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Is Chymase 1 A Therapeutic Target in Cardiovascular Disease?

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Abstract

Introduction: Non-angiotensin converting enzyme mechanisms of angiotensin II production remain underappreciated in part due to the success of current therapies to ameliorate the impact of primary hypertension and atherosclerotic diseases of the heart and the blood vessels. This review scrutinize the current literature to highlight chymase role as a critical participant in the pathogenesis of cardiovascular disease and heart failure.

Areas covered: We review the contemporaneous understanding of circulating and tissue biotransformation mechanisms of the angiotensins focusing on the role of chymase as an alternate tissue generating pathway for angiotensin II pathological mechanisms of action.

Expert opinion: While robust literature documents the singularity of chymase as an angiotensin II-forming enzyme, particularly when angiotensin converting enzyme is inhibited, this knowledge has not been fully recognized to clinical medicine. This review discusses limitations of clinical trials' that explored the benefits of chymase inhibition in accounting for the failure to duplicate in humans what has been demonstrated in experimental animals.

Keywords

Angiotensin Converting Enzyme; Angiotensin II; Angiotensin peptides; Angiotensin-(1-12); Blood Pressure; Chronic Kidney Disease; Chymase; Diabetes Mellitus; Heart Failure; Myocardial Infarction; Primary Hypertension; Vascular Disease

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1. Introduction

More people die each year from cardiovascular diseases than any other cause [1]. High blood pressure remains a significant risk factor for premature cardiovascular mortality. The magnitude of this problem is reflected in epidemiological surveys revealing that hypertension is present in 1.4 billion people worldwide, with less than one in five people with a hypertension diagnosis having their blood pressure under control [1]. The global antihypertensive drug market is forecast to increase from USD 20 billion in 2021 to USD 40 billion by 2031 [2]. The expansion in market size reflects a staggering rise in the global incidence of hypertension, in part attributable to a rise in cardiovascular disease, increased salt consumption, expansion of the geriatric population, and obesity. While the adoption of combination therapies or single-pill combinations (polypill) may afford greater therapeutic efficacy and increased adherence [3,4], hypertension treatment management remains suboptimal. Angiotensin converting enzyme (ACE) inhibitors and angiotensin II (Ang II) receptor blockers (ARBs) are the most common antihypertensive prescription drug classes in the United States [5]. Both types of medicines are recommended as first-line agents for initiating antihypertensive therapy based on the highest level of evidence [6]. The importance of these drugs in managing hypertension, type 2 diabetes, and heart failure are reflected by a projected market increase in the use of ARBs from 7.85 billion in 2020 to 9.95 billion in 2028. While ACE inhibitors and ARBs demonstrate comparable effects on blood pressure control, randomized clinical trials (RCT) and large meta-analyses indicate that the magnitude of the relative benefit in terms of reducing all-cause and cardiovascular mortality, stroke, heart failure, myocardial infarction, composite cardiovascular events, kidney disease, or diabetes remains uncertain [7-9]. While comparative assessment of the two drugs classes in terms of clinical outcome yields similar effectiveness [10], those analyses do not take into consideration the magnitude of the absolute risk reduction achieved with either of the two drug classes compared to placebo or other antihypertensive medications [9,11,12]. Evidence that blood pressure-independent mechanisms contribute to the adverse cardiovascular remodeling associated with high blood pressure is often not appreciated. Retrospective analysis of clinical endpoints with drugs that block the generation or activity of Ang II by us [9,11] and others [13-15] reveals non-superior efficacy of ACE inhibitors and ARBs over other antihypertensive agents for reducing the risk of myocardial infarction, heart failure (HF) or cardiovascular death. The quantification of the lifetime ***residual risk*** of cardiovascular events in hypertensive patients is several orders of magnitude greater than the risk reduction [9]. This is a disconcerting finding, given the strength of the research implicating the renin angiotensin system (RAS) in the pathogenesis of experimental and human hypertension.

Both extrinsic and intrinsic factors explain the limitations of RAS inhibitors to attain greater reductions in adverse clinical outcomes beyond what is attributed to blood pressure control [9]. A systemic chronic inflammatory response is present in non-communicable diseases such as cardiovascular disease, cancer, diabetes mellitus, chronic kidney disease, non-alcoholic fatty liver disease, autoimmune, and neurodegenerative disorders [16]. The non-superiority of RAS inhibitors over other agents may reflect the failure of these drugs to reach the intracellular sites where Ang II can be generated [17]. Angiotensin II (Ang

II) formation occurs in multiple tissues because RAS genes in these target tissues generate functional proteins [18-20]. Therefore, the production of Ang II intracellularly *(intracrine pathway)* or in the environmental milieu of the interstitial spaces of tissues *(paracrine pathway)* appears critical in mediating the pathophysiology of cardiovascular diseases in specific tissues.

Furthermore, there is a general ignorance of the interplay among the RAS, the kallikrein-kinin system (KKS), and chymase [21]. These peptidergic systems exert proinflammatory and procoagulant effects via the Ang II type 1 receptor (AT_1-R) and the bradykinin type 1 (BKB-1R) receptor, respectively. In addition, bradykinin vasodilator, natriuretic, and antithrombotic effects induce chymase upregulation via activation of BKB-2R [21]. Interwoven interactions among the RAS, K_KS, and chymase play a critical role in regulating cardiovascular function. This review article summarizes the potential criticality of alternate mechanisms of Ang II production via a chymase/Ang II pathway in human heart disease. Furthermore, the material presented here updates a previously published article that recapitulated the patent literature on chymase inhibitors for treating cardiovascular disease between 2010 and 2018 [22].

2. Current understanding of angiotensins forming mechanisms

Today's generally accepted view of the biochemical cascade that accounts for the generation of angiotensin peptides possessing biological activity did not become apparent until the 1970s. The classic literature of that time had concluded that Ang II biological activity was the product of sequential reactions initiated by the generation of the decapeptide angiotensin I (Ang I) through the hydrolysis of hepatic angiotensinogen (AGT) and the subsequent Ang I cleavage by ACE into the active octapeptide pressor hormone Ang II [23,24]. However, it was not until the 1980s that seminal publications from Ferrario and colleagues uncovered the presence of the heptapeptide -angiotensin-(1-7) [Ang-(1-7)] in rat cerebrospinal fluid (CSF) [25] and the concurrent demonstration of its biological activity in the release of neurohypophysial vasopressin [26]. The further demonstration that Ang-(1-7) possessed vasodilator activity in areflexic rats [27] strengthened the potential for Ang-(1-7) to exert a role in the control of arterial pressure. While these discoveries did not immediately alter conceptualizations of the RAS, persistence in the study of Ang-(1-7) biological activity and its potential role in the pathogenesis of hypertension culminated with the discovery that the heptapeptide functions as an endogenous "inhibitor" of Ang II pressor and trophic mechanisms of action [28]. Increased recognition of Ang-(1-7) as an integral component of the RAS was accelerated by the identification of an ACE homolog -ACE 2 (ACE2) [29,30]- functioning as a mono peptidase with high catalytic efficiency for conversion of Ang II into Ang-(1-7) [31,32].

The complexity of enzymatic mechanisms with the ability to generate the angiotensins is influenced by the composition of the tissue sites at which the AGT substrate can be processed to generate Ang I, Ang II, Ang-(1-7), Ang III, the pentapeptide Ang-(1-5), and the alanine substituted angiotensins [Angiotensin A [33]and Alamandine [34]] (Figure 1). These discoveries have enriched our understanding of both the homeostatic control and RAS's dysregulation in human diseases by revealing that the vasoconstrictive, pro-proliferative, and

proinflammatory actions of the ACE/Ang II/AT_1-R are counterbalanced by the vasodilator, antiproliferative, and anti-inflammatory actions of ACE2/Ang-(1-7)/Mas-R axis [35]. A potential third axis is constituted by Angiotensin-A/Alamandine/MrgD-receptor [36].

Figure 2 documents the principal cleavage sites of the angiotensins. The diversity of the branching mechanisms within the RAS that can be activated to generate the different angiotensins subserving endocrine, paracrine, autocrine, and intracrine mechanisms is being further expanded with the more recent identification of a catalytic pathway upstream of Ang I and Ang-(1-9) [9,35]. In 2006, Nagata and colleagues [37] at the University of Miyazaki, Japan, reported the existence of a peptide composed of the first 12 amino acids of the AGT protein in the blood and tissues of a Japanese strain of Wistar rats. Proangiotensin-12 [Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Phe^8-His^9-Leu^{10}-Leu^{11}-Tyr^{12}], as named by the Japanese investigators, was reported to be present in highest concentrations in the gastrointestinal tract. Nevertheless, a substantial concentration of this alternate substrate was detected in the brain, heart, kidney, adrenal gland, and blood [37]. The biological activity of Proangiotensin-12 was evidenced by the abolition of the pressor response induced by intravenous injection of the dodecapeptide by either the ACE inhibitor captopril or the ARB candesartan [37]. The existence of an alternate source for Ang II generation from a molecule composed of 12 amino acids rather than the 425 amino acids contained in AGT was of interest to Ferrario's laboratory because, in past collaboration with one of the authors of this review (RS), we had reported the existence of a family of endogenous Ang II precursors in canine cerebrospinal fluid (CSF) [38]. Since the Nagata et al. [37] report of proangiotensin-12, we and others have provided extensive evidence for the functional role of this alternate substrate as an endogenous source for the production of Ang II., Proangiotensin-12 was renamed as angiotensin-(1-12) [Ang-(1–12)] to maintain consistency with the recommendations of the Nomenclature for Angiotensin Receptors Committee of the American Heart Association [39].

Ang-(1-12) is a precursor for renin-independent Ang II formation in the circulation[40], the human and rat heart [41-46], kidney[47], and bone marrow [48]. Ang-(1-12), cleaved from AGT by a kallikrein-like enzyme [49,50], is preferentially metabolized by endothelial ACE into Ang I in the circulation [43] and cleaved directly into Ang II by chymase in the human heart, rat kidney, and rat bone marrow [41,44,48] (Figure 1). The rate of Ang II formation from the Ang-(1-12) substrate is several-fold higher than from Ang I in plasma membranes isolated from the heart of adult spontaneously hypertensive rats (SHR) [51]. Ang-(1-12) augments rat cardiac myocyte contractility via increasing intracellular K^+ currents in WKY[52] and intracellular Ca^{2+} mobilization in SD rats[53] and TGR(hAGT)L1623 hypertensive rats[53]. In a humanized model of hypertension expressing the human AGT gene (Tg^+-hAGT), cardiac hypertrophy and systolic dysfunction were accompanied by a 4-fold increase in left ventricular (LV) Ang II content that failed to be reversed following a fortnight treatment with valsartan[54]. These data implicate a compensatory activation of intracellular Ang II generation from internalized AGT, Ang-(1–12), or both. This interpretation is in keeping with: (a)- internalization of AGT and Ang-(1–12) in human pigment retina cells by a non-AT_1-R mechanism [45,55]; (b)- augmented incorporation of Ang-(1–12) in neonatal cardiac myocytes in culture[51]; (c)- mobilization of intracellular Ca^{2+}[53] and increase in K^+ currents[52] in primary cardiac

myocyte cultures during superfusion with Ang-(1–12); (d)- the demonstration that inotropic stimulation of cardiomyocytes by Ang-(1–12) is inhibited by superfusion of a chymase inhibitor [56] or intracellular application of valsartan[52].

3. Chymase functions and inhibition

The original demonstration that chymase is the major Ang II forming enzyme in the human heart dates to 1990 [57,58] based upon the inhibition of 75% of Ang I conversion to Ang II by serine protease inhibitors. In 2010, Park et al. [59] noted that while healthy mouse kidneys depended primarily upon ACE to form Ang II from Ang I, diabetic mouse (db/db) kidneys relied primarily upon a serine protease to form Ang II based upon the ability of the non-selective serine protease inhibitor phenylmethylsulfonylfluoride (PMSF) to inhibit a renal arteriolar vasoconstrictor response to Ang I. In an accompanying editorial, Lorenz [60] cited work indicating chymase involvement in human diabetic nephropathy and in the formation of Ang II in human arteries.

Kaltenecker et al. [61], also reported that chymase played a major role in the elevation of renal Ang II levels in chronic kidney disease compared to those of healthy kidney donors.

Chymase is the predominant Ang II forming enzyme in failing hearts as well as in the right ventricles of healthy transplanted hearts [62]. Indeed, no ACE activity was observed in the failing hearts. Of note, Pavo et al. [62] inability to detect Ang I in the failing human hearts suggest that plasma renin did not directly influence myocardial RAS regulation while supporting the conclusion that chymase is the main enzyme responsible for tissue Ang II formation. We have proposed [63] that Ang-(1–12) might be the Ang II precursor in the heart, an interpretation that is keeping with high cardiac levels of Ang-(1–12) in the heart of SHR [64] and transgenic hypertensive rats expressing the human *Agt* gene in their genome [54,65].

Chymase, a member of serine proteases primarily secreted by mast cells, exhibits a twenty-fold higher catalytic efficiency for conversion of Ang I into Ang II [66,67] by its preference for phenylalanine or leucine being in the P1 position (proximate to the scissile bond) according to the Schechter and Berger nomenclature system [68]. Of note, while the action of chymase to form Ang II from Ang I mimics that of ACE, it does not mimic the proficiency of ACE to metabolize Ang-(1-7) to Ang-(1-5) since isoleucine in the P1 position is less optimal for binding to chymase. Additional chymase proteolytic properties include the lysis of extracellular proteins (laminin and fibronectin), activation of transforming growth factor-β_1 (TGF-β_1), interleukin-1β (IL-1β), generation of endothelin (ET)-1(1-31), and degradation of high-density lipoprotein-3 (HDL3) [67,69,70]. The newly identified potency of chymase in direct tissue generation of Ang II from Ang-(1–12) strengthens the importance of chymase as a critical enzymatic pathway for the intracellular actions of the hormone [17,41-43].

Mammalian chymases are classified into two subfamilies, α-chymase and β-chymase [71-73]. A single α-form of mast cell (MC) chymase is expressed in humans while both α- and β-isoforms of chymases (also known as mast cell proteases, rMCP in rat and mMCP

in mice) are found in rodents. The α-chymases in rodents (rats and mice) are elastase-like proteases, and the β-chymases are chymotrypsin-like proteases [74]. While both α- and β-chymases generate Ang II, only a β-form (preferentially rMCP-1) degrades Ang II by cleaving the Tyr^4-Ile^5 bond of the peptide [75]. More recently, a rat vascular chymase (RVC, a β-form) expressed primarily in vascular smooth muscle cells of SHR has been reported to convert Ang I into Ang II [76]. Upregulation of this novel RCV chymase in hypertensive rats may significantly affect vascular proliferation and blood pressure regulation [70,76]. The two β-chymases (rMCP-1 and rMCP-2) expressed in rats MCs have been widely studied; both enzyme isoforms weakly convert Ang I into Ang II compared to human α-form chymase [77,78]. Except for rMCP-1, rMCP-2, and RVC, the substrate specificity of rMCP-3 and rMCP-4 for generating Ang II from Ang I remains unknown [22].

Because chymase has many substrates other than angiotensins, a dysregulated processing of chymase substrates is predominantly detrimental [79]. For example, chymase activates matrix metalloproteases and releases transforming growth factor (TGF)-β1 from its latent state, both of which are implicated in albuminuria, mesangial cell expansion, and interstitial fibrosis, hallmarks of diabetic kidney disease (DKD) [80]. So there are multiple reasons why chymase inhibitors have therapeutic potential.

Chymase can be inhibited by a large number of serine protease inhibitors such as chymostatin, 4-(2-Aminoethyl)benzene sulfonyl fluoride hydrochloride (AEBSF), soybean trypsin inhibitor, SoybeanBowman–Birk protease inhibitor (BBI), Eglin c, secretory leukocyte protease inhibitor [(SLPI), also known as mucus proteinase inhibitor [81]], and several endogenous serpins (see below) that are described in various protein databases [81].

The endogenous serpins (SERineProteaseINhibitors) that inhibit the activity of chymase include α-1-antitrypsin (AAT, α-1-proteinase inhibitor) [68]; α-1 antichymotrypsin (ACT, Serpin A3) [68]; seminal human inhibitor I (HUSI-I), anti leukoprotease (ALP) [82]; and squamous cell carcinoma antigen 2 (SCCA2, serpin B4) [83]. Other serpins can inhibit chymase, but their low potency makes them physiologically inconsequential.

Fulacimstat (BAY1142524) is the only specific chymase-inhibiting drug to be developed for potential clinical application. It is reported to have an IC_{50} of 4 nM for human chymase [84]. In hamsters with cardiac fibrosis, Fulacimstat dose-dependently reduced the extent of the fibrosis. Additionally, hamsters with experimentally induced myocardial infarctions treated with Fulacimstat had a significantly lower end-diastolic pressure than the placebo group [84].

3.1. Chymase inhibitors and cardiovascular disease

Chymase has been called the “chameleon of host defense and tissue remodeling due to its many effects on tissue remodeling [67,85]. Numerous chymase inhibitors have been tested in preclinical animal models of acute and chronic injury targeting various organs with largely beneficial effects (Table 1). However, despite this great success in preclinical studies, only one clinical trial of a chymase inhibitor is reported in the literature. Recently, a Phase II clinical trial in patients with ST-segment elevation myocardial infarction reported

no improvement in adverse LV remodeling and LV systolic function when Fulacimstat was added to conventional post-myocardial infarction medical therapy [86,87].

In the (CHIARA MIA) 2 trial, patients were randomized to the chymase inhibitor Fulacimstat (n=54) or placebo (n=53) five to nine days after an ST Elevation Myocardial Infarction [86,87]. Left ventricular ejection fraction (LVEF) obtained by cardiac magnetic resonance imaging was < 45% in all patients, with the change in LVEF at 6 months being equivalent between treated and placebo groups. There were no differences in cardiovascular demographics or the use of beta-blockers, ACE inhibitors, or Ang II type 1 receptor antagonists. In addition, no significant differences were reported between the treatment groups in LVEF, LV end-diastolic or end-systolic volume index, or infarct size at baseline or after 6 months of treatment in this patient cohort with a high risk of LV remodeling. These findings contradict the beneficial effects of various chymase inhibitors in preclinical in vivo animal models of ischemia-reperfusion injury or coronary ligation. Comparing the timing of chymase inhibitor administration in the preclinical animal studies and this human study provides insight into this disappointing result.

We have shown a significant influx of chymase into cardiomyocytes and an increase in interstitial fluid chymase-mediated Ang II formation within two hours of coronary ligation in the dog [88]. Pretreatment with the chymase inhibitor significantly decreases interstitial fluid Ang II formation and troponin release [88]. Studies in the pig [89], hamster [90-92], rat [93], and dog [88] demonstrate that early treatment with a chymase inhibitor within 24 hours after coronary artery ligation decreases infarct size resulting in improved LV remodeling and LV function. Other studies of ischemia-reperfusion injury show a decrease in infarct size when a chymase inhibitor is administered shortly after induction of ischemia/reperfusion injury [94]. Treatment with a chymase-specific inhibitory RNA aptamer during coronary occlusion in the hamster also improves LV remodeling and function in four weeks [95]. Finally, the absence of mast cell protease in the mouse following coronary artery ligation results in decreased infarct size and improved LV size and function compared to wild-type mice [96-98].

Taken together, blockade or absence of chymase from the outset of coronary ligation or ischemia/reperfusion confers acute tissue protection that reduces infarct size, improves LV remodeling, and LVEF at one month. In addition to a decrease in Ang II, a significant effect of chymase inhibition in these studies is the significant decrease in MMP-9 [88,89,99], a major component of the polymorphonuclear cells that are essential mediators of acute injury of ischemia or infarction. In support of acute chymase activation in the human with ischemia/reperfusion, we have shown a 4-fold increase in pericardial fluid chymase activity within 4 hours after cardiopulmonary bypass [100]. This increase in chymase activity relates to intraoperative cross-clamp and total operative time. In another study of patients with myocardial infarction, early peak values of chymase- and Cathepsin G-dependent angiotensin II formation in circulating mononuclear leukocytes correlate with elevations of creatine kinase [101]. These studies may explain the failure of chymase inhibition to improve LV remodeling and function in the recent CHIARA MIA 2 clinical trial, where the chymase inhibitor was started six to 12 days post-myocardial infarction [86,87].

Preclinical studies in chronic heart failure report beneficial effects of chymase inhibition in the dog with pacing tachycardia [102] and with experimentally induced mitral regurgitation [103]. The nature of these two models of heart failure is a chronic state of inflammation due to the extreme nature of the stress that is unabated throughout its course until animal euthanasia. The dog model is especially clinically relevant because instead of multiple α- and β- isoforms of mast cell chymase in the rodent, the dog possessed only the alpha isoform that is very similar to the human. The silica and bleomycin models of acute pulmonary injury and subsequent pulmonary fibrosis are other models where the acute stress is treated within onset or 24 hours of inception. Chymase inhibition started within 24 hours results in reduced pulmonary fibrosis at 14 and 30 days after administration of these toxic agents [104,105].

One area of potential use of a chymase inhibitor in a chronic setting is preventing atrial fibrillation [106]. Chymase activity is four-fold higher in the left atrium compared to the right atrium and LV [99,107]. In patients with Primary Mitral Regurgitation (PMR) chymase activity was associated with extensive fibrosis, increased left atrial volume, and depressed left atrial function. Chymase-mediated Ang II formation also has an important potential intracellular electrophysiological effect. Rapid field electrical stimulation of HL-1 atrial cells induced a sustained augmentation of intracellular Ca^{2+} associated with increases in ACE and chymase activities and AGT expression [107]. Furthermore, transmission electron microscopy reveals chymase presence within rat cardiomyocytes [108] and human left atrial myocytes [99]. Intracellular administration of Ang (1-12), the preferred chymase substrate [41,43], into adult rat cardiomyocytes caused depolarization of the cell surface membrane with an increase of duration of the action potential followed by the generation of early afterdepolarizations due to a decrease in the potassium current [52]. Administration of the chymase inhibitor -chymostatin- (10^{-9} M), abolished the effect of intracellular Ang-(1-12) on the potassium current[52]. Thus, extracellular effects of chymase on inflammation and fibrosis and its intracellular effects of potassium current combine for important mechanisms underlying the genesis of atrial fibrillation.

The large presence of circulating serine protease inhibitors has clouded a potential chymase-mediated mechanism of ACE escape in the intravascular space and for blood pressure control. Urata and coworkers demonstrated a positive correlation between circulating mononuclear cell chymase-mediated Ang II formation and atrial fibrillation [109] and blood pressure in large cohorts of patients undergoing routine cardiovascular examination. Furthermore, chymase presence in endothelial cells can also serve as a means of chymase-mediated intracellular Ang II formation and secretion into the circulation. Urata and coworkers [110] have reported a major role of chymase in salt-sensitive elevation of blood pressure. A chymase inhibitor (TPC-806) prevented the elevation of blood pressure in mice with excessive salt intake [111]. In a follow-up study in humans, TSP-806 decreased blood pressure with mild hypertension after one dose [111]. Responders had a higher estimated salt intake than the non-responders, suggesting that chymase inhibition is more effective for salt-dependent hypertension. In addition, chymase inhibition prevented vascular inflammation and dysfunction in angiotensin I-infused and stroke-prone hypertensive rats, there were important anti-inflammatory vascular effects of chymase inhibition, improving survival without blood pressure reduction [112,113].

3.2. Chymase inhibition, diabetes, and cardiometabolic disease

Experimental observations suggest renoprotective and anti-diabetic actions of chronic chymase inhibition [114]. In a phase II trial (NCT03412006), Fulacimstat was administered for six months to type II diabetes mellitus (T2DM) patients diagnosed with diabetic kidney disease. The outcome measure was a reduction in the urinary albumin to creatine ratio (UACR). In addition, the trial assessed the safety and tolerability of Fulacimstat. While Fulacimstat was well tolerated and safe, the 19.6% reduction in UACR relative to the placebo group fell short of the anticipated 30% reduction for statistical significance. One of the conclusions made by the authors was that the animal models of diabetic kidney disease (DKD) that showed marked elevation of chymase activity and improvements with chymase inhibition might not be representative of DKD in humans [115]. However, some preliminary data was obtained from humans, including a demonstration of 10 - 15-fold increases in chymase activity in diabetic kidneys [116], and upregulation of chymase activity in human mesangial cells cultured in a high-glucose medium [117]. It may also be of note that chymase activity is also increased in polycystic kidney disease patients [118]. Another chymase inhibitor under development was JNJ-10311795/RWJ-355871. This drug inhibited inflammatory responses to glycogen-induced peritonitis and lipopolysaccharide-induced bronchial inflammation by reversing increases in the proinflammatory cytokines IL-1α, IL-1ß and TNFα- [119]. RWJ-355871 also showed anti-inflammatory effects in several additional rat models of inflammation, with therapeutic promise for treating airway inflammatory diseases [87,120]. The potential translation of these experimental findings to the clinical management of type-2 diabetes mellitus awaits resolution, as no clinical trials are registered for any chymase-inhibiting drugs on ClinicalTrials.gov as of June 2023.

Mast cell degranulation inhibitors may be an alternate, albeit indirect, way to suppress chymase contribution to diseases. These drugs, acting to prevent mast cell degranulation, impede the liberation of chymase to act upon its extracellular substrates. The first mast cell degranulation inhibitor, sodium cromoglycate disodium 5,5’-[(2-hydroxytrimethylene)dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylate], also known as cromolyn sodium (Intal®) was marketed as an inhalant for the treatment of asthma in the early 1970’s. Subsequently, it was marketed as a nasal spray (Nasalcrom®) for the treatment of allergic rhinitis and eye drops (Opticrom®) for allergic irritation of the eyes. A subsequent preparation was developed as an enema to treat inflammatory bowel disease with limited efficacy. An orally administered concentrated solution form of cromolyn sodium (Gastrocrom®) is currently marketed for the treatment of mastocytosis. The “membrane stabilizing” mechanism of action of cromolyn sodium remains uncertain but has many proposed mechanisms based upon a relatively recent review [121]. Additional approaches to inhibiting mast cell degranulation block the post-receptor signaling pathways leading to mast cell degranulation [122]; inhibition of Bruton’s tyrosine kinase with ibrutinib (Imbruvica®) [123] to antagonize the substance P receptor [124]; coumarins possessing high affinity agonism for GPR35 [125]; and flavonoids [126]. No information exists on the potential effect of these drugs on heart and blood vessel diseases or type 2 diabetes mellitus.

4. Conclusion

An abundance of evidence indicates that mast cell chymase plays a crucial role in tissue Ang II formation from Ang I and upstream precursors. Mast cell-derived chymase activity contributes to tissue damage, cardiac remodeling, and cardiovascular disease. However, clinical trials of chymase inhibitors have not yet led to their implementation as cardiovascular therapeutics despite promising preclinical results. The importance of chymase in contributing to Ang II pathological actions may be most critical in conditions in which ACE activity is suppressed, so investigation of the therapeutic benefits of chymase inhibitors should continue.

5. Expert opinion

Chymase inhibition remains largely an obscure therapeutic concept in 21st-century medicine. However, its relative novelty and therapeutic potential have made this a promising area of research. Interestingly, with all the chymase inhibitors developed to date, no chronic human study has demonstrated their efficacy in treating hypertension, heart failure, and diabetes. However, it is tempting to speculate that given the many proinflammatory effects of chymase, in addition to Ang II forming capacity, the plan for any clinical trial should be highly contextual concerning the timing of administration and type of remodeling process. In that respect, there may be a role for a chymase inhibitor in patients with resistant hypertension who cannot reduce a high salt intake and have a high risk for atrial fibrillation. In addition, preclinical studies suggest that a trial of chymase inhibitor in the acute phase of myocardial infarction may attenuate acute myocardial injury and result in improved LV remodeling and function over time.

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Article highlights

- The therapeutic potential of chymase in the treatment of cardiovascular disease remains to be appreciated.
- Chymase represents an important enzymatic mechanism for the formation of Ang II from either Ang-(1–12) or Ang I.
- Chymase has multiple substrates other than angiotensins, the metabolism of which is predominantly detrimental.
- As a member of the serine protease family, chymase can be inhibited by many serine protease inhibitors.

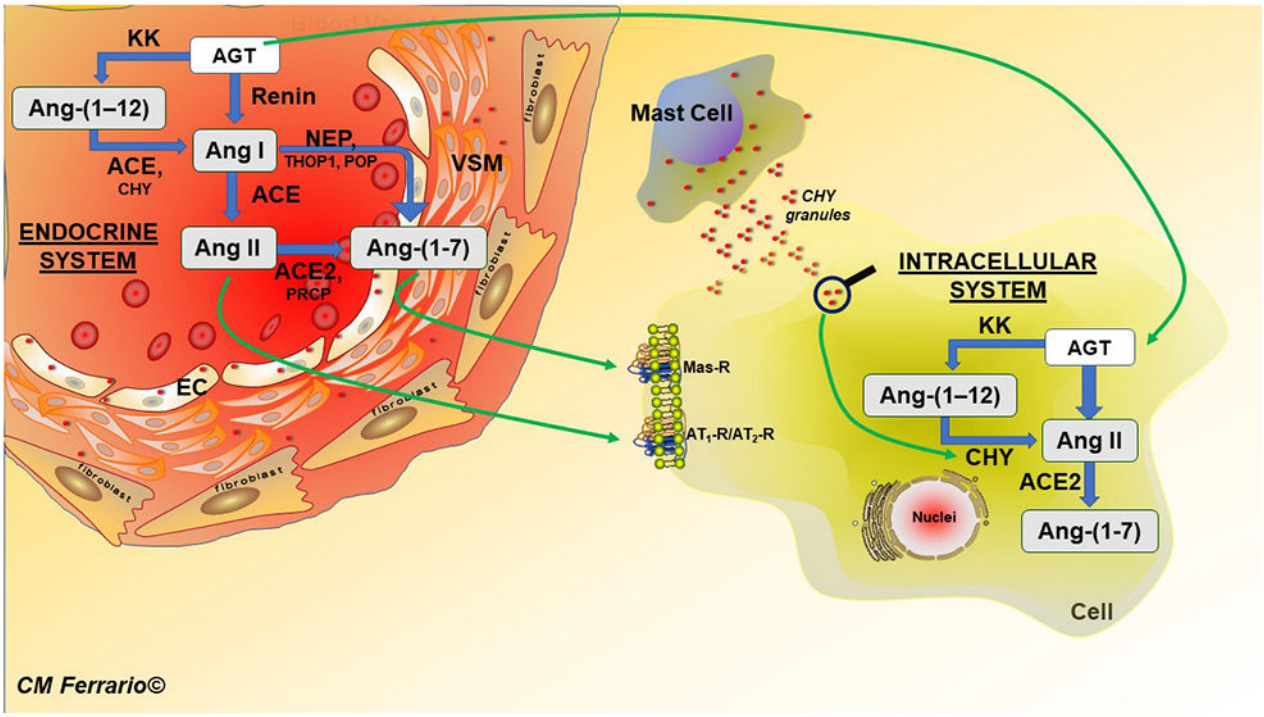

Figure 1.

Schematic diagram of the main biochemical pathways of the angiotensin peptide production via the circulation (**endocrine system** depicted in the insert as a blood vessel segment) and intracellularly. The illustration depicts a role of mast cell degranulation of chymase for intracellular incorporation and direct metabolism of Ang-(1–12) into Ang II. Abbreviations, EC, endothelial cell; VSM, vascular smooth muscle. POP, prolyl oligopeptidase; THOP1, thimet oligopeptidase. Other abbreviations as in text.

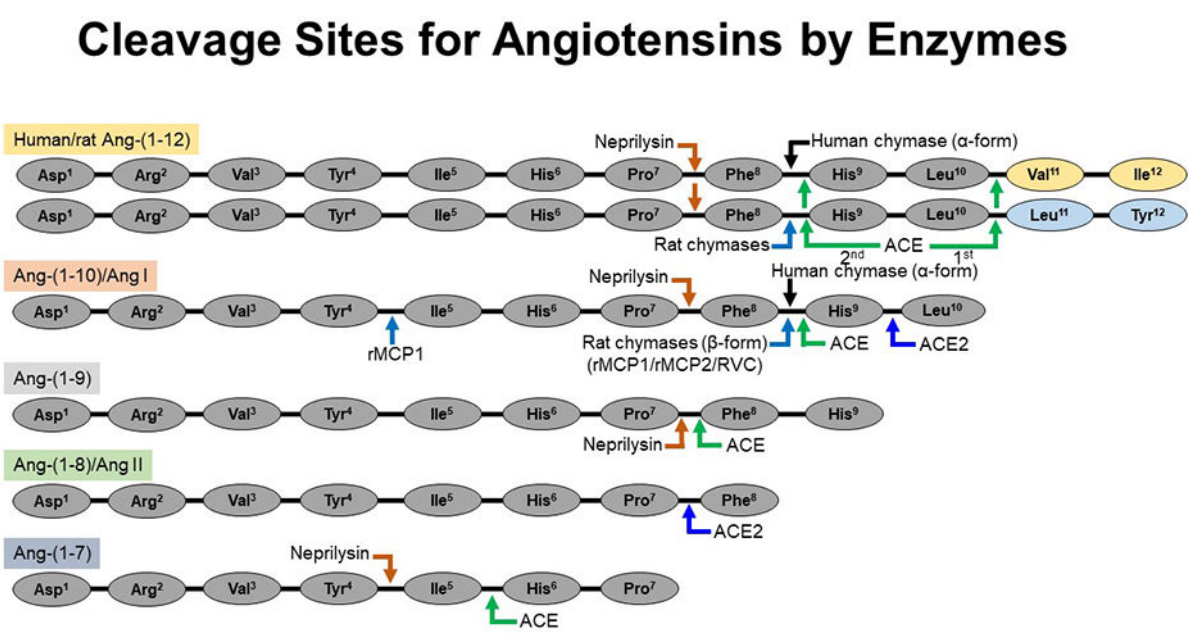

Figure 2.

Cleavage sites for the processing of angiotensin peptides by chymase, ACE, ACE2, and neprilysin. Humans (α-chymase) and rat (β-chymases) directly cleave the Phe^8-His^9 bond of the Ang-(1-12) to generate Ang II directly. Similarly, rat β-chymases (rMCP-1, rMCP-2 and RVC) cleave the Phe^8-His^9 bond of the Ang I to generate Ang II directly. ACE cleaves Ang-(1-12) [sequentially, 1st Leu^{10}-Val^{11} (human)/Leu^{10}-Leu^{11} (rat) and then Phe^8-His^9 bonds] and Ang I [Phe^8-His^9 bond] to generate Ang II. ACE2 also cleaves the Ang I [Phe^8-His^9 bond] to generate Ang-(1-9). Ang-(1-12), Ang I and Ang-(1-9) are cleaved by neprilysin (an endopeptidase) at Pro^7-Phe^8 bond to generate Ang-(1-7). Rat β-chymase (rMCP-1) may hydrolyze Ang II at the Tyr^4-Ile^5 bond. Ang II is not further cleaved by human α-chymase but, ACE2 cleaves the Pro^7-Phe^8 bond of Ang II to generate Ang-(1-7). Ang-(1-7) is cleaved by ACE and neprilysin to generate Ang-(1-5) and Ang-(1-4), respectively. Abbreviations: rat mast cell protease-1, rMCP-1; rat mast cell protease-2, rMCP-2 and rat vascular chymase, RVC.

Table 1.

Studies of Chymase Inhibition in Mice, Rats, Hamsters and Humans

Condition	Model	Chymase inhibition effects	Reference
Intermittent Hypoxia	mouse	Decreased perivascular fibrosis and cardiomyocyte hypertrophy, inflammatory cytokines, oxidative stress, Ang II, and superoxide production in LV myocardium	[127]
Dextran sodium sulfate-induced colitis	mouse	Decreased neutrophil infiltration and MMP-9 activity resulting in improved disease activity index and histological scores	[128]
Ang II induced aneurysm	ApoE-deficient mouse	Decreased MMP-9 activity and macrophage infiltration. Prevented Ang II-induced aortic aneurysm formation in ApoE-deficient mice	[129]
Increased salt diet	mouse	Suppressed hypertension and decreased plasma Ang II and aldosterone	[110]
Myosin-immunized myocarditis	rat	Decreased MMP-9 activation, TGF-β expression, and macrophage infiltration; Improved survival and LV function 4 weeks after immunization	[130]
Stroke-Prone SHR	rat	Decreased aortic MMP-9 activity, TNF-α, MCP-1 levels and macrophage infiltration; Improved vascular function *in vitro* and survival	[113]
Indomethacin-induced colitis	rat	Decreased intestinal wall MMP-9 activation and myeloperoxidase activity; Decreased intestinal lesions and damage	[131]
Lipopolysaccharide induced liver injury	hamster	Improved liver function and reduced liver necrosis and fibrosis; Decreased liver MMP-9 activation and myeloperoxidase and TNF-α levels	[132]
Carbon tetrachloride-induced chronic liver failure	hamster	Decreased liver myofibroblasts and fibrosis and decreased liver Ang II levels	[133]
Elastase-induced aneurysm formation	hamster	Decreased abdominal aortic aneurysm size and mast cell infiltration	[134]
Streptozotocin-induced diabetes	hamster	Decreased LV NOX4-induced oxidative stress, malonaldehyde levels, and interstitial fibrosis; Attenuated kidney oxidative stress, decreased renal fibrosis and TGF-β, and improved renal function	[135-137]
Cigarette smoking and bleomycin-induced lung injury	hamster	Decreased lung TGF-β signaling, ET-1 levels, and pulmonary hypertension and fibrosis	[138, 139]
Coronary artery ligation	hamster	Improved LV systolic function and hemodynamics, hypertrophy and fibrosis; Improved survival	[90,91,140]
Obstructed kidney	hamster	Attenuated tubulointerstitial fibrosis and TGF-β and α-smooth muscle actin expression	[141]
Prolonged high-fat diet	hamster	Prevented lipid deposition in the aortas	[142]
Cardiac Ischemia Reperfusion	mouse	Decrease infarction size and improved LV function	[94]
Cardiac Ischemia Reperfusion	pig	Decreased myocardial infarction size and tissue MMP-9 activity	[89]
Cardiac Ischemia Reperfusion	dog	Decreased troponin, MMP-9 activity, and cardiac interstitial chymase activity and attenuated mitochondrial damage	[88]
Post-Transmural Myocardial Infarction	human	Did not improve LVEF or LV volumes six months after drug initiation 5-10 days after myocardial infarction	[86,87]
Hypertension	human	Polygonum inhibition of chymase caused a depressor effect especially in hypertensive subjects with excessive salt intake	[143]
Carotid artery bypass	dog	Inhibited vascular proliferation in grafted vein	[144]
Orally active chymase inhibitor	hamster	Suppressed heart chymase activity	[145]

Abbreviations: LV - Left ventricular, LVEF - Left ventricular ejection fraction, TNF – Tumor necrosis factor, MMP-9 Matrix metallopeptidase 9, MCP-1 - Mast cell protease 1, NOX4 - NADPH oxidase 4, ET-1 – Endothelin 1.