Original article

Molecular mechanism of imidapril for cardiovascular protection via inhibition of MMP-9

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Abstract

To investigate the inhibitory specificity of angiotensin converting enzyme (ACE) inhibitors to matrix metalloproteinase (MMP)-9, we predicted molecular interactions between an ACE inhibitor imidapril and MMP-9 active site based on recent X-ray structural analyses. Two binding modes differing in the orientation of imidapril on the active site were identified, and its hydrophobic group appeared to preferentially interact with the S1 site compared with the S1′ site. Compared with the lisinopril-MMP-9 model in our previous study, imidapril was stabilized effectively on the active site with less of molecular distortions. We also measured ACE and MMP-9 inhibitory activities of imidapril and lisinopril after myocardial infarction. Imidapril had a stronger inhibitory activity against MMP-9 than lisinopril. These findings show that imidapril inhibits MMP-9 directly like lisinopril and its hydrophobic interactions with the S1 site of MMP-9 would be important for enhancing inhibitory activity.

1. Introduction

Matrix metalloproteinase (MMP)-9 is a Zn^{2+}-dependent endopeptidase, and levels of this enzyme significantly increase after myocardial infarction in human and animals [1,2]. Cardiac dysfunction and mortality are significantly suppressed by MMP-9 inhibitors and in MMP-9 null mice after myocardial infarction [3,4]. Therefore, MMP-9 inhibition is considered useful for suppressing cardiac dysfunction and mortality after myocardial infarction. On the other hand, angiotensin II is closely involved not only in the pathogenesis of hypertension but also in tissue remodeling such as that associated with atherosclerosis and cardiac dysfunction [5].

Both angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers have been used clinically to prevent the angiotensin II-related actions, and numerous clinical studies of ACE inhibitors and angiotensin II receptor blockers have demonstrated that they are both useful for preventing stroke and cardiac dysfunction. However, ACE inhibitors significantly reduce the onset and mortality in myocardial infarction, whereas angiotensin II receptor blockers do not [6,7]. These findings are controversial [8,9], and the mechanism remains unclear.

The ACE inhibitors captopril and lisinopril dose-dependently inhibit MMP-9 activity in rat kidney extract [10], and captopril, lisinopril and ramiprilat also inhibited MMP-9 activity in extracts of human cardiac tissues, and an excess of zinc blunted such inhibition [11]. This finding suggested direct inhibition of MMP-9 activity, but the inhibitory mechanism remained unknown. We recently predicted the

inhibitory specificity of a typical ACE inhibitor, lisinopril, for MMP-9 activity by molecular modeling of these complexes based on recent X-ray structural analyses [12]. Two binding modes differing in the orientation of the inhibitor on the active site have been identified, and lisinopril was effectively stabilized by specific interactions in the active site of MMP-9. Moreover we recently found that lisinopril attenuated not only ACE, but also MMP-9 activity at 1 day after myocardial infarction, whereas an angiotensin II receptor blocker did not [13].

Here we investigated molecular interactions between an ACE inhibitor imidapril and the MMP-9 active site, and measured ACE and MMP-9 inhibitory activities of imidapril after myocardial infarction. These results were compared with those of lisinopril to show the molecular mechanism of ACE inhibitors for cardiovascular protection.

2. Methods

2.1. Molecular modeling of the complex between imidapril and MMP-9 active domain

For modeling of MMP-9 molecules complexed with imidapril, a model of MMP-9 active domain was prepared from the coordinate sets of 1GKC chain B [14] in the Protein Data Bank (PDB) including Ca^{2+} and Zn^{2+} ions and crystal waters. The binding structure of imidapril to the Zn^{2+} ion of the MMP-9 active center was constructed by referring to the ACE active center inhibited by lisinopril (PDB code: 1O86) [15]. Two interaction modes were modeled for the complex as described in the Results section. Crystal waters that overlapped with the inhibitor molecule were deleted. Initial optimization was performed by energy minimization using the MMFF94x force field [16–20] under conditions of fixed metal ions. The energy cutoff distance was set at 10 Å, and the dielectric constant was distance dependent based on a value of 1.0 for the protein complex and 80.0 for the solvent.

We performed 100-picosecond (ps) molecular dynamics simulations on each model at 300 K using a 0.001-ps time step, the MMFF94x force field and the NVT method [21,22]. Before these equilibrium iterations, 1-ps heating iterations were used to consider the stable equilibration. The metal ions were all fixed. To relax the charge interactions between surface residues, the later 50-ps simulations were conducted with water molecules randomly distributed in a 5Å shell around each model. Finally, the most stable structure of each model during the later simulations was optimized by energy minimization. The potential energy of each molecular system after final optimization was -1.78×10^4 kcal/mol including 912 water molecules for mode-A, and -1.76×10^4 kcal/mol including 925 water molecules for mode-B.

All modeling operations were performed using a package for molecular structure analyses (MOE; Molecular Operating Environment, Chemical Computing Group Inc., Québec, CA http://www.chemcomp.com/).

2.2. Agents and animals

Lisinopril was purchased from Sigma (St. Louis, MO, USA) and Tanabe Seiyaku Co., Ltd. (Osaka, Japan) donated imidapril. Male Syrian hamsters (Japan SLC, Shizuoka, Japan) aged 6 weeks and weighing 90–110 g were fed with regular hamster chow, had free access to tap water and were housed in a temperature-, humidity- and light-controlled room. The experimental procedures for animals were conducted in accordance with the guidelines of Osaka Medical College for medical experiments approved by the ethics committee included outside members, and this research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health.

2.3. Myocardial infarction

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A left-sided thoracotomy was performed via the fourth intercostal space and the lungs were retracted to expose the heart. After opening the pericardium, the left coronary artery was ligated near its origin using a 7-0 silk suture [23]. Coronary ligation was considered successful when the anterior wall of the left ventricle turned pale, and then the thoracotomy site was closed in layers. Infarcted left ventricles were obtained under the anesthesia for measuring ACE and MMP-9 activities 1, 3 and 7 days after myocardial infarction. To evaluate the inhibitory effects of lisinopril and imidapril upon MMP-9 activity 1 day after myocardial infarction, we administered the animals with placebo, lisinopril (20 mg/kg per day) and imidapril (20 mg/kg per day) (each group, $n=8$). Normal hamsters served as controls ($n=8$). The doses of lisinopril and imidapril showed equal hypotensive effects based on our preliminary study (data not shown).

2.4. Assessment of infarct size

Four 5-μm sections were cut from each slice of heart. To measure infarct size, every section was stained with azan Mallory stain and the infarct size was determined by using a computerized morphometry system, MacSCOPE Ver 2.2 (Mitani Co., Fukui, Japan).

2.5. Enzyme activities of ACE and MMP-9

Activities of ACE and MMP-9 in the infarcted left ventricle were measured as described [24]. In brief, tissues were minced and homogenized in 5 vol (w/v) of 20 mM Tris–HCl buffer, pH 8.3, containing 5 mM $Mg(CH_3COO)_2$, 30 mM KCl, 250 mM sucrose and 0.5% Nonidet P-40. The supernatant was used for the measurement of ACE and MMP-9 activities.

We measured ACE activity by incubating the tissue extracts for 30 min at 37 °C with 5 mM hippuryl–His–Leu in 250 μl of 10 mM phosphate buffer, pH 8.3, containing 600 mM NaCl [24]. In the present study, the level of ACE activity in hamster heart extracts was high and it was determined using the