-(1-7) concentrations. Construction of the recombinant probiotics secreting Ang-(1-7) was performed using plasmid as a backbone with an Ang-(1-7)-expression vector. The plasmid was then electroporated into Lactobacillus paracasei, and after incubation, bacteria were harvested, washed, and aliquoted for further use (Carter et al., 2020). After three weeks of either LP-A or S.C. Ang-(1-7) administration, data showed that LP-A statistically increased the circulating Ang-(1-7) and decreased Ang II. Results also demonstrated that three weeks of dosing was the most efficacious regimen to be used for further preclinical studies. Despite the beneficial effects of LP-A, the research group did not compare the impact of LP-A with LP alone. LP-A could induce beneficial changes in all dosing regimens in the fecal microbiome, including overall microbiota community structure and α-diversity. LP-A also significantly reduced neuro-inflammatory gene expression in the prefrontal cortex. Subcutaneous delivery of Ang-(1-7) increased circulating Ang-(1-7) and reduced Ang II, but most gut-brain parameters remained unchanged. Oral but not subcutaneous Ang-(1-7) altered the physiologic parameters related to the gut-brain axis, with the majority of effects observed in the 3×/week oral dosing regimen in older rats (Buford et al., 2020).

In line with the approach of enhancing the systemic and local activity of the protective RAS, Shil et al. bioencapsulated Ang-(1-7) in plant cells and tested its efficacy on ocular inflammation as a highly efficient and cost-effective approach. The nucleotide sequences of Ang-(1-7) and ACE2 were fused with the cholera nontoxic B subunit (CTB) gene and then cloned into the chloroplast transformation vector to express CTB-ACE2-Ang-(1-7) complex. The lyophilized plant cells, containing the therapeutic proteins complex formulated in the capsule, have been administered orally to mice. In the mouse GI tract, plant cells were consumed by commensal bacteria as an energy source resulting in the release of the bioencapsulated ACE2/Ang-(1-7) in the intestine. Consequently, the transmucosal carrier, CTB, binds to the intestinal epithelium and internalizes the CTB-fused proteins to deliver ACE2/Ang-(1-7) into blood circulation. Overall, using this
method, this group observed an increased level of ACE2/Ang-(1-7) in circulation and retina, which effectively reduced retinal inflammation in an animal model of endotoxin-induced uveitis (Shil et al., 2014).

Utilizing the same approach, other groups tested the oral CTB-fused Ang-(1-7) on pulmonary hypertension (PH). Their data demonstrated that administration of this fusion protein in rats prevented the induction and progression of PH and proinflammatory cytokines formation (Shenoy et al., 2014; Lahm et al., 2018). None of these studies compared the efficacy or stability profile of the CTB-Ang-(1-7) with native peptide.

Recently, Daniell et al. invented a composition comprising plant (lettuce, carrot, cauliflower, cabbage, grass, low-nicotine tobacco, spinach, kale, and cilantro) chloroplast fused with Ang-(1-7) using transplastomic technology. Following oral administration, this compound produced an effective patient response. The therapeutic protein is a fusion protein made of Ang-(1-7) and CTB, which provides a cardioprotective effect. CTB stabilizes Ang-(1-7) by pentamer formation resulting in increased Ang-(1-7) half-life found in patient's sera. This approach could prevent and treat pulmonary hypertension, experimental-induced ocular disease, and autoimmune disorders by inhibiting proinflammatory cytokines and autophagy (Daniell et al., 2019).

5 Conclusion

Since Tigerstedt and Bergmann's initial discovery of the RAS at the end of the 19th century (Tigerstedt and Bergman, 1898), many laboratories have contributed to the understanding of this essential physiological system. Growing evidence indicates that the stimulation of the RAS's protective arm represents a novel, powerful therapeutic approach to treating a multitude of diseases and disorders. This realization has motivated many research groups to evaluate Ang-(1-7)'s role in a range of pathological conditions. This review has highlighted many relevant roles that Ang-(1-7) and its analogs play in a broad range of physiological and
pathophysiological states as well as current delivery systems that use the RAS protective arm for therapeutic advantage.

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Authorship contributions

Wrote or contributed to the writing of the manuscript: Khajehpour, Aghazadeh-Habashi
References


Daniell H, Li Q and Raizada MK (2019) Oral delivery of angiotensin converting enzyme 2 (ACE2) or angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension, cardiac dysfunction and development of autoimmune and experimental induced ocular disorders, in, Google Patents.


Footnotes

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Conflict of interest

No author has an actual or perceived conflict of interest with the contents of this article.

1 Sana Khajehpour
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Figure legends

**Figure 1.** Schematic depiction of the RAS components and selected actions. Gray arrows show newly described enzymatic pathways, and receptors are shown in boxes.

**Figure 2.** A. Chemical structure of AVE 0991, 5-formyl-4-methoxy-2-phenyl-1((4-(2(ethylaminocarbonylsulfonamido)-5-isobutyl-3-thienyl-phenyl)-methyl)-imidazole. B. Hypothetical chemical structure of cAng-(1-7). The thioether bridge from positions 4 to 7 (N terminus on the left) is depicted in the D-L configuration expected based on the DL configuration of the thioether bridges in nisin and other lantibiotics. C. Addition of a cyclic non-natural amino acid to the Ang-(1-7) backbone at the site of ACE and DPP3 hydrolysis.

**Figure 3.** Signaling pathways for nitric oxide (NO) formation. Ang-(1-7)/MasR and Ang II/AT$_2$Rs induce NO formation via phosphatidylinositol 3-kinase (PI3K)/Akt signaling. Alamandine/Mas-related G protein-coupled receptor D (MrgD) leads to NO formation through AMPK.

**Figure 4.** A and B enhanced proteolytic resistance of cAng-(1-7). Proteolytic resistance was measured of natural (△, ○, □, ▽) and cyclized Ang-(1-7), (▲, ●, ■, ▼), against ACE (A, △, ▲), plasma (A, ○, ●), liver (B, □, ■), and kidney homogenate (B, ▽, ▼) at pH 7.4. Each point represents the mean ± SEM, generated from at least three separate experiments (with permission from reference (Kluskens et al., 2009)).
### Table 1. Ang-(1-7) peptide drug delivery approaches

<table>
<thead>
<tr>
<th>Delivery approach</th>
<th>Key achievements</th>
<th>Therapeutic use</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Novel drug delivery systems</strong></td>
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<tr>
<td>Ang-(1-7) eluting stent</td>
<td>Control the release of Ang-(1-7) from the polymer coated device</td>
<td>Reduces restenosis and improves endothelial cell function</td>
<td>(Tijmsa et al., 2007)</td>
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<td>βCD/Ang-(1-7) complex</td>
<td>Feasible oral formulation for long-term oral administration of heptapeptide</td>
<td>Modulation of MI proteome dysregulation in an animal model of MI</td>
<td>(Lula et al., 2007) (Marques et al., 2012)</td>
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<td>HPβ-CD/Ang-(1-7) Complex</td>
<td>The inhaled formulation is achieved by incorporation of Ang-(1-7) in HPβ-CD</td>
<td>Lung protective effect in chronic asthma</td>
<td>(Magalhães et al., 2018)</td>
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<td>PCL-Ang-(1-7)</td>
<td>Highly porous three-dimensional biodegradable scaffold from BCP, PCL, and Ang-(1-7) was fabricated</td>
<td>Improvement of degenerative diseases and damaged bone tissue</td>
<td>(Macedo et al., 2012)</td>
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<td>Liposomal Ang-(1-7)</td>
<td>Potential of liposomes as a tool for the sustained release of the short half-life Ang-(1-7) was studied</td>
<td>Modulation of circadian rhythm, MAP, and heart rate</td>
<td>(Silva-Barcellos et al., 2001)</td>
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<td>Ang-(1-7)/PAMAM-OH</td>
<td>Neutral dendrimers as therapeutic peptide carriers were fabricated</td>
<td>Anti-atrophic effect in disuse skeletal muscle tissue in mice</td>
<td>(Márquez-Miranda et al., 2017)</td>
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<td>Ang-(1-7) Conjugate</td>
<td>Sustained release of the peptide, improved PK and prolonged biological half-life</td>
<td>Increasing the RAS protective arm components in an animal model of arthritis</td>
<td>(Habashi et al., 2020)</td>
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<td><strong>Structurally-modified homologs</strong></td>
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<td>Glycosylated Ang-(1-7)</td>
<td>Improved PK and half-life</td>
<td>Modulation of VCID</td>
<td>(Hay et al., 2019)</td>
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<td>Lanthionine-stabilized Ang-(1-7) (cAng-(1-7))</td>
<td>Improved PK and half-life</td>
<td>Modulation of diabetes in animal model</td>
<td>(Kluskens et al., 2009)</td>
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<td>(Cassis et al., 2019)</td>
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<tr>
<td>Acetylation and Amination of Ang-(1-7)</td>
<td>Increased peptide stability, improved PK and half-life</td>
<td>Anti-proliferative and anti-invasive properties in lung cancer cell line and mice model</td>
<td>(Ma et al., 2018)</td>
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<td>Cyclized Ang-(1-7)</td>
<td>Increased peptide stability, improved PK and half-life</td>
<td>Anti-proliferative property in breast cancer and fibrosarcoma cells</td>
<td>(Wester et al., 2017)</td>
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<td>NorLeu³-Ang-(1-7)</td>
<td>The topical administration</td>
<td>Treating diabetic foot ulcers (in Phase III clinical trial) faster than current approved medication</td>
<td>(Rodgers et al., 2005)</td>
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<td><strong>Bio expressing Ang-(1-7)</strong></td>
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<td>Lactobacillus Paracasei (LP) modified to expressing Ang-(1-7)</td>
<td>Subcutaneous delivery of Ang-(1-7) increased circulating Ang-(1-7) and reduced angiotensin II, but most gut-brain parameters were unchanged in response.</td>
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<td>(Carter et al., 2020)</td>
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<td>Bioencapsulated plant cells expressing CTB-Ang-(1-7)</td>
<td>Increased shelf-life, improved PK and half-life</td>
<td>Decreasing retinal inflammation in mouse model inhibiting both progression and commencement of pulmonary hypertension</td>
<td>(Shenoy et al., 2014)</td>
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Angiotensinogen

Ang-(1-12)

Ang-(1-7)

Ang I

Ang II

AT₁R

Vasoconstrictor
Fibrotic
Inflammatory
Cancer progression

AT₂R

Vasodilator
Antifibrotic
Anti-inflammatory
Anti-angiogenesis

MasR

Vasodilator
Antifibrotic
Anti-inflammatory

MrgD

Vasodilator
Antifibrotic

Chymase (Heart)

ACE

ACE2

Renin

Cathepsin B

PPR

Prorenin

Unknown

Figure 1
Figure 2
Figure 3

Ang II → p-PI3K → p-AKT
p-AMPK
p-LKB1

Ang-(1-7) → p-eNOS

AT₂R
MasR

NO
MrgD
Alamandine
Figure 4