Jpn. J. Pharmacol. **86**, 124 – 126 (2001) Short Communication

An Orally Active Chymase Inhibitor, BCEAB, Suppresses Heart Chymase Activity in the Hamster

Shinji Takai*, Denan Jin, Masato Sakaguchi, Kazuyoshi Kirimura and Mizuo Miyazaki

Department of Pharmacology, Osaka Medical College, Takatsuki City, Osaka 569-8686, Japan

Received January 29, 2001 Accepted March 19, 2001

ABSTRACT—We investigated the effects of a novel chymase inhibitor, BCEAB (4-[1-{[bis-(4-methyl-phenyl)-methyl]-carbamoyl}-3-(2-ethoxy-benzyl)-4-oxo-azetidine-2-yloxy]-benzoic acid). The IC_{50} value of BCEAB for purified human chymase was 5.4 nM, whereas BCEAB did not inhibit the angiotensin-converting enzyme, elastase and tryptase. In isolated dog arteries, the IC_{50} value of BCEAB for the angiotensin I-induced contraction in the presence of 1 μM lisinopril was 2.8 μM. 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 of BCEAB/kg of body weight, respectively. In conclusion, BCEAB is a useful chymase inhibitor for studying the role of chymase in vivo.

Keywords: Chymase, Inhibitor, Oral administration

Chymase is a chymotrypsin-like serine protease contained in mast cells. Chymases have been isolated and their enzymatic characteristics have been studied in humans, monkeys, dogs, hamsters and rats (1 – 5). In general, these chymases hydrolyze the C-terminal side of proteins after aromatic amino acids such as Phe, Tyr and Trp. Using the substrate angiotensin (Ang) I, human, monkey, dog and hamster chymases cleave the Phe^8-His^9 bond of Ang I to yield Ang II (1 – 4). On the other hand, rat chymase cleaves the Tyr^4-Ile^5 bond to form inactive fragments, suggesting that rat vascular tissues could not contain chymase as an Ang II-forming enzyme (5). In fact, the Ang I-induced vasoconstriction in isolated rat arteries was completely inhibited by an angiotensin-converting enzyme (ACE) inhibitor, whereas Ang I-dependent vasoconstriction in isolated human, dog and monkey arteries was not completely suppressed by an ACE inhibitor (6). The remaining vasoconstriction was blocked by chymostatin, which inhibits chymase (6). Recently, we demonstrated that a chymase inhibitor, Suc-Val-Pro-$Phe^P(OPh)_2$, prevented the vascular proliferation of dog grafted veins by infiltrating the drug into blood vessels that were extirpated during operation (7). However, this inhibitor could not be administered orally. In this study, we investigated whether oral administration of a chymase inhibitor suppresses tissue chymase activity.

A novel chymase inhibitor, 4-[1-{[bis-(4-methyl-phenyl)-methyl]-carbamoyl}-3-(2-ethoxy-benzyl)-4-oxo-azetidine-2-yloxy]-benzoic acid (BCEAB), was a gift from Shionogi Co. (Osaka). Purified human chymase and monkey tryptase were obtained as described previously (8, 9). Purified porcine elastase was purchased from SERVA Electrophoresis (Heidelberg, Germany). Four beagle dogs and 20 hamsters were anesthetized with sodium pentobarbital in a dose of 50 mg/kg, i.v. and i.p., respectively. The experimental procedure for the animals was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

The dog carotid arteries were cut into helical strips, 15 mm in length and 2.0 mm in width. The artery strip was placed on a myograph under a resting tension of 2.0 g. The bathing medium was Tyrode's solution consisting of 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 12 mM $NaHCO_3$ and 5.7 mM glucose, pH 7.4 (6). The medium was maintained at 37°C and bubbled continuously with O_2/CO_2 (95:5). The medium was washed out twice for 15 min each time with fresh Tyrode's solution, and 1 μM lisinopril was added to the bathing medium and incubated for 30 min, and then 100 nM Ang I was added to the bathing medium. The step for the Ang I response was repeated twice, and the third Ang I response was regarded as the Ang I control response in the presence of an ACE inhibitor. After the response, the medium was washed out twice for 15 min each time with fresh Tyrode's solution. BCEAB was added and preincubation was con-

*Corresponding author. FAX: +81-726-84-6518
E-mail: pha010@art.osaka-med.ac.jp

ducted for 30 min, and the Ang I response was observed. The IC_{50} values were determined by regression of dose-response curves.

Hamsters were administered oral doses of 30, 100 and 300 mg of BCEAB/kg of body weight. The heart was removed at 3 h after the oral administration and homogenized in 20 mM Na-phosphate buffer, pH 7.4. The homogenate was centrifuged at 15,000 rpm for 30 min and the pellets were homogenized in 20 mM phosphate buffer, pH 7.4, containing 2 M KCI and 0.1% Nonidet P-40. The supernatant was used as the tissue extract of ACE and chymase.

The ACE activity was measured using a synthetic substrate, hippuryl-His-Leu (Peptide Institute, Inc., Osaka). The tissue extract was incubated for 30 min at 37°C with 5 mM hippuryl-His-Leu in 10 mM phosphate buffer, pH 8.3, containing 600 mM NaCl (7). The reaction was terminated with 3% metaphosphoric acid, and then the mixture was centrifuged at 15,000 rpm for 5 min. The supernatant was applied to a reversed-phase column. Hippuric acid was detected by ultraviolet absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 μmol hippuric acid/min.

The chymase activity was measured by incubating tissue extracts for 30 min at 37°C with 770 μM Ang I in 150 mM borax-borate buffer, pH 8.5, containing 8 mM dipyridyl, 770 μM diisopropyl phosphorofluoridate and 5 mM ethylenediaminetetraacetic acid (7). The reaction was terminated with 15% trichloroacetic acid, and the mixture was centrifuged at 15,000 rpm for 5 min. The quantitation of His-Leu cleaved from an Ang I substrate was determined using 10% *o*-phthaldialdehyde. One unit of chymase activity was defined as the amount of enzyme that cleaved 1 μmol His-Leu/min.

All data are expressed as means ± S.E.M. Statistical significance was determined with one-way ANOVA and Fisher's test. Differences were considered statistically significant at a value of $P<0.05$.

At 5.4 nM, BCEAB inhibited chymase activity by 50% (IC_{50} value). On the other hand, ACE, elastase and tryptase activities were not inhibited by concentrations up to 100 μM of BCEAB.

Figure 1 shows the effects of BCEAB on the vasoconstriction induced by 100 nM Ang I in the presence of an ACE inhibitor in isolated dog arteries. BCEAB suppressed the Ang I-induced vasoconstriction in isolated dog arteries, and the IC_{50} value of BCEAB was 2.8 μM.

In hamsters, after oral administration of 30, 100 and 300 mg of BCEAB/kg of body weight, heart chymase activities were suppressed to 80.2%, 42.0% and 26.9%, respectively, and the chymase activities treated with BCEAB concentrations of 100 and 300 mg/kg were significant compared with the control level (Fig. 2). However, heart ACE activities were not inhibited by BCEAB (Fig. 2).

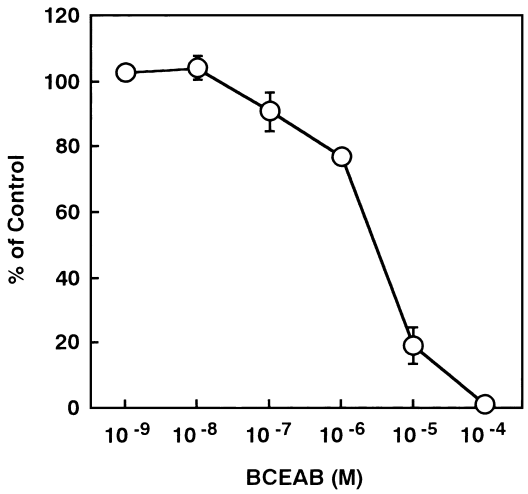

Fig. 1. Effects of BCEAB on the vasocontriction of 100 nM Ang I in the presence of 1 μM lisinopril in isolated dog artery (n = 4).

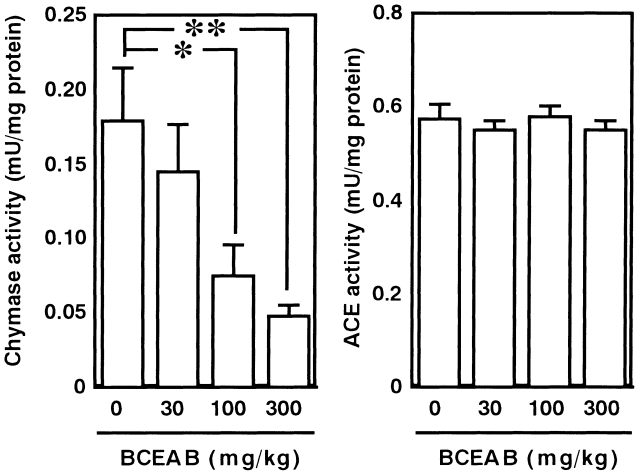

Fig. 2. Effects of BCEAB on the activities of chymase (left panel) and ACE (right panel) in the extract from hamster heart 3 h after an oral administration of BCEAB (30, 100 and 300 mg/kg) (n = 5). *$P<0.05$ and **$P<0.01$ vs placebo-treated group.

In the present study, BCEAB inhibited purified chymase activity with an IC_{50} value of 5.4 nM. However, this inhibitor did not suppress ACE, elastase and tryptase activities. In human, monkey, dog and hamster tissues, two Ang II-forming enzymes, ACE and chymase, exist, but BCEAB is thought to inhibit only chymase-dependent Ang II formation. Furthermore, mast cells predominantly contain two serine proteases, chymase and tryptase, and this inhibitor did not inhibit tryptase activity. Therefore, BCEAB may specifically inhibit chymase activity. In isolated dog arteries, BCEAB suppressed the Ang I-induced vasoconstriction in the presence of an ACE inhibitor, thus indicating the presence of a chymase-dependent Ang II formation. Although BCEAB inhibited purified chymase activity with

an IC_{50} value of 5.4 nM, it suppressed the Ang I-induced vasoconstriction at an IC_{50} value of 2.8 μM. This discrepancy may result from the transition probability of this inhibitor. In this study, we demonstrated that an oral administration of BCEAB could prevent heart chymase activity, but not ACE activity, in hamsters. This finding suggests that an oral administration of BCEAB can specifically inhibit tissue chymase-dependent Ang II-forming activity.

Chymase-dependent Ang II formation may play a pathophysiological role in tissue remodeling. For example, an Ang II antagonist was effective in preventing intimal formation of dog arteries injured by a balloon catheter, but an ACE inhibitor was ineffective (10). We also reported that a chymase inhibitor Suc-Val-Pro-$Phe^{P}(OPh)_2$, which could not be administered orally, prevented the vascular proliferation of dog grafted veins by infiltrating the drug into blood vessels that were extirpated during operation (7). In this model, an Ang II antagonist also suppressed the development of vascular proliferation (11). These reports suggest that the chymase-dependent Ang II formation may contribute to the development of intimal formation in vessels.

Oral administration of BCEAB could inhibit tissue chymase activity, and this inhibition may be useful for preventing tissue remodeling in vivo.

Acknowledgment

We thank Shionogi Co. for kindly supplying BCEAB.

REFERENCES

1 Takai S, Shiota N, Sakaguchi M, Muraguchi H, Matsumura E and Miyazaki M: Characterization of chymase from human vascular tissues. Clin Chim Acta **265**, 13 – 20 (1997)

2 Takai S, Shiota N, Kobayashi S, Matsumura E and Miyazaki M: Induction of chymase that forms angiotensin II in the monkey atherosclerotic aorta. FEBS Lett **412**, 86 – 90 (1997)

3 Caughey GH, Raymond WW and Wolters PJ: Angiotensin II generation by mast cell alpha- and beta-chymases. Biochim Biophys Acta **1480**, 245 – 257 (2000)

4 Takai S, Shiota N, Yamamoto D, Okunishi H and Miyazaki M: Purification and characterization of angiotensin II-generating chymase from hamster cheek pouch. Life Sci **58**, 591 – 597 (1996)

5 Wintroub BU, Schechter NM, Lazarus GS, Kaempfer CE and Schwartz LB: Angiotensin I conversion by human and rat chymotryptic proteinases. J Invest Dermatol **83**, 336 – 339 (1984)

6 Okunishi H, Oka Y, Shiota N, Kawamoto T, Song K and Miyazaki M: Marked species-difference in the vascular angiotensin II-forming pathways: humans versus rodents. Jpn J Pharmacol **62**, 207 – 210 (1993)

7 Takai S, Yuda A, Jin D, Nishimoto M, Sakagichi M, Sasaki S and Miyazaki M: Inhibition of chymase reduces vascular proliferation in dog grafted veins. FEBS Lett **467**, 141 – 144 (2000)

8 Takai S, Sumi S, Aoike M, Sakaguchi M, Itoh Y, Jin D, Matsumura E and Miyazaki M: Characterization of recombinant human chymase expressed in *Escherichia coli*. Jpn J Pharmacol **82**, 144 – 149 (2000)

9 Sakaguchi M, Takai S, Jin D, Yamada M and Miyazaki M: Characteristics of monkey tryptase purified from cheek pouch vascular tissues. Jpn J Pharmacol **84**, 375 – 380 (2000)

10 Miyazaki M, Wada T, Shiota N and Takai S: Effect of an angiotensin II receptor antagonist, candesartan cilexetil, on canine intima hyperplasia after balloon injury. J Hum Hypertens **13**, Suppl **1**, S21 – S25 (1999)

11 Yuda A, Takai S, Jin D, Sawada Y, Nishimoto M, Matsuyama N, Asada K, Kondo K, Sasaki S and Miyazaki M: Angiotensin II receptor antagonist, L-158,809, prevents intimal hyperplasia in dog grafted veins. Life Sci **68**, 41 – 48 (2000)