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Review

Vascular chymase: pathophysiological role and therapeutic potential of inhibition

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Abstract

Chymase is a chymotrypsin-like serine protease secreted from mast cells. Mammalian chymases are classified into two subgroups (α and β) according to structure and substrate specificity; human chymase is an α-chymase. An important action of chymase is the ACE-independent conversion of Ang I to Ang II, but chymase also degrades the extracellular matrix, activates TGF-β1 and IL-1β, forms 31-amino acid endothelins and is involved in lipid metabolism. Under physiological conditions, the role of chymase in blood vessels is uncertain. In pathological situations, however, chymase may be important. In animal models of hypertension and atherosclerosis, chymase may be involved in lipid deposition and intimal and smooth muscle hyperplasia, at least in some vessels. In addition, chymase has pro-angiogenic properties. In human diseased blood vessels (e.g. atherosclerotic and aneurysmal aorta; remodeled pulmonary blood vessels), there are increases in chymase-containing mast cells and/or in chymase-dependent conversion of Ang I to Ang II. These findings have raised the possibility that inhibition of chymase may have a role in the therapy of vascular disease. The effects of chymase can theoretically be attenuated either by reducing availability of the enzyme, with a mast cell stabiliser, or alternatively with specific chymase inhibitors. The mast cell stabiliser, tranilast, was shown to be beneficial in animal models of atherosclerosis, where a prevention protocol was used, but was not effective in clinical trials where it was administered after angioplasty. Chymase inhibitors could have the advantage of being effective even if used after injury. Several orally active inhibitors, including SUN-C8257, BCEAB, NK3201 and TEI-E548, are now available. These have yet to be tested in humans, but promising results have been obtained in animal models of atherosclerosis and angiogenesis. It is concluded that orally active inhibitors of chymase may have a place in the treatment of vascular diseases where injury-induced mast cell degranulation contributes to the pathology.

Keywords: Angiotensin; Vascular disease; Chymase; Chymase inhibitors; Mast cells

1. Introduction

Chymase is a chymotrypsin-like serine protease, stored in a macromolecular complex with heparin proteoglycan within the secretory granules of mast cells. On release, chymase remains complexed and binds to the extracellular matrix, and continues to function for several weeks.

Chymase is involved in the synthesis of angiotensin II (Ang II), but the Ang II formed in this pathway is probably not involved in the short-term regulation of blood pressure. Ang II from the chymase pathway is, however, probably involved in the structural remodeling associated with disease of the cardiovascular system. In addition, chymase has many other actions including degrading the extracellular matrix, activating transforming growth factor-β (TGF-β), and promoting the synthesis of 1–31 amino acid-length endothelins, all of which may contribute to the vascular response to injury.

Inhibition of chymase might be an important development for the treatment of vascular injury associated with mast cell degranulation. In this review, initially we consider the characteristics of chymase, particularly chymase in the blood vessels, before outlining the actions of chymase. These actions underlie the effects of chymase on the normal vascular system, and the pathophysiological effects of

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Table 1
Selected chymase subgroups [4]

α-Chymase	Human, monkey, dog chymase
	Sheep chymase 2
	Hamster chymase 2
β-Chymase	Rat mast cell chymases 1 and 2
	Mouse chymases 1, 2 and 4
	Gerbil chymase 1

chymase in the vasculature, which are discussed in the following sections. Finally, we discuss what, for us, is the most interesting development, the findings with inhibitors of chymase in animal models of vascular disease. We show that the physiological role of chymase on the vascular system is uncertain, but that chymase has a clearly defined role in some vascular injuries. Inhibitors of chymase may be an important development for the treatment of vascular injury associated with mast cell degranulation.

2. Characteristics of chymases

Unlike ACE and ACE2, which are carboxypeptidases, chymase is an endopeptidase [1]. It is secreted from mast cells in a complex with heparin proteoglycan. Prior to secretion, mast cell chymase is activated intracellularly [2]. The pro-chymase precursor dipeptides are similar to those of cathepsin G, group B granzymes and neutrophil elastases, most of which can be activated by dipeptidyl peptidase I [2]. Dipeptidyl peptidase I is particularly abundant in mast cells [2].

Once secreted, chymase is surrounded by extracellular fluid containing endogenous inhibitors of chymase. However, recent evidence suggests that when chymase is bound to heparin it is resistant to these endogenous inhibitors. The experiments showing this used chymase from rat serosal mast cells, and characterised the ability of chymase to degrade high-density lipoprotein-3 (HDL_3) [3]. Chymase, when bound to the heparin proteoglycans of mast cell granule remnants, but not when released from them, was resistant to inhibition by the endogenous protease inhibitors [3]. Furthermore, the heparin proteoglycan-bound chymase, but not the unbound chymase, degrades its inhibitor α_1-antitrypsin [3].

Mammalian chymases can be classified into two subgroups (α and β) according to their structure and substrate specificity (Table 1; Ref. [4]). Both sub-groups of chymases convert Ang I to Ang II, but only the β-chymases degrade Ang II (Fig. 1). The α-chymases cleave the Phe^8–His^9 bond of Ang I [4], and are widely expressed in mammalian mast cells. The closest known genetic relative to the human α-chymase is cathepsin G [2]. The genes for

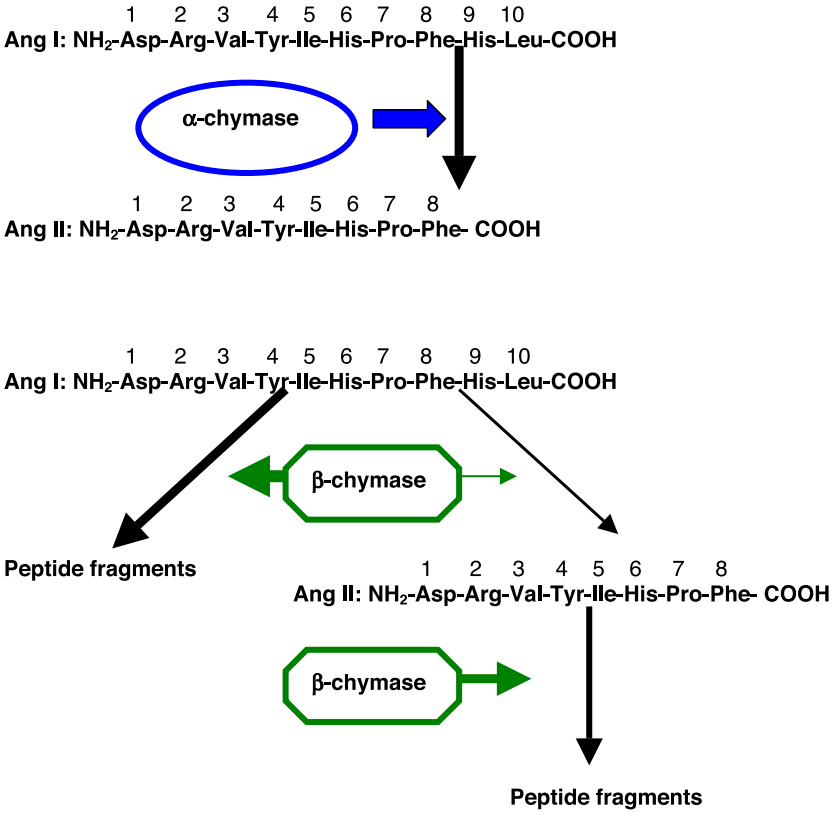

Fig. 1. Effect of chymases on Ang I: Top α-chymase catalyses the synthesis of the pathophysiologically active Ang II. Bottom β-chymase preferentially degrades Ang I and Ang II to inactive peptide fragments.

β-chymases, which are predominantly found in rodents (Table 1), appear to have been lost in human evolution [2]. β-Chymases, like α-chymases, can convert Ang I to Ang II but preferentially hydrolyse the Tyr^4–Ile^5 bond of Ang I and Ang II, yielding inactive peptide fragments (Fig. 1) [5].

Chymase is found in human blood vessels, heart, and many other tissues [6], but not plasma [7]. In blood vessels, chymase is predominantly localised to the adventitia, whereas ACE is predominantly localised in the vascular endothelium. Thus, in the human saphenous vein, mast cell chymase is present mainly in the adventitial layer of the vessel, with only a few cells staining positive for chymase in the media [8]. Similarly, in human radial and internal thoracic arteries, chymase is co-localised with mast cells in the adventitia, and occasionally in the medial layer of the radial artery [9]. The implication of this is that intravascular Ang I is more accessible to ACE than chymase. Interestingly, the chymase activity in human saphenous vein is almost double that in human internal thoracic artery, consistent with a larger number of chymase containing cells in the saphenous vein [10]. Since ACE activity was the same in the two arteries [10], this suggests possible heterogeneity between vessel types with respect to the relative importance of these two Ang II-generating pathways.

Chymase had a higher ability than ACE to generate Ang II in homogenates of hamster, marmoset and human aorta [11]. Chymase has a lesser role in the generation of Ang II in some rat blood vessels, since the β-chymase found in rats may also degrade Ang II, leaving ACE the dominant Ang II forming enzyme. Thus, the ability of the homogenates of rat carotid artery to convert Ang I to II is due to ACE only, as it is inhibited by lisinopril but not by the chymase inhibitor, chymostatin [12]. The contractions of rat carotid arteries to Ang I are abolished with the ACE inhibitor lisinopril, indicating no involvement of chymase in this response [13]. Chymase also has little or no role in the contractions of rabbit resistance arteries to Ang I, as the contractions are almost completely inhibited by enalaprilat, but not altered by the chymase inhibitor CH 5450 [14].

3. Actions of chymase

Chymase was previously known as angiotensin I-convertase, because the conversion of Ang I to Ang II was one of the first reactions it was shown to catalyse. However, it is now known that chymase has diverse actions, which are discussed below, and inhibitors of chymase will have the potential to inhibit all of these actions.

Chymase degrades the extracellular matrix [15–17]. More recently, it has been demonstrated that serosal mast cell-derived chymase induces apoptosis of cultured rat aortic smooth muscle cells by a mechanism involving the degradation of fibronectin, with subsequent disruption of focal adhesions [18].

Chymase may have a role in the release and activation of latent TGF-β1. TGF-β1 has a role in atherosclerotic lesions where it regulates the differentiation, migration, and proliferation of smooth muscle cells, and the synthesis and secretion of extracellular matrix. On exposure to rat serosal mast cell chymase, the latent TGF-β1, associated with the extracellular matrix of cultured rat aortic smooth muscle cells, was released and activated [19]. This activated TGF-β1 could, in turn, inhibit the growth of the cultured cells [19].

Chymase has actions on lipoproteins that may be atherogenic. The proteases from mast cells are involved in the proteolysis of low-density lipoprotein (LDL), which may precede foam cell formation [20]. In macrophages, the efficient efflux of cholesterol to HDL prevents the accumulation of cholesterol. Chymase from mast cells degrades the apolipoproteins of HDL_3, rendering cholesterol-loaded macrophages (i.e. foam cells) unable to mediate cholesterol efflux from the foam cells [21].

Endothelins (1–31) are formed by chymase cleavage of big endothelins at the Tyr^{31}–Gly^{32} bond, with no further degradation products by chymase cleavage [22]. IL-1β is a pro-inflammatory cytokine. Human chymase converts the precursor of IL-1β to active IL-1β [23].

4. Effects of chymase on blood vessels

Evidence for a physiological role for chymase in blood vessels has been sought from biochemical determinations of the conversion of Ang I to Ang II (in vitro) and from the study of vasocontractile responses to chymase substrates (in vitro and in vivo). Findings in isolated tissues or homogenates have been useful in determining the biochemical characteristics of chymase. However, these findings probably do not represent physiological effects in that the isolation of tissues may be associated with injury leading to the degranulation of mast cells and increased chymase levels. In addition, studies with the selective chymase substrate [Pro^{11},D-Ala^{12}]Ang I are useful in demonstrating chymase activity but not physiological effects. Thus, in determining if chymase has a physiological role on the vasculature, the results of in vivo studies on the effect of chymase inhibitors on contractions to the endogenous substrate Ang I are the most relevant.

4.1. Conversion of Ang I to Ang II

In homogenates of human internal thoracic artery or gastroepiploic arteries, the formation of Ang II is reduced by 95% with the chymase inhibitor chymostatin (internal thoracic artery, [24]; gastroepiploic artery, [12]). Slices, homogenates and extracts of human gastroepiploic arteries convert Ang I to Ang II [25]. In the slices, the ACE inhibitor lisinopril and chymostatin inhibited Ang II formation by 5% and 90%, respectively [25]. The natural protease inhibitor α-

antitrypsin reduced the Ang II formation by only 8% in the slices [25]. Contractions of the arteries to Ang I, in the presence of lisinopril (i.e. contractions mediated by chymase-derived Ang II) were likewise not inhibited by α-antitrypsin [25]. The lack of effect of α-antitrypsin may indicate that chymase is still bound to heparin in slice preparations. When the heparin chymase complex was removed from homogenates of human gastroepiploic arteries, there was a much-reduced ability of the homogenate to generate Ang II. This residual generation of Ang II was sensitive to lisinopril, indicating that it was catalysed by ACE [26].

4.2. Contractions to Ang I and [Pro^{11},D-Ala^{12}]Ang I

4.2.1. In vitro

Ang I and [Pro^{11},D-Ala^{12}]angiotensin I contract the isolated hamster aorta, and these contractions are inhibited by captopril and chymostatin, respectively [27]. Chymostatin alone had no effect on the hamster aortic responses to Ang I, suggesting that chymase has a limited physiological role in the generation of Ang II [27]. When the response to Ang I was inhibited 87% by captopril, the residual response was sensitive to chymostatin [27].

Similarly, chymase inhibition alone has no effect on the responses of dog or monkey vessels to Ang I, but does cause inhibition in the presence of ACE inhibition. Thus, on the isolated canine superior mesenteric artery, chymostatin alone had no effect on the response to Ang I, but eliminated the response when added to a concentration of captopril that did not fully inhibit the response [28]. The responses of other dog arteries and monkey arteries were also inhibited by lisinopril and further inhibited on the addition of chymostatin [13].

Taken together, these animal studies may suggest that chymase has a minor role under physiological conditions or that in the presence of ACE inhibition, the Ang I is diverted to the chymase pathway.

Some of the studies on human vasculature indicate a possible physiological role for chymase. Thus, chymase inhibition alone reduces the responses to Ang I to a small extent in coronary arteries and internal mammary arteries. On human coronary artery rings obtained from donor beating hearts, where the donor had a non-cardiac death, chymase inhibition with C41 or chymostatin inhibited responses to Ang I to a small extent [29]. Captopril at 10 μM inhibited the responses to Ang I to a similar degree to the chymase inhibitors, and had an additive inhibitory effect with C41 [29]. On application of Ang I to the organ bath, chymostatin reduced the release of Ang II [29]. When perfused human coronary artery segments were exposed luminally or adventitially to Ang I, Ang II was released, and this release was also blocked by chymostatin [29].

Segments of internal mammary arteries contract to Ang I [30]. These responses were inhibited to a small extent by captopril or chymostatin alone [30]. The responses were inhibited by the combination of captopril and chymostatin to a greater extent, which was more than the addition of the effects of captopril and chymostatin alone [30].

In other studies on isolated human blood vessels, ACE inhibitors or chymase inhibitors had no effect alone, but the combination reduced the responses to Ang I (saphenous vein [8], radial and internal thoracic artery [9], resistance vessels from subcutaneous gluteal fat biopsy [14]). Similar findings were obtained using resistance vessels from patients with coronary heart disease; the responses to Ang I were not altered by either enalaprilat or chymostatin alone, but were inhibited by the combination [31]. In contrast, the responses of the resistance vessels from patients with chronic heart failure were inhibited by enalaprilat alone, but not by chymostatin alone [31]. The inhibitory effect of enalaprilat and chymostatin in combination was much greater than the effect of enalaprilat alone in the resistance vessels from the heart failure patients [31]. The reason for the difference with enalaprilat alone in the heart failure patients is not known but may be related to the heart failure or to the use of ACE inhibitors for treatment of the heart failure [31].

4.2.2. In vivo

In conscious hamsters, the intravenous injections of both Ang I and [Pro^{11},D-Ala^{12}]Ang I produced pressor responses, which were inhibited by the ACE inhibitor captopril and the chymase inhibitor chymostatin, respectively [27]. In dorsal veins of patients with coronary heart disease, the ACE inhibitor captopril abolished responses to Ang I, which suggests that ACE is the enzyme predominantly being used in the formation of Ang II [32]. When [Pro^{11},D-Ala^{12}]Ang I was used as the substrate, contractions to formed Ang II were observed suggesting that chymase activity is present in the human dorsal vein in vivo [32]. To demonstrate a clear physiological role for chymase, chymostatin would have to inhibit the responses to the physiological substrate Ang I. However, the effects of chymostatin on the pressor responses to Ang I were not reported in this in vivo study [27]. Thus, the physiological role, if any, of chymase on the vasculature remains uncertain.

5. Pathophysiological role of chymase on the vasculature

In contrast to the normal vasculature, where it seems unlikely that chymase has a major physiological role, there is good evidence that chymase has a pathophysiological role in vascular disease, especially that associated with injury.

5.1. Animal models of disease

5.1.1. Ageing and atherosclerosis

Monkey vascular chymase has a high homology to human chymase [33]. Chymase is present in the adventitia of the aorta of 20-year-old, but not 6-year-old monkeys

[34]. The role of this chymase in the aged vasculature has not been ascertained.

In the atherosclerotic aorta of monkeys fed a high cholesterol diet for 6 months, mRNA levels of aortic chymase were increased [33]. In addition, in hamsters fed a high-cholesterol diet, there were increases in aortic chymase activity and the development of marked lipid deposits in the aortic intima, and both of these effects were reduced with chymase inhibition [35]. This suggests that arterial chymase may participate in the acceleration of lipid deposition in arterial walls exposed to high plasma cholesterol. If so, inhibition of arterial chymase may potentially retard the progression of atherosclerosis [35].

5.1.2. Injury and proliferation

Balloon injury to the dog carotid artery results in hypertrophy of the vessel. In these hypertrophied vessels, there are increased levels of mRNA for ACE and chymase, and also increased activities of both enzymes [36], but the increase is greater for chymase than ACE activity [37]. In contrast, balloon injury to the dog femoral artery is associated with an increase in ACE, but not chymase activity [37].

There is probably also a role for chymase-dependent Ang II formation in the vascular proliferation associated with vein grafts. Chymase activity, but not ACE activity, was increased when jugular veins were grafted to the ipsilateral carotid artery [38]. This was associated with increases in mRNA levels of fibronectin and collagens I and III and in the intima–media ratio of the vessels, all of which were reduced by local treatment with a chymase inhibitor [38].

Recently, a novel vascular smooth muscle β-chymase has been cloned from rat pulmonary arteries and this may have a role in vascular proliferation [39]. This chymase may only be active in proliferating cells as it can be detected in cultured pulmonary and aortic smooth muscle cells of normotensive and spontaneously hypertensive rats, but not in freshly prepared pulmonary artery and aorta or other tissues (heart, lung, kidney, liver) [39]. There were higher chymase mRNA levels in aortic and pulmonary artery smooth muscle cells from the spontaneously hypertensive than the normotensive rats [39]. The smooth muscle cells from spontaneously hypertensive rats were able to generate Ang II from Ang I, and this generation was inhibited by chymostatin but not by captopril [39].

5.1.3. Angiogenesis

Angiogenesis is responsible for the progression of pathological conditions such as cancer, diabetic retinopathy and rheumatoid arthritis. Chymase is a pro-angiogenic factor. In the hamster sponge model of angiogenesis, in vivo transfection of human pro-chymase cDNA or a direct injection of purified chymase into the sponge implants increased the angiogenesis [40]. Ang II was involved in the basic fibroblast growth factor induced angiogenesis, as an AT_1-receptor antagonist inhibited the response [40]. Chymase was involved in the angiogenic responses to Ang I and basic fibroblast growth factor, as chymostatin inhibited angiogenesis to both these inducers [40]. Subsequent studies have shown that compound 48/80 activated the mast cells in this model inducing granulomas and angiogenesis [41], with an up-regulation of vascular endothelial growth factor (VEGF) mRNA [42]. The angiogenesis was inhibited by chymostatin, and to a lesser extent by the histamine H_1-receptor antagonist pyrilamine, but not by the tryptase inhibitor leupeptin [41]. Chymostatin also inhibited the up-regulation of VEGF mRNA, and this suggests a chymase–Ang II–VEGF pathway may operate in granulation tissue as the primary mediator of angiogenesis [42].

5.2. Human blood vessels in disease

An association of mast cells and early atherosclerotic plaques has been observed on aortas and coronary arteries of 115 young subjects (15–34 years) who had traumatic deaths, suggesting a role of mast cell products in the evolution of the atherosclerotic plaque [43]. Aortic and coronary segments with raised lesions had greater numbers of mast cells in the adventitia than in normal segments [43]. In addition, in the aortic segments, greater numbers of mast cells were located in the dorsal portion, which is considered to be lesion-prone, than in the lesion-resistant ventral half [43].

Chymase localisation and activity have been studied in the atheromatous human aorta using samples of normal ($n=9$), atherosclerotic ($n=8$) and aneurysmal ($n=6$) vessels from autopsy or cardiovascular surgery [44]. Chymase-positive mast cells were located in the tunica adventitia of normal and atheromatous aortas [44]. In contrast, ACE was localised to the endothelial cells of normal aorta and in macrophages of atheromatous neointima [44]. Although the density of chymase-positive cells was slightly higher in atherosclerotic lesions than normal aorta, this was not significant [44]. However, the number of activated mast cells in the aneurysmal lesions (18%) was higher than in the atherosclerotic (5%) or normal (1%) aortas [44]. Total Ang II forming activity was higher in atherosclerotic and aneurysmal lesions than in normal tissue, and most of the activity was chymase-dependent (normal, 82%: atherosclerotic, 90%) [44]. The differences between chymase activity in aneurysmal and normal aorta have been confirmed [45]. In addition, in aneurysmal compared to normal aorta, increases in the numbers of total mast cells and chymase-positive cells were detected in the medial area in addition to the adventitia [45].

Studies with coronary artery homogenates from heart transplant patients have shown that chymase can generate Ang II. Thus, in homogenates of coronary arteries from recipient hearts (described as having the least disease), Ang II formation was inhibited by chymostatin (100 μM),

but not by captopril (100 μM) [46]. Contractility studies performed with intact arteries showed that the contractions to Ang I were inhibited by the ACE inhibitor cilazaprilat or by chymostatin [46]. However, Ang II may not be an important mediator of the effects of chymase in intact coronary arteries as chymase and Ang II do not co-localise in human coronary atherosclerotic lesions. In the co-localisation study, coronary arteries obtained at autopsy were histological characterised before the localisation of chymase, ACE and Ang II. Chymase was expressed in the cytosol of mast cells, and the expression was greater in the intima of atheromatous plaques than in normal coronary arteries [47]. However, chymase did not co-localise with Ang II, whereas ACE did [47]. This suggests that in human coronary atherosclerotic lesions, Ang II is generated from ACE rather than chymase.

It is possible that mast cells/chymase from the coronary arteries may be involved in myocardial infarction. Preceding the majority of acute coronary syndromes, erosion and rupture of coronary atheroma occur. The site at which erosion or rupture is most likely to occur is the shoulder region of the atheroma, which is the site where mast cells accumulate. From the hearts of patients who had died of myocardial infarction, thrombosed coronary arteries with atheromatous erosion or rupture were removed [48]. At the immediate site of erosion or rupture, mast cells were 6% of all nucleated cells but only 1% in the adjacent atheromatous area, and 0.1% in the unaffected intimal area [48]. The proportion of these mast cells that had been stimulated to degranulate and release some of their chymase and tryptase content was 86% at the site of erosion, 63% in the adjacent atheromatous area, and 27% in the unaffected intima [48]. Subsequently, it was shown that some of these mast cells contain tumour necrosis factor (TNF)-α. Thus, 14 of 24 atheromas found in human coronary arteries, contained TNF-α-positive mast cells [49]. The majority of the TNF-α-positive mast cells were found in the shoulder region of the atheroma [49].

Internal thoracic (mammary) arteries are used in coronary artery bypass surgery. The levels of chymase activity in generating Ang II from homogenates of non-atherosclerotic human internal thoracic artery are positively correlated with plasma LDL-cholesterol levels [24]. Chymase activity did not correlate with a variety of other parameters including HDL-cholesterol, triglycerides, blood pressure, fasting blood glucose and body mass index [24]. Thus, it is possible that the increase in plasma LDL-cholesterol is linked to the increase in chymase activity in some way [24].

The degeneration of venous coronary bypass grafts has been linked to a polymorphism in the chymase gene [50]. In a follow-up study of 101 patients after bypass surgery, there was an association between the degree of bypass graft degeneration and the presence of the G allele of the chymase gene, but no association with polymorphisms of other genes, including ACE [50].

There is indirect evidence that chymase (probably via production of Ang II) may be involved in the development of pulmonary vascular disease associated with congenital heart disease. In lung biopsies from 23 patients with atrial or ventricular septal defects, or both, there were increases in the proportion of mast cells containing chymase [51]. The chymase containing mast cells were present in interstitial lung connective tissue, and in the media and adventitia of small pulmonary vessels, and Ang II was co-localised with chymase. The number of mast cells correlated positively with pulmonary vascular resistance suggesting a possible link between chymase and early pulmonary vascular disease [51].

6. Mast cell stabilisation

The effects of chymase can theoretically be attenuated either by reducing availability of the enzyme, with a mast cell stabiliser (this section), or alternatively with specific chymase inhibitors (Sections 7 and 8). Mast cell stabilisers, in addition to inhibiting the secretion of chymase, also prevent the secretion of the other contents of the mast cells (e.g. chemotactic factors, cytokines, growth factors and other serine proteases). These stabilisers have been used both to demonstrate a role for mast cells in cardiovascular injury, and as a potential treatment for the injury. Tranilast, *N*-(3,5-dimethoxycinnamoyl) anthranilic acid, the mast cell stabiliser most commonly used in these studies, may also act as an antagonist at AT_1-receptors in high concentrations (36 μM) [52].

Pretreatment or concurrent treatment with tranilast inhibits vascular proliferation in animal models of vascular injury/disease, but tranilast treatment after angiography is not beneficial. Tranilast, 2 weeks before and 4 weeks after balloon injury to the dog carotid artery, abolished the increases in mast cell count and chymase activity in the injured vessel [53]. Tranilast reduced the injury-induced increases in chymase mRNA and neointimal thickening [53]. Tranilast, 3 days before and 14 days after balloon injury to the rat carotid artery, also reduced the degree of neointima/media thickening [54].

Tranilast is beneficial in preventing heart transplant-associated coronary atherosclerosis in a mouse model. Thus, the oral administration of tranilast, when started 3 days before transplantation, markedly reduced luminal occlusion at 14 and 28 days [55]. In another study, tranilast from the time of the surgery reduced the development of coronary atherosclerosis [56].

Clinical trial showed that tranilast decreased the rate of restenosis after percutaneous transluminal coronary angioplasty [TREAT-2, 57], but this did not translate into improved clinical outcomes. In TREAT-2, there were 297 patients with de nova or restenotic native lesions and they were given tranilast or placebo from the day after balloon angioplasty for 3 months [57]. The per lesion restenosis

rate, defined as a loss of 50% or more of the initial gain with balloon inflation, was lower in the tranilast group (19%) than the placebo group (44%) [57]. The per lesion restenosis rate, defined as a percent stenosis of $\geq 50\%$ at follow-up examination was also lower in the tranilast group (26%) than in the placebo group (42%) [57]. However, there was only a small improvement in percent stenosis (placebo, 48%; tranilast, 42%) [57]. In a 12-month follow-up, there were similar revascularization rates between groups (placebo, 31%; tranilast, 28%), and bypass surgery was performed in two patients, both of whom had been treated with tranilast [57]. One suggestion for these poor clinical results was that tranilast is delaying rather than preventing the restenosis [57].

In the Prevention of REStenosis with Tranilast and its Outcomes (PRESTO) trial, 11,484 patients were enrolled within 4 h of percutaneous coronary intervention to placebo or tranilast for 1 or 3 months [58]. Tranilast did not decrease neointimal hyperplasia nor improve the quantitative measures of restenosis (angiographic and intravascular ultrasound) [58]. Tranilast also had no effect on the primary clinical end point of the composite of the first occurrence of death, myocardial infarction, or ischemia-driven target vessel revascularization [58]. This raises questions as to whether inhibition of chymase will be effective in preventing the clinical sequelae of restenosis.

In both TREAT-2 and PRESTO, tranilast was administered after the angiography, with disappointing results. In contrast, in animal models of cardiovascular injury, tranilast was given prior to or at the time of the injury, and was effective. In the treatment of asthma, mast cell stabilisers are considered to be preventative treatment as they are ineffective after the mediators have been released. Hence, tranilast may have been effective in clinical trial if it had been administered prior to angioplasty, and this needs to be tested. This limitation of having to be used as a preventative agent may not apply to specific chymase inhibitors.

7. Chymase inhibitors

Chymostatin, the standard chymase inhibitor used in experimental studies, is unsuitable for in vivo studies. Recently, chymase inhibitors have been developed that are suitable for testing in animal models of cardiovascular disease. Initially, Suc–Val–Pro–Phe^{P}(OPh)$_2$ was used as a potent inhibitor of chymase activity with a half-degradation time of 20 h in human plasma [59]. Subsequently, a number of orally active inhibitors of chymase have been developed. These include BCEAB, 4-[1-{[bis-(4-methyl-phenyl)-methyl]-carbomoyl}-3-(2-ethoxy-benzyl)-4-oxooazetidine-2-yloxyl]benzoic acid and SUN-C8257, (3-[(3-amino-4-carboxy) phenylsulfonyl]-7-chloroquinazoline-2,4(1H,3H)-dione, which inhibit human chymase with IC_{50} values of 5.4 nM [60] and 0.31 μM [61], respectively. BCEAB does not suppress ACE, elastase or tryptase activities [60]. Other examples of orally active chymase inhibitors include NK3201, 2-(5-Formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl-*N*-[{3,4-dioxo-1-phenyl-7-(2-pyridyloxy)}-2-heptyl]acetamide, and TEI-E548, 4-[1-napthylmethyl)benzimidazol-2-ylthio]butanoic. NK3201 inhibits recombinant human chymase, and dog and hamster chymases isolated from vascular tissue with IC_{50} values of 2.5, 1.2 and 28 nM, respectively [62]. TEI-E548 inhibits human and hamster chymase with IC_{50} values of 6.2 and 30.6 nM, respectively [63].

8. Chymase inhibitors in animal models of vascular disease

Chymase inhibitors may prevent or slow the progression of atherosclerosis. In hamsters fed a diet supplemented with cholesterol and coconut oil for 12 weeks, aortic chymase activity increased, and there were marked lipids deposits in the aortic intima [35]. SUN-C8257, added to the drinking water, decreased the chymase, but not the ACE, Ang II forming activity of the aorta [35]. SUN-C8257 reduced the adventitial Ang II-immunoreactivity [35]. SUN-C8257 decreased the aortic lipid deposition without changing body weight, blood pressure, plasma LDL-cholesterol and plasma Ang II levels [35].

The potential of chymase inhibition in grafting was demonstrated with Suc–Val–Pro–Phe^{P}(OPh)$_2$. Prior to grafting, the isolated dog jugular veins were treated for 20 min with saline with or without Suc–Val–Pro–Phe^{P}(OPh) [60]. One month after the graft, the activities of chymase and ACE were increased 15- and 2-fold in control grafts, whereas chymase activity was decreased in the grafts that had been treated with Suc–Val–Pro–Phe^{P}(OPh)$_2$[60]. The mRNA levels of fibronectin, and collagens I and III were increased in the grafted veins, and these increases were suppressed by the chymase inhibitor [10]. The intimal thickening observed in the normal graft was reduced by 64% by Suc–Val–Pro–Phe^{P}(OPh)$_2$ [60].

Subsequently, it has been shown that a similar benefit on grafted veins can be achieved with the oral administration of NK3201. In dogs, the plasma concentration of NK3201 is about 10 μM, 24 h after the oral administration of 5 mg/kg [62]. When dogs were treated with NK3201 for 5 days prior to the grafting procedure, both the increase in chymase activity and also the vascular proliferation normally associated with grafting were suppressed [62].

The potential benefit of chymase inhibition on balloon injury was demonstrated with NK3201. Dogs were given NK3201 or placebo, beginning 5 days before balloon injury to the carotid artery, and continuing throughout the experiment [63]. NK3201 had no effect on the mean blood pressure of the dogs, or plasma levels of renin, ACE and Ang II [63]. As there were no increases in the circulating levels of Ang II or blood pressure, this suggests that any generation of angiotensin II by chymase is

localised to the area of injury. There was a 2-fold increase in the chymase activity in the injured arteries, but no significant difference in ACE activity [63]. NK3201 reduced the increased chymase activity in the injured arteries but did not affect basal levels of chymase activity in control arteries [63]. Neointimal thickening was observed in the injured arteries, and NK3201 reduced this [63].

BCEAB has been used to demonstrate the potential of chymase inhibitors in angiogenesis. The ability of basic fibroblast growth factor to induce angiogenesis in the hamster sponge granulomas was inhibited by the simultaneous administration of BCEAB [64]. BCEAB attenuated the basic fibroblast growth factor-induced increase of local blood flow around the granuloma [64]. BCEAB also attenuated the increased chymase activity and VEGF mRNA observed with basic fibroblast growth factor [64].

9. General conclusions

The physiological role, if any, for chymase in the vasculature is uncertain. However, in the context of disease and injury, where chymase-containing mast cells may increase in number and be activated, chymase almost certainly has a pathophysiological role in blood vessels. The role of chymase in vascular disease may involve not only conversion of Ang I to Ang II but also degradation of the extracellular matrix, proteolysis of LDLs and activation of TGF-β. Chymase appears to contribute to the development of atherosclerosis. Inhibition of chymase can be achieved either by preventing mast cell degranulation with a mast cell stabiliser, thus reducing chymase availability, or by inhibiting the action of the enzyme with a selective chymase inhibitor. The former would require administration before injury but the latter would not necessarily be subject to this limitation. Results obtained so far with the mast cell stabiliser, tranilast, have been more encouraging in animal models of vascular disease than in clinical trials, but better outcomes in the clinic may well be achieved if a preventative protocol is used. The advent of several orally active selective chymase inhibitors has facilitated the testing of these compounds in cardiovascular disease. Although these drugs have yet to be trialed in the clinic, encouraging results have been obtained in animal models of atherosclerosis and angiogenesis. It is concluded that orally active chymase inhibitors may have a place in the treatment of vascular diseases where injury-induced mast cell degranulation contributes to the pathology.

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