

## FORUM MINIREVIEW

### Development and Application of Chymase Inhibitors

## Development of a Chymase Inhibitor: Pharmacological Characterization of a Chymase Inhibitor in Inflamed Tissue Remodeling and Fibrosis

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**ABSTRACT**—Chymase, a chymotrypsin-like serine protease, has not only alternative angiotensin II-generating activity but also various activities involving inflammatory responses. However, little is known of its contribution to physiological functions. Therefore, chymase inhibitors are thought to be potentially useful as tools for elucidating the physiological functions of chymase and therapeutic agents. Within the last five years, many patents on non-peptide chymase inhibitors have been published. We developed a potent non-peptide chymase inhibitor BCEAB (4-[1-{{[bis-(4-methyl-phenyl)-methy]-carbamoyl}-3-(2-ethoxy-benzyl)-4-oxo-azetidine-2-yloxy]-benzoic acid) and examined its effect on inflamed tissue remodeling and fibrosis using a hamster sponge implant model. BCEAB has high inhibitory activity against human chymase but not against angiotensin-converting enzyme, elastase and tryptase. In the hamster sponge implant model, oral administration of BCEAB for 15 days dose-dependently suppressed both the dry weight of granuloma tissues in the sponge discs and the amounts of hydroxyproline in the tissues gradually increased during the experimental period. These results suggest that chymase, at least in part, participates in the growth of granuloma tissues of inflammatory regions by stimulating fibroblast growth and extracellular matrix collagen deposition. Chymase inhibitors for oral administration, such as BCEAB, might be useful for clarifying the pathophysiological roles of chymase in vivo.

**Keywords:** Non-peptide chymase inhibitor, BCEAB, Human chymase, Fibrosis, Tissue remodeling

Chymase is a chymotrypsin-like serine protease, which is present mostly in the secretory granules of mast cells and is released into the extracellular matrix by degranulation when mast cells are activated (1). After being released, the enzyme displays a range of important proinflammatory effects in cooperation with histamine. Chymase affects the processing of many physiologically active substances, such as stem cell factor (2), and also participates in the activation of collagenase (3), the formation (4) and degradation (5) of extracellular matrix, and the enhancement of cutaneous vascular permeability (6–8). In addition to these effects, one of the major roles of human chymase is thought to be the production of angiotensin II (Ang II) from angiotensin I (Ang I) (9, 10). Thus, chymase is speculated to play an important role in congestive heart failure, hypertension, allergy, dermatitis, rheumatoid arthritis, asthma and chronic

inflammation following fibrosis, such as cardiac and pulmonary fibrosis.

#### Synthesis of chymase inhibitors

Given the variety of pathophysiological processes in which chymase may be involved, chymase inhibitors are thought to be potentially useful tools for elucidating the physiological and pathological roles of chymase and therapeutic agents. Therefore, we tried to develop orally available chymase inhibitors. Screening our company's compound collection led to identification of the 1-oxacephem and 1,3-diazetidione-2,4-dione derivative as a chymase inhibitor (Fig. 1). After optimizations, we synthesized three series of  $\beta$ -lactam chymase inhibitors (11–15). The first series consists of 1-oxacephems, a group of derivatized compounds of formula 1. Preferred examples are illustrated by structure 2 ( $IC_{50}$  values of 6 nM). The second series consists of 1,3,7  $\beta$  positions of cephalosporine sulfone derivatives displaying a similar tendency

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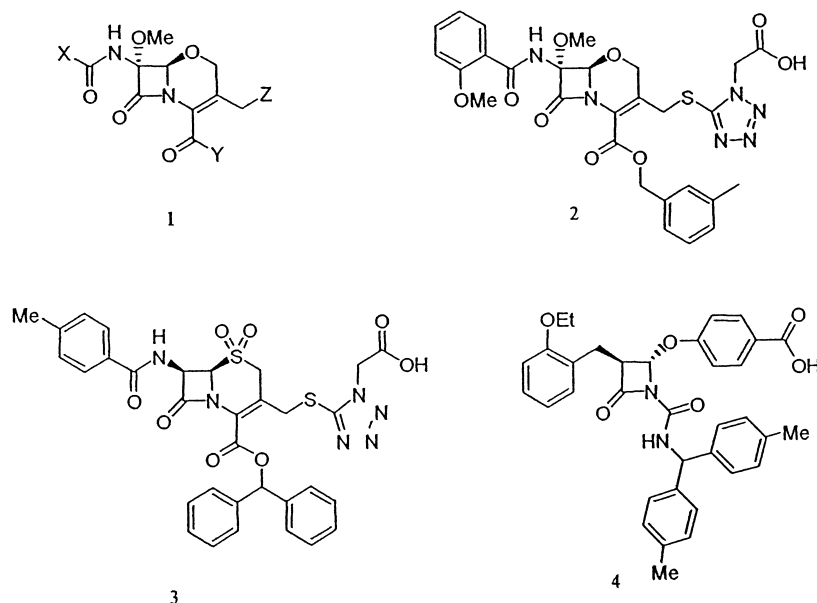


Fig. 1. Chemical structures of chymase inhibitors. Structure 4 shows BCEAB.

to those of 1-oxacephems and includes the preferred compound 3 ( $IC_{50}$  values of 28 nM). The third series consists of derivatives of monocyclic  $\beta$ -lactams, 3-benzylazetidine-2-ones having 1-benzylurea and 4-*para*-carboxyphenoxy. The preferred example is illustrated by structure 4 (4-[1-{[bis-(4-methyl-phenyl)-methyl]-carbamoyl}-3-(2-ethoxy-benzyl)-4-oxo-azetidine-2-yloxy]-benzoic acid: BCEAB), which possesses high potency ( $IC_{50}$  values of 5.4 nM).

Within the last five years, in addition to our reports, various chymase inhibitors have been reported, such as phenylalanine-containing dihydropyrimidiones and tetrahydropyrazinones, arylsulfonamide derivatives, imidazo [1,2-*d*][1,2,4]triazines and benzimidazoles, indoles, hydrazide group-containing isoxazoles, and 2*H*-indene spirp-(3'-cyclohexene) compounds (16).

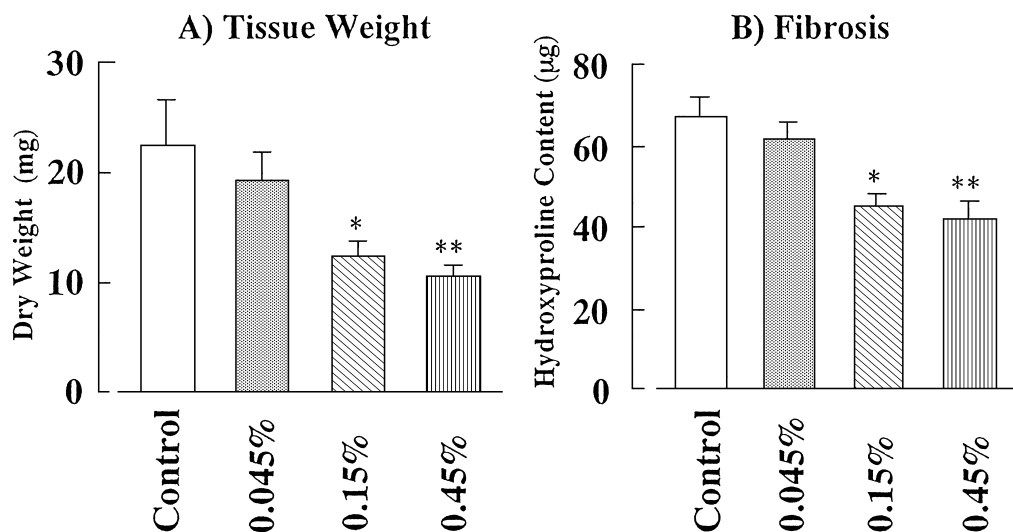
#### Anti-fibrotic effect of chymase inhibitor

Chymase has been reported to have the ability to directly degrade connective tissue proteoglycans (17) and basement membrane collagen (type IV) (18). Moreover, this enzyme indirectly activates human interstitial pro-collagenase (3), thus causing increased collagen degradation, which may be partly related to its neutrophil chemoattractant activity in vitro (8). It has also been reported that type I collagen molecules cleaved by chymase are initiated to form collagen fibrils (5). Chymase released from mast cells may be effectively involved in tissue remodeling through stimulation of the cell growth of mesenchymal fibroblasts, up-regulation of the synthetic cycle of the extracellular matrix collagen, and induction of attraction and invasion of in-

flammatory cells (8). Furthermore, heparin released from mast cells by degranulation can interact with chymase molecules to stabilize and maintain the activity for a long time (19). These previous reports led us to speculate that chymase accelerates the turnover of collagen fibrils and stimulates the growth and remodeling of inflamed tissue.

In a hamster sponge implant model, the dry weight of tissue and the total amounts of the hydroxyproline contents of granuloma tissues gradually increased over the 15-day experimental period, showing fibrosis and collagen deposition progress along with the growth of granuloma tissues (20). To ascertain the possibility of chymase being involved in this cell growth and/or fibrosis, the chymase activity in the granuloma tissue was measured. The chymase activity emerged in the tissues by 5 days after implantation of the sponge discs and gradually increased up to a maximal level at about day 10. The increases of chymase activities in the granuloma tissues are thought to result from accumulation of mast cells in the tissues accompanying the inflammatory process (20). This was supported by the result that the accumulation of mast cells was actually observed in the sponge tissues. These results strongly suggest that chymase, which is produced by infiltrated mast cells, is involved in the growth and fibrosis of inflamed tissues.

We therefore, examined whether the chymase inhibitor BCEAB can attenuate the cell growth and fibrosis of inflamed tissues in vivo using the hamster sponge implant model as a model of inflammatory remodeling and fibrosis. BCEAB has high inhibitory activity of human chymase but not of angiotensin-converting enzyme, elastase and tryptase. BCEAB effectively inhibited angiotensin I-in-



**Fig. 2.** Effects of BCEAB on tissue dry weight (A) and hydroxyproline content (B) in the hamster sponge implant model. \* $P < 0.05$ , \*\* $P < 0.01$  vs vehicle.

duced contraction in isolated dog arteries pretreated with 1  $\mu$ M lisinopril (21). In hamster, heart chymase activity was significantly suppressed 3 h after oral administration of BCEAB (21).

As shown in Fig. 2A, administration of BCEAB (0.045%, 0.15% and 0.45% in diet) dose-dependently suppressed the dry weights of granuloma tissues in sponge discs. Furthermore, as shown in Fig. 2B, the increase of hydroxyproline content in the granuloma tissues was dose-dependently and effectively suppressed by oral administration of BCEAB (0.15% and 0.45%). Using BCEAB, an orally available chymase inhibitor, we demonstrated that human-type chymase participates in the progress of tissue remodeling through stimulation of cell growth and extracellular matrix accumulation in the inflammatory region. In addition to our findings, Muramatsu et al. reported that direct injection of purified hamster chymase into the implanted sponges elicits angiogenesis and that daily injection of chymostatin, a peptide inhibitor for chymotrypsin-type proteases, into the implanted sponge suppressed basic fibroblast growth factor-induced angiogenesis (22).

## Conclusion

In the present study, we demonstrated that chymase plays an important role in tissue remodeling in inflammatory regions through stimulation of fibroblast growth and extracellular matrix collagen deposition. Recently, Nishimoto et al. reported that treatment with a peptide chymase inhibitor, Suc-Val-Pro-Phe<sup>P</sup>(OPh)<sub>2</sub>, suppressed the development of vascular proliferation of grafted vein in the dog (23). This peptide chymase inhibitor has also been reported to suppress adhesion formation in a hamster experimental model (24). In addition to these recent reports, our results

strongly suggest that chymase contributes to tissue remodeling. Orally available chymase inhibitors, such as BCEAB, might be useful for clarifying the pathophysiological roles of chymase in vivo.

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