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ORIGINAL ARTICLE

Increase of chymase-dependent angiotensin II-forming activity in circulating mononuclear leukocytes after acute myocardial infarction chymase activity after acute myocardial infarction

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Abstract
A previous clinical study revealed elevation of chymase- and cathepsin G-dependent angiotensin II-forming activity (AIIFA) in the myocardium after acute myocardial infarction (AMI). This study examined the time course of chymase- and cathepsin G-dependent AIIFA in circulating mononuclear leukocytes (CML) after AMI. Consecutive patients with AMI were recruited. Chymase- and cathepsin G-dependent AIIFA in CML were assayed using a modified angiotensin I substrate with Nma/Dnp fluorescence quenching. The changes of CML AIIFA were monitored over time in the patients. Fifteen consecutive AMI patients admitted to our hospital were recruited. At 1 day after the admission, CML chymase- and cathepsin G-dependent AIIFA were 2.9- and 1.7-fold higher than at discharge, respectively. The ratio of chymase-dependent AIIFA to total AIIFA was significantly increased. AIIFA gradually decreased over time after the admission. The peak value of chymase- and cathepsin G-dependent AIIFA was significantly correlated with the maximum levels of aspartate aminotransferase ($r=0.53$, 0.64), lactate dehydrogenase ($r=0.57$, 0.62), and creatine kinase ($r=0.60$, 0.65). This is the first evidence that chymase- and cathepsin G-dependent AIIFA is elevated in CML after AMI. Our data suggested that chymase-dependent AIIFA is increased in CML as well as in the myocardium after AMI, and that the level of chymase-dependent AIIFA might reflect the severity of infarction.

Keywords Chymase · Cathepsin G · Acute myocardial infarction · Angiotensin II-forming activity · Renin–angiotensin system

Introduction

The tissue angiotensin II (AII) generation system includes serine proteinases such as chymase, cathepsin G, and kallikrein, as well as angiotensin-converting enzyme (ACE) [1–4]. ACE only accounts for approximately 10–15% of AII generation in human left ventricular homogenate and the majority of the AII-generating activity is not blocked by ACE inhibitors (ACI-I), while it is blocked by serine proteinase inhibitors [5]. Thus, these serine proteinases such as chymase and cathepsin G are very important for AII generation. In humans, chymase is produced by mast cells and stored in secretory granules, after which it is released from these cells in response to stimulation and generates AII in the extracellular and interstitial regions. This system is activated in the cardiomyocytes with hypertrophic cardiomyopathy and in the arteriosclerotic lesions of hypertensive patients [6–10]. Immunostaining of coronary arteriosclerotic lesions has demonstrated elevation of ACE activity, which is considered to partly explain how the prognosis of coronary artery disease can be improved by ACE-I therapy [11]. Although there have been few investigations of non-ACE serine proteinases other than chymase, it was similarly reported that chymase activity is increased in aortic arteriosclerotic lesions. We previously reported that, while ACE activity was also increased in such tissues, angiotensin generation via chymase was markedly elevated and was especially prominent at sites of aneurysm formation [12, 13].

It was also reported that total AII generation is increased in human myocardial tissues obtained at autopsy after myocardial infarction (MI), with not only the activity of ACE

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but also that of other serine proteinases being elevated, and it has been suggested that tissue levels of angiotensin-generating enzymes may vary over time after MI [14, 15]. Based on autopsy studies, AII generation seems to be associated with infiltration of neutrophils in the early period after acute MI (AMI), and non-ACE/non-chymase serine proteinases (probably cathepsin G) are most important at this time. Since infiltration of mast cells into the myocardium becomes prominent from the 5th day onwards, chymase then takes on the chief role in AII generation, and different serine proteinases may be dominant in the angiotensin system at different times [14, 15].

Based on these reports, we previously created a hamster model of MI, and showed that the post-infarction survival rate and cardiac remodeling were improved by oral administration of specific chymase inhibitor (TEI-E548) [16]. Therefore, it is possible that chymase is increased in the human myocardium during the subacute phase after MI and is involved in cardiac remodeling and fibrosis, thus potentially influencing the prognosis of MI patients.

We previously found a positive correlation between chymase-dependent AIIFA in circulating mononuclear leukocytes (CML) and blood pressure in hypertensive patients who were not on antihypertensive therapy [17]. In this study, we investigated the changes of chymase-dependent AIIFA in CML over time after AMI in humans.

Patients and methods

In consecutive patients who presented to Fukuoka University Chikushi Hospital and were diagnosed with AMI, we measured total AIIFA, chymase-dependent AIIFA, and cathepsin G-dependent AIIFA in CML at four different time points: at the admission, 1 day after the admission, 5 days after the admission, and at discharge. Routine laboratory tests were also performed at the same times.

Measurement of AIIFA in CML

We measured chymase-dependent AIIFA in human CML, as reported previously [17]. Because intrinsic inhibitors of serine proteinases in the plasma would inhibit chymase activity [18], we measured AIIFA in peripheral white blood cells instead. Our previous research has shown that chymase activity and ACE activity are both low in neutrophils, while cathepsin G activity is high [17]. On the other hand, the levels of ACE, chymase, and cathepsin G activity are similar in mononuclear leukocytes (approximately 30% of total activity each), so we used CML for measurement of AIIFA. Blood was collected into a heparinized blood collection tube and the same volume of normal saline was added for dilution, after which 6 mL of the diluted blood was poured over 3 mL of Lymphoprep (Daiichi Kagaku, Tokyo) and centrifuged at 800*g* for 20 min at room temperature. Lymphocytes/monocytes were pipetted carefully, washed and agitated once with normal saline, centrifuged at 2000 rpm for 10 min, and stored at − 80 °C until assay. Using Nma/Dnp-modified angiotensin I, which emits fluorescence when the Phe8–His9 bond of angiotensin I is cleaved, Nma-AII was synthesized and used to create a standard curve for measurement of Nma-AII generated by CML (Fig. 1). AIIFA in the presence of a cathepsin G inhibitor (2 nM aprotinin) was subtracted from total AIIFA to calculate cathepsin G activity. Then, chymase activity was calculated as AIIFA inhibited by a chymase inhibitor (E-548; Teijin Pharma, 2 nM, which inhibits both chymase and cathepsin G) minus cathepsin G activity.

The protocol for this study was approved by the institutional review board of Fukuoka University Chikushi Hospital (approval no. R07-005 and R18-042), and written and oral informed consent were obtained from the patients for collection of blood samples.

Items investigated

First, we observed the changes of AIIFA over time after the admission. Next, we determined whether the maximum AIIFA was related to the severity of MI in each patient. To assess the severity of MI, we used the maximum values of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatinine kinase (CK) during hospitalization, as well as the ejection fraction (EF) and left ventricular diastolic dimension (LVDd) measured by echocardiography before discharge. We also investigated the correlation between the severity of MI and the maximum AIIFA.

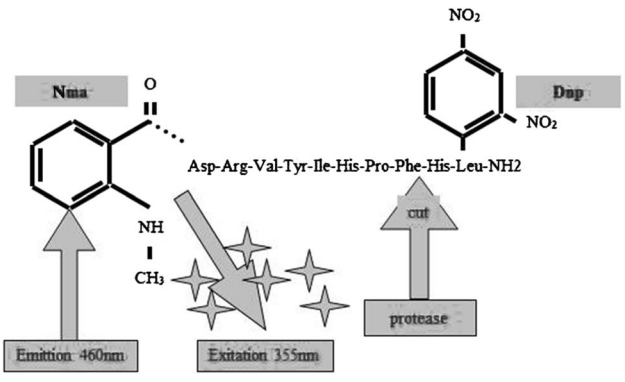

Fig. 1 The structure of the Nma/Dnp-type fluorescence-quenching substrate of the modified angiotensin I

Statistical analysis

Statistical analysis was performed at Fukuoka University using IBM SPSS Statistics 23. To assess the significance of differences, we used the *t* test for variables with a normal distribution with Levene's test when variance was equal and Welch's test when variance was not equal. For items where variables that did not show a normal distribution, we examined changes over time using the Wilcoxon signed-rank test. We performed Spearman's rank correlation coefficient analysis to test correlations. Numerical data are presented as the mean with standard deviation (SD), the median with interquartile range (IQR), or as the frequency (percentage). A p value < 0.05 was considered to indicate significance.

Results

The study period was from December 2007 to September 2008.

Table 1 shows the characteristics of the 15 AMI patients who were registered. All cases visited to our hospital in the acute phase of AMI.

Their mean age was 64 ± 15 years, and 14 patients (93%) were men. The most common infarct-related artery was the left anterior descending coronary artery (LAD), which was involved in seven patients (46%). All patients except one underwent emergency percutaneous coronary intervention (PCI) during the acute phase of AMI. One patient died of cardiac rupture during hospitalization, but the others underwent cardiac rehabilitation and were discharged uneventfully.

Table 1 Patient characteristics ($n = 15$)

Age, mean (SD), years	64 (15)
BMI, mean (SD), kg/m^2	24 (4)
Male gender, *n* (%)	14 (93)
HT, *n* (%)	12 (80)
DL, *n* (%)	4 (27)
DM, *n* (%)	2 (13)
Medications CCB, *n* (%)	3 (20)
RAS-I, *n* (%)	4 (27)
Culprit vessel, *n* (%)	RCA 5 (33), LAD 7 (46), LCX 3 (20)
PCI, *n* (%)	Emergency 14 (93), elective 1 (7)
Max CK, median (IQR), IU/L	1335 (534–2062)
LVDd, mean (SD), mm	52 (6)
EF, mean (SD), %	55 (12)
In-hospital survival, *n* (%)	14 (93)

SD, standard deviation; BMI, Body Mass Index; *n*, number of patients; HT, hypertension; DL, dyslipidemia; DM, diabetes mellitus; CCB, calcium channel blocker; RAS-I, renin–angiotensin system inhibitor; PCI, percutaneous coronary intervention; CK, creatine kinase; IQR, interquartile range; LVDd, diastolic left ventricular dimension; EF: ejection fraction; RCA, right coronary artery; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery

Figure 2 shows the changes of AIIFA components over time after AMI.

CML total AIIFA, CML chymase-dependent AIIFA and CML cathepsin G-dependent AIIFA were measured at the time of discharge, which is when patients are considered to be stable, and the data were compared with measurements at the admission, 1 day after the admission, and 5 days after the admission. Compared with that at discharge, total AIIFA was significantly higher at the admission ($p < 0.05$) and 1 day after the admission ($p < 0.05$) (Fig. 2a). CML chymase-dependent AIIFA was also significantly higher at the admission ($p < 0.05$) and 1 day after the admission ($p < 0.05$) compared with that at discharge (Fig. 2b). Furthermore, CML cathepsin G-dependent AIIFA was significantly higher at the admission ($p < 0.05$), 1 day after the admission ($p < 0.05$), and 5 days after the admission ($p < 0.01$) compared with that at discharge (Fig. 2c). Thus, all components of CML AIIFA were maximal at 1 day after the admission, and gradually decreased thereafter. At 1 day after the admission, CML chymase- and cathepsin G-dependent AIIFA were, respectively, 2.9 times and 1.7 times higher than at discharge.

Figure 3 shows the changes over time of CML chymase-dependent AIIFA and CML cathepsin G-dependent AIIFA relative to CML total AIIFA. Values measured at discharge when patients are stable were compared with data obtained at the admission, 1 day after the admission, and 5 days after the admission. Compared with at discharge, the ratio of CML chymase-dependent AIIFA to CML total AIIFA was significantly higher at the admission ($p < 0.05$) and 1 day after the admission ($p < 0.05$) (Fig. 3a), and it was maximal at 1 day after the admission. In contrast, there was no change over time in the ratio of CML cathepsin G-dependent AIIFA to CML total AIIFA compared with at discharge when it was measured at the admission, 1 day after the admission, and 5 days after the admission (Fig. 3b).

In each patient, the maximum value of CML total AIIFA (Max CML Total AIIFA) was compared with the maximum levels of common laboratory markers that increase after MI, including the white blood cell count (WBC), AST, LDH, CK, and C-reactive protein (CPR) (Max WBC, Max AST, Max LDH, Max CK, and Max CRP, respectively) (Fig. 4). There was no significant correlation between Max WBC and Max CRP, which are markers of inflammation, and Max CML Total AIIFA. However, a positive correlation was observed between Max CML Total AIIFA and Max LDH ($r = 0.54$, $p < 0.05$), while there was a weak positive correlation with Max CK ($r = 0.49$, $p = 0.06$).

The correlations between MAX CML chymase-dependent AIIFA or Max CML cathepsin G-dependent AIIFA and

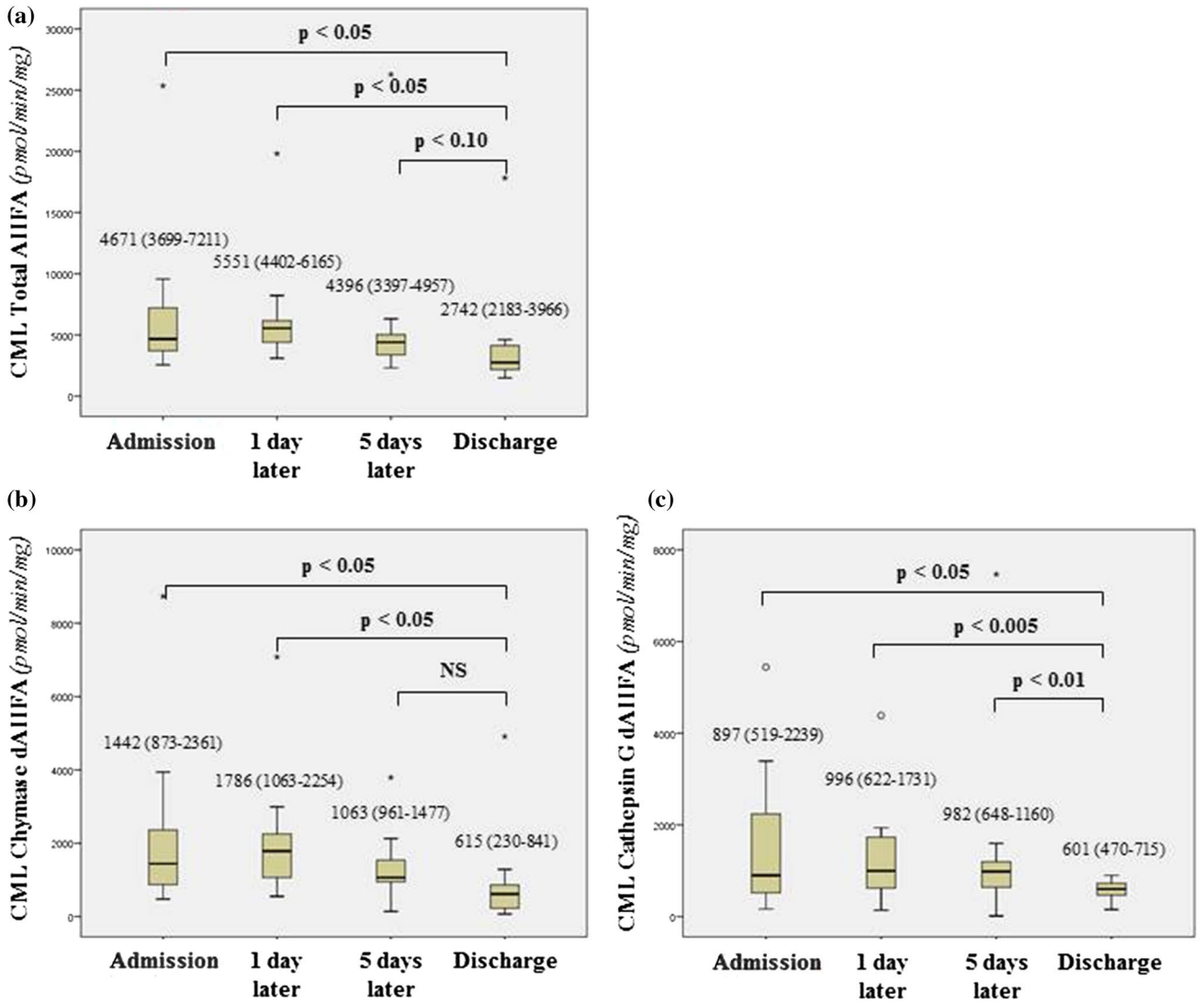

Fig. 2 Changes of AIIFA over time after MI ($n=14$). CML total AIIFA, CML chymase-dependent AIIFA, and CML cathepsin G-dependent AIIFA were measured at the time of discharge, which is when patients are considered to be stable. Data were compared with measurements obtained at the admission, 1 day later, and 5 days later using the Wilcoxon signed-rank test. One patient with cardiac rupture was excluded. Total AIIFA was significantly higher at the admission and 1 day later compared with that at discharge (**a**). CML chymase-dependent AIIFA was significantly higher at the admission and 1 day later compared with that at discharge (**b**). CML cathepsin G-dependent AIIFA was significantly higher at the admission, 1 day later, and 5 days later compared with that at discharge (**c**).All components of AIIFA were maximal at 1 day after the admission and gradually decreased thereafter. CML, circulating mononuclear leukocytes; AIIFA, angiotensin II-forming activity; dAIIFA, dependent AIIFA; AMI, acute myocardial infarction; NS, not significant

MI markers were also examined (Figs. 5, 6). Max CML chymase-dependent AIIFA showed a significant correlation with Max AST ($r=0.53$, $p<0.05$), Max LDH ($r=0.57$, $p<0.05$), and Max CK ($r=0.60$, $p<0.05$), but not with Max WBC or Max CRP (Fig. 5). Max CML cathepsin G-dependent AIIFA also showed a significant correlation with Max AST ($r=0.64$, $p<0.05$), Max LDH ($r=0.62$, $p<0.05$), and Max CK ($r=0.65$, $p<0.05$), but not with Max WBC or Max CRP (Fig. 6).

When CML total AIIFA, CML chymase-dependent AIIFA, CML cathepsin G-dependent AIIFA, and Max CK were compared with stratification by the culprit artery [LAD, left circumflex coronary artery (LCS), or right coronary artery (RCA)] using the Kruskal–Wallis test, no significant correlations were found (data not shown). Additionally, correlations of Max CML Total AIIFA, Max CML chymase-dependent AIIFA, Max CML cathepsin G-dependent AIIFA, or Max CK with EF and LVDd were investigated using Spearman's rank correlation coefficient analysis, but no significant correlations were observed (data not shown).

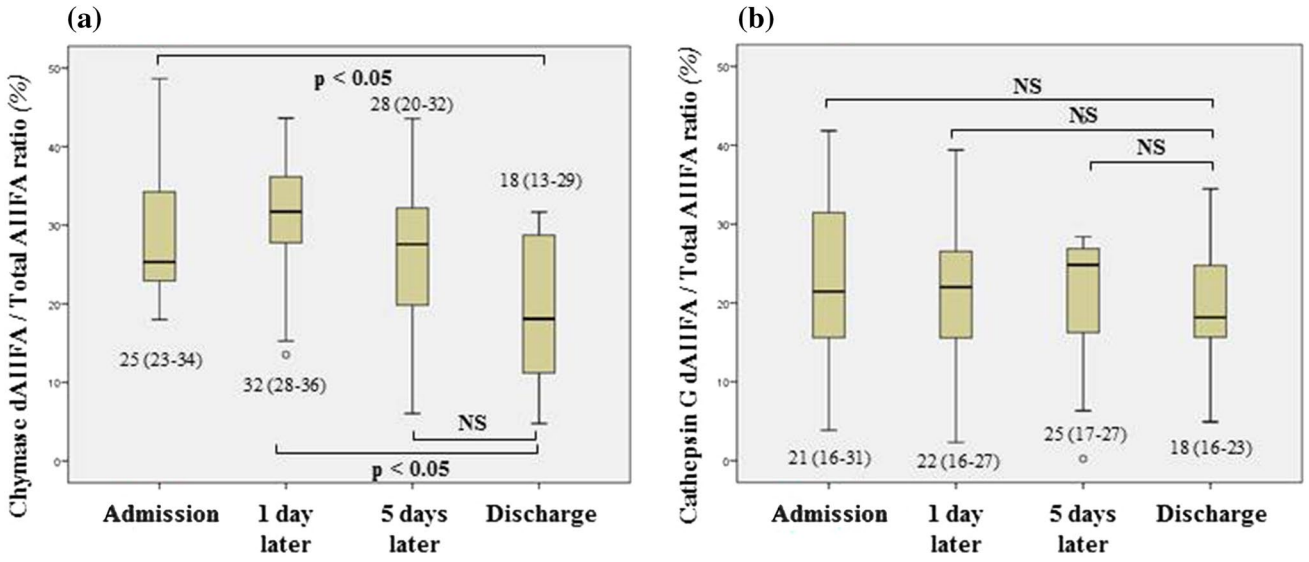

Fig. 3 Changes over time in the ratio of CML chymase-dependent AIIFA and CML cathepsin G-dependent AIIFA to CML total AIIFA ($n = 14$). The ratios measured at discharge, when patients are considered to be stable, were compared with those measured at the admission, 1 day later, and 5 days later using the Wilcoxon signed-rank test. One patient with cardiac rupture was excluded. The ratio of CML chymase-dependent AIIFA to CML total AIIFA was significantly higher at the onset of AMI and 1 day later compared with that at discharge (**a**). However, there were no changes over time in the ratio of CML cathepsin G dAIIFA to CML total AIIFA (**b**). dAIIFA, dependent AIIFA; For other abbreviations, see the previous figure

Discussion

The main findings of the present study were as follows: (1) CML total AIIFA, CML chymase-dependent AIIFA, and CML cathepsin G-dependent AIIFA reached the maximum levels at 1 day after the admission and gradually decreased thereafter. (2) CML chymase-dependent AIIFA accounted for a higher proportion of Total AIIFA at 1 day after the admission, but there was no significant change in the ratio of CML cathepsin G-dependent AIIFA to CML total AIIFA over time. (3) Max CML chymase-dependent AIIFA and Max CML cathepsin G-dependent AIIFA were significantly correlated with Max AST, Max LDH, and Max CK, but were not correlated with Max WBC or Max CRP.

This is the first study to show that CML total AIIFA, CML chymase-dependent AIIFA, and CML cathepsin G-dependent AIIFA are increased in the acute phase after MI.

Tissue AIIFA varies significantly between species and organs [14] while chymase-like activity cannot be detected in the plasma because of the influence of serine proteinase inhibitors [18]. Because circulating chymase is captured by inhibitors, it is difficult to measure using common antibody methods. Although it is possible to measure tissue chymase activity with HPLC by directly observing AII generation using AI as a substrate, this requires tissue sample collection at autopsy or surgery and is complex and time-consuming, limiting the number of samples that can be measured [14]. We previously reported the establishment of a simpler method for measuring chymase-dependent AIIFA, which is a convenient clinical assay based on a blood test [17], allowing us to examine the clinical importance of chymase after MI in this study.

It is considered that both plaque rupture and cardiac remodeling, which contribute to the development of MI, are involved in the mechanism that increases CML chymase-dependent AIIFA after AMI. We previously reported that chymase-dependent AII production was enhanced in human internal thoracic arteries used for coronary artery bypass grafts and was correlated with the blood level of low-density lipoprotein cholesterol [12], suggesting a relationship between lipid metabolism and chymase. We also reported that significant AII generation is observed, mainly due to increased chymase activity, in the aorta of patients with arteriosclerosis, while immunohistochemistry reveals the existence of chymase-positive mast cells at sites of arteriosclerosis, indicating a relationship between arteriosclerosis and mast cell chymase [13]. In addition, it was reported that activated mast cells containing chymase exist in human coronary artery plaques, suggesting that chymase has a role in plaque development [19]. Moreover, significant accumulation and degranulation of mast cells was reported in human myocardium after MI [20], as well as accumulation of inflammatory cells such as macrophages and T cells at sites of plaque, suggesting that chymase may have a role in plaque rupture [21]. Therefore, it can be postulated that chymase

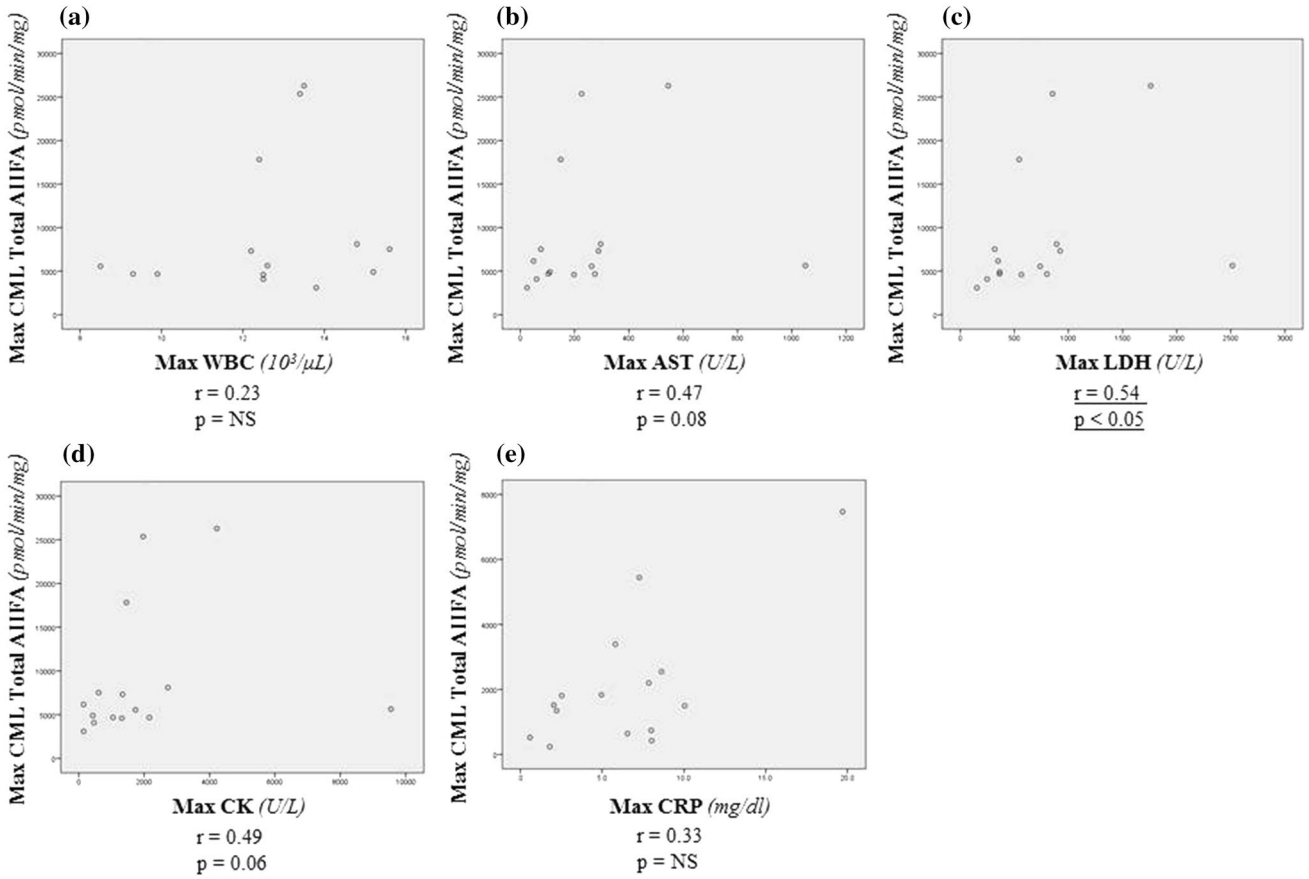

Fig. 4 Correlations of Max CML Total AIIFA with Max cardiac enzymes ($n = 15$). In each patient, the correlations between the maximum value of CML Total AIIFA (Max CML Total AIIFA) and the maximum levels of white blood cells (WBC), AST, LDH, CK, and C-reactive protein (CPR) (Max WBC, Max AST, Max LDH, Max CK, and Max CRP, respectively) were investigated by Spearman's rank correlation coefficient analysis. Although no significant correlation was observed with Max WBC or Max CRP, which are inflammatory markers, a positive correlation was observed between Max CML Total AIIFA and Max LDH. WBC, white blood cells; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; for other abbreviations, see the previous table and figure

has a role in various processes leading to the occurrence of MI, including lipid metabolism, development of arteriosclerosis, and plaque formation and rupture. Considering that CML chymase-dependent AIIFA indicates the trends of tissue chymase activity, the increase of CML chymase-dependent AIIFA observed in this study after AMI supports a strong relationship between chymase and occurrence of MI. While Max CML chymase AIIFA and Max CML cathepsin G AIIFA showed a significant positive correlation with Max AST, Max LDH, and Max CK in the present study, there was no significant correlation with Max WBC or Max CRP. The increase of these CML AIIFA components was correlated with elevation of cardiac enzymes instead of inflammatory markers related to plaque rupture, suggesting a relationship of AIIFA with the extent of myocardial damage or the severity of AMI. Because plaque rupture occurs locally in a coronary artery, the systemic effect would be relatively small and, therefore, the majority of the increase in CML chymase-dependent AIIFA was probably related to ischemic myocardial damage rather than plaque rupture. Most patients were treated with PCI during the acute phase and the peak levels of myocardial enzyme might not necessarily reflect the infarcted size. Originally it should be quantified with myocardial scintigraphy. This is the limitation of this research.

We previously reported that administration of a chymase inhibitor inhibited the development of aortic atherosclerotic plaque in hamsters on a high-fat diet [22], so it is possible that chymase inhibitor therapy may be able to prevent AMI due to plaque rupture.

ACE activity is increased in the myocardium after MI [23], and HPLC has shown that chymase activity and cathepsin G activity are also increased [14, 15]. In this study, CML total AIIFA, CML chymase-dependent AIIFA, and CML cathepsin G-dependent AIIFA all increased after AMI, reaching the maximum at 1 day after the onset and decreasing thereafter. Compared to the mean values of CML total AIIFA, CML chymase-dependent AIIFA, and CML

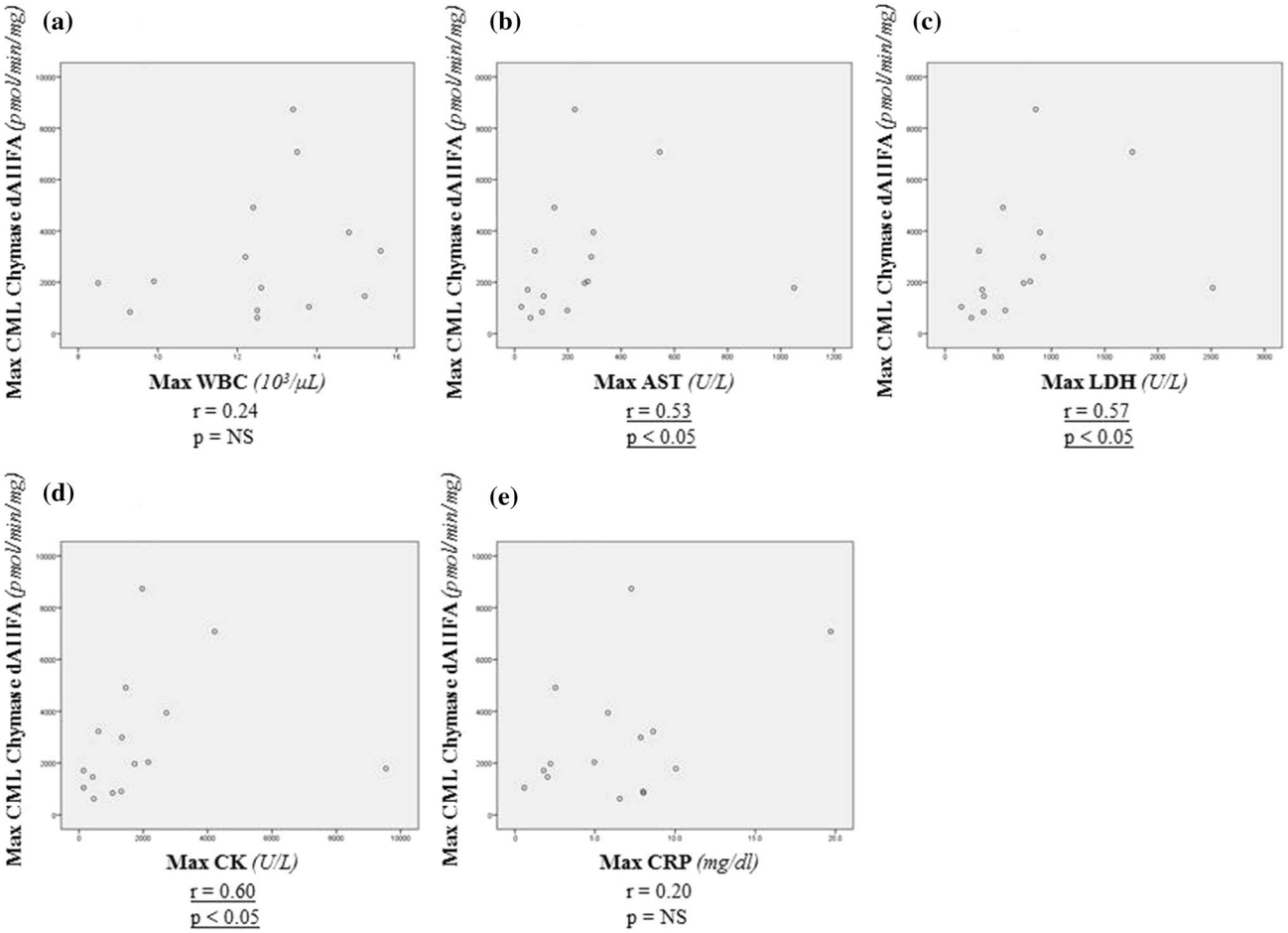

Fig. 5 Correlations of Max CML chymase-dependent AIIFA with Max cardiac enzymes ($n = 15$). Max CML chymase-dependent AIIFA was significantly correlated with Max AST, Max LDH and Max CK, but not with Max WBC or Max CRP. For abbreviations, see the previous table and figure

cathepsin G-dependent AIIFA previously reported in 170 cardiovascular outpatients [17], the levels determined during the acute phase of MI in this study were higher by 1.3-, 3.4-, and 2.2-fold, respectively, and the increase of CML chymase-dependent AIIFA was significant. We previously reported that elevation of cathepsin G activity is prominent in the early phase after AMI, while chymase is more important in the mid-phase onwards, suggesting that different AII-generating enzymes may be involved in remodeling at different periods after AMI [14, 15]. While the ratio of CML cathepsin G-dependent AIIFA to total AIIFA did not change in the early period after AMI, the increase in the ratio of CML chymase-dependent AIIFA was significant. These results suggested that chymase may be most strongly related to remodeling after MI among the various AIIFAs.

It has been reported that chymase activity is increased in ischemic myocardium following AMI [24], and that chymase activates matrix metalloproteinase-9 (MMP-9) [25]. In an AMI model, MMP-9 activity was found to be correlated with the infarct size and myocardial fibrosis [26], while chymase-dependent activation of MMP-9 was involved in myocardial remodeling after AMI [27]. Expression of transforming growth factor-beta (TGF-beta) also increases following MI [28] and promotes myocardial fibrosis, and TGF-beta is activated by chymase [29]. Therefore, MMP-9 and TGF-beta could be activated by chymase in MI patients, and may contribute to cardiac remodeling.

In a hamster MI model, chymase activity was increased in the infarct zone [30], while treatment with a chymase inhibitor significantly reduced mortality [16]. In an AMI model, administration of a chymase inhibitor inhibited induction of MMP-9 [25], interleukin-18 [31], and intercellular adhesion molecule-1 in the myocardium after MI and activated endothelial nitric-oxide synthase (eNOS), thus inhibiting fibrosis caused by chymase activation and providing myocardial protection [32]. Another mechanism of myocardial protection involves accumulation of bradykinin, which is degraded by chymase. Bradykinin accumulates due to

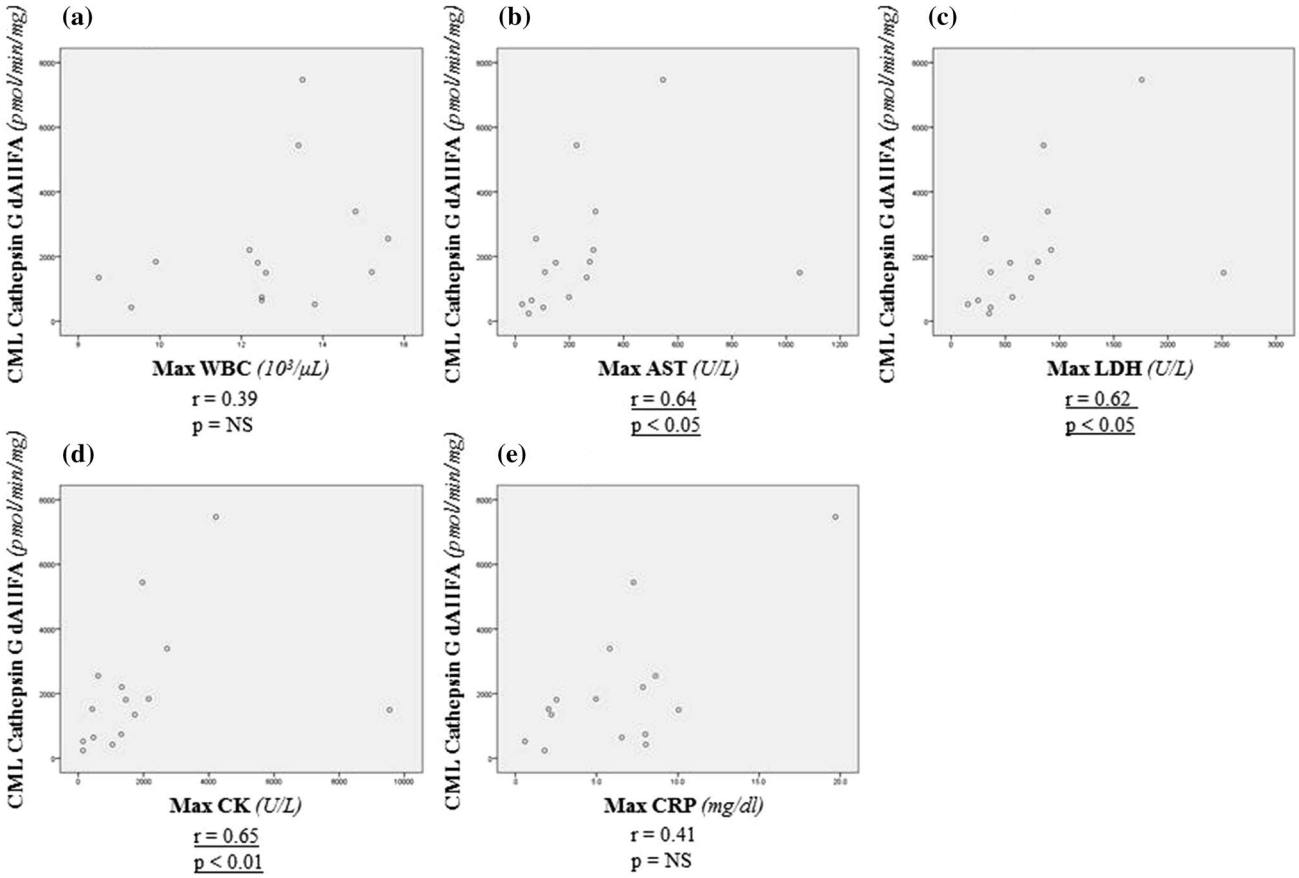

Fig. 6 Correlations of Max CML cathepsin G-dependent AIIFA with Max cardiac enzymes ($n = 15$). Max CML cathepsin G-dependent AIIFA was significantly correlated with Max AST, Max LDH and Max CK, but not with Max WBC or Max CRP. For abbreviations, see the previous table and figure

administration of a chymase inhibitor, leading to activation of eNOS that generates NO and protects the myocardium [29].

It has been reported that chymase inhibitor therapy does not improve fractional shortening or enlargement of LVDd, and that chymase-dependent TGF-beta production has an important role in myocardial fibrosis and cardiac dysfunction rather than in left ventricular dilation after MI [33]. This report is consistent with the results of our current research, in which there was no correlation between CML chymase-dependent AIIFA and EF or LVDd before discharge.

In hypertensive mice on a high-salt diet (2% salt solution), skin chymase activity was increased and a chymase inhibitor decreased the blood pressure, indicating that salt loading may activate chymase [34]. In MI patients, intravenous infusion in the acute phase may increase the sodium load, but patients are placed on a low-sodium diet prior to discharge. Activation of tissue chymase is considered to be involved in the mechanism of sodium-dependent hypertension, and it is also thought that circulating CML chymase-dependent AIIFA changes with sodium loading. Therefore, it is possible that salt loading via intravenous infusion contributed to the rapid increase of CML chymase-dependent AIIFA after AMI in this study. However, our results make it unlikely that sodium loading was the only cause of the change in AIIFA, and it seems more reasonable to conclude that the majority of the change after AMI reflected the increase of chymase in white blood cells recruited by cytokines in association with myocardial damage. Also, it was reported that the levels of brain natriuretic peptide and chymase-dependent AIIFA are correlated [17]. Therefore, heart failure caused by MI may have influenced the dynamics of CML chymase-dependent AIIFA that we observed, but this could not be clarified in the present observational study.

In this study, BNP and NT-proBNP were not necessarily measured in all cases because of medical insurance limits. In addition, even among the cases we measured those markers, the timing of measurement was different. Therefore, it is difficult to evaluate on time correlation between chymase-dependent AIIFA and the BNP or NT-proBNP levels in this study. If we measure BNP or NT-proBNP in clinical time course

and compare it with chymase dAIIFA, this relationship may become obvious.

Typically, a balanced AIIFA was observed in mononuclear leukocytes. Therefore, we decided to use circulating mononuclear, but not polynuclear, leukocyte fraction for the following experiments. Murakami et al. reported that the levels of ACE, chymase and cathepsin G activity were 25, 31 and 44 (%), respectively, in mononuclear leukocytes [35]. According to current report, the combined proportions of chymase and cathepsin G activity to total AIIFA were about 47% (admission), 53% (1 day later), 50% (5 days later) and 35% (discharge). Although these results were similar to the previous report, the value of AIIFA varies depending on individual cases and circumstances. Unfortunately, we did not measure CML ACE-dependent AIIFA, directly in current report. Therefore, total AIIFA − (chymase-dependent AIIFA + cathepsin G), which was considered to correspond to ACE-dependent AIIFA, was calculated and the estimate of ACE-dependent AIIFA was indirectly evaluated. These value were 1917 (1764–2919) pmol/min/mg (admission), 2389 (2193–3222) pmol/min/mg (1 day later), 2122 (1776–2945) pmol/min/mg (5 days later) and 1862 (1076–2465) pmol/min/mg (discharge). But this change was not significant by repeated measure ANOVA. Although renin–angiotensin system (RAS) inhibitor such as ACE-inhibitor might affect value of CML chymase-dependent AIIFA, it is unclear how CML AIIFA changes by administering RAS inhibitor such as ACE-inhibitor so far.

Based on the above findings, we propose the following hypothesis. Occurrence of MI increases chymase activity in the myocardium, and myocardial remodeling is promoted by MMP-9, interleukin-18, intercellular adhesion molecule-1, and TGF-beta. Chymase is not only activated in the myocardium but also in CML, and chymase-dependent AIIFA might reflect the severity of AMI. At the very least, there is a possibility that chymase contributes to the pathology of AMI along with the activation of the renin–angiotensin system (RAS).

Conclusion

This is the first report on elevation of CML chymase- and cathepsin G-dependent AIIFA after AMI. Our data suggested that chymase-dependent AIIFA was not only increased in the myocardium but also in CML after AMI and that its level reflected the severity of infarction.

Study limitations

This was a cross-sectional observational study that simply examined the relation between CML AIIFA and AMI. Patients with MI undergo PCI and receive medications such as RAS inhibitors, statins, and aspirin, which may have potentially influenced the dynamics of chymase-dependent AIIFA. To further analyze the role of chymase in the pathology of MI, it would be necessary to conduct a prospective study in MI patients with and without chymase inhibitor therapy.

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