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

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ABSTRACT

The in vivo application and efficacy of many therapeutic peptides is limited because of 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 classic and protective arms. The balance between these two arms is critical for the homeostasis of the body's physiologic function. Activation of the RAS results in the suppression of its protective arm, which has been reported in inflammatory and pathologic conditions such as arthritis, cardiovascular diseases, diabetes, and cancer. Clinical application of 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 renin-angiotensin 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.

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, which link the RAS to the initiation, development, and progression of several diseases (Ko and Bakris, 2018).

The RAS has two opposing arms: the classic arm, composed of angiotensin-converting enzyme (ACE), Ang II, and Ang II type 1 receptor (AT_1R), and the protective arm, composed of ACE2, Ang-(1–7), and Mas receptor (MasR) (Fig. 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 AT_1R. 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 classic 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 AT_1R 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 NADPH oxidase activity via the AT_1R in endothelial and vascular smooth muscle cells (Zafari et al., 1998), stimulating reactive oxygen species (ROS) and nitrogen species formation (Lacolley et al., 2012; Li, 2012; Kaschina and Unger, 2019). Many signaling pathways (e.g., superoxide and H_2O_2) may be activated by ROS products and

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ABBREVIATIONS: ACCA, *cis*-3(aminomethyl)cyclobutane carboxylic acid; ACE, angiotensin-converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; Ang, angiotensin; Ang Conj., Ang-(1–7) conjugate; ARB, angiotensin receptor blocker; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor; Ang-AA, Acetylated and aminated Ang-(1-7); βCD, β-cyclodextrin; CTB, cholera nontoxic B subunit; CV, cardiovascular; cAng-(1-7), Cyclic Ang-(1-7); DFU, diabetic foot ulcer; DPP3, dipeptidyl peptidase 3; GI, gastrointestinal; HF, heart failure; HPβCD, hydroxypropyl β-cyclodextrin; LAng, liposomal Ang-(1–7); LP-A, Lactobacillus *paracasei* expressing Ang-(1-7); MAP, mean arterial pressure; MasR, Mas receptor; MI, myocardial infarction; MrgD, Mas-related G protein–coupled receptor D; NO, nitric oxide; OVA, ovalbumin; PAMAM-OH, hydroxyl-terminated poly(amidoamine); RAS, renin-angiotensin system; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; VCID, Vascualr cognitive impairment and dementia.

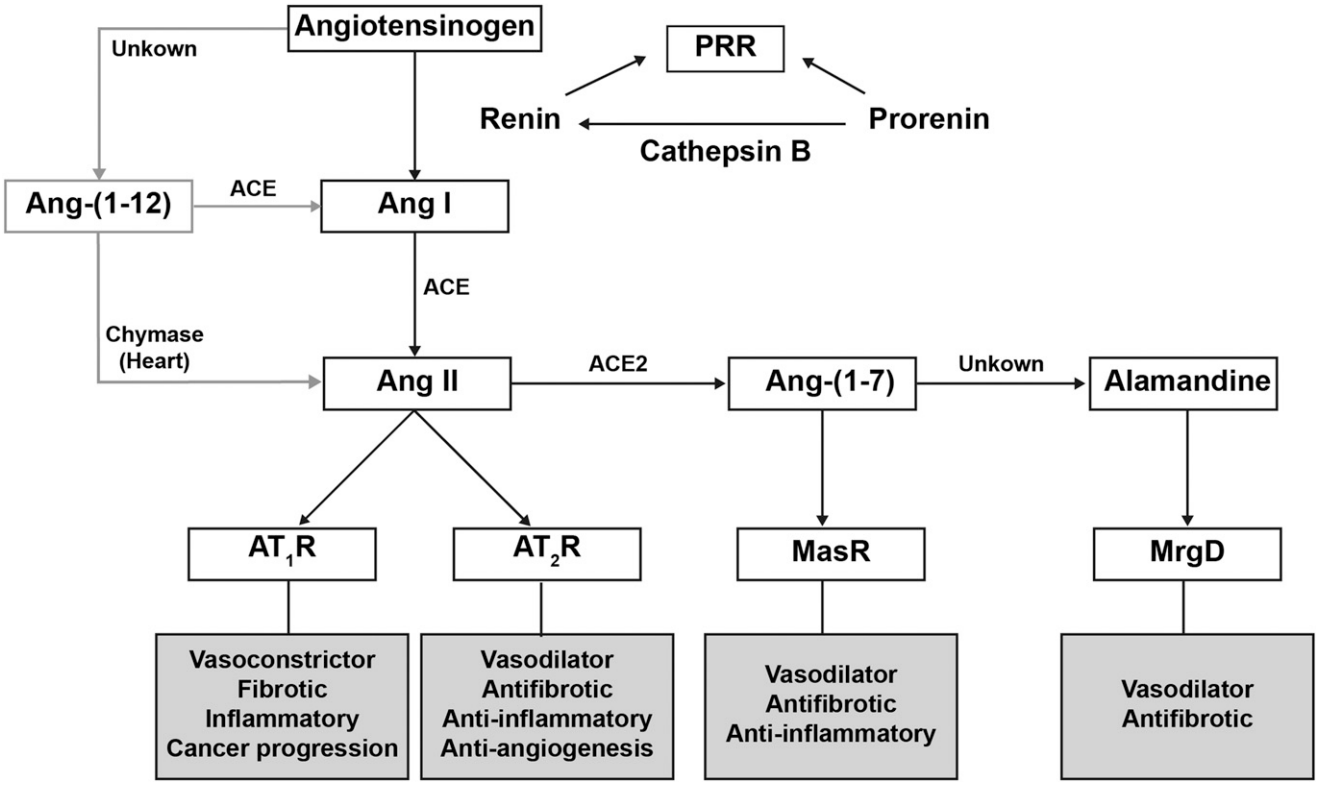

Fig. 1. Schematic depiction of the RAS components and selected actions. Gray arrows show newly described enzymatic pathways, and receptors are shown in boxes, PRR; Prorenin receptor.

subsequently trigger mitogen-activated protein kinases, 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 (Fig. 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 biologic 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), and MasR, the protective arm regulates many body functions, including the cardiovascular (CV), central nervous system, 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 family (Solinski et al., 2014). As the research evolves (Jackson et al., 1988), scientists have determined that Ang II is not an MasR ligand (Dong et al., 2001) and that the Mas proto-oncogene, which codifies MasR, has fewer tumorigenic properties than initially believed (Kostenis et al., 2005).

Like the RAS's protective arm, the classic 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, antiproliferation, and anti-inflammatory and antifibrotic actions. 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 classic arm has resulted in angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) 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). Because of the rapid systemic clearance of Ang-(1–7), its short half-life (3–15 minutes) 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 particular (Chappell et al., 1998). This review updates the RAS conceptual development and discusses different Ang-(1–7) structural modifications and delivery approaches.

Targeting the RAS Classic 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 disease (Ohrui et al., 2004); and cancer (Guo et al., 2015). The ability to suppress the classic 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 phenomenon (Antman et al., 2004). Another means of blocking Ang II from acting on AT_1R 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 classic RAS is suppressing AT_1R gene expression. Guo et al. (2015) used MicroRNA-410, a microRNA, to silence the gene and suppress AT_1R 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.

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 AT_1R and AT_2R, 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. De Gasparo et al. (de Gasparo et al., 2000) and Miura et al. (2010) provide excellent detail on Ang II receptor activation. Also, there are several peptides and nonpeptide compounds that are under development for targeting AT_2R, which have been discussed previously (Unger et al., 2015).

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). Santos et al. (2000) reported that Ang-(1–7) interacts with putative receptors such as the losartan-sensitive receptor, and $AT_{1-7}R$. Santos further concluded that AT_1R is not a target receptor for this peptide. In 2003, the same group reported using A-799 [D-Ala^7-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 physiologic 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 after an acute water load (Santos et al., 2003). Activation of MasR induces the phosphatidylinositol 3-kinase/ Protein kinase B 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 AT_1R (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; inhibits Na/K/ATPase; and leads to vasorelaxation, natriuresis, diuresis, and down-regulation of AT_1R, 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; Simões 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. (2018) provided a detailed review of the benefits of Ang-(1–7) to the body.

An effective approach for reversing the imbalance between the classic and protective arms of the activated RAS is to increase the Ang-(1–7) peptide concentration. Wysocki et al. (2010) reported targeting the protective arm using recombinant ACE2. 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).

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 nonpeptide 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.

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. (2020), for example, tested an analog of Ang-(1–7) in a diabetic mouse model to determine whether the analog showed similar benefits to 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. 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 with the parent Ang-(1–7) can be made.

MasR Agonist AVE 0991. AVE 0991 (Fig. 2) is an orally active nonpeptide 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. (2007) demonstrated that it attenuates myocardial infarction (MI)-induced heart failure. In mouse models, AVE 0991 produces antidiuretic effects that could prevent end-organ damage and morphologic 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

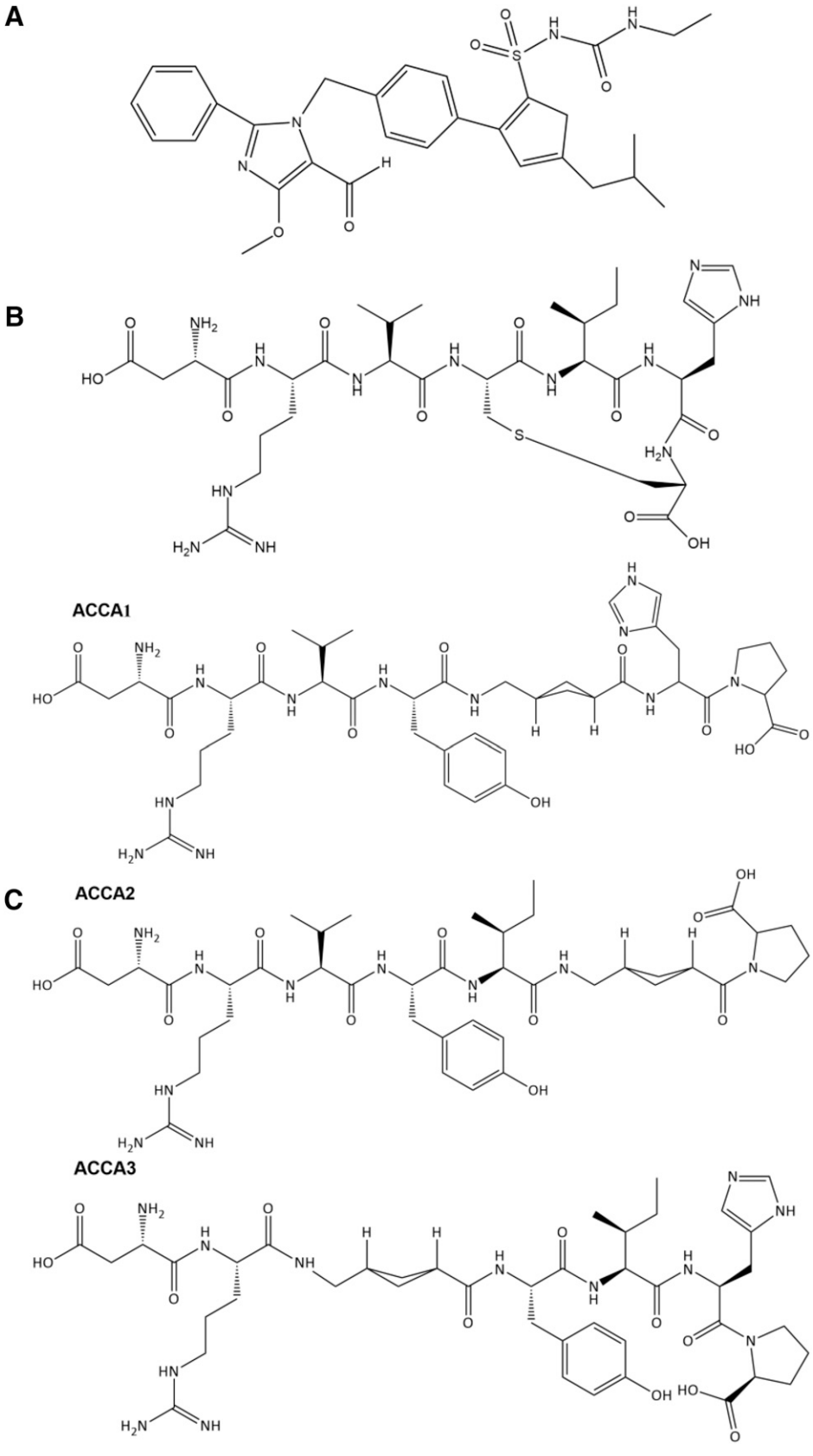

Fig. 2. (A) Chemical structure of AVE 0991, 5-formyl-4-methoxy-2-phenyl-1((4-(2 (ethylaminocarbonylsulfonamido)-5-isobutyl-3-thienyl-phenyl)-methyl)-imidazole. (B) Hypothetical chemical structure of cAng-(1–7). The thioether bridge from positions 4 to 7 (N terminus on the left) is depicted in the D-L configuration expected based on the D-L 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.

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.

MrgD Agonist Alamandine. Etelvino et al. (2014) recently introduced alamandine [Ala–Arg–Val–Tyr–Ile–His–Pro], a RAS component that forms through either of two pathways: Ang A hydrolysis by ACE2 at the C terminus or decarboxylation of aspartate at the N terminus of Ang-(1–7). Based on functional data, alamandine interacts with the Mas-related G protein–coupled receptor D (MrgD) (Fig. 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 mitogen-activated protein kinase 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 AT_2R, these observations suggest that alamandine may act as a neuronal excitatory molecule in the brain (Marins et al., 2014). Activation of MrgD by alamandine leads to NO release using a different mechanism than Ang-(1–7) by targeting AMP-activated protein kinase, primarily to induce NO formation (Fig. 3) (Jesus et al., 2018).

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.

Application of Ang-(1–7) Complex Formulation for Various Diseases

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 because of its anti-inflammatory and antifibrotic properties in cardiac ischemia (Rodrigues Prestes et al., 2017).

Tijsma et al. (2007) 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

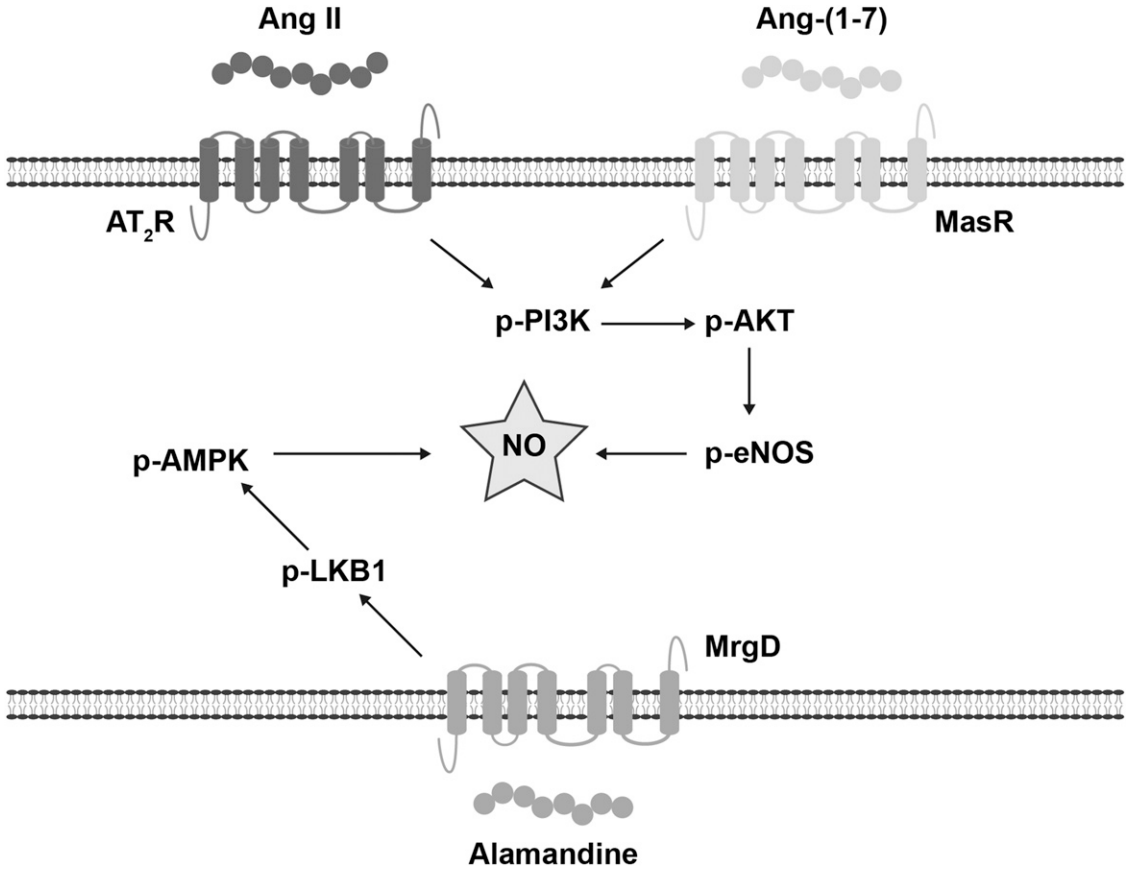

Fig. 3. Signaling pathways for NO formation. Ang-(1–7)/MasR and Ang II/AT_2Rs induce NO formation via phosphatidylinositol 3-kinase (PI3K)/Akt signaling. Alamandine/MrgD leads to NO formation through AMP-activated protein kinase, p-AKT: phosphorylated protein kinase Bp-eNOS: phosphorylated endothelial nitrix oxide synthasep-AMPK: phosphorylated adenosine mono phosphate-activated protein kinasep-LKB1: phosphorylated liver kinase B1.

TABLE 1
Ang-(1–7) peptide drug delivery approaches

Delivery Approach	Key Achievements	Therapeutic Use	References
Novel drug delivery systems			
Ang-(1–7) eluting stent	Control the release of Ang-(1–7) from the polymer-coated device	Reduces restenosis and improves endothelial cell function	Tijsma et al. (2007)
βCD/Ang-(1–7) complex	Feasible oral formulation for long-term oral administration of heptapeptide	Modulation of MI proteome dysregulation in an animal model of MI	Lula et al. (2007); Marques et al. (2012); Gómez-Mendoza et al. (2019)
		Modulation of diabetes in animal model; modulation of pulmonary emphysema in animal model	Santos et al. (2014); Bastos et al. (2020)
HPβCD/Ang-(1–7) complex	The inhaled formulation is achieved by incorporation of Ang-(1–7) in HPβCD	Lung protective effect in chronic asthma	(Magalhães et al., 2018)
PCL[f]-Ang-(1–7)	Highly porous three-dimensional biodegradable scaffold from BCP[g], PCL, and Ang-(1–7) was fabricated	Improvement of degenerative diseases and damaged bone tissue	Macedo et al. (2012)
Liposomal Ang-(1–7)	Potential of liposomes as a tool for the sustained release of the short half-life Ang-(1–7) was studied	Modulation of circadian rhythm, MAP, and heart rate	Silva-Barcellos et al. (2001)
Ang-(1–7)/PAMAM-OH	Neutral dendrimers as therapeutic peptide carriers were fabricated	Antiatrophic effect in disuse skeletal muscle tissue in mice	Márquez-Miranda et al. (2017)
Ang-(1–7) conjugate	Sustained release of the peptide; improved PK[h] and prolonged biologic half-life	Increasing the RAS protective arm components in an animal model of arthritis	Habashi et al. (2020)
Structurally modified homologs			
Glycosylated Ang-(1–7)	Improved PK and half-life	Modulation of VCID	Hay et al. (2019)
Lanthionine-stabilized Ang-(1–7) [cAng-(1–7)]	Improved PK and half-life	Modulation of diabetes in animal model	Kluskens et al. (2009); de Vries et al. (2010); Kuipers et al. (2019)
		Improving type 2 diabetic nephropathy	Cassis et al. (2019)
Acetylation and amination of Ang-(1–7)	Increased peptide stability; improved PK and half-life	Antiproliferative and anti-invasive properties in lung cancer cell line and mice model	Ma et al. (2018)
Cyclized Ang-(1–7)	Increased peptide stability; improved PK and half-life	Antiproliferative property in breast cancer and fibrosarcoma cells	Wester et al., (2017)
NorLeu^3-Ang-(1–7)	The topical administration	Treating diabetic foot ulcers (in phase III clinical trial) faster than current approved medication	Rodgers et al. (2005, 2011, 2015); Balingit et al. (2012)
Bio expressing Ang-(1–7)			
L. paracasei modified to express Ang-(1–7)	Subcutaneous delivery of Ang-(1–7) increased circulating Ang-(1–7) and reduced angiotensin II, but most gut-brain parameters were unchanged in response	Anti-inflammatory effect in animal model	Buford et al. (2020); Carter et al. (2020)
Bioencapsulated plant cells expressing CTB-Ang-(1–7)	Increased shelf-life; improved PK and half-life	Decreasing retinal inflammation in mouse model, inhibiting both progression and commencement of pulmonary hypertension	Shenoy et al. (2014); Lahm et al. (2018); Shil et al. (2014); Daniell et al. (2019)

[f]Polycaprolactone
[g]Beta-tricalcium phosphate-hydroxyapatite bioceramic
[h]Pharmacokinetics

open-ended cylindrical insert that has both the outer and inner surfaces coated with a controlled-release matrix comprising 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 poststent implantation restenosis and improves vascular endothelial functioning.

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 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 with the pure peptide. However, the effects of LAng on blood pressure [mean arterial 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 after vehicle microinjection. The data prove that this novel technique can be used in chronic conditions and reveal a new physiologic role (i.e., modulation of the circadian rhythms of MAP and heart rate) for Ang-(1–7) at the RVLM (Silva-Barcellos et al., 2001).

In an innovative delivery approach, Lula et al. (2007) formed and characterized an inclusion of Ang-(1–7) with β-cyclodextrin [βCD/Ang-(1–7)] using a freeze-drying method. Santos et al. (2014) 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.

Furthermore, Ang-(1–7) enclosed in hydroxypropyl β-cyclodextrin [HPβCD/Ang-(1–7)] has also been tested in an experimental MI rat model as a means of reversing cardiac tissue dysregulation after oral administration (Marques et al., 2011). HPβCD/Ang-(1–7) treatment improved the rats' postinfarction 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 after experimental MI significantly downregulated the C-X-C chemokine receptor type 4 (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 after oral administration.

Musculoskeletal Disease. A new strategy for degenerative diseases and damaged bone tissue includes autografts, allografts, xenografts, and artificial materials (metals and bioceramics). Fabricating scaffolds for meeting the needs of specific repair sites is challenging because of bone tissue's complexity and property variability. Macedo et al. (2012) combined β-tricalcium phosphate-hydroxyapatite bioceramic, polycaprolactone, and Ang-(1–7) composite to form a porous, three-dimensional, biodegradable scaffold using solvent casting and particulate leaching methods. This innovative combination joins a mechanical anchor for osteoblastic cells and a more favorable surface for cell attachment betatricalcium phosphate-hydroxyapatite with a semicrystalline, bioresorbable polymer and is well known for its slow degradation rate polycaprolactone 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 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.

A noncytotoxic hydroxyl-terminated poly(amidoamine) (PAMAM-OH) dendrimer has been used as an Ang-(1–7) carrier [Ang-(1–7)/PAMAM-OH] by Márquez-Miranda et al. (2017). Molecular dynamics simulation data suggest this dendrimer could protect Ang-(1–7) and form a stable complex because it retards peptide mobility. When administered intraperitoneally, the Ang-(1–7) dendrimer, but not plain Ang-(1–7), could demonstrate antiatrophic properties in skeletal muscle tissue. In vivo toxicity studies showed no significant toxic effect on male mice. The authors attribute the improvement of antiatrophic 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).

Pulmonary Disease. Investigations showed the role of Ang-(1–7) in the prevention of chronic allergic lung inflammation. Resolving eosinophilic inflammation in an asthmatic model characterized by inflammation, pulmonary remodeling, and bronchial hyperresponsiveness was achieved after the inclusion of Ang-(1–7) in HPβCD 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 9, matrix metallopeptidase 12, and α-smooth muscle actin. 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. (2020) 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.

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 have 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 disease and related dementia result from decreased brain blood flow, increased production of ROS, and proinflammatory mechanisms (Pavol et al., 2018). Hay et al. (2019) tested their novel glycosylated Ang-(1–7) peptide Ang-1-6-*O*-Ser-Glc-NH2 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, in which proline is substituted with serine and a β-D-glucose is bound to serine-side methyl. Ang-1-6-*O*-Ser-Glc-NH2 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 because of its preserved MasR activation activity. Decreased VCID/HF-induced activation of brain microglia/macrophages could dramatically reduce circulating tumor necrosis factor α, interleukin-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)] (Fig. 4), which has been shown to be fully resistant to ACE as well as other peptidases (Fig. 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 et al. (2019) tested cAng-(1–7) in the streptozotocin-induced mouse 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.

In another study, de Vries et al. (2010) investigated cyclized thioether bridge Ang-(1–7) compound delivery through both oral and pulmonary administration. Stability data show 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.

Cassis et al. (2019) investigated the impact of cAng-(1–7) in Black and Tan, Brachyuric lacking the leptin hormone known as BTBR *ob*/*ob* mice suffering from type 2 diabetic nephropathy. The BTBR ob/ob mouse 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 antiproteinuric effect compared with lisinopril alone because of better preservation of podocyte proteins and capillary density amelioration. Adding cAng-(1–7) to ACEI therapy could benefit patients with diabetes who have responded unsatisfactorily to ACEI therapy (Cassis et al., 2019).

Ma et al. (2018) used an alternative Ang-(1–7) analog peptide with N and C termini 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 to 238.7 ± 61.3 minutes. 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. (2017) 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 (DPP3)

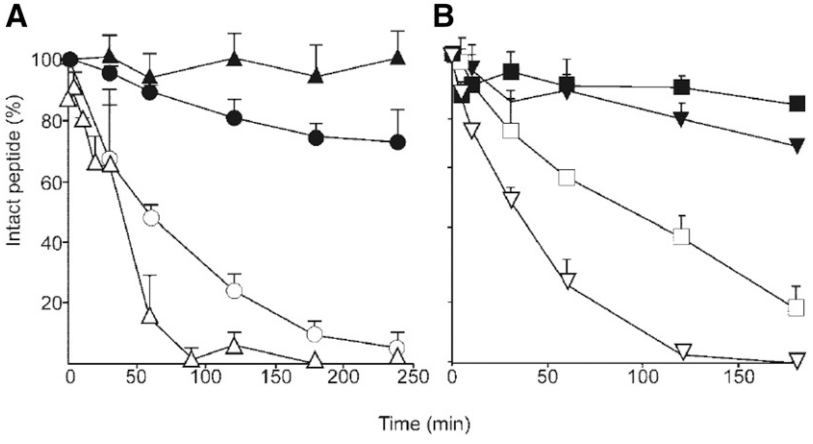

Fig. 4. (A and B) Enhanced proteolytic resistance of cAng-(1–7). Proteolytic resistance was measured of natural (△, ○, □, ▽) and cyclized (▲, •, ■, ▼) Ang-(1–7) against ACE (A,△, ▲), plasma (A, ○,•), liver (B, □,■), and kidney homogenate (B, ▽, ▼) at pH 7.4. Each point represents the means ± S.E.M., generated from at least three separate experiments [with permission from reference Kluskens et al. (2009)].

enzymes. ACCA substitution used solid-phase peptide synthesis on Ile^5, His^6, and Val^3 positions, named ACCA 1, 2, and 3, respectively. ACCA 1 and 2 showed complete resistance to ACE, and ACCA analog 1 was resistant to DPP3 hydrolysis. All analogs had preserved activity against breast cancer and fibrosarcoma cells (Fig. 3).

Diabetes is a disorder that can delay wound repair, which may result in colonized chronic wounds (Rodgers et al., 2011). Patients with diabetes 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, which 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 Nor-Leu3-Ang-(1–7), is an analog of Ang-(1–7) that induces proliferation and 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).

Bioexpressing Ang-(1–7)

Recently, Carter et al. (2020) studied the effect of multiple doses of an oral formulation of modified Ang-(1–7)–expressing probiotic bacteria *Lactobacillus paracasei* (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 *L. paracasei*, and after incubation, bacteria were harvested, washed, and aliquoted for further use. After 3 weeks of either LP-A or subcutaneous Ang-(1–7) administration, data showed that LP-A statistically increased the circulating Ang-(1–7) and decreased Ang II. Results also demonstrated that 3 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 *L. paracasei* 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 neuroinflammatory 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 three times per 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. (2014) 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 protein 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.

Utilizing the same approach, other groups tested the oral CTB-fused Ang-(1–7) on pulmonary hypertension. Their data demonstrated that administration of this fusion protein in rats prevented the induction and progression of pulmonary hypertension and proinflammatory cytokine 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. (2019) 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. After 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 patients' sera. This approach could prevent and treat pulmonary hypertension, experimentally induced ocular disease, and autoimmune disorders by inhibiting proinflammatory cytokines and autophagy.

Conclusion

Since the initial discovery of the RAS at the end of the 19th century by Tigerstedt and Bergman (1898), many laboratories have contributed to the understanding of this essential physiologic 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 the role of Ang-(1–7) in a range of pathologic conditions. This review has highlighted many relevant roles that Ang-(1–7) and its analogs play in a broad range of physiologic and pathophysiological states as well as current delivery systems that use the RAS protective arm for therapeutic advantage.

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