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Review

Therapeutic applications of chymase inhibitors in cardiovascular diseases and fibrosis

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Abstract

Chymase activates not only angiotensin I to angiotensin II but also latent transforming growth factor-β-binding protein to transforming growth factor-β. In dog grafted veins, chymase activity and angiotensin II concentration along with vascular proliferation were significantly increased, while they were significantly suppressed by a chymase inhibitor. After balloon injury in dog arteries, chymase activity was significantly increased in the injured artery, and a chymase inhibitor and an angiotensin AT_1 receptor antagonist were effective in preventing the vascular proliferation, but an angiotensin-converting enzyme inhibitor was ineffective. In fibrotic models, the tissue fibrosis was reduced by chymase inhibitors. In adhesion models, the transforming growth factor-β concentration and adhesion formation were suppressed by chymase inhibitors. Therefore, chymase inhibitors may be useful for preventing cardiovascular diseases and fibrosis via inhibition of angiotensin II formation and transforming growth factor-β activation.

Keywords: Angiotensin II; Chymase; Inhibitor; Transforming growth factor-β

Contents

1. Introduction

Chymase (EC 3.4.21.39) is a chymotrypsin-like enzyme that is expressed in the secretory granule of mast cells. Chymase is stored as an inactive enzyme in secretory

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granules, because the pH within the granule is regulated at pH 5.5, in which chymase has no enzymatic activity (De Young et al., 1987; McEuen et al., 1995). The optimal pH of chymase is between 7 and 9, and its activity shows almost a maximum level immediately upon release into the extracellular matrix (pH 7.4), when the mast cells are activated in injured or inflammatory tissues (Urata et al., 1990b; Takai et al., 1996, 1999). However, strong chymase inhibitors such as serine protease inhibitors are contained in blood, and the activity of chymase is immediately inhibited. Therefore, chymase has enzymatic activity only in local tissues.

Angiotensin II is a vasoconstricting peptide derived from angiotensinogen by renin and angiotensin-converting enzyme, and the latter is a well-known enzyme for conversion from angiotensin I to angiotensin II. However, chymase can produce angiotensin II from angiotensin I (Urata et al., 1990b; Takai et al., 1996). Angiotensin II also plays an important role in vascular proliferation (Kim and Iwao, 2000). In clinical studies, an angiotensin AT_1 receptor antagonist was successful in preventing restenosis after percutaneous coronary intervention, but an angiotensin-converting enzyme inhibitor was not (MERCATOR Study Group, 1992; Peters et al., 2001). These studies suggest that chymase-dependent angiotensin II formation may play an important role in vascular proliferation. In homogenate of human cardiac tissues, 75% and 25% of angiotensin II-forming activities are dependent on chymase and angiotensin-converting enzyme, respectively (Urata et al., 1990a,b). In homogenates of human gastroepiploic and internal thoracic arteries, angiotensin II-forming activity is inhibited by about 90% with a chymase inhibtor (Takai et al., 1998, 2001c; Uehara et al., 2000). Therefore, chymase-dependent angiotensin II formation may function rather than angiotensin-converting enzyme-dependent angiotensin II formation, when chymase is released from mast cells existed in cardiovascular tissues.

Chymase also contributes to the release of latent transforming growth factor (TGF)-β from latent TGF-β-binding proteins of the extracellular matrix of human epithelial and endothelial cells (Taipale et al., 1995). In human dermal fibroblasts, chymase was found to a significantly increase cell proliferation in fibroblasts (Takai et al., 2003a). In media supernatants of the cultured fibroblasts, the concentration of TGF-β was significantly increased after the injection of chymase, but the increase in TGF-β was inhibited by a chymase inhibitor (Takai et al., 2003a). Anti-TGF-β neutralizing antibodies completely suppressed cell proliferation induced by human chymase, indicating that chymase induced the cell proliferation through TGF-β activation (Takai et al., 2003a). Thus, chymase activates not only angiotensin I to angiotensin II but also latent TGF-β-binding protein to TGF-β, resulting in inducing cardiovascular diseases and fibrosis. In this review, we propose that chymase inhibitors may be useful for preventing cardiovascular diseases and fibrosis.

2. Peptidic and non-peptidic chymase inhibitors

A peptidic chymotrypsin inhibitor chymostatin has been used as a standard chymase inhibitor. However, chymostatin inhibits chymotrypsin-like enzymes such as cathepsin G which can produce angiotensin II from angiotensin I, and it is unsuitable for in vivo studies (Oleksyszyn and Powers, 1994; Caughey, 1994). A peptidic chymase inhibitor Suc-Val-Pro-Phe^{P}(Oph)$_2$ is a non-competitive inhibitor and was developed as a potent inhibitor of chymase activity (Oleksyszyn and Powers, 1991). The half-degradative time of Suc-Val-Pro-Phe^{P}(Oph)$_2$ is about 20 h in human plasma. In isolated dog arteries, Suc-Val-Pro-Phe^{P}(OPh)$_2$ dose-dependently suppressed the angiotensin I-induced vascular contraction in the presence of an angiotensin-converting enzyme inhibitor (Takai et al., 2000). The remaining response in the presence of an angiotensin-converting enzyme inhibitor is dependent on chymase-dependent angiotensin II formation, because the remaining response was completely inhibited by the combination of an angiotensin-converting enzyme inhibitor and chymostatin. In this system, the IC_{50} value of Suc-Val-Pro-Phe^{P}(OPh)$_2$ in isolated dog arteries was 2.8 nM, and the value was about 35 times higher than that of chymostatin (Takai et al., 2000). Local infiltration, and not oral administration, of Suc-Val-Pro-Phe^{P}(OPh)$_2$ could prevent vascular proliferation in dog grafted veins or adhesion formation after surgery.

Non-peptidic chymase inhibitors have been developed as orally active chymase inhibitors, and these chemical structures are shown in Fig. 1. 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, is a competitive inhibitor. NK3201 inhibits human, dog and hamster chymases by IC_{50} at concentrations of 2.5, 1.2 and 28 nM, respectively, but it has hardly inhibitory activity to other types of serine proteases, tryptase, thrombin, elastase, plasmin and plasminogen activator (Takai et al., 2001a). In dog, the concentration of NK3201 in plasma, heart and aorta are about 470, 195 and 78 nM, respectively, 8 h after oral administration of 1 mg/kg of NK3201 (Takai et al., 2003b). BCEAB, 4-[1-[[bis-(4-methyl-phenyl)-methy]-carbamoyl]-3-(2-ethoxy-benzyl)-4-oxo-azetidine-2-yloxy]-benzoic acid, inhibits human chymase by IC_{50} at concentrations of 5.4 nM, but it does not inhibit angiotensin-converting enzyme, elastase and tryptase (Takai et al., 2001b). In the hamster, the heart chymase activities were significantly suppressed to 42.0% and 26.9% 3 h after oral administration of 100 and 300 mg/kg of BCEAB, respectively (Takai et al., 2001b). SUN-C8257, 3-[(3-amino-4-carboxy) phenylsulfonyl]-7-chloroquinazoline-2,4(1H,3H)-dione inhibits human and hamster chymases by IC_{50} at concentrations of 310 and 680 nM, respectively, but it inhibits human cathepsin G by IC_{50} at concentration of 5.5 μM (Uehara et al., 2002). TY-51184, 2-[4-(5-Fluoro-3-methylbenzo[*b*]thiophen-2-yl)sulfonamido-3-methanesulfonylphenyl]oxazole-4-carboxylica-cid inhibits human, dog and hamster chymases by IC_{50} at

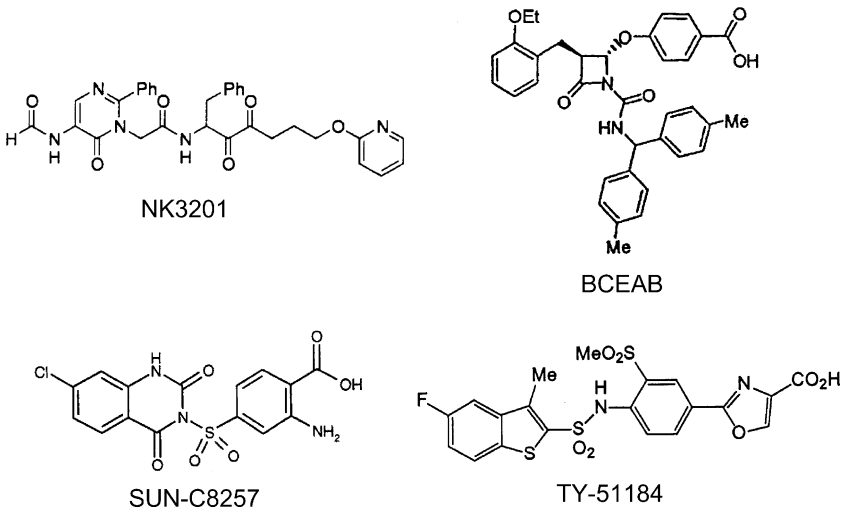

Fig. 1. Chemical structures of non-peptidic chymase inhibitors.

concentrations of 37, 58 and 128 nM, respectively, but it does not suppress cathepsin G even at 100 μM (Takai et al., 2004).

3. Inhibition of angiotensin II formation

3.1. Vascular proliferation in vein graft

Patients with ischemic heart disease are offered with coronary artery bypass grafting or percutaneous coronary intervention. In coronary artery bypass grafting, the internal thoracic artery and saphenous vein have been frequently used as coronary artery bypass conduits. However, the poor performance of the saphenous vein compared with the internal thoracic artery is well known (Fuchs et al., 1978; Lytle et al., 1985). In isolated human saphenous vein, the contractile response of angiotensin II is greater than that in the internal thoracic artery, suggesting that the saphenous vein exhibits greater angiotensin II-mediated action than the internal thoracic artery (Borland et al., 1998). Moreover, the chymase activity and total angiotensin II-forming activity, but not the angiotensin-converting enzyme activity, is significantly higher in human saphenous vein than in the internal thoracic artery (Nishimoto et al., 2001b). This high level of total angiotensin II-forming activity in the saphenous vein is thought to be dependent on the upregulated chymase activity. In a dog grafted model, the angiotensin-converting enzyme activity in the grafted veins was significantly decreased up to 7 days after grafting, and especially after 1 and 3 days, it was suppressed in the grafted veins to less than 10% of the control value (Nishimoto et al., 2001a). The reason why the angiotensin-converting enzyme activity was decreased at acute periods after the operation is thought to be dependent on the loss of the endothelium. Because the endothelium in grafted veins is put under arterial pressure, thus resulting in the loss of the endothelium including angiotensin-converting enzyme (Fukuyama et al., 1996). On the other hand, 7 days after the operation, the chymase activity was significantly increased in the grafted veins. Considering these findings, up to 7 days after the operation, the angiotensin II formation in the grafted veins is thought to depend mainly on the chymase-dependent angiotensin II-forming pathway. In fact, 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 7 days after the operation, while they were significantly suppressed by a chymase inhibitor Suc-Val-Pro-Phe^{P}(Oph)$_2$ (Nishimoto et al., 2001a). We also confirmed the long-term effect of chymase inhibitors, Suc-Val-Pro-Phe^{P}(Oph)$_2$, NK3201 and TY-51184, in the prevention of vascular proliferation in this dog grafted veins (Takai et al., 2000, 2001a, 2004; Tsunemi et al., 2002b).

3.2. Vascular proliferation after balloon injury

In clinical studies, an angiotensin AT$_1$ receptor antagonist valsartan was successful in preventing restenosis after percutaneous coronary intervention, but an angiotensin-converting enzyme inhibitor cilazapril was not (MERCATOR Study Group, 1992; Peters et al., 2001). In a dog balloon-injury model, chymase activity, but not angiotensin-converting enzyme activity, was significantly increased in the arteries injured by a balloon catheter (Takai et al., 2003b). In this model, an angiotensin AT$_1$ receptor antagonist candesartan significantly suppressed the formation of intimal hyperplasia in the injured arteries, while an angiotensin-converting enzyme inhibitor enalapril did not (Miyazaki et al., 1999b). The difference in the inhibitory action of candesartan and enalapril is thought to be that angiotensin-converting enzyme inhibitor suppresses only the angiotensin II action produced by angiotensin-converting enzyme but that angiotensin AT$_1$ receptor antagonist can suppress the angiotensin II action produced by chymase in

addition to that by angiotensin-converting enzyme. 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). We propose that chymase inhibitors may be useful for preventing restenosis after percutaneous coronary intervention.

3.3. Atherosclerosis and aneurysm

In human atherosclerosis, the number of activated mast cells was increased in atherosclerotic lesions and a chymase gene variant was associated with atherosclerosis (Kaartinen et al., 1994; Ortlepp et al., 2001). In animal atherosclerotic models, the chymase activities and mRNA levels were increased in atherosclerotic legions, whereas a chymase inhibitor SUN-C8257 significantly suppressed the development of atherosclerosis (Takai et al., 1997; Uehara et al., 2002). Angiotensin II is well known to induce atherosclerosis, and angiotensin AT_1 receptor antagonists prevent the development of atherosclerosis in animal atherosclerotic models (Miyazaki et al., 1999a; Strawn et al., 2000). Thus, an increase of local angiotensin II formation by chymase may play an important role in the development of atherosclerosis.

Aneurysmal aorta which represents a chronic degenerative condition associated with atherosclerosis is characterized by segmental weakening and dilatation of the aortic wall, and carries a life-threatening risk of rupture (Thompson, 1996). The pathophysiology of aneurysmal aorta includes aortic atherosclerosis, chronic inflammation within the outer aortic wall, and an imbalance between the production and degradation of structural extracellular matrix proteins (White et al., 1993). In human abdominal aortic aneurysms, chymase activity is significantly increased (Nishimoto et al., 2002; Tsunemi et al., 2002a). Chymase-positive mast cells are hardly detected in the normal vessels, and only in the adventitial area (Nishimoto et al., 2002). However, in the abdominal aortic aneurysms, 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). The increased chymase activity in the abdominal aortic aneurysms is thought to be dependent on the accumulation of chymase-positive mast cells. The number of macrophages is also increased in the abdominal aortic aneurysms, and angiotensin II activates macrophages (Hernandez-Presa et al., 1997; Schieffer et al., 2000). The activated macrophages induce nuclear factor-κB, and this in turn induces an inflammatory cytokine, interleukin-1, and a chemokine, monocyte chemoattractant protein (MCP)-1 (Collins et al., 1995). Interleukin-1 produced by activated macrophages induces tissue damage, and MCP-1 induces the activation and migration of monocytes, resulting in an accumulation of macrophages (Chen et al., 1998; Mabuchi et al., 2000). Angiotensin AT_1 receptor antagonists were found to reduce gene expression of MCP-1, and reduced the accumulation of macrophages (Kato et al., 1999; Hilgers et al., 2000). Daugherty et al. (2000) demonstrated that infusion of angiotensin II leads to development of aortic aneurysm in apolipoprotein E-deficient mice. In contrast, an angiotensin AT_1 receptor antagonist can suppress progression of the abdominal aortic aneurysm (Daugherty et al., 2001). In a hamster aneurysmal model, chymase activity in abdominal aortic aneurysms was significantly higher than that in normal aorta, whereas a chymase inhibitor NK3201 significantly suppressed both the chymase activity and the aortic diameter (Tsunemi et al., 2004). Thus, the suppression of chymase-dependent angiotensin II formation may also be useful for preventing the progression of abdominal aortic aneurysm.

3.4. Hypertension

Angiotensin II plays an important role in the regulation of blood pressure. However, the renin- and angiotensin-converting enzyme activities in plasma were apparently normal or low in the chronic stage of hypertensive rat models, such as spontaneously hypertensive rats and two-kidney, one clip hypertensive rats (Miyazaki et al., 1986; Nakamura et al., 1988; Okunishi et al., 1991). On the other hand, vascular angiotensin-converting enzyme was activated to increase local production of vascular angiotensin II in all these hypertensive models, but the high blood pressure was reduced by angiotensin-converting enzyme inhibitors (Nakamura et al., 1988; Okunishi et al., 1991). These findings suggest that the increase of angiotensin II generated by angiotensin-converting enzyme in vascular tissues plays a crucial role in the pathogenesis of hypertension. To clarify whether chymase is involved in take part in, we studied a two-kidney, one clip hypertensive model in hamster which vessels, like humans, contain chymase-dependent angiotensin II-forming pathway (Jin et al., 1998). The blood pressure in the two-kidney, one clip hamster increased significantly 2 weeks after clipping (acute stage), and was sustained at the high level until 32 weeks after clipping (chronic stage). Plasma renin activity increased significantly during the acute stage, but returned to the normal level at the chronic stage. In the chronic stage, the vascular angiotensin-converting enzyme activity, but not the chymase activity, increased significantly, and an angiotensin-converting enzyme inhibitor and an angiotensin AT_1 receptor antagonist showed equipotently hypotensive effects at the acute and chronic stages (Jin et al., 1998). Angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists are known not only to reduce blood pressure but also to increase plasma renin activity, while NK3201 did not affect both blood pressure and plasma renin activity in dog (Takai et al., 2003b). These findings suggest that chymase inhibitors, but not angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists, may be hardly

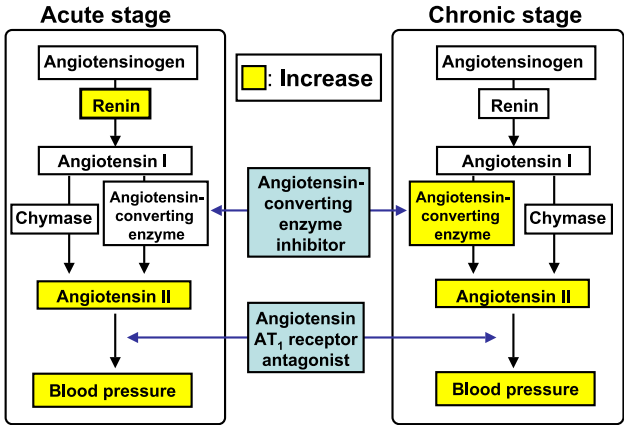

Fig. 2. Pathophysiological role of renin, angiotensin-converting enzyme and chymase on acute and chronic stages in two-kidney, one clip hypertensive hamsters. Increase of plasma renin and vascular angiotensin-converting enzyme, but not chymase, play an important role in the pathophysiology of hypertension in acute and chronic stages in two-kidney, one clip hypertensive hamsters, respectively. In both stages, angiotensin-converting enzyme-dependent angiotensin II formation is predominant, and angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists equally reduce the blood pressure.

involved in the regulation of blood pressure (Fig. 2) and may be more useful for prevention of local angiotensin II formation only in cardiovascular damages than angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists.

4. Inhibition of transforming growth factor-β activation

4.1. Pulmonary fibrosis

In idiopathic pulmonary fibrosis in patients, TGF-β is increased, and in animal models of bleomycin-induced pulmonary fibrosis, TGF-β may also play an important role in the development of pulmonary fibrosis (Broekelmann et al., 1991; Zhang et al., 1996). For example, administration of anti-TGF-β antibodies or an antagonist of TGF-β signaling could reduce bleomycin-induced pulmonary fibrosis via reduction of collagen mRNA levels (Giri et al., 1993; Nakao et al., 1999). A chemotherapeutic agent bleomycin is known to develop lung fibrosis in humans and animal experimental models. In bleomycin-induced pulmonary fibrosis in mice, both the chymase activity and hydroxyproline content in pulmonary tissues were significantly increased after bleomycin-treatment, whereas intraperitoneal administration of a chymase inhibitor, 7-chloro-3-(3-amynophenyl) quinazoline-2, 4-dione methanesulfonate (SUN-C8077), significantly decreased not only chymase activity but also hydroxyproline content (Tomimori et al., 2003). In a hamster model, oral administration of NK3201 significant reduced increases of chymase activity and fibrotic area in pulmonary tissues after bleomycin-treatment (Sakaguchi et al., 2004). Therefore, chymase inhibitors may be promising for treatment of pulmonary fibrosis.

4.2. Cardiomyopathy

In cardiac tissue of cardiomyopathic patients and experimental animal models, TGF-β is also increased (Eghbali et al., 1991; Li et al., 1997). 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 in the cardiomyopathic hamsters (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 cardiocmyopathic hamsters, while BCEAB significantly suppressed the mRNA levels and improved cardiac function (Takai et al., 2003a). However, BCEAB could not reduce the cardiac hypertrophy. These reports are very similar to results for treatment with anti-TGF-β neutralizing antibodies. The increase in cardiac chymase activity may induce TGF-β activation, and this inhibition may play an important role in reducing cardiac fibrosis and dysfunction via induction of the expression of collagen I and collagen III genes.

5. Adhesion formation

Postoperative adhesions are a well-known complication of surgery, and mast cells may be involved in adhesion formation, which is closely related to fibrotic formation (Persinger et al., 1983; Liebman et al., 1993; Langer et al., 1995). The number of mast cells is increased around

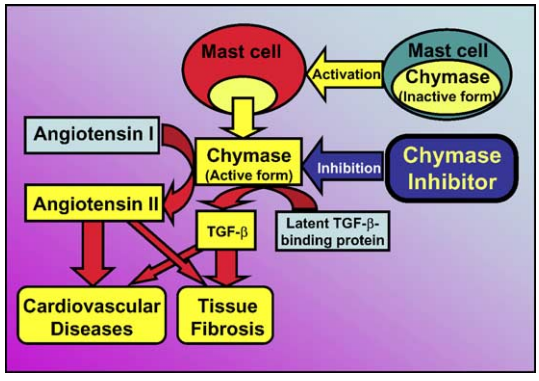

Fig. 3. Mechanism of chymase inhibitor for prevention of the cardiovascular diseases and fibrosis. After mast cells have been activated, chymase produces angiotensin II and TGF-β, both of which induce the cardiovascular diseases and fibrosis. Chymase inhibitor can prevent the cardiovascular diseases and fibrosis via the reduction of angiotensin II and TGF-β.

wounds in the late stages of the healing process (Persinger et al., 1983; Liebman et al., 1993). In contrast, mast-cell stabilizers, which inhibit the activation and accumulation of mast cells, are effective in attenuating adhesion formation in rat models (Persinger et al., 1983; 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). These reports suggest that mast cells is involved in adhesion formation. On the other hand, Chegini (1997) demonstrated evidence for a key role for TGF-β in adhesion formation. 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), and the intraperitoneal injection of TGF-β accelerates adhesion formation (Williams et al., 1992). 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). These reports suggest that TGF-β may play an important role in the development of adhesion formation. In a hamster adhesion model, chymase activity was significantly increased at the adhesion lesion, while it was significantly reduced by treatment with chymase inhibitors, Suc-Val-Pro-Phe^{P}(Oph)$_2$, NK3201, BCEAB and TY-51184, along with reduction in adhesion formation (Okamoto et al., 2002a,b,c, 2004). 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^{P}(Oph)$_2$ (Soga et al., 2004). Therefore, chymase inhibition might be related to the reduction of TGF-β activation, and its mechanism may play an important role in preventing adhesion formation.

6. Conclusion

Chymase-dependent angiotensin II formation is involved in the development of vascular proliferation and aneurysm and chymase-dependent TGF-β activation is involved in the developments of fibrotic formation such as tissue fibrosis and adhesion formation. Therefore, chymase inhibitors may be useful for preventing cardiovascular diseases and fibrosis via inhibition of angiotensin II formation and TGF-β activation (Fig. 3).

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