

Therapeutic Potential of Chymase Inhibitors in Cardiovascular Diseases: An Overview

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ABSTRACT

Chymase belongs to the family of serine proteases and is mainly warehoused in a heparin proteoglycan macromolecular complex within the mast cells. Extensive studies have been carried out in the last few decades to assess the role of chymase in human diseases. Recent studies have shown the significance of chymase in blood pressure regulation owing to its efficient angiotensin II forming activity. Angiotensin II-generation routes that are associated with human cardiovascular diseases have pathophysiological importance which is still argumentative. Chymase inhibitors play a distinctive role in regulating the renin-angiotensin system as compared to the inhibitors of angiotensin-converting enzyme and type 1 angiotensin II receptor. Therefore, this mechanism may have a role in medical applications of chymase inhibitors by inactivation of the local renin-angiotensin system to avoid cardiovascular diseases. This review highlights the significant role of chymase inhibitors as a potential approach for the management of cardiovascular diseases such as atherosclerosis, hypertension, vascular proliferation, myocardial infarction and heart failure.

Key words: Chymase inhibitors, Angiotensin II, Angiotensin-converting enzyme, Mast cells, Blood pressure regulator, Cardiovascular diseases.

INTRODUCTION

Chymase was first recognized and localized in mast cell granules in 1959. It is chymotrypsin-like serine-class peptidases of the trypsin family, which has a significant role in angiotensin II development. This protein is the primary enzyme-producing angiotensin II (Ang II) in the human heart and possesses a catalytic potency which is 20 folds better than the angiotensin-converting enzyme (ACE). However, the angiotensin II produced via the chymase pathway (non-ACE pathway) had a limited role for the short-term effects on hypertensive patients. Serine proteases also contribute to the degradation of the extracellular matrix, apolipoproteins and activation of transforming growth factor- β (TGF- β) and the production of endothelins, all these

effects are responsible for the vascular rupture. Within mast cell, chymase doesn't exhibit enzymatic activity. As a result of robust stimulation (vascular injury via balloon catheter), chymase released out of mast cell into the interstitial tissues and become active.¹ In addition, chymase activation is associated with vascular diseases as shown in Figure 1. The notion that chymases are available in various tissues and they cut various substrates suggests that chymases may perform several specific roles in physiological and pathological situations.

CHYMASE BIOLOGY

Primarily chymase is produced as ineffectual precursors and stocked as active enzymes in the secretory glands. Before secretion

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of chymase, it is activated intracellularly via a type of thiol proteinase called dipeptidyl peptidase I.^{2,4} In general, chymase remains inactive in mast cells because of differences in the pH values. The optimum pH for functionally active dipeptidyl peptidase I is slightly acidic with a pH around 6.0. However, in more acidic conditions (pH = 5.5) the granules are in the secretory phase and the most favorable chymase pH values in the human vascular system range from 7 to 9 (Figure 2). Consequently, after vascular inflammation or injury, chymase will be released into the interstitial tissues and begin its enzymatic activities.^{5,6} When chymase is released, it will be circumfluent by extracellular fluid that contains endogenous inhibitors, as alpha-1 antitrypsin⁶ while its complex (heparin-chymase) is more resistant against serine protease inhibitors.^{3,6} Specific chymases were defined in different organisms and according to the structure and specificity of chymase, they are categorized into two subgroups namely; alpha and beta⁷ and are depicted in Figure 3. Indeed, both chymase subgroups produce angiotensin II from angiotensin I. In human, where the alpha subgroup is found, it produces angiotensin II by breaking the Phe⁸-His⁹ bond of the non-bioactive peptide angiotensin I.⁷⁻⁹ On the other hand, the beta subgroup is absent in humans, it hydrolyzes the Tyr⁴-Ile⁵ bond of angiotensin I and II producing inefficient metabolites.^{3,5,7} The beta subgroup causing degradation of angiotensin II is presented in Figures 4 and 5.

In human, chymases are present in mast cells throughout the blood vessels and heart, but not found in plasma. Mast cell adventitia in blood vessels mainly have chymase, whereas vascular endothelium mainly contains ACE^{10,11} and circulating angiotensin I cannot easily penetrate the interstitial compartment.

PHYSIOLOGICAL/ PATHOPHYSIOLOGICAL EFFECTS

Chymase has additional effects other than the transformation of angiotensin I into angiotensin II. These include degrading the extracellular matrix *via* apoptosis process stimulation, which leads up into laminin and fibronectin degradation which are essential for cell survival; gap junction proteins that are crucial in regulating intestinal permeability and insulin-derived growth factor 1, which contradicts the favorable effects in ischemia/ reperfusion injury, activation of precursors to peptides and enzymes, namely metalloproteinases matrix such as matrix metalloproteinases-9, kallikrein and interleukin-6.^{2,12,13} Chymase has the ability to up-regulate the TGF- β expression from latent TGF- β -binding proteins, which play a significant role in both walling off injured areas as well as negative regulation of tissue inflammation. Thus, TGF- β functions as a primary modulator of tissue repair. The atherosclerotic effect of chymase has been previously demonstrated by the degradation of apolipoprotein A1 of high-density lipoprotein 3, thus impairing the efflux

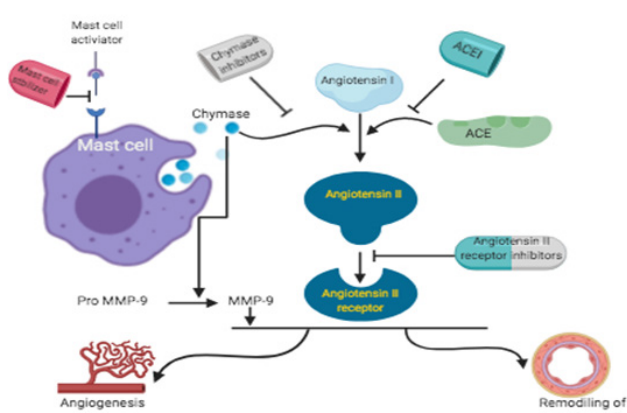


Figure 1: Schematic representation of chymase activity. Chymase has no enzymatic activity in normal blood vessels. It is stored in mast cell and acts as preventer for vascular disease through three pathways: Mast cell stabilizing; inhibitor for chymase; and angiotensin II receptor inhibitor. The use of mast cell stabilizer inhibits activation of mast cell and reduces the release of chymase, whereas blocking chymase by chymase inhibitors inhibits the angiotensin II production and MMP-9 activation. The use of angiotensin II receptor blockers inhibits the effect of angiotensin II produced throughout chymase pathway.

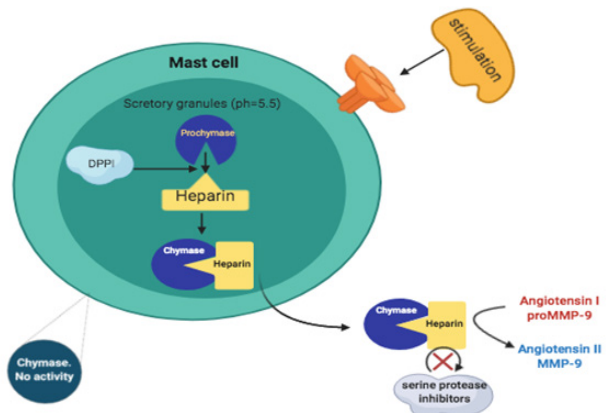


Figure 2: Schematic representation of synthesis and chymase activity. Mast cells contain secretory granules synthesize prochymase which then converted to chymase by optimal activity of dipeptidyl peptidase I at pH=6. Chymase then complex with intracellular heparin proteoglycan. When mast cells are strongly activated, this complex is released into the interstitial tissues, it exhibits direct activation via prompting in vascular tissues and stimulates the production of angiotensin II and activate metalloproteinase 9. Heparin-chymase complex enzyme is resistant to serine protease inhibitors despite of chymase lonely is blocked via these inhibitors.

of intracellular cholesterol. These proteases include certain matrix metalloproteases, plasmin and kallikrein and chymase. Hence, chymase inhibits elimination of cholesterol by high-density lipoproteins and might progress to atherosclerosis.^{14,15} Endothelins are mainly associated with various cardiovascular diseases including hypertension, heart failure, atherosclerosis, restenosis, idiopathic cardiomyopathy and renal failure. It exhibits various biological properties, such as vasoconstriction, stimulation of cell proliferation both inside and outside the cardiovascular system. Synthesis of endothelins-1 takes place with the help of endothelin converting enzymes, chymases and non-endothelin converting enzyme metalloproteases. Regulation of endothelins-1 both in vascular and nonvascular cells takes place in an autocrine fashion. Chymase can convert pro-IL-1 β into a biologically active form which is found to be different from its N-terminus (three amino acids longer) as compared to the mature protein produced by caspase-1, which is a pro-inflammatory cytokine.^{2,12}

VASOCONSTRICTOR ACTIVITY

One of the main regulatory mechanisms of blood pressure is through angiotensin II.¹⁶⁻¹⁸ Chymase also demonstrates a substantial role in the formation of angiotensin II from angiotensin I, which is evident from both the *in vitro* and *in vivo* clinical researches by utilizing a specific chymase substrate (Pro¹¹D-Ala¹²)

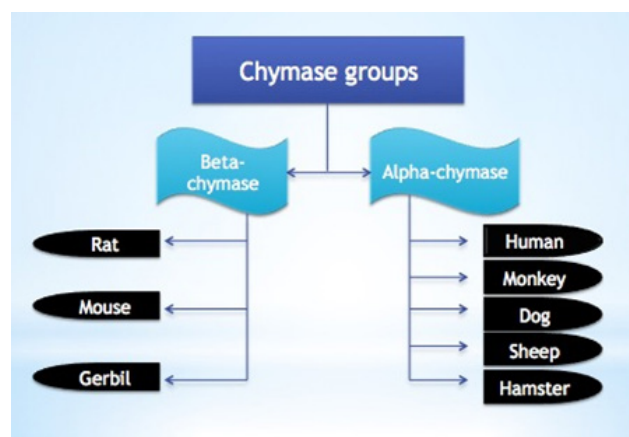


Figure 3: Classification of chymase subgroups.

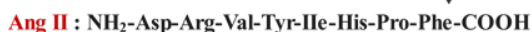
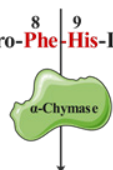


Figure 4: Conversion of angiotensin I to angiotensin II via alpha-chymase action.

and tissue homogenates.^{3,10,19} In a study that carried out in 1998 by Takai *et al.*²⁰ to confirm that chymase is the angiotensin II-generating enzyme, the arteries have shown to be contracted by chymase specific substrate in homogenates of human gastroepiploic arteries by the effect of chymase inhibitor chymostatin in the rate of 90%.

Literature also suggests that ACE predominantly transformed angiotensin I to angiotensin II in the human vascular tissue extraction.²⁰ Nishimura *et al.* also explain the mechanism of synthesis of angiotensin II from a route other than ACE pathway in the cardiovascular system.²¹ This study concluded that when injected chymotrypsin without additives by the existence of angiotensin I, the result indicates no effect by chymase inhibitor on aortic responses to angiotensin I. On the other hand, the addition of Pro,¹¹ D-Ala¹² angiotensin I with chymostatin, the contraction was inhibited.²¹ Mangiapane *et al.* assessed the role of chymostatin and ACE inhibitor on angiotensin I response wherein it was found that there is no effect of the chymostatin alone on the angiotensin I response. However, with the presence of ACE inhibitor, chymostatin is capable of eliminating the residual response, despite of limited physiological effect of chymase of angiotensin II production. Moreover, it has been observed that there is a residual angiotensin II production with the presence of ACE inhibitors, this is due to the influence of chymase.²² A clinical study was carried out in 2003 on human vasculature by Tom *et al.* to evaluate the differences of ACE versus chymase activity in human coronary arteries. The study showed that angiotensin II generation is more dependent on ACE comparing to the chymase pathway.²³

DISTRIBUTION AND ACTIVITY IN CARDIAC TISSUE

The cardiac chymase converts approximately 80% of angiotensin I into angiotensin II compared to ACE.²⁴

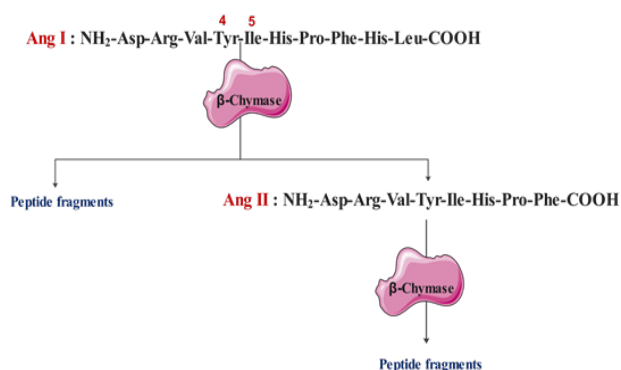


Figure 5: Degradation of angiotensin I and angiotensin II to inactive fragments.

The distribution of chymase and ACE were first recognized by Urata *et al.* who tested these enzymes in patients with cardiovascular diseases.²⁵ The right side of the heart is more abundant with ACE, the right ventricle had about three folds compared to the left ventricle, the right atrium is about two folds higher to left atrium.²⁵ These data illustrated the pivotal function of chymase in the case of left ventricular hypertrophy and the significance reaction of cardiac chambers to ACE therapy.

Chymases are localized in the interstitium and myocardium; it might be accountable for extracellular matrix angiotensin II production, which resulted in collagen sedimentation and fibrosis.²⁶ Cardiac chymase is recognized to up-regulate the TGF- β from latent TGF- β -binding proteins that cause of myocardial fibrosis.²⁷ A study was performed by Nussberger *et al.* to determine the production of angiotensin II and its metabolites. It was demonstrated that the chymase-heparin complex was resisted to serum serine proteinase.²⁸ This correctly elucidated the cause of remained chymase, as active form with exists of serine inhibitors in the extracellular matrix. Systolic heart failure is one of the sequela to remodeling induced by angiotensin II.²⁹ In a 2008 report, Amir *et al.* studied the relationship between chymase and ACE gene polymorphisms linked to genotype- interactions in victims with diminished ejection fraction heart failure.³⁰ The study showed that chymase 1 polymorphism, with particular homozygous G allele, is mainly associated with left ventricular systolic dysfunction. The effect of these polymorphisms may be mediated by an acceleration of cardiac remodeling in patients with heart failure. This remodeling process was mainly linked to the inflammatory mechanism mediated by mast cell chymase.³⁰ Matsumoto *et al.* also studied the function of chymase in left ventricular remodeling induced by oxidative stress via angiotensin II upregulation by the initiation of chymase, leading to the overexpression of inflammatory cytokines and TGF- β , causing progress of left ventricular remodeling.³¹ It was noticed that the acute myocardial infarction resulted in increased cardiac chymase and ACE activities significantly in three days showing that the activation of cardiac chymase after myocardial infarction could have an important effect in post-myocardial infarction pathology.³²

DISTRIBUTION AND ACTIVITY IN VASCULAR TISSUE

Hypertension and vascular disorders are mainly related to vasoconstriction;³³ angiotensin II implicates vasoconstriction in acute hypertension.³⁴ Vascular angiotensin II is obtained from circulating angiotensin

II produced by ACE³⁵ whereas the localized angiotensin II in the vessel is produced by the action of both the ACE and chymase.³⁶ A study was performed to determine the chymase and ACE effects on angiotensin II production. It was found that a high formation rate reached (~70%) when captopril was injected into vascular tissues. Additionally, the vasoconstrictive condition was also successfully developed suggesting the feasibility of an alternative route of angiotensin II production.³⁷ Thus, chymase may be safely used in normotensive patients, owing to its cardiac and vascular anti remodeling advantages with limited hypotension as an adverse effect.

CHYMASE AND THE RISK OF CARDIOVASCULAR DISEASES

Atherosclerosis

A study was done in 2001 by Ortlepp *et al.* explained some hindrance regarding the potential of activated mast cells to damage and deactivate enzymes helps in lipoprotein metabolism and its association with atherosclerosis and consequently increasing prevalence of venous coronary artery bypass grafts.³⁸ Chymase inhibitor, SUN-C8257 (3-[(3-amino-4-carboxy) phenylsulfonyl]-7-chloroquinazoline-2,4 (1H,3H)-dione), is studied by Uehara *et al.* in an animal model to determine the chymase inhibitor effect on atherosclerotic. The study concluded that the process of atherosclerotic in animal model was blocked.¹⁵ The interesting study carried out by Ohishi *et al.* to understand the relative localization of ACE, chymase and angiotensin II in human coronary atherosclerotic lesions. This study confirmed that chymase is upregulated in activated mast cells, which in turn leads to higher formation in coronary atherosclerotic lesions, co-localization of angiotensin II and ACE, except for chymase, was recognized in the ruptures.³⁹

Aortic aneurysm

The presence of aortic aneurysms in the human abdomen is associated with an increment in the number of activated mast cells and the activity of chymase. The progression of aortic aneurysm could results in extracellular structural degradation of matrix proteins caused by the inflammatory process and atherosclerotic lesion. A study by Nishimoto *et al.* to compare normal aortic vessel and aortic aneurysms and resulted in the distribution of mast cells in vascular adventitia in normal vessels comparing to the higher distribution of mast cells in the medial area besides vascular adventitia seen in aortic aneurysms.⁴⁰ Angiotensin II is an inducer

for macrophages in an abdominal aortic aneurysm. Schieffer explained that nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) can be stimulated by activated macrophages and consequently induces an inflammatory cytokine, interleukin-1 and a chemokine, monocyte chemoattractant protein-1.⁴¹ The formation of monocyte chemoattractant protein-1 and the consequent buildup of macrophages were decreased in the presence of angiotensin AT1 receptor antagonists as illustrated by Mabuchi *et al.*⁴² Tsunemi *et al.* proved that the use of an injectable NK3201, a chymase inhibitor, will suppress the activity of chymase and the diameter of aorta was reduced in a hamster aneurysmal model.⁴³ Accordingly, chymase inhibitors have a significant contribution to the prevention of aortic aneurysms.

Hypertension

The valuable mechanism to control cardiovascular diseases, mainly blood pressure, is through interfering with the renin-angiotensin system. ACE inhibitors (ACEIs) may suppress this system and therefore provided a therapeutic cardiac effect. ACEIs could not completely block the renin-angiotensin system, as a consequence of which the activity and plasma concentration of angiotensin II was turned back to normal regardless of ACEI therapy.⁴⁴ Another study signifies that an additional benefit in reducing blood pressure was achieved when an angiotensin AT1 receptor antagonist is combined with ACE inhibitors.⁴⁵ One of the key reasons for ineffective ACE inhibitors in hypertension therapy controlling is the presence of chymase. Another study in canine models, signifies that another chymase inhibitor (NK3201; chemically 2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-N-[(3,4-dioxo-1-phenyl-7-(2-pyridyloxy))-2-heptyl] acetamide), unable to lower blood pressure.⁴⁶ These results show the inadequate effect of chymase in hypertension in the absence of vascular rupturing; chymase inhibitors may play a limited role in controlling hypertension.

Vascular proliferation

The most effective way of treating ischemic heart disease is by coronary artery bypass grafting. Nishimoto *et al.* elucidate that in the saphenous vein the levels of chymase and angiotensin II are greater in comparison to the internal thoracic artery,⁴² this indicates that chymase plays big role in saphenous vein intimal hyperplasia after grafting. In Shiota's study, the chymase activity was higher in comparison to ACE activity for patients with carotid artery balloon catheterization and it is confirmed

by a high level of ACE and chymase messenger RNA. This result clearly indicates that the balloon injury in the femoral artery has increased the ACS level alone and has not influenced the chymase activity.⁴⁷ Another study has shown that utilizing the candesartan (an angiotensin AT1 receptor antagonist) and NK3201 (a chymase inhibitor) could block the vascular creation in the damaged arteries, while it was inactive when ACE inhibitor enalapril was used.⁴⁸ This variation in the effectiveness is related to the target of inhibition. Certainly, the ACEI blocked angiotensin II act formed by ACE-dependent pathway whereas angiotensin AT1 receptor antagonist prevented angiotensin II act formed by chymase along with that by ACE. This indicates that local expression of angiotensin II via chymase in case of balloon catheter injury is included in an intimal arteries hyperplasia. Thus, chymase inhibitors could be a beneficial agent as a prophylactic method of restenosis following percutaneous coronary intervention.

Myocardial infarction

Chymase which is localized in the coronary arteries perhaps implicated in myocardial infarction.⁴⁹ Kovanen *et al.* tested thrombosed coronary arteries with atheromatous corrosion, which is isolated from human victims deceased due to myocardial infarction.⁵⁰ Cardiac chymase in human hearts accounts for >90% of Ang II formation and inhibition of the increase in cardiac chymase activity that follows myocardial infarction is associated with significant improvements in cardiac function, structural remodeling and survival rate.³² Another animal study revealed a post-myocardial infarction anti-arrhythmic impact following intravenous chymase inhibitor administration (TY51184); the decreasing of angiotensin II levels by the action of TY51184 could be attributed to the antiarrhythmic effect.⁵¹ In another trial that exploited chymase hamster model, signifies that the cardiac chymase will increase its activity in an infarction area. The increase in activity occurred earlier and remained for a longer time compared to ACE action in the case of myocardial infarction in the hamster model. By using candesartan cilexetil which blocked angiotensin II, cardiac functions are enhanced compared to the use of ACE inhibition with lisinopril which did not achieve positive results.⁵² Data demonstrated wherein elevation of angiotensin II production by cardiac chymase may have a pathophysiological effect following myocardial infarction in the hamster model. In order to improve these findings and apply them to humans in safe techniques, clinical research is important.

Heart failure

Cardiac chymase is resistant to ACEI therapy and heart chymase stimulates interstitial fibrosis by disturbing collagen metabolism through TGF- β . Employing another chymase inhibitor (SUNC8257) in dogs with heart failure has revealed to suppress cardiac fibrosis whilst developing diastolic dysfunction.⁵³ A study was carried out by Zisman *et al.* in 1995 on a healthy heart and showed that 90% of angiotensin II could be inactivated by the action of the ACE inhibitor.⁵⁴ In order to achieve chymase activity in cardiac mast cells, they should be potentiated to de-granulate; in the case of chronic inflammation, this stimulation can occur due to intracellular chymase accumulation in secretory granules of these cells.⁵⁵ In case of *in vitro* heart extracts experiments, it is necessary to rupture existing mast cells in the myocardial tissue to enable intracellular chymase to reach its normal substrates as angiotensin I. Transformation of cardiac chymase into angiotensin II occurs in the interstitial fluid that includes protease inhibitors which can prevent cardiac chymase angiotensin II conversion that lost in case of preparation heart extracts.

A study was carried out to determine the possibility of decreasing angiotensin cardiac metabolism in an interstitial fluid. For this, a tissue of human heart extracts with angiotensin I was incubated *in vitro* and found that chymase has sensitivity against protease inhibitors in the interstitial fluid.⁵⁶ Despite of high chymase concentrations that reach to angiotensin I in the heart extracts, more than 95% of the cardiac chymase was inactivated. Adding to this, the utilization of angiotensin II type-1 receptor blockers in the major clinical experiments have not confirmed greater to ACE inhibitors.^{57,58} A study was performed by Matsumoto *et al.* in 2003 to explain the role of chymase inhibition in avoiding cardiac fibrosis and to improve diastolic dysfunction in the advancement of heart failure. In this trial, chymase in dog model has used because of large similarities to human chymase in angiotensin I conversion into angiotensin II. By using a certain chymase inhibitor in dog model with heart failure, a positive result was obtained which indicates that chymase is predicted to have a pathophysiological effect in heart failure progressing in humans.⁵⁶ In 2002, another research carried out by Hara and others demonstrated that there is a significant role of mast cell in heart failure development, due to the inability of chymase to produce angiotensin.⁵⁹ The authors concluded that there is a prophylaxis strategy in cardiac remodeling and the development of heart failure in humans.⁵⁹

Role in cardiovascular diseases

Chymase can influence the pathophysiology of blood vessels. In vascular disease state or injury, chymase-containing mast cells are activated and associated with the development of cardiac remodeling and left ventricular dysfunction. The role of chymase is not only restricted to the conversion of angiotensin I to angiotensin II but also inflammatory progression throughout the activation of TGF- β and oxidative stress, degradation of extracellular matrix and proteolysis of low-density lipoproteins. Inhibition of chymase has a promising role in cardiovascular disease which can be attained either by mast cell stabilizer mediated prevention of mast cell degranulation, thereby reducing the availability of chymase or by inhibition of the enzyme action with the help of a selective chymase inhibitor.

CONCLUSION

Various studies about the pathological effects of chymase and the role of chymase inhibitors in the prevention of cardiovascular diseases have been discussed. Cardiovascular diseases and injury are associated with chymase release and consequently pathophysiological progression. The role of chymase is not only involved in the transformation of angiotensin I to angiotensin II but has oxidative, inflammatory and dyslipidemic effects. Usages of mast cell stabilizer or a selective chymase inhibitor will suppress the activity of chymase. In summary, chymase plays a significant role in cardiovascular diseases and could be a potential option to treat by chymase inhibition.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

ACE: Angiotensin-converting enzyme; **ACEI:** Angiotensin-converting enzyme inhibitors; **TGF- β :** Transforming growth factor- β .

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