1   **Review Article**

2   **Angiotensin (1-7) and Alamandine: similarities and differences**

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Abstract. A primary peptide of the renin angiotensin system (RAS), Angiotensin (Ang) II, is a vasoconstrictor and promotor of atherosclerosis. To counter this, the RAS also consists of peptides and receptors which increase nitric oxide release from the endothelium and decrease nicotinamide adenine dinucleotide phosphate oxidase-related superoxide production. Two peptides, Ang (1-7) and alamandine are vasodilators, by activating the nitric oxide pathway via different receptors in the endothelium. Thus, herein we focus on the similarities and differences between alamandine and Ang (1-7) and the counterbalancing hypothesis on Ang II during endothelial dysfunction and atherosclerosis.

Keywords: Renin angiotensin system; Angiotensin (1-7); Alamandine; Endothelial dysfunction

Chemical compounds mentioned in this article:

Alamandine (44192273); Angiotensin 1-7 (123805); Angiotensin II (172198); Angiotensin I (3081372)

Abbreviations:

Angiotensin A (Ang A); Angiotensin converting enzyme 1 (ACE 1); Angiotensin converting enzyme 2(ACE2); Angiotensin II type I receptor (AT_1); Angiotensin II type II receptor (AT_2); Cardiovascular disease (CVD); Chinse Hamster Ovary (CHO); Endothelial nitric oxide synthase (eNOS); Mas-related G couple protein receptor member D (MrgD); Nicotinamide adenine dinucleotide phosphate (NADPH); Nitric oxide (NO); Protein kinase A (PKA); Protein kinase C (PKC); Reactive oxygen species (ROS); Renin angiotensin system (RAS);Unilateral ureteral obstruction (UUO).

1.1 Introduction

Cardiovascular disease (CVD) accounts for 1 in 3 deaths worldwide [1] with increasing prevalence in the Western world. Despite the therapeutic advances for treating CVD [2], novel interventions are necessary to further reduce this burden. The renin angiotensin system (RAS) is a hormone system that regulates blood pressure, electrolyte balance and plays a crucial role in atherogenesis and thus CVD.

Atherosclerosis is a progressive process of arterial wall thickening and is characterised by the deposition of lipoproteins, cholesterol and white blood cells on the innermost layer of blood vessels [3]. Atherogenesis is complex, however 3 distinct stages have been identified. (1) Endothelial dysfunction, which occurs throughout all stages of atherosclerosis; (2) plaque formation, and, (3) thrombosis [4-6]. These stages occur at the luminal surface of blood vessels and are time-dependent [7]. Endothelial dysfunction is a pathophysiological shift towards a vasoconstrictive and pro-inflammatory state. This is believed to occur mainly due to a reduction in the bio-availability of nitric oxide, possible due to an altered activity of endothelial nitric oxide synthase (eNOS) [8] or an over-activation of the protein kinase C (PKC) signalling pathway [9] coupled with an overproduction of superoxide [10, 11]. Over-activation of RAS has been linked to endothelial dysfunction and may be involved in altering protein kinases, eNOS activity and superoxide production, ultimately regulating atherogenesis. Thus, drugs regulating RAS are a promising therapeutic modality against CVD.

The RAS includes a number of peptides and enzymes, including Ang II, angiotensin converting enzyme 1 (ACE1), ACE2, angiotensin A (Ang A, where the A is abbreviated for alanine), angiotensin (1-7) (Ang (1-7)), Mas receptor, Mas-related G coupled protein receptor member D (MrgD) and alamandine [12-14]. The RAS is present in a number of tissues including, blood vessels, myocardium, kidney and brain [15-18]. The pathway begins with angiotensinogen, which is formed in the liver and released into the circulation. It is proteolyzed by renin resulting in a the decapeptide, Ang I. Ang I binds to the endothelial cell layer of blood vessels, and ACE1 cleaves the C-terminal dipeptide (L-histidyl-L-leucine) of Ang I to form Ang II [19-21] (Figure 1).

70   Ang II and Ang A have vasoconstrictive properties, which act through the angiotensin II type I receptor

71   (AT_1) [14, 22]. AT_1 is a 41 kDa transmembrane receptor highly expressed in the cardiovascular system

72   and regulates aldosterone secretion and controls blood pressure [23]. Binding of Ang II to AT_1 receptor

73   stimulates an array of pathophysiological actions including, vasoconstriction, which elevates blood

74   pressure, endothelial dysfunction (inhibition of NO production) and increases nicotinamide adenine

75   dinucleotide phosphate (NADPH) oxidase activation for superoxide production; leading to the

76   development of cardiac fibrosis, inflammation associated with atherosclerosis and atherosclerotic plaques

77   [24-28]. In addition, Ang II may act through the angiotensin II type II receptor (AT_2) resulting in

78   vasodilatory effects. AT_2 is highly expressed in the developing fetus, however, its expression is low in the

79   cardiovascular system but is increased in inflammation, hypertension and atherosclerosis and is

80   vasoactive in human radial arteries [29]. Likewise, alamandine, is hydrolyzed by ACE2 from Ang A or

81   decarboxylated from Ang (1-7), also exerts vasodilatory and anti-hypertensive actions, through the MrgD

82   receptor [12, 30]. Ang (1-7) is also a vasodilator which acts via the Mas receptor, which is related to the

83   MrgD receptor. Therefore, the localization of both the MrgD and the Mas receptor on endothelial cells

84   suggests that both alamandine and Ang (1-7) may play important roles in blood vessel physiology [22,

85   31]. In addition, Ang (1-7) exerts an anti-angiogenic role by decreasing proangiogenic hormones such as

86   vascular endothelial growth factor-A [12, 32]. However, there are no reports to demonstrate the effect of

87   alamandine on angiogenesis. Furthermore, ingestion of alamandine/cyclodextrin decreases the

88   accumulation of collagen I, III, and fibronectin in isoproterenol-treated rats, contributing to a decrease in

89   cardiac fibrosis [12]. Interestingly, research performed in our laboratory showed a marked reduction in

90   rat aortic wall elastic fibers after an atherogenic diet for 15 weeks [33], and thus it would be interesting to

91   determine if alamandine can reduce this pathological effect. Therefore, Ang (1-7) and potentially

92   alamandine acting via their specific receptors, may be able to decrease endothelial cell proliferation and

93   migration, resulting in a decrease in endothelial cell permeability, and thus an attenuation of

94   atherogenesis.

95   Both the Mas and the MrgD receptors are proto-oncogenes. The Mas proto-oncogene was first identified

96   in 1986 where it was noted to facilitate tumorigenesis, and similarly for the overexpression of the MrgD

97   receptor in murine fibroblast cell lines [34, 35]. Thus, if alamandine and Ang (1-7) are to be used as a

98   treatment for CVD, these peptides could affect oncogenesis. Thus, it is important to determine a possible

99   carcinogenic/oncogenic effect in animal models using alamandine and Ang (1-7) in a combined

100   CVD/cancer model.

101   Ang (1-7) counterbalances the effects of Ang II exerted on human cerebral smooth muscle cells [25].

102   These effects reduce Ang II-induced apoptosis and proliferation [25]. Indeed, *in vitro* studies show that

103   administration of Ang (1-7) to rat aorta prevents Ang II-stimulated reactive oxygen species (ROS)

104   production [24] and increases NO release resulting in increased vasodilation [36], and reduces endothelial

105   oxidative stress. We are unaware of studies that demonstrate alamandine counterbalancing Ang II effects

106   on vascular smooth muscle cells and on endothelial cells. It is possible that alamandine may mimic the

107   effects of Ang (1-7), due to the close homology of their amino-acid sequence (Figure 1 and 2). Indeed,

108   alamandine stimulates NO release potentially by eNOS in endothelial cells to induce vasodilation through

109   the MrgD receptor [12]. Thus, combined Ang (1-7)/alamandine would be viable pathways to reduce Ang

110   II-associated endothelial dysfunction (i.e. low NO release from endothelium), Ang II-associated vascular

111   proliferation, and Ang II-associated inflammation during atherosclerosis.

1.2 Biosynthetic pathway for of Ang (1-7) and Alamandine

113   The RAS exists in the circulatory and tissue system and circulatory-Ang II is synthesized when ACE

114   cleaves the C terminal dipeptide of the renin-catalyzed-angiotensin I [37]. An intracellular form of renin,

115   as well as angiotensin I and both forms of ACE expressed in tissues have been associated with increased

116   intracellular Ang II production [38]. Therefore, the formation Ang (1-7) and alamandine, can also be

117   biosynthesized at a circulatory and tissue level. As revised by Zhang et al (2015), ACE2 catalyzes

118   angiotensin (1-9) from angiotensin I, so that angiotensin (1-9) can be cleaved by ACE into Ang (1-7).

119   Moreover, Ang II can be directly converted into Ang (1-7) via ACE2 [39]. Also, carboxylase can convert

120   Ang (1-7) to alamandine. Similarly to Ang (1-7), alamandine can be synthesized at the circulatory and

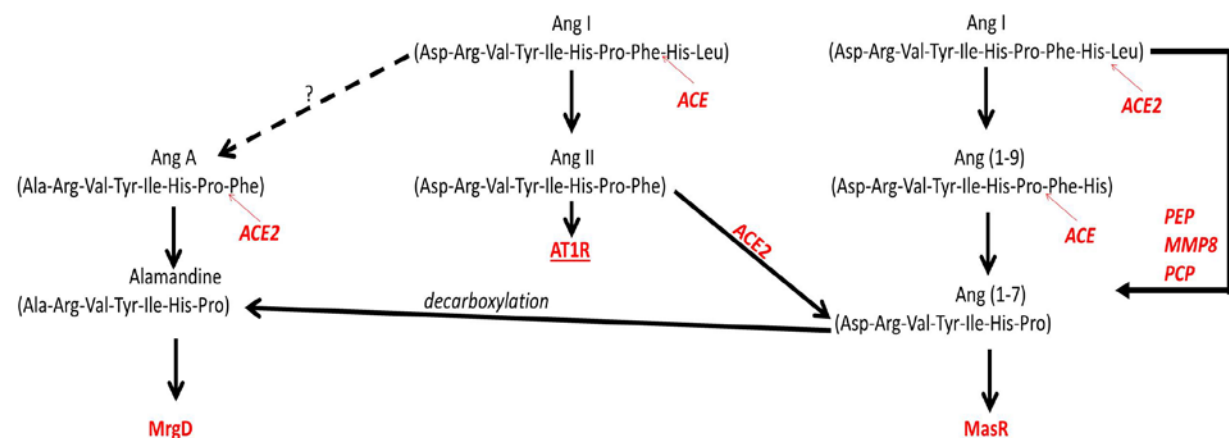121   tissue system (Figure 1).

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Figure 1: The pathway for both alamandine and Ang (1-7). Whether Ang A is formed from Ang I remains unknown. However, Ang A has been demonstrated through chromatographic purification and structural analysis that it is mostly likely generated enzymatically from Ang II via decarboxylase [14]. Ang I can be cleaved into Ang (1-7) by prolyl endopeptidase (PEP) [40], matrix metalloproteinase-8 (MMP8) [41] and prolyl carboxypeptidase (PCP) [42].

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1.3 Factors involved in vascular dysfunction, leading to atherosclerosis

129   The widely studied vasodilator produced by the endothelial layer of blood vessels is the free radical gas,

130   NO [43]. NO contributes to normal vascular homeostasis and reduced NO bioavailability results in

131   endothelial dysfunction [44, 45]. CVD risk factors, such as type 2 diabetes, hypertension, and

132   hypercholesteremia exacerbate the reduction of NO bioavailability thus promoting atherosclerosis. Since

133   atherosclerosis is the primary cause of CVD, the pharmacological drive to reverse endothelial

134   dysfunction, by improving NO release, should improve vascular health and reduce the burden of CVD. In

135   addition, ROS released by cells, including endothelial cells, is a byproduct of oxygen in normal

136   homeostasis. During stress however, ROS is produced in excessive levels leading to cell damage (cellular

137 oxidative stress). In fact, overproduction of Ang II leads to dramatic increase in ROS production by

138 endothelial cells, through the NADPH oxidase enzyme inducing cellular oxidative stress, leading to

139 endothelial dysfunction, endothelial apoptosis and promotion of thrombosis formation [46-48]. Hence,

140 cellular oxidative stress and reduction in NO, contribute to endothelial dysfunction [49-51]. Furthermore,

141 RAS peptides and enzymes are increased in cardiovascular disease in tissues, such as, blood vessels [22],

142 kidney [52], cardiomyocytes [53], skeletal muscle cells [54] and the colon [55], hence treatment

143 modalities are targeted to reduce the systemic over-activation of the RAS.

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145 **1.4 Trophic effects of RAS peptides on endothelial cell function and vascular tone regulation**

146 Vascular endothelial cells release trophic factors such as NO, neutral endopeptidase, prostaglandin I2 and

147 endothelin-1 that regulate vascular tone. An imbalance of these vasoactive factors may lead to an

148 imbalance of vascular tone and induce proliferation of VSMCs, leading to hypertension and

149 atherosclerotic plaque accumulation. Although physiological levels of Ang II, upon stimulation of the

150 AT_1 and AT_2 receptors on endothelial cells signal vascular tone and proliferation, pathophysiological

151 levels of Ang II disrupts this homeostasis and induces excess VSMC proliferation in rodents, rabbits and

152 humans [56-58]. In mice and rat, two weeks of Ang II infusion via mini-osmotic pumps induces

153 hypertension, cardiac and VSMC hypertrophy, and endothelial dysfunction [59]. Indeed, in humans, an

154 imbalance between Ang (1-7)/Ang II ratio has been shown in systemic sclerosis-induced endothelial

155 dysfunction [60]. Furthermore, Ang(1-7) infusion inhibits VSMC proliferation in balloon-injured carotid

156 arteries of rats [61]. Although further evidence is required to fully elucidate the mechanistic pathway

157 elicited by Ang (1-7), most *in vitro* studies conducted on human or rodent cultured aortic endothelial

158 cells show that after Ang (1-7) and its associated receptor signalling on the endothelial cell surface, an

159 array of enzymes are phosphorylated, such as phosphoinositide 3-kinase/AKT, protein kinase A,

160 phospholipase A2 phosphorylation leading to increased production of eNOS-induced NO and cyclic

161 AMP production [36, 62]. Ang (1-7) has also been shown to cause a reduction of mitogen-activated

162 protein kinase (MAPK) phosphorylation concomitant with a reduction of extracellular-signal-regulated

163 kinase (ERK1/2) phosphorylation within endothelial cells [62, 63], which is likely to be a major anti-

164 proliferation pathway induced by Ang (1-7).

165 A critical role for Ang (1-7)/Ang II has been established in endothelial dysfunction and cellular

166 proliferation. Considering research from this laboratory is clearly providing a vasoactive role for both

167 alamandine and Ang A [22], a trophic or anti-trophic effect on endothelial cells has not as yet been

168 demonstrated. Therefore, future experiments similar to studies conducted for Ang (1-7) will provide the

169 essential information necessary to compare these two heptapeptides.

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171 **1.5 Ang (1-7) and Mas receptor counterbalance the effects of Ang II and AT$_1$ receptor**

172 The Mas receptor is a G-coupled protein receptor which is expressed in brain, kidneys, testes, heart and

173 blood vessels of both humans and mice [36, 64-67]. It is conceivable that Ang (1-7) mediated through the

174 Mas receptor [68], may have beneficial effects in the treatment for CVD. Indeed, Ang (1-7) at 80nM has

175 been shown to protect against hypoxia and cardiomyocyte apoptosis by preventing ROS-induced

176 mitochondrial dysfunction in rat cell lines [69]. This suggests that Ang (1-7) may be protective in specific

177 tissues and that it may act on mitochondria. Furthermore, a number of RAS blockers have been developed

178 in order to reduce the effects of Ang II; such blockers (renin inhibitors, ACE inhibitors and Ang II

179 receptor blockers) all have anti-hypertensive effects [15]. Interestingly, inhibition of Ang II via ACE1

180 blockers increases Ang (1-7) by 5–25-fold in blood [70] and Ang (1-7) may also be involved in the anti-

181 depressant effect of ACE1 treatment in hypertensive rats [71]. This suggests that Ang (1-7) alone has an

182 important role in blood pressure regulation via inducing vasodilation protecting endothelial function and

183 hence, cardiovascular related diseases. Since Ang (1-7) is formed by ACE1, increasing its levels could be

184 a viable alternative to inhibition of ACE1 in order to achieve desired plasma Ang (1-7) concentration.

185 Likewise, alamandine has anti-hypertensive properties, enhances vasodilation and reverses endothelial

186 dysfunction in spontaneous hypertensive rats and homocysteine-induced endothelial dysfunction rabbits

187 [12, 72]. Hence, as Ang (1-7) can reverse the negative effects of Ang II on endothelial dysfunction,

188 superoxide production, as well as lowering Ang II-induced atherosclerotic lesions and plaque formation,

189 further studies with alamandine could show similar findings, and together these heptapeptides could

190 reduce the progression of CVD.

191 However, controversial results on Ang (1-7)-beneficial physiological effects have been reported. In Mas

192 knockout mice induced with unilateral ureteral obstruction (UUO) kidneys, the administration of Ang (1-

193 7) worsened renal lesions compared to untreated Mas knockout UUO mice [73], suggesting that Ang (1-

194 7) administration can be harmful in individuals with obstructed kidneys. However, one must take into

195 account that Ang (1-7) has only been shown to exert beneficial physiological effects, such as reducing

196 atherosclerotic plaque and inducing vasodilation, in the presence of the Mas receptor. Additionally, it has

197 been shown that mice with UUO already develops severe renal inflammation with significant

198 upregulation of inflammatory chemokines and cytokines such as MCP-1 and IL-6 respectively [74],

199 suggesting that Ang (1-7) may not be a beneficial treatment at severe stages of disease, and that it may

200 potentially exacerbate the development of disease instead. However, more studies report that chronic

201 treatment of Ang (1-7) is able to reduce fat accumulation and inflammation in animal models of liver

202 disease, suggesting Ang (1-7) as a strong anti-inflammatory agent [75]. Thus the model chosen plays a

203 role in the controversial reports of any experiments related to Ang (1-7) as a treatment.

204 Of interest, Ang (1-7) and Ang II both stimulated ERK1/2 phosphorylation and enhanced mesangial cell

205 proliferation [76], further conflicting most reports that demonstrate the opposing effects of Ang (1-7)

206 compared to Ang II [77, 78]. However, when co-administered to rat renal mesangial cells, Ang (1-7)

207 inhibited the stimulatory effects induced by Ang II, which could be blocked by a Mas receptor blocker,

208 A779, yet anti-AT_1 or anti-AT_2 receptor blockers had no effect [76]. Hence, although interacting with

209 each other [79], Ang (1-7) specifically acts on the Mas receptor and Ang II specifically acts on the AT_1

210 receptor. In human umbilical vein endothelial cells (HUVEC), Ang (1-7) at 1µM had no effect on

211 endothelial cell apoptosis, however when HUVEC are treated with Ang II, Ang (1-7) suppresses

212 apoptosis [80]. This indicates not only the crucial role on endothelial cell survival [62], but also that Ang

213 (1-7) either interacts with the AT_1 receptor by dislodging Ang II binding or interacts with the Mas

214 receptor. Similar findings were observed in aortic vascular cells of ACE2-knockout mice [81].

215 Furthermore, in Ang II induced endothelial dysfunction, blocking of the AT_1 receptor augments the

216 production of NO and improves vasodilation, which are crucial factors in the prevention of hypertension

217 and atherosclerosis [82, 83]. Since, blocking of the AT_1 receptor is associated with an increase of Ang (1-

218 7), the addition of Ang (1-7) may counterbalance the effects of Ang II induced endothelial dysfunction

219 and may aid in the prevention of atherosclerosis. To this effect, *ex vivo* treatment of murine aortae with

220 Ang II impairs vasodilation which is reversed by Ang (1-7) [31]; these effects are diminished following

221 treatment with the Mas receptor antagonist, A779. These findings, suggest that endothelial dysfunction

222 induced by Ang II (reduced NO and vasodilation), could be counterbalanced by Ang (1-7) acting through

223 the Mas receptor. Moreover, the studies suggest that the counterbalancing effects caused by Ang (1-7)

224 through the Mas receptor are a compensatory system that is important to balance the over-activity of Ang

225 II through the AT_1 receptor. However, the cellular mechanism underlying this theory is not yet clear,

226 although some studies suggest a specific non-competitive inhibition of Ang (1-7) on the AT_1 receptor [84,

227 85]. Others suggest that the AT_1 receptor and Mas receptor form a hetero-oligomer in order for their

228 effects to be counterbalanced [76, 86]. Further research to establish these initial results are necessary.
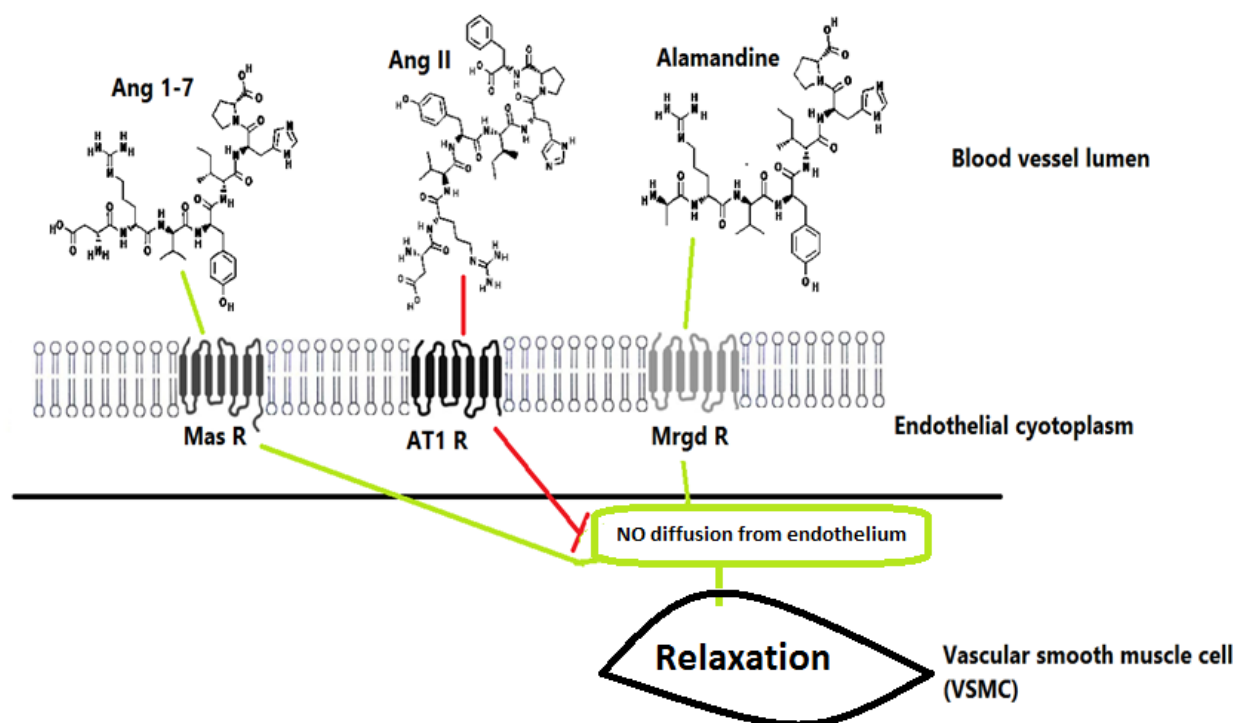
Figure 2: The chemical structure of Ang II, Ang (1-7) and alamandine peptides as ligands and their corresponding receptors. Both Ang (1-7) and alamandine have similar amino acid sequence and they both function as ligands for the proto-oncogene receptors, Mas and MrgD, respectively. Upon stimulation of these receptors there is upregulation of NO from the endothelium of blood vessels. The release of NO diffuses into the vascular smooth muscle cell (VSMC) layer (as NO is a soluble gas) and induces relaxation. However, Ang II counters these effects upon stimulation of the AT_1 receptor. (NO, nitric oxide; VSMC, vascular smooth muscle cell; Red line, blocking effect; Green line, activation).

1.6 Alamandine and the MrgD receptor: similarities to Ang (1-7)

Alamandine, a heptapeptide, is closely related to the vasodilator Ang (1-7), with only one amino acid difference, whereby the first amino acid of Ang (1-7) is aspartate, and alanine is for alamandine. Therefore, alamandine can be synthesized from Ang (1-7) by decarboxylation of aspartate into alanine. As these peptides have similar amino acid sequence and structure (Figure 1 and 2), their effects are highly

243 likely to be similar. Through chromatographic approach, it was demonstrated that a peptidase exists in the

244 medulla of sheep brain which converts Ang (1-7) into Ang (1-4) [87]. Although the peptidase was not

245 identified, it was suggested that metalloendopeptidase may have been responsible. More recently, it was

246 noted that the hydrolysis of alamandine may also occur via metalloendopeptidase for Ang (1-7) in kidney

247 tissue [88].

248 Interestingly, Chinese Hamster Ovary (CHO) cells transfected with Mas receptor and incubated with Ang

249 (1-7) resulted in elevated NO levels and eNOS activity, which was inhibited by the Mas receptor blocker ,

250 A779. In addition, wortmannin, a specific phosphatidylinositol 3 (PI3) kinase inhibitor reduced NO levels

251 and eNOS activity in the absence of A779 [36]. Similar effects were also noted in human aortic

252 endothelial cells (HAEC), which constitutively express the Mas receptor [36], as well as in rat hearts [89].

253 In addition, Ang (1-7) through the Mas receptor activates the protein kinase B pathway (PKB) in

254 pancreatic islet endothelial cells [62], increasing eNOS activity and NO production. However, in

255 mesangial cells Ang (1-7) activates Mas receptor via a different protein kinase pathway, PKA, and

256 correlates with increased cyclic AMP [90].

257 To further understand the similar roles amongst Ang (1-7) and alamandine, we recently demonstrated that

258 *ex vivo* treatment of alamandine to isolated rabbit aortas reversed homocysteine-induced endothelial

259 dysfunction by activating the PKA pathway for vasorelaxation [72]. Although we demonstrated the

260 presence of MrgD receptor using immunohistochemistry in another study [22], others have shown that

261 CHO cells transfected with MrgD receptor and cultured with alamandine, high levels of NO is produced

262 but not in normal CHO cells, suggesting that alamandine binds to MrgD receptor to induce NO

263 production via increasing eNOS activity [12]. This specific finding establishes that alamandine binds to

264 MrgD receptor and that the outcome is enhanced NO production through eNOS, the enzyme that produces

265 NO within endothelial cells. Whilst these studies were conducted in non-diseased cells [12], we

266 previously demonstrated that alamandine was not able to prevent endothelial dysfunction in atherogenic-

267 diet fed rabbits, however alamandine is likely to exert more acetylcholine-mediated vasodilation in

268   thoracic and carotid artery compared to acetylcholine-exerted vasodilation alone in these atherogenic

269   vessels [22]. These studies clearly suggest that the role of alamandine may exert beneficial vasodilatory

270   effects on the initial steps of atherosclerosis and may not play a role in advanced stages of atherosclerosis.

271   Possibly, the function and expressions of MrgD receptor on the endothelial cell layer may not be

272   sufficient or efficient when the vessel is completely dysfunctional and, therefore, alamandine has no

273   beneficial effects in such conditions. Hence, early intervention needs to be considered when treating

274   atherosclerosis with alamandine.

275   Moreover, in organ bath experiments, individual incubations of alamandine and Ang (1-7) were not able

276   to reduce Ang II mediated vasoconstriction [22]. Conversely, Ang (1-7) has been shown to reverse Ang

277   II-mediated endothelial dysfunction in mouse aorta [31]. Although the results are conflicting, differing

278   experimental conditions exist. In our study [22], the vessels were incubated with the RAS peptides and

279   then a dose-response curve to Ang II was constructed, whereas in [31], the vessels were initially

280   incubated with Ang II in organ cultures for 24 hours and then Ang (1-7) was incubated, where 30 minutes

281   after this point a dose-response curve to acetylcholine was constructed [31]. These differences suggest

282   that further studies are required to elucidate the roles of alamandine and Ang (1-7) during diseased states

283   such as atherosclerosis.

284

285   **1.7 Conclusion**

286   Studies from our and other laboratories show that Ang (1-7) and alamandine are able to promote

287   beneficial effects on the cardiovascular system, such as, counterbalancing Ang II-associated endothelial

288   dysfunction and therefore, may reduce the development of Ang II associated atherosclerosis.

289

290   *Table 1: A summary of the novel renin angiotensin system (RAS) components and their function and*
291   *mechanisms on endothelial cells*

RAS peptides	Reference	Induce or	Other cardiovascular	Mechanism

and receptors		prevent endothelial dysfunction?	harmful or protective effects?	
Ang II	[46-48]	Induce	Involved in hypertension, smooth muscle cell proliferation	Through NADPH oxidase, reduction in NO
Ang A	[14, 22]	Remains unknown	Vasoconstrictor	Possibly through the AT_1 receptor
Ang 1-7	[22, 31]	Prevent	Anti-proliferative effects, vasodilation	Counterbalance Ang II actions by increasing eNOS activity, and thus NO production
Alamandine	[12, 22, 72]	Prevent	Anti-hypertensive effects, vasodilation	May potentially counterbalance Ang-II actions
AT1 receptor	[82, 83]	Upon agonistic stimulation; induce	Upon agonistic stimulation, vasoconstriction	Superoxide production, increase in NADPH oxidase expression
Mas receptor	[36]	Prevent	Upon agonistic stimulation, vasodilation	Increase in eNOS activity, NO production, and reduction in superoxide production
MrgD receptor	[12, 72]	Prevent	Upon agonistic stimulation, vasodilation	Increase in eNOS activity, NO production via PKA pathway

292 Ang, Angiotensin; AT_1, Angiotensin II type I receptor; eNOS, endothelial nitric oxide synthase; NADPH,
293 nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PKA, protein kinase A.

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