Chymase is a Target Enzyme for Prevention of Organ Damage

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Abstract: Background: Organ damage is closely related in the upregulation of various key factors. Especially, angiotensin II and Transforming Growth Factor (TGF)-β play a crucial role in the cardiovascular remodeling and tissue fibrosis. Matrix metalloprotease (MMP)-9 is also related to the development and progression of various tissue remodeling. Chymase converts angiotensin I to angiotensin II and this enzyme can also convert precursors of TGF-β and MMP-9 to their active forms and its enzymatic function may play a crucial role in the development and the progression of organ damage. Results: Angiotensin II regulates blood pressure and plays an important role in cardiovascular remodeling. Chymase activities are augmented in the lesion of cardiovascular remodeling and its inhibition has shown the prevention of its remodeling via the reduction of angiotensin II. Moreover, chymase-activated MMP-9 also plays a crucial role in the cardiovascular remodeling and chymase inhibitors have shown the prevention of the remodeling via the suppression of MMP-9 activity. TGF-β is converted from its precursor by chymase and its inhibition results in the prevention of tissue fibrosis. Conclusion: Chymase is an important processing enzyme for angiotensin II, TGF-β and MMP-9, all of which are key factors for organ damage. Thus, chymase may be a key enzyme for organ damage and its inhibition may be useful for preventing various organ damages. We propose that chymase inhibitor may become a useful agent for prevention of organ damage in clinical settings.

Key words: Chymase, inhibitor, cardiovascular remodeling, inflammation, fibrosis, organ damage

INTRODUCTION

Angiotensin II is derived from angiotensinogen by renin and Angiotensin-Converting Enzyme (ACE) and the latter is a well-known enzyme for conversion from angiotensin I to angiotensin II. On the other hand, cardiovascular tissues, chymase can produce angiotensin II from angiotensin I (Urata et al., 1990; Takai et al., 1996). Chymase activates not only angiotensin II but also TGF-β, a major stimulator of tissue fibrosis (Takai et al., 2003a). In human liver fibrosis, both chymase and angiotensin II levels were significantly increased and significant correlations between chymase and angiotensin II levels, between chymase level and fibrotic degree and between angiotensin II level and fibrotic degree were observed (Kameda et al., 2008). Chymase also converts pro-matrix metalloprotease (proMMP)-9 to MMP-9. For example, in human Abdominal Aortic Aneurysms (AAA), chymase-positive mast cells are detected in the medial area, in addition to the adventitial area and the number of mast cells is obviously increased in comparison with the normal aorta (Nishimoto et al., 2002). In human AAA extracts, the conversion of proMMP-9 to MMP-9 was significantly augmented after incubation than before incubation; this suggests that the extract included proMMP-9-activating enzymes (Furubayashi et al., 2008). Using the human AAA extract, chymase inhibition decreased by about 50% in the conversion of proMMP-9 to MMP-9 (Furubayashi et al., 2008).

Chymase is a chymotrypsin-like enzyme that is expressed in the secretory granule of mast cells and this enzyme may be a key enzyme for progression of organ damage. In this review, we propose the usefulness of chymase inhibitors for prevention of various diseases.

VASCULAR REMODELING

Patients with ischemic heart disease are offered with Coronary Artery Bypass Grafting (CABG) or PCI. In CABG, the internal thoracic artery and saphenous vein have been frequently used as coronary artery bypass conduits but the poor performance of the saphenous vein compared with the internal thoracic artery is well known (Lytle et al., 1985). Both chymase activity and total angiotensin II-forming activity was significantly higher in human saphenous vein than in the internal thoracic artery.
In a dog grafted model, the chymase activity was significantly increased in the grafted veins. Moreover, the angiotensin II concentration and the mRNA levels of fibronectin, collagen I and collagen III, all of which are induced by an increase of angiotensin II action (Nishimoto et al., 2001a), were significantly increased in the grafted veins, while they were significantly suppressed by a chymase inhibitor Sue-Val-Pro-Phe\(\beta\)(Oph), (Nishimoto et al., 2001a).

Vascular proliferation after injury by a balloon catheter is recognized as a restenosis model after PCI. In a dog balloon-injury model, chymase activity but not ACE activity, was significantly increased in the arteries injured by a balloon catheter (Takai et al., 2003b). In this model, an ARB candesartan significantly suppressed the formation of intimal hyperplasia in the injured arteries, while an ACE inhibitor enalapril did not (Miyazaki et al., 1999). The difference in the inhibitory action of candesartan and enalapril is thought to be that ACE inhibitor suppresses only the angiotensin II action produced by ACE but that ARB can suppress the angiotensin II action produced by chymase in addition to that by ACE. These results indicated that local angiotensin II production by chymase is involved in the intimal hyperplasia seen in the injured arteries. In fact, in this dog model, a chymase inhibitor NK3201 significantly reduced intimal hyperplasia in the injured arteries (Takai et al., 2003b).

In patients with AAA, chymase-positive mast cells were detected in the medial area in addition to the adventitial area and the number of mast cells was obviously increased in comparison with the normal aorta (Nishimoto et al., 2002). A significant correlation between serum chymase level and AAA expansion rate has been observed in patients with AAA (Sun et al., 2009). In animal models in which AAA was induced by elastase, chymase, angiotensin II-forming and MMP-9 activities were significantly augmented (Tsunemi et al., 2004; Furubayashi et al., 2007). A chymase inhibitor significantly attenuated not only chymase activity but also angiotensin II-forming and MMP-9 activities in these AAs and prevented AAA development (Tsunemi et al., 2004; Furubayashi et al., 2007). In angiotensin II-infused apolipoprotein E-deficient mice, the aortic diameter was expanded and significant augmentation of chymase and MMP-9 activities was observed (Inoue et al., 2009). Both chymase and MMP-9 activities were significantly attenuated by treatment with a chymase inhibitor and AAA development was prevented. In this model, MMP-9 expression was induced by angiotensin II and the target of chymase inhibitors might be downstream of angiotensin II action, such as inhibition of MMP-9 activation. In fact, using an extract of AAA, the MMP-9 activity was reduced to normal levels by a chymase inhibitor (Inoue et al., 2009). Furthermore, in chymase-deficient mice, the development of AAA was significantly attenuated along with reduction of MMP-9 concentration (Sun et al., 2009). Thus, the mechanism by which a chymase inhibitor prevents AAA development might depend on attenuation of angiotensin II formation and MMP-9 activation.

ARBs, ACE inhibitors and statins may contribute to the attenuation of MMP-9 activity. Both ARBs and statins attenuate the gene expression of proMMP-9 and ACE inhibitors directly bind to the active center of MMP-9, causing direct inhibition of MMP-9 activity (Cipollone et al., 2004; Wang et al., 2000; Yamamoto et al., 2007). Therefore, the combination of chymase inhibitors with ARBs, ACE inhibitors, or statins may be more useful for potent inhibition of angiotensin II and MMP-9 and may promise the powerful prevention of vascular remodeling.

**NEOVASCULARIZATION**

Chymase is associated with deposition of Advanced Glycation End products (AGEs) and chymase expression was induced by AGEs via its receptor-ERK1/2 MAP kinase pathway in cultured Vascular Smooth Muscle Cells (VSMCs) (Otani et al., 2000). In these cells, high glucose levels also upregulated chymase-dependent angiotensin II formation via ERK1/2 MAP kinase activation (Muramatsu et al., 2000). In patients with diabetes, Vascular Endothelial Growth Factor (VEGF) also plays a major role in the initiation and development of diabetic retinopathy. VEGF is an important regulator of vascular permeability, angiogenesis and endothelial cell proliferation. Angiotensin II promotes the expression of VEGF on the pericytes surrounding endothelial cells (Muramatsu et al., 2002). In the retina, NADPH oxidase induced by angiotensin II might be involved in VEGF expression in diabetic rats (Schalkwijk et al., 1999). VEGF stimulates MMP-9 expression in human VSMCs (Stitt et al., 1997). On the other hand, AGEs increase level of NADPH oxidase and induce retinal VEGF expression (Koka et al., 2006). An ARB inhibited AGEs and reduced VEGF gene expression in diabetic rats (Lavrentyev et al., 2007). This finding shows that an inhibitory mechanism related to ARB treatment might be involved in the inhibition of AGEs, suggesting that angiotensin II may increase AGE formation. MMP inhibitors might also prevent retinal neovascularization in a mouse model.
Therefore, chymase inhibition may prevent diabetic retinopathy via reduction of VEGF and MMP-9 levels.

CARDIAC REMODELING

Chymase may be involved in cardiac dysfunction after myocardial infarction. In hamsters, chymase- and angiotensin II-positive cells in the cardiac tissues were significantly increased 1 day after myocardial infarction (Jin et al., 2001). Chymase inhibitors attenuated the chymase activity and the cardiac dysfunction which extended survival (Jin et al., 2002, 2003). Thus, chymase-dependent angiotensin II formation may accelerate cardiac dysfunction after myocardial infarction. On the other hand, in cultured human fibroblasts, chymase was found to significantly increase cell proliferation in fibroblasts (Takai et al., 2003a). This increased cell proliferation was completely suppressed by a chymase inhibitor but not by an ARB (Takai et al., 2003a). Anti-TGF-β neutralizing antibody completely suppressed cell proliferation induced by human chymase, indicating that chymase induced the cell proliferation through TGF-β activation in vitro.

In the cardiomyopathic hamsters, TGF-β is known to induce the expression of collagen I and collagen III genes (Lijnen et al., 2003), the expression of collagen I and collagen III genes in cardiac tissues were significantly increased (Dixon et al., 1997). In pressure-overloaded rats, the administration of anti-TGF-β neutralizing antibodies prevented both the expression of collagen genes and myocardial fibrosis but not myocyte hypertrophy (Kuwahara et al., 2002). In cardiomyopathic hamsters, the chymase activity in heart was significantly increased compared with that in control hamsters, whereas a chymase inhibitor BCEAB significantly reduced not only the chymase activity but also the fibrotic area in heart (Takai et al., 2003a). The mRNA levels of collagen I and collagen III and the fibrotic area of cardiac tissues are also increased in cardiomyopathic hamsters, while BCEAB significantly suppressed the mRNA levels and improved cardiac function (Takai et al., 2003a).

TISSUE FIBROSIS

The chemotherapeutic agent bleomycin is known to cause lung fibrosis in humans and animal experimental models. Administration of anti-TGF-β antibodies could reduce bleomycin-induced pulmonary fibrosis via reduction of collagen mRNA levels and TGF-β may play an important role in the development of bleomycin-induced pulmonary fibrosis in animal models (Giri et al., 1993). In hamsters, significant increases of chymase activity and fibrotic areas in pulmonary tissues after bleomycin treatment were significantly reduced by treatment with a chymase inhibitor (Sakaguchi et al., 2004). In silica-induced pulmonary fibrosis in mice, a chymase inhibitor led to a significant reduction of chymase activity and pulmonary fibrosis as well as the number of neutrophils and the levels of macrophage inflammatory protein-2, monocyte chemoattractant protein-1 and TGF-β (Takato et al., 2011). Chymase inhibitors significantly attenuated tetrachloride-induced liver fibrosis in hamsters (Komeda et al., 2010). Nonalcoholic steatohepatitis (NASH) is accompanied by metabolic syndrome comprising obesity, type-2 diabetes and hypertension. NASH is a distinct clinical entity characterized by varying degrees of progressive steatosis, lobular inflammation and fibrosis of the liver (Powell et al., 1999). Patients with NASH have a high risk for developing advanced fibrosis, cirrhosis and hepatocellular carcinoma (Bugianesi et al., 2002). Mast cell number increases in human chronic liver diseases associated with fibrosis (Farrell et al., 1995; Ambrust et al., 1997). A mast cell stabilizer inhibited the activation of mast cells and prevented the development of hepatic fibrosis in a rat NASH model (Uno et al., 2008). In ahamster NASH model, the levels of the biochemical markers total bilirubin, hyaluronic acid and triglycerides, all of which are known to increase in patients with NASH, were significantly increased (Tashiro et al., 2010). In addition, marked steatosis and fibrosis were observed in this model on histological examination (Tashiro et al., 2010). However, all the biochemical markers and the histological changes were significantly attenuated by treatment with a chymase inhibitor (Tashiro et al., 2010). In this model, we observed a significant reduction of angiotensin II-forming and MMP-9 activities, both of which are involved in the steatosis and fibrosis in the liver (Tashiro et al., 2010). Thus, chymase may become a target for prevention of NASH.

POSTOPERATIVE ADHESION

Postoperative adhesions are a well-known complication of surgery and may be involved in mast cell accumulation (Persinger et al., 1983; Liebman et al., 1993). The number of mast cells is increased around wounds in the late stages of the healing process (Persinger et al., 1983; Liebman et al., 1993). In fact, mast-cell stabilizers which inhibit the activation and accumulation of mast
cells, are effective in attenuating adhesion formation in rat models (Langer et al., 1995). In mast-cell-deficient mice, adhesion formation was significantly less severe than that in normal control mice (Yao et al., 2000). On the other hand, in a rat model, although intact peritoneal/fascial tissue contains a very low level of TGF-β, the level of TGF-β was significantly increased within the fibrosis adhesion after peritoneal wall injury (Williams et al., 1992). In a mouse adhesion model, the level of TGF-β in peritoneal fluid was significantly higher during the first week postsurgery than in uninjured controls (Chegini et al., 1994). In contrast, the intraperitoneal injection of a neutralizing antibody to TGF-β decreased adhesion formation in a rat adhesion model (Lucas et al., 1996; Crowe et al., 2000).

In a hamster adhesion model, chymase activity was significantly increased at the adhesion lesion, while it was significantly reduced by treatment with chymase inhibitors along with reduction in adhesion formation (Okamoto et al., 2002a-c). TGF-β concentrations in the pleural fluid were significantly increased after the cardiac surgery in hamsters, while the increased TGF-β concentrations were significantly reduced by treatment with a chymase inhibitor Suc-Val-Pro-Phe(Oph) (Soga et al., 2004). Thus, chymase inhibition may become a useful strategy for prevention of postoperative adhesion.

CONCLUSION

Chymase increases angiotensin II formation and activates MMP-9 and TGF-β in various tissues and the enzymatic function plays a crucial role in the progression of organ damages (Fig. 1). Therefore, chymase inhibition may become a useful strategy for prevention of organ damages.

REFERENCES


