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Short communication

Significance of matrix metalloproteinase-9 in cardiac dysfunction during the very acute phase after myocardial infarction in hamsters

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Abstract

Matrix metalloproteinase-9 activity is dramatically increased during the acute phase after myocardial infarction. However, the relationship between matrix metalloproteinase-9 activity and cardiac dysfunction is unclear. In 1-day post-myocardial infarction hamsters, matrix metalloproteinase-9 activity was significantly increased, while matrix metalloproteinase-2 activity was not increased. A selective matrix metalloproteinase inhibitor, [2*S*,4*S*]-*N*-Hydroxy-5-ethoxymethyloxy-2-methyl-4-[4-phenoxybenzoyl] aminopentanamide (ONO-4817), significantly suppressed matrix metalloproteinase-9 activity 1 day after myocardial infarction. ONO-4817 also significantly prevented the development of cardiac dysfunction and left-ventricular dilatation. Matrix metalloproteinase-9 might play a crucial role in cardiac dysfunction and left-ventricular dilatation during the very acute phase after myocardial infarction.

Keywords: Cardiac dysfunction; Inhibitor; Myocardial infarction; Matrix metalloproteinase; Left-ventricular dilatation

1. Introduction

Matrix metalloproteinase (MMP)-9 is a Zn^{2+}-dependent endopeptidase. Clinically, MMP-9 activity is dramatically increased during the acute phase after myocardial infarction (Kai et al., 1998; Kameda et al., 2006). We recently reported that MMP-9 activity was significantly increased 1 day after myocardial infarction in hamsters, and suggested that an angiotensin-converting enzyme (ACE) inhibitor might directly suppress MMP-9 activity (Takai et al., 2007). On the other hand, an angiotensin II receptor blocker could not attenuate MMP-9 activity (Takai et al., 2007). ACE, like MMP-9, is a Zn^{2+}-dependent endopeptidase; ACE inhibitors directly inhibit MMP-9 activity in tissue extracts (Sorbi et al., 1993; Reinhardt et al., 2002). We recently characterized the inhibitory specificity of ACE inhibitors for MMP-9 activity; ACE inhibitors were found to be effectively stabilized by specific hydrogen bonds and hydrophobic interactions in the active site of MMP-9 (Yamamoto et al., 2007). In clinical studies, ACE inhibitors have been found to significantly reduce the onset and mortality of myocardial infarction; this effect could be the result of inhibition of MMP-9 during the very acute phase after myocardial infarction.

Cardiac dysfunction and left-ventricular dilatation are significantly suppressed by MMP-9 inhibition, as well as in MMP-9 null mice, after myocardial infarction (Lindsey et al., 2002, 2006). Therefore, MMP-9 inhibition is considered to be useful for decreasing cardiac dysfunction and left-ventricular dilatation after myocardial infarction. However, these reports evaluated the effect of MMP-9 inhibition on cardiac dysfunction 1 week or several weeks after myocardial infarction (Lindsey et al., 2002, 2006). We recently demonstrated that MMP-9 activity was significantly increased even 1 day after myocardial infarction and that this activity progressively declined to the pre-infarction level 3 and 7 days later (Takai et al., 2007). On the other hand, MMP-2 activity was significantly increased from 3 days after myocardial infarction (Takai et al., 2007). Therefore, MMP-9, but not MMP-2, may play an important role in the very acute phase after

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myocardial infarction. However, it is unclear whether inhibition of MMP-9 activity during the very acute phase after myocardial infarction attenuates cardiac dysfunction.

[2*S*,4*S*]-*N*-Hydroxy-5-ethoxymethyloxy-2-methyl-4-[4-phenoxybenzoyl] aminopentanamide (ONO-4817) had a high inhibitory activity against MMP-2 and MMP-9; this inhibition is specific for MMPs, since ONO-4817 has almost no inhibitory activity against other proteases (Mori et al., 2001). In the present paper, the effect of MMP-9 inhibition on cardiac dysfunction and dilatation during the very acute phase after myocardial infarction was assessed.

2. Materials and methods

2.1. Agents and animals

A selective matrix metalloproteinase inhibitor, ONO-4817, was donated by ONO Pharmaceutical Co. (Osaka, Japan). Six-week-old, male Syrian hamsters (Japan SLC, Shizuoka, Japan), 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 on the animals were conducted in accordance with the guidelines of the Osaka Medical College for medical experiments, which were approved by the college ethics committee that includes outside members; 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.

2.2. Myocardial infarction

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A left-sided thoracotomy was done 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 (Jin et al., 2003). Coronary ligation was considered successful when the anterior wall of the left-ventricle turned pale; the thoracotomy site was then closed in layers. Under anesthesia, the infarcted left-ventricles were harvested to measure MMP-9 activity 1 day after myocardial infarction. To evaluate the effects of ONO-4817, the animals were orally given placebo or ONO-4817 (100 mg/kg per day) (each group, $n=6$). Normal hamsters served as controls ($n=6$).

2.3. MMP-9 activity

MMP-9 activity in the infarcted left-ventricle was measured as described previously (Takai et al., 2007). In brief, the 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 MMP-9 activity. MMP-9 activity was detected using standard gelatin zymography on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels containing 1 mg/ml gelatin (Fang et al., 1997).

2.4. Echocardiographic study

One day after myocardial infarction, echocardiography (Nemio 30, Toshiba Co., Tokyo, Japan) was done according to previously described methods (Jin et al., 2003). In brief, after intraperitoneal injection of ketamine HCl (25 to 50 mg/kg) and xylazine (5 to 10 mg/kg), M-mode tracings and pulse-wave Doppler spectra (E and A waves) of the mitral inflow were recorded in each group.

2.5. Hemodynamic measurement

After the echocardiographic study, the trachea was intubated. A polyethylene catheter was introduced into the right carotid artery. The catheter was then connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo, Japan) and the mean arterial blood pressure (MABP) was measured. After this procedure, the thorax was opened under positive-pressure respiration and a catheter was inserted into the left-ventricular chamber via its apex, where maximal positive and negative rates of pressure development ($+dP/dt$ and $-dP/dt$, respectively) were measured. Finally, hearts were harvested for later biochemical assay and infarct size assessments.

2.6. Assessment of infarct size

Four 5-μm sections were cut from each slice. 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).

2.7. Statistical analysis

Significant differences among the mean values of multiple groups were evaluated using 1-way ANOVA followed by Fisher's test. Values were considered statistically significant at $P<0.05$. Data are expressed as mean±standard error of the mean.

3. Results

The ratio of the infarct area to the left-ventricular area was 44.0%+2.8% in placebo-treated hamsters and 42.9%+2.8% in

Table 1
Hemodynamic and echoradiographic parameters

	Normal	Placebo	ONO-4817
MABP (mm Hg)	108±2.4	87±5.6[a]	102±6.3[c]
$+dP/dt$ (mm Hg/s)	3817±170	2600±216[b]	3233±208[c]
$-dP/dt$ (mm Hg/s)	2367±169	1467±167[b]	1950±109[c]
EF (%)	82±1.5	50±3.8[b]	65±2.5[b,d]
FS (%)	43±1.6	21±2.0[b]	30±1.6[b,d]
LVDd (mm)	3.62±0.08	4.08±0.12[b]	3.66±0.09[d]

MABP, mean arterial blood pressure, $+dP/dt$, maximum positive rates of pressure development, $-dP/dt$, maximum negative rates of pressure.
Development, EF, ejection fraction; FS, fractional shortening; LVDd, left-ventricular dimension end diastole.
[a]$P<0.05$ and [b]$P<0.01$ vs. normal. [c]$P<0.05$ and [d]$P<0.01$ and vs. placebo.

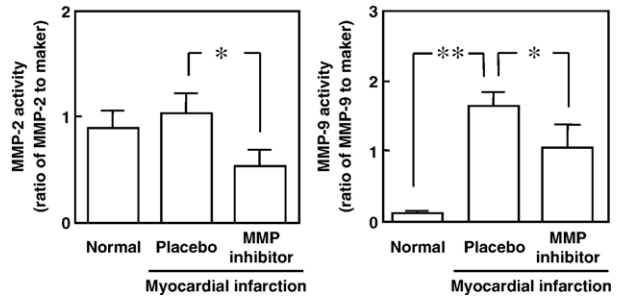

Fig. 1. MMP-2 and MMP-9 activities of the normal, placebo-, and ONO-4817-treated groups 1 day after myocardial infarction (each group, $n=6$). *$P<0.05$ and **$P<0.01$ vs. placebo.

ONO-4817-treated hamsters; the difference between the two groups was not statistically significant.

Table 1 shows the hemodynamic and echocardiographic parameters. The mean arterial blood pressure was significantly lower in the placebo-treated group than in the normal group, while the MABP was significantly higher in the ONO-4817-treated group than in the placebo-treated group. Both the $+dP/dt$ and $-dP/dt$ were significantly lower in the placebo-treated group than in the normal group, both were significantly higher in the ONO-4817-treated group than in the placebo-treated group. The ejection fraction (EF) and fractional shortening (FS) on echocardiography were significantly lower in the placebo-treated group than in the normal group, while they were significantly higher in the ONO-4817-treated group than in the placebo-treated group. The left-ventricular end-diastolic dimension (LVDd) was significantly larger in the placebo-treated group than in the normal group. However, there was no significant difference in the left-ventricular end-diastolic dimension between the normal group and the ONO-4817-treated group 1 day after myocardial infarction.

One day after myocardial infarction, MMP-9 activity was significantly increased in the placebo-treated group, while MMP-9 activity was significantly lower in the ONO-4817-treated group than in the placebo-treated group (Fig. 1). However, there was no difference in MMP-2 activity between the control and placebo-treated groups. Furthermore, ONO-4817 also significantly attenuated MMP-2 activity 1 day after myocardial infarction (Fig. 1).

4. Discussion

In the present study, MMP-9, but not MMP-2, activity was significantly increased 1 day after myocardial infarction in a hamster model. A specific MMP inhibitor, ONO-4817, significantly reduced not only MMP-9 activity but also MMP-2 activity during the very acute phase after myocardial infarction. ONO-4817 used in the present study suppresses the MMP-2 and MMP-9, but not MMP-1 or MMP-3 (Mori et al., 2001). ONO-4817 has no inhibitory effect of ACE activity (data not shown). The administration of 20 mg/kg per day of ONO-4817 has been reported to result in the suppression of neointimal thickening in a hamster arteriosclerosis model (Matsuno et al., 2004). In the present study, ONO-4817 was administered at a dose of 100 mg/kg per day; in a pre-experiment in normal hamsters, this concentration significantly suppressed MMP-2 and MMP-9 activity in hearts extracted 24 h after a single oral dose. In our model, on 1 day after myocardial infarction, MABP significantly decreased in the placebo-treated group compared to the normal group and significantly increased in the ONO-4817-treated group compared to the placebo-treated group; this shows that there were no significant differences between the ONO-4817-treated and the normal groups. Significant increases of $+dP/dt$ and $-dP/dt$ were observed by treatment with ONO-4817. In addition, although EF and FS levels significantly decreased in the placebo-treated group compared to the normal group, they were significantly increased in the ONO-4817-treated group. Furthermore, LVDd, an indicator of cardiac dilatation, significantly increased in the placebo-treated group compared to the normal group, but significantly decreased in the ONO-4817-treated group compared to the placebo-treated group; the ONO-4817-treated group had values similar to those of the normal group. In contrast, no differences in infarct area were observed between treated and non-treated groups. These results indicate that during the acute phase 1 day after myocardial infarction, ONO-4817 prevented cardiac dysfunction by suppressing cardiac dilatation, rather than by cardiac remodeling. One day after myocardial infarction, MMP-9 activity increased, but no changes were observed in MMP-2 activity. Therefore, the increase in MMP-9 during this acute phase after myocardial infarction may play a crucial role in the cardiac dysfunction that is associated with cardiac dilatation.

Previous animal studies, including our own, showed that MMP-9 activity was significantly increased one day after myocardial infarction and then declined progressively to pre-infarction levels a few days to 1 week later (Lindsey et al., 2001; Takai et al., 2007). In a rat myocardial infarction model, Villarreal et al. (2003) reported that the administration of an MMP inhibitor from 48 h prior to myocardial infarction to 36 h after myocardial infarction suppressed cardiac dysfunction and dilatation 2 and 4 weeks after myocardial infarction. These findings suggest that the inhibition of MMP during the acute phase after myocardial infarction play an important role in subsequent cardiac remodeling. Clinical studies also have shown that MMP-9 activity is significantly increased during the very acute phase after acute myocardial infarction and that it then subsequently decreases gradually (Kai et al., 1998; Squire et al., 2004). Cardiac dysfunction and left-ventricular dilatation were significantly decreased 1 week or several weeks after myocardial infarction through the use of an MMP inhibitor, as well as in MMP-9 null mice (Lindsey et al., 2002, 2006). In the present study, ONO-4817 prevented cardiac dysfunction and left-ventricular dilatation 1 day after myocardial infarction. Meanwhile, Mukherjee et al. (2003) reported that, in pigs, the administration of the MMP inhibitor PD166793 from 5 days after myocardial infarction resulted in significant reductions in LVDd and infarct area after 2 weeks. In this porcine myocardial infarction model, MMP-2 activity did not change 5 days after myocardial infarction, but significantly increased after 2 weeks; this increase was significantly suppressed by the MMP inhibitor.

Thus, not only MMP-9 but also MMP-2 may also play an important role in the cardiac dysfunction after myocardial infarction.

On the other hand, a recent clinical study on the use of an MMP inhibitor PG116800 in myocardial infarction patients yielded negative results (Hudson et al., 2006). Cardiac remodeling may be more greatly affected by factors such as angiogenesis than by MMP inhibition (Carluccio et al., 2006). However, it is worth noting that in the clinical study, which was conducted at multiple institutions in the United States (18%), Canada (14%), and Poland (68%), 94% of patients in both the MMP inhibitor-treated and control drug-treated groups were given ACE inhibitors or angiotensin receptor blockers as concomitant drugs. Since ACE inhibitors are generally prescribed in these countries, a considerable proportion of patients was likely to have been taking ACE inhibitors. The direct inhibitory effect of ACE inhibitors on MMP has previously been confirmed in tissue extracts (Sorbi et al., 1993; Reinhardt et al., 2002). We also demonstrated that an ACE inhibitor can be stabilized to the MMP-9 active site, and that its hydrophobic group appeared to preferentially interact with the S1 site compared with the S1′ site (Yamamoto et al., 2007). Therefore, in patients given ACE inhibitors, MMP was likely to have already been inhibited by ACE inhibitors. This may explain the absence of intergroup differences in the effects of MMP inhibitors noted in the clinical study. In future clinical studies, it may be important to take into account the concomitant use of ACE inhibitors on the effects of MMP inhibitors.

In conclusion, MMP-9 inhibition may be a useful strategy for preventing cardiac dysfunction during the very acute phase after myocardial infarction.

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