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FORUM MINIREVIEW

Development and Application of Chymase Inhibitors

Development of the Chymase Inhibitor as an Anti-Tissue-Remodeling Drug: Myocardial Infarction and Some Other Possibilities

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ABSTRACT—Chymase leading to tissue remodeling is expected to be a potent pharmaceutical target. Its functions in vivo are still unclear, because of lack of orally available inhibitors. Recently, however, the chymase inhibitor NK3201 (2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidin-1-yl)-*N*-[{3,4-dioxo-1-phenyl-7-(2-pyridyloxy)}-2-heptyl] acetamide) was demonstrated to have oral activity against neointimal hyperplasia in dog models (Takai S. et al., Life Sci **69**, 1725 – 1732 (2001)). In this review, by showing the efficacy of NK3201 in some hamster models, chymase functions in vivo are summarized, and the potency of this chymase inhibitor is introduced. In vitro study, NK3201 showed potent chymase specific inhibitory activity, and Dixon plot analysis indicated competitive inhibition. Oral administration of NK3201 into normal rats resulted in rapid spread over every tissue except the brain, and sufficient activity to inhibit tissue chymase was detected even after 24 h. In passive cutaneous anaphylaxis, myocardial infarction and bleomycin-induced pulmonary fibrosis models, orally administered NK3201 showed potent inhibition of inflammatory response, tissue angiotensin II formation, and fibrosis, respectively. These data suggest that chymase has a vital role in tissue remodeling through promotion of the inflammatory response, tissue angiotensin II and tissue fibrosis. Our recent data indicated chymase participation in bladder fibrosis, like interstitial cystitis. Therefore, the orally active chymase inhibitor NK3201 may have protective effects on tissue remodeling in several diseases.

Keywords: Chymase, Inhibitor, Myocardial infarction, Tissue remodeling

Research to find chymase inhibitors has been continued for over a decade, but suitable compounds for in vivo use have not been obtained yet. Chymase stocked in mast cells was originally noticed as an inflammatory protease. In 1985, anti-chymase antibody or chymase inhibitor was reported to inhibit mast-cell degranulation (1). Therefore, it was thought that a chymase inhibitor would be effective as an anti-asthma or anti-allergic drug. In the early 1990s, some investigators observed angiotensin (Ang) II forming activity of chymase in heart and artery (2, 3). Hence, chymase-inhibitor development focused on cardiovascular diseases. As a result of overall studies, chymase is thought to participate in tissue remodeling in not only cardiac diseases but also other chronic diseases (4, 5), and the chymase inhibitors are expected to have broad target diseases.

On the other hand, development of chymase inhibitors has made slow progress. Several compounds showed strong inhibitory activity in vitro but not in vivo. Recently, we succeeded to demonstrate the oral activity of NK3201 (2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidin-1-yl)-*N*-[{3,4-dioxo-1-phenyl-7-(2-pyridyloxy)}-2-heptyl] acetamide) in dog bypass graft model (6) and carotid-artery balloon injury model (7). The details of these anti-hypertrophic activities are summarized in another review by Takai and Miyazaki (8). In this review, results of enzyme kinetic analysis for NK3201 are introduced, and its efficacy in myocardial infarction in the hamster is demonstrated. Further possibilities for its use in other clinical fields are discussed with preliminary data.

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Enzyme kinetic analysis for NK3201

NK3201 shows strong competitive inhibition against

chymase in the mode of formation of the acyl-intermediate between the active serine residue of the enzyme and the diketone structure of NK3201 by computer 3D calculation. Dixon plot analysis indicates that NK3201 shows competitive inhibition, and its Ki values against human and dog chymases are 1.9 and 0.23 nM, respectively. NK3201 also has sufficient inhibitory activity against other chymases (IC_{50} values for monkey, 0.38 nM; for hamster, 11 nM; for rat, 31 nM). It has been shown that NK3201 has no inhibitory activity against other types of serine proteases.

After oral administration, NK3201 concentration in tissues or plasma, which was not measured directly, was estimated from in vitro dog chymase activity in extracted tissues or plasma samples. Using extract from tissue or plasma samples obtained from NK3201-administered rats, in vitro dog chymase inhibitory activity was measured and NK3201 concentration was calculated. The results indicated that NK3201 was absorbed rapidly into plasma after oral administration of 1 mg/kg NK3201. NK3201 activity was still left at the equivalent of approximately 100 nM in plasma at 24 h after administration. NK3201 activity was also detected in almost every tissue, i.e., heart, aorta, lung, liver, kidney, muscle and skin, but not in the brain. In all examined tissues except the brain, over 10 nM equivalent of NK3201 was left 24 h later, which is sufficient activity to inhibit human or dog chymase completely. Hence, orally administered NK3201 is expected to inhibit tissue chymase in vivo.

Hypothesis of chymase function in vivo

What are the clinical targets of an orally active chymase inhibitor? A number of studies indicated that chymase might cause tissue remodeling in some chronic diseases. Tissue remodeling caused by chymase might be brought by way of inflammatory response promotion, tissue Ang II formation, and tissue fibrosis after tissue degradation (Fig. 1).

Chymase produced mainly by mast cells was reported to promote mast-cell degranulation (2) and to activate some cytokines, such as interleukin (IL)-1β (9), transforming growth factor (TGF)-β (10) and c-kit ligand (11). Thus, chymase must initiate inflammation through promotion of mast-cell degranulation and aggravates inflammation through cytokine activation and mast-cell migration by c-kit ligand activation.

In 1990s, chymase was reported as highly specific Ang II forming enzyme (3), rather than Ang I converting enzyme (ACE). ACE exists mainly on the vascular intimal surface and in blood, and it controls blood pressure. On the other hand, chymase localizes in tissues. Tissue Ang II will be a hypertrophic or hyperplastic signal, but have no effects on blood pressure. Therefore, chymase must be a hypertrophic or hyperplastic factor of cardiac tissues rather than a blood pressure controller.

Other chymase substrates have been reported. One of the chymase functions must be degradation of extracellular matrix proteins. Chymase specifically processes some pro-

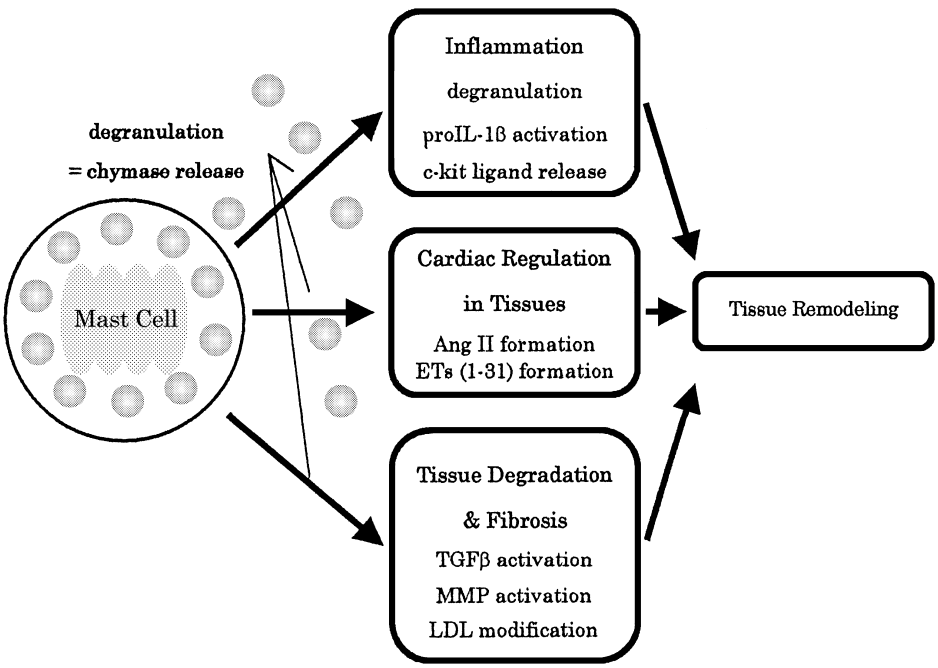

Fig. 1. Scheme for chymase function leading to tissue remodeling. IL, interleukin; Ang, angiotensin; ET, endothelin; TGF, transforming growth factor; MMP, matrix metalloproteinase; LDL, light-density lipoprotein.

matrix metalloproteinases (pro-MMPs), such as pro-collagenase (12, 13), and activates them. Chymase also activates MMPs by degradation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) (14), which are the natural inhibitors of MMPs. Excessive MMPs activation often causes inadequate tissue degradation that leads to fibroblast migration and tissue fibrosis. In addition, tissue fibrosis formation is assisted by TGF-β activation. Thus, chymase causes tissue fibrosis via tissue degradation.

Demonstration of the hypothesis

To confirm each function of chymase, effects of NK3201 were evaluated in some hamster experiments, because hamster has an α type of chymase as the dominant type (15). Only α-chymase exists in humans, but rodents except hamster have β types of chymase dominantly. Therefore, selection of α-chymase dominant animals is an essential step to studying human chymase function in animal experiments.

For evaluating a role in mast-cell degranulation, hamster passive cutaneous anaphylaxis (PCA) was conducted. In the PCA model, intravenously injected Evans' blue was extravasated to skin by mast-cell released histamine. In our experiment, hamster anti-ovalbumin IgE serum prepared in advance was injected intradermally into abdominal skin with various dilutions. At 2 days after IgE injection, 100 mg/kg NK3201 was orally administered, and 3 h later, the mixed solution of Evans' blue and ovalbumin was intravenously injected into the jugular vein. At an hour after the injection of the mixed solution, extravasated dye area on the abdominal skin was measured. The dye extravasation by anaphylaxis was completely suppressed by oral administration of NK3201. This result shows that NK3201 can suppress mast-cell degranulation in vivo and indicates that chymase participates in mast-cell degranulation.

An Ang II suppressive effect was confirmed in the hamster myocardial infarction model. In this model, an Ang II type 1 receptor blocker, TCV-116, reduced mortality rate but the ACE inhibitor lisinopril failed to do so (16). These results suggest that Ang II formed by chymase, rather than formed by ACE, has an important role in failing heart after ischemia. In this model, 30 mg/kg per day NK3201 or vehicle was orally administered from 3 days before ligation of coronary artery. At days 3 and 14 after the operation, hemodynamic functions were measured (D. Jin et al., submitted). Myocardial infarction resulted in significant decreases in heart rate (HR), mean arterial blood pressure (MABP), positive rates of pressure development (+dP/dt) and negative dP/dt (–dP/dt). NK3201 treatment significantly increased +dP/dt and –dP/dt on both days 3 and 14. In contrast, there were no statistically significant differences in HR and MABP between NK3201- and vehicle-treated groups. As a result, NK3201 treatment reduced the 14-day mortality rate from 60.8% (vehicle treatment) to 16.8% (NK3201 treatment). These effects are quite similar to the effects of TCV-116 in the former experiment (16). Results of these two experiments support that NK3201 can improve hemodynamics and survival rate through suppression of tissue Ang II formation. Our findings also suggest that NK3201 has potency for clinical treatment of patients with myocardial infarction or chronic heart failure.

In the next experiment, NK3201 successfully suppressed pulmonary fibrosis induced by bleomycin, a representative model for tissue fibrosis. In our experiment, 0.5 mg bleomycin was treated intratracheally at day 0, and NK3201 was orally administered at the dose of 30 mg/kg per day from day 0 to day 20. At day 28, lung tissues were obtained, and histological analysis was performed with their section stained by Azan's stain. In the analysis, pulmonary fibrosis was observed in the bleomycin-treated group (Fig. 2a). In contrast, fibrosis formation by bleomycin was dramatically suppressed in the NK3201-admnistered group (Fig. 2b). In comparison with the vehicle-treated group, oral administration of NK3201 led to 36.5% reduction of fibrotic area. Lung weight/body weight rate was also reduced from 0.777 ± 0.128 to 0.692 ± 0.120 by NK3201 treatment. These findings show that NK3201 can inhibit the formation of tissue fibrosis. It demonstrates that chymase has an important role in tissue degradation and the formation of tissue fibrosis.

All these experiments indicate that the chymase inhibitor NK3201 has suppressive effects on inflammation, tissue Ang II formation and tissue fibrosis after degradation. Thus, chymase may participate in tissue remodeling via these three functions shown in Fig. 1, and NK3201 is expected to have potency for anti-tissue remodeling activity.

Potency of NK3201 in clinical use

As described before, the chymase inhibitor has broad potency for clinical use. Tissue remodeling that often occurs at the chronic phase in diseases is thought to be an irreversible change and problematic phenomenon. Data in some experiments show that NK3201 can protect cardiac tissues from tissue remodeling. Intimal hyperplasia after vein graft or balloon injury is remodeling in the vascular wall (6, 7). NK3201 also reduced mortality rate in the hamster myocardial infarction model. In infarcted heart, myocytes are injured, and tissue remodeling leading to mal-function and death occurs. Thus, NK3201 will protect against tissue remodeling in failing heart. These data suggest that NK3201 has inhibitory activity against cardiac tissue remodeling, such as restenosis after cardiac intervention, vein-graft disease and chronic heart failure.

Furthermore, NK3201 prevented bleomycin-induced pulmonary fibrosis. This shows that NK3201 can protect

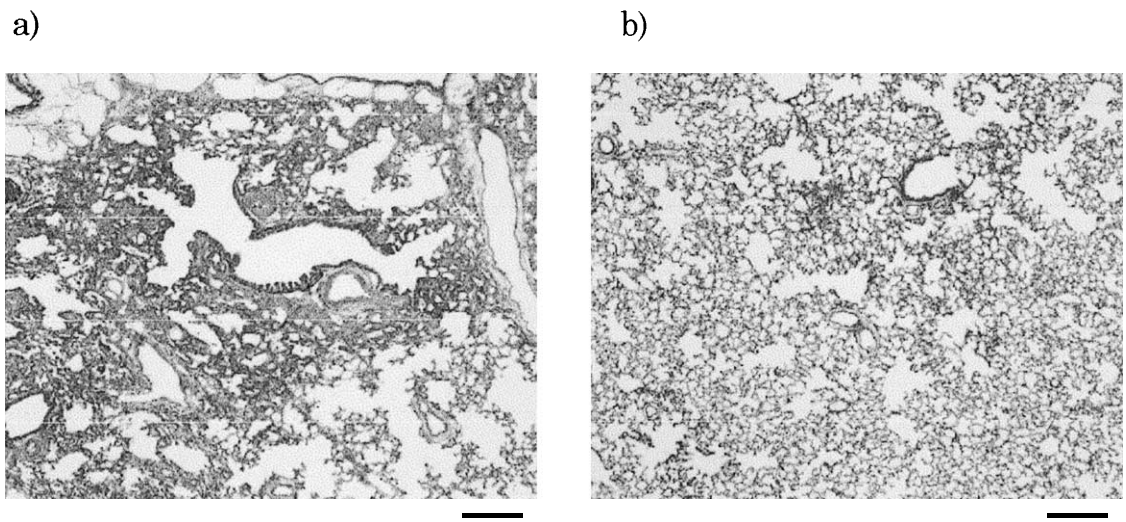

Fig. 2. Staining of fibrotic area in bleomycin-treated lung tissues by Azan's stain. Lung was collected from vehicle (a)- or 30 mg/kg per day NK3201 (b)-treated hamsters 28 days after bleomycin injection. Fromalin-fixed tissue sections were stained by Azan's stain. Bars under photographs represent 200 μm.

not only cardiac tissues but also other tissues from tissue remodeling in chronic diseases. Some data indicate the possibility that chymase participates in other tissue remodeling. Chymase up-regulation was reported in adhesion in the injured region (4), transplanted kidneys (5), rheumatoid arthritis regions (17), lung tissues of pulmonary hypertension (18), atherosclerotic arteries (19), and others. Recently we demonstrated that chymase was accumulated in fibrotic bladders. In the HCl-induced interstitial cystitis model (20), fibrosis in the interstitium was observed in the bladder at 2 weeks after HCl induction. In the same experiment, chymase activity clearly increased to 5.4-fold of saline-injected non-induced bladders. Thus, chymase may cause interstitial cystitis whose cause has not been clarified yet, and interstitial cystitis is one of the potential targets of the chymase inhibitor, NK3201.

In summary, our results suggest that NK3201 may have potency to prevent irreversible tissue damage in several chronic diseases. Now, the potency of NK3201 is being further evaluated in other disease models.

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