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Dose-Dependent Inhibitory Effect of Rosuvastatin in Japanese Patients with Acute Myocardial Infarction on Serum Concentration of Matrix Metalloproteinases–INVITATION Trial–

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Aim: Matrix metalloproteinases (MMPs) play critical roles in acute myocardial infarction (AMI). This trial was conducted to determine the potential effects of higher-dose rosuvastatin on circulating MMP levels in patients with AMI.

Methods: This was a multicenter, open-label, 1:1 randomized, parallel-group study. Patients with AMI were randomly assigned to the appropriate-dose group (10 mg rosuvastatin once daily) or the low-dose group (2.5 mg rosuvastatin once daily) within 24 hours after percutaneous coronary intervention. MMP-2 and MMP-9 levels were measured on day 1 and at week 4, 12, and 24 after enrollment. The primary endpoint was the change in MMP levels at 24 weeks after enrollment. The secondary endpoints were change in MMP levels at day 1 and weeks 4 and 12 after enrollment.

Results: Between August 2017 and October 2018, 120 patients with AMI from 19 institutions were randomly assigned to either the appropriate-dose or the low-dose group. There were 109 patients who completed the 24-week follow-up. The primary endpoint for both MMP-2 and MMP-9 was not significantly different between the two groups. The change in the active/total ratio of MMP-9 at week 12 after baseline was significantly lower in the appropriate-dose group compared with the low-dose group (0.81 [−52.8–60.1]% vs. 70.1 [−14.5–214.2]%, $P=0.004$), while the changes in MMP-2 were not significantly different between the two groups during the study period.

Conclusions: This study could not demonstrate the superiority of appropriate-dose of rosuvastatin in inhibiting serum MMPs levels in patients with AMI.

Key words: Matrix metalloproteinases, Statins, Acute myocardial infarction

Introduction

Intensive treatment with high-intensity statins reduces the incidence of cardiovascular events after acute myocardial infarction (AMI)[1]. AMI is mainly characterized by the rupture of a lipid-rich vulnerable atherosclerotic plaque[2]. Matrix metalloproteinases (MMPs), namely MMP-2 and MMP-9, have been localized in atherosclerotic plaques, which are characterized by a lipid core covered with a fibrous cap in which inflammatory cells such as macrophages and T-lymphocytes are accumulated[3-5],with the metalloproteinases being a family of zinc-dependent proteases that are produced by a variety of cell types, including endothelial cells, smooth muscle cells, and monocytes. The accumulation of macrophage-derived foam cells in the vulnerable shoulder regions of atherosclerotic plaques correlates with increased local MMP release. MMPs are secreted as an inactive form and then are transformed into the active forms. Because plaque rupture or vulnerable plaque can exist in addition to the culprit AMI lesion, regulation of MMPs could be a potential target in secondary prevention of AMI, with released MMPs making the fibrous cap tissue weaken, resulting in this plaque rupture[6].

Cardiac remodeling followed by AMI causes reduced cardiac performance, and it is associated with morbidity and mortality after AMI, with MMPs having also been reported to be associated with cardiac remodeling after AMI[7, 8]. The benefit of anti-inflammatory treatment using canakinumab, an anti-interleukin-1β inhibitor, on cardiovascular disease has been reported[9], and the anti-inflammatory strategy including statins has also been refocused.

Statins are known to have pleiotropic effects besides lipid-lowering effects, with previous studies reporting that statins inhibited the increase in MMP-2 and MMP-9 levels in AMI[7, 10], and high-intensive statin therapy is the recommended lipid-lowering therapy for the secondary prevention of AMI worldwide. However, there are few comparison studies between the different dosages of the same statin and circulating MMPs levels and their activities in AMI. It is important to evaluate the concentration levels of active MMPs, because it is known that total and active MMPs are biologically different[11, 12]. To elucidate the pathophysiological mechanisms underlying the clinical benefit of higher-dose statin administration in patients with AMI, this study preliminarily investigated the potential effects of low-dose and appropriate-dose rosuvastatin on circulating MMP levels and their activities.

Methods

INVITATION Trial Study Design

The detailed protocol was described previously[13]. Briefly, the "Dose-dependent INhibitory effect of rosuVastatin In Japanese patienTs with Acute myocardial infarcTION on serum concentration of matrix metalloproteinases" (INVITATION) trial was a multicenter, open-label, randomized, parallel-group study that was conducted to compare appropriate or low-dose of rosuvastatin on the effect of serum inflammatory marker levels in patients with AMI. Enrolled patients were allocated to either the low-dose group or the appropriate-dose group. The low-dose group was treated with rosuvastatin 2.5 mg once daily during a follow-up period. The appropriate-dose group initially received rosuvastatin 5 mg once daily, and the dose of rosuvastatin was titrated to 10 mg within four weeks. Exercise and diet combined-therapy regimens were allowed, but concomitant use of other lipid-lowering drugs was prohibited during the study, and the allocation results were immediately communicated to the investigators, with the investigators starting the participants on the appropriate study treatment (start administration) within 24 h after allocation. Data were collected at day 0 (at enrollment) and day 1, and at weeks 4, 12, and 24.

Patients were enrolled and followed at 19 institutes in Japan. The first patient was randomized on August 8, 2017 and the last patient was randomized on October 30, 2018.

This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. This study was approved by the Institutional Review Board or Independent Ethics Committee of Kumamoto University Graduate of Medicine, and all of the participating institutes. This study was registered at the UMIN protocol registration system (identification number UMIN000016780), in accordance with the statement of the International Committee of Medical Journal Editors. The investigators explained the study to patients who were

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considered to be appropriate subjects for the study and confirmed their willingness to participate in the study. Written consent was obtained from all patients.

Study Subjects

Patients with AMI (ST-segment elevation myocardial infarction [STEMI] or non-STEMI [NSTEMI]) who were 20 years of age or older and who underwent percutaneous coronary intervention within 48 h of onset were screened. Serum low-density lipoprotein-cholesterol (LDL-C) levels greater than 100 mg/dL (2.59 mmol/L) met the eligibility criteria. Patients were excluded if they took lipid-lowering drugs including statins within three months prior to trial registration; severe hypertension (systolic blood pressure ≥ 180 mmHg or diastolic blood pressure > 110 mmHg); familial hypercholesterolemia; serum triglyceride level ≥ 400 mg/dL; previous onset of cardiovascular or cerebrovascular disease within six months prior to registration in this study; hypersensitivity to statins; history of drug-induced myopathy; active liver disease or elevated liver enzymes (alanine aminotransferase or aspartate transaminase > 3 times the upper limit of normal or bilirubin >2 times the upper limit of normal); nephrotic syndrome or renal dysfunction (creatinine clearance <30 mL/min/1.73 m^2 or serum creatinine >2.0 mg/dL); administered cyclosporin; serious concurrent disease such as malignancy; patients with severely limited lifespan; pregnant; or judged by the investigators to be ineligible for participation in the study for any other reason.

Definition of Myocardial Infarction

STEMI was defined as the presence of chest symptoms (e.g., chest pain or dyspnea) that was suspected to be a result of myocardial ischemia with a detected ST elevation of ≥ 1 mm in two contiguous leads, new left bundle branch block, or pathological Q wave on electrocardiogram with an elevation of cardiac troponin or creatine phosphokinase-MB above the 99th percentile of the upper limit of normal[14]. NSTEMI was defined as the presence of chest symptoms (e.g., chest pain or dyspnea) that were suspected to be a result of myocardial ischemia without ST elevation, new left bundle branch block, or pathological Q wave on electrocardiogram, but with elevation of cardiac troponin or creatine phosphokinase-MB above the 99th percentile of the upper limit of normal[14].

Endpoints

The primary endpoint was the change rate of MMP levels at 24 weeks after enrollment. Secondary endpoints were the change in MMP levels, lipid profiles, and inflammatory markers at day 1 and weeks 4, 12, and 24 weeks after enrollment.

Measurement of MMP-2 and MMP-9

Enzymatic activity of human MMP-2 and total human MMP-2 in serum were determined using ELISA kits (GE Healthcare, Buckinghamshire, UK), in accordance with the manufacturer's instructions. Enzymatic activity of active human MMP-9 and total human MMP-9 in serum were determined using ELISA kits (QuickZyme Biosciences B.V., Leiden, The Netherlands), in accordance with the manufacturer's instructions. These assays were performed by Kyushu Pro Search LLP (Fukuoka, Japan).

Measurement of TIMP-1, TIMP-2, IL-6, and PTX3

Interleukin (IL)-6, and pentraxin 3 (PTX3) were quantified using an ELISA kit (IL-6: QuantiGlo® ELISA Human IL-6 Immunoassay/R&D Systems, Inc., PTX3: Human Pentraxin3/TSG-14 ELISA System/Perseus Proteomics Inc.) in Kyushu Pro Search LLP (Fukuoka, Japan), according to the instructions in the manufacturer's manual. Tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 levels were measured by ELISAs using the kits Quantikine® (R&D Systems, Abingdon, United Kingdom), in accordance with the manufacturer's instructions in the laboratory of the Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University.

Statistical Analysis

In addition to this being a preliminary pilot study, there are few studies that have evaluated the effect of several statin dosages to reduce MMPs. Therefore, the minimum sample size was calculated to show the different trend of effect between two groups for exploration. For the primary hypothesis, sample size estimation was based on detecting a difference of 7% in the rate of MMP-9 change from baseline between rosuvastatin 10 mg and 2.5 mg. The average rate of change in the MMP-9 level after administering rosuvastatin 10 mg or 2.5 mg was assumed to be 22% and 15%, respectively[15-18]. There was no report on the standard deviation (SD) for the MMP-9 percent change. Therefore, if the SD was assumed to be 10%, the required number of patients was calculated as 44 for each group when the significance level (two-sided) was $\alpha=0.05$ and with power $(1-\beta)=0.9$. We estimated that 16-20% of participants would drop out of this study. Therefore, we decided that the target sample size of 120 patients (60 patients for each

group) was required to satisfy the principal study hypotheses that rosuvastatin 10 mg is superior to 2.5 mg (primary study objective) with respect to the average rate of change in MMP-9 from baseline. A Student's paired *t*-test was used to compare the mean values before and after each treatment. Differences between groups were compared using the Student's *t*-test, the Mann–Whitney *U*-test, or an analysis of variance with repeated measurements. Within-group comparisons for continuous variables were made by Friedman test and Wilcoxon signed rank test. Multiplicity of comparisons among groups was accounted by using Bonferroni correction. A value of $P<0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS 23 for Macintosh (SPSS Inc., Tokyo, Japan).

Results

One-hundred twenty patients with AMI who were enrolled at 19 institutes were randomly assigned to the appropriate-dose group (rosuvastatin 10 mg daily) or the low-dose group (rosuvastatin 2.5 mg daily) in Japan between August 2017 and October 2018. Finally, 109 patients completed a 24-week follow-up visit **(Supplementary Fig. 1)**. AMI-related deaths were observed, as follows: one in the appropriate-dose group and two in the low-dose group. Three patients were lost to follow-up and one patient withdrew their consent in the appropriate-dose group. Three patients in the appropriate-dose group and one patient in the low-dose group had protocol violations.

Baseline characteristics are shown in **Table 1**. Age, male gender, coronary risk factors, previous histories of myocardial infarction, percutaneous coronary intervention, and coronary artery bypass grafting were not statistically different between two study groups. AMI culprit lesions were similar between the two study groups (left anterior descending [LAD] artery, 56% in the appropriate-dose group and 46% in the low-dose group, $P=0.34$). Baseline lipid profiles and baseline total and active MMP-2 and MMP-9 levels were not statistically different between two groups **(Table 1, and 2, and Figs. 1A–D and 2A–F)**.

MMP levels and their changes at week 24 after enrollment were shown in **Table 2, Figs. 1A–D, 2A-F, and 3A–B**. Primary outcomes defined as the change rate of total and active MMP-2 and MMP-9 at 24 weeks after enrollment were not statistically different between the two study groups. Although the statistical difference in total and active MMP-2 and MMP-9 levels was not significantly different at each visit point **(Table 2 and Figs. 2A–D)**, the active MMP-9 levels in the appropriate-dose group tended to be lower compared with the low-dose group at day 1 and at weeks 4 and 12 after enrollment **(Table 2 and Figs. 2A–F)**. The change in the active/total ratio of MMP-9 at week 12 from baseline was significantly lower in the appropriate-dose group compared with the low-dose group (0.81 [–52.8– 60.1]% vs. 70.1 [–14.5– 214.2]%, $P=0.004$; **Fig. 3B**), while the changes in the MMP-2 active/total ratio were not significantly different between two groups the during study period **(Fig. 3A)**. Levels of TIMP-1 and 2, which regulate MMP-2 and 9, were not significantly different between the two study groups during the study period **(Table 2)**. Although MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios were not different in each visiting point, the percent change in the active MMP-9/TIMP-1 ratio was significantly decreased in the appropriate-dose group compared with the low-dose group at 12 weeks after enrollment (–10.3 [–60.3–90.5] % vs. –35.8 [–75.7–20.6] %, $P=0.04$]. The change in the active MMP-9/TIMP-1 ratio of 24 weeks from baseline was not significantly different between the appropriate-dose group and the low-dose group (–16.0 [–61.7–68.6] % vs. –19.9 [–70.7–84.0], $P=0.54$, **Table 2**). LDL-C levels were significantly lower in the appropriate-dose group compared with the low-dose group (67 [58–84] mg/dL vs. 77 [66–91] mg/dL, $P=0.02$). C-reactive protein (CRP), IL-6, and PTX3, B-type natriuretic peptide (BNP) levels at week 24 after enrollment and their percent change from baseline to week 24 were not different between the two groups **(Table 1)**, although all of them at 24 weeks were improved from baseline.

Discussion

This study did not support the primary endpoint, which was defined as the superiority of higher dose of rosuvastatin in inhibiting serum MMP-2 and MMP-9 levels at 24 weeks after enrollment in patients with AMI. We observed a dose-dependent effect of rosuvastatin in the change of the active/total MMP-9 ratio from baseline to 12 weeks after enrollment. Although levels of TIMP-1 and TIMP-2, which are known to regulate MMPs[19], were not significantly different between the appropriate-dose group and the low-dose group at each visit point, active MMP-9/TIMP-1 ratio was significantly decreased in the appropriate-dose group compared with the low-dose group at 12 weeks after enrollment, which may affect the result regarding the change of active/total MMP-9 at 12 weeks. Although there was some bias because of the results of secondary

Table 1. Baseline characteristics and laboratory data at 24-week follow-up visit

	Low-dose group $n = 57$	Appropriate-dose group $n = 52$	P value
Age, years	65.5 ± 11.0	67.7 ± 11.7	0.33
Male, *n* (%)	48 (84)	42 (81)	1.00
Body mass index, kg/m^2	24.2 ± 3.8	23.9 ± 3.9	0.74
Hypertension, *n* (%)	30 (53)	25 (48)	0.70
Diabetes Mellitus, *n* (%)	19 (33)	17 (33)	1.00
Dyslipidemia, *n* (%)	20 (35)	12 (23)	0.21
Current smoking, *n* (%)	23 (40)	20 (38)	0.85
Culprit vessel			
LAD, *n* (%)	26 (46)	29 (56)	0.34
LCX, *n* (%)	10 (18)	8 (15)	0.80
RCA, *n* (%)	21 (37)	15 (29)	0.42
History of PCI, *n* (%)	0 (0)	3 (6)	0.11
History of CABG, *n* (%)	0 (0)	0 (0)	-
History of MI, *n* (%)	0 (0)	1 (2)	0.48
History of stroke, *n* (%)	5 (9)	2 (4)	0.44
History of PAD, *n* (%)	0 (0)	1 (2)	0.48
eGFR, mL/min/1.73 m^2	55.2 ± 17.9	53.1 ± 14.2	0.50
peak creatinine kinase, U/L	1733 (586 – 2311)	1557 (760 – 2697)	0.79
Medication			
Aspirin, *n* (%)	37 (65)	36 (69)	0.89
Beta-blockers, *n* (%)	20 (35)	16 (31)	0.69
Calcium channel blockers, *n* (%)	12 (21)	10 (19)	1.00
ACE inhibitors or ARBs, *n* (%)	33 (58)	28 (54)	0.70
Insulin, *n* (%)	5 (9)	1 (2)	0.21
Laboratory data			
Hemoglobin A1c, baseline, %	5.9 (5.6 – 5.0)	6.0 (5.8 – 6.9)	0.39
Hemoglobin A1c, 24 weeks, %	5.9 (5.7 – 6.2)	6.2 (5.9 – 6.7)	0.24
ΔHemoglobin A1c, 24 weeks, %	0 (-2.7 – 5.3)	-1.6 (-4.8 – 3.3)	0.62
Triglycerides, baseline, mg/dL	111 (72 – 180)	120 (88 – 148)	0.95
Triglycerides, 24 weeks, mg/dL	125 (95 – 161)	103 (89 – 149)	0.65
ΔTriglycerides, 24 weeks, %	2.7 (-13.0 – 28.8)	-1.3 (-27.8 – 35.5)	0.79
HDL-C, baseline, mg/dL	45 (39 – 57)	47 (40 – 53)	0.83
HDL-C, 24 weeks, mg/dL	51 (40 – 58)	47 (40 – 52)	0.60
ΔHDL-C, 24 weeks, %	2.9 (-5.7 – 16.7)	-4.0 (-13.2 – 9.7)	0.21
LDL-C, baseline, mg/dL	135 (113 – 158)	139 (125 – 153)	0.41
LDL-C, 24 weeks, mg/dL	77 (66 – 91)	67 (58 – 84)	0.02
ΔLDL-C, 24 weeks, %	-40.0 (-45.1 – -29.9)	-52.4 (-59.7 – -44.1)	0.002
CRP, baseline, mg/dL	0.185 (0.080 – 0.420)	0.240 (0.100 – 0.710)	0.77
CRP, 24 weeks, mg/dL	0.060 (0.035 – 0.100)	0.100 (0.030 – 0.150)	0.85
ΔCRP, 24 weeks, %	-47.1(-70.0 – 0)	-65.7 (-83.6 – -21.2)	0.30
PTX3, baseline, ng/mL	3.2 (2.2 – 5.0)	3.6 (2.5 – 5.8)	0.34
PTX3, 24 weeks, ng/mL	1.9 (1.5 – 2.7)	2.3 (1.7 – 3.1)	0.17
ΔPTX3, 24 weeks, %	-45.4 (-67.6 – -16.9)	-40.3 (-55.8 – -6.9)	0.68
IL-6, baseline, pg/mL	5.9 (3.8 – 11.8)	5.7 (3.4 – 15.2)	0.75
IL-6, 24 weeks, pg/mL	1.7 (1.1 – 3.1)	1.7 (1.2 – 2.6)	0.91
ΔIL-6, 24 weeks, %	-69.9 (-89.5 – -42.9)	-73.6 (-84.6 – -43.4)	0.92
BNP, baseline, pg/mL	38.8 (13.9 – 152.2)	35.7 (13.9 – 112.5)	0.64
BNP, 24 weeks, pg/mL	29.2 (15.7 – 95.0)	37.2 (14.8 – 86.8)	0.75
ΔBNP, 24 weeks, %	-20.6 (-58.7 – 151.9)	2.9 (-57.1 – 118.1)	0.86

Data are presented as the mean ± SD, *n* (%), or median (IQR). Δ indicated the percent relative change from baseline. LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; MI, myocardial infarction; PAD, peripheral artery disease; eGFR, estimated glomerular filtration rate; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; CRP, C-reactive protein; PTX3, pentraxin 3; IL-6, interleukin-6; and BNP, B-type natriuretic peptide.

Table 2. The changes and differences of MMPs and TIMPs at each visit point

	Low-dose group ($n=57$)	Appropriate-dose group ($n=52$)	*P* value intergroup	*P* value_Bonferroni adjustment	
				intragroup Low-dose group	intragroup Appropriate-dose group
Total MMP-2, ng/mL					
Baseline	354.3 [279.4, 436.0]	357.4 [297.1, 440.1]	0.93	-	-
1-day	306.3 [276.6, 364.6]	309.4 [238.3, 386.4]	0.74	-	-
4-week	375.4 [321.6, 479.8]	430.7 [345.3, 497.3]	0.17	-	-
12-week	413.3 [366.5, 490.9]	448.4 [356.5, 532.7]	0.29	-	-
24-week	396.6 [362.0, 451.9]	416.3 [382.8, 526.1]	0.40	-	-
% change rate from baseline					
1-day	-13.0 [-24.1, 2.6]	-13.8 [-22.4, -5.5]	0.56	<0.001	<0.001
4-week	17.7 [-4.4, 31.5]	17.4 [1.2, 41.6]	0.28	0.004	<0.001
12-week	17.2 [2.5, 36.4]	23.7 [8.4, 40.0]	0.31	<0.001	<0.001
24-week	12.2 [-2.3, 30.6]	17.9 [1.2, 44.7]	0.18	0.004	<0.001
Active MMP-2, ng/mL					
Baseline	7.1 [5.6, 8.9]	6.4 [5.3, 9.1]	0.57	-	-
1-day	6.8 [5.6, 9.0]	6.3 [4.5, 8.1]	0.16	-	-
4-week	7.7 [5.6, 9.7]	7.8 [5.8, 10.6]	0.83	-	-
12-week	7.9 [5.1, 9.7]	7.7 [5.4, 10.7]	0.53	-	-
24-week	7.8 [6.0, 9.0]	7.5 [6.0, 10.1]	0.71	-	-
% change rate from baseline					
1-day	-4.4 [-22.9, 15.8]	-7.3 [-27.8, 11.2]	0.37	1.00	0.20
4-week	6.5 [-12.7, 27.1]	8.0 [-0.3, 37.8]	0.22	0.52	0.002
12-week	3.5 [-26.1, 27.3]	11.7 [-10.6, 31.7]	0.14	>0.99	0.07
24-week	5.7 [-10.5, 24.0]	12.8 [-7.1, 37.6]	0.37	0.57	0.07
Total MMP-9, ng/mL					
Baseline	23.8 [14.6, 42.5]	22.6 [13.1, 43.0]	0.70	-	-
1-day	15.1 [9.9, 26.0]	12.8 [8.6, 21.1]	0.21	-	-
4-week	13.8 [10.9, 22.3]	15.3 [11.6, 27.2]	0.48	-	-
12-week	15.4 [9.8, 26.0]	16.2 [12.6, 21.4]	0.95	-	-
24-week	14.9 [10.9, 20.2]	15.5 [11.7, 23.8]	0.47	-	-
% change rate from baseline					
1-day	-30.3 [-67.2, 10.2]	-33.1 [-62.5, 4.2]	0.78	0.005	<0.001
4-week	-41.8 [-66.4, 22.6]	-17.9 [-69.8, 35.1]	0.46	<0.001	0.16
12-week	-35.2 [-60.3, 15.2]	-29.4 [-66.2, 35.5]	0.68	0.001	0.040
24-week	-32.1 [-70.1, 4.6]	-8.7 [-71.6, 42.7]	0.43	<0.001	0.11
Active MMP-9, ng/mL					
Baseline	1.5 [0.7, 3.7]	1.9 [1.1, 4.1]	0.28	-	-
1-day	1.7 [0.5, 3.8]	1.7 [0.8, 3.2]	0.61	-	-
4-week	1.0 [0.7, 2.5]	1.2 [0.6, 2.8]	0.72	-	-
12-week	1.4 [0.9, 3.4]	1.4 [0.7, 2.7]	0.45	-	-
24-week	1.9 [0.8, 3.1]	1.4 [0.7, 3.1]	0.82	-	-
% change rate from baseline					
1-day	4.3 [-58.1, 81.2]	-14.4 [-66.3, 82.4]	0.29	>0.99	0.74
4-week	-6.0 [-70.5, 86.9]	-41.2 [-81.4, 37.8]	0.23	>0.99	0.21
12-week	17.7 [-54.5, 113.3]	-32.9 [-73.2, 32.1]	0.03	>0.99	0.09
24-week	14.6 [-48.9, 91.5]	-16.7 [-68.1, 127.5]	0.48	>0.99	0.78
Active MMP-2/Total MMP-2 (%)					
Baseline	2.0 [1.6, 2.6]	1.9 [1.4, 2.5]	0.63	-	-
1-day	2.1 [1.7, 2.9]	2.1 [1.4, 2.9]	0.46	-	-
4-week	1.8 [1.5, 2.5]	1.9 [1.4, 2.2]	0.59	-	-
12-week	1.8 [1.2, 2.4]	1.8 [1.3, 2.3]	0.90	-	-
24-week	2.0 [1.5, 2.2]	1.9 [1.5, 2.1]	0.29	-	-
% change rate from baseline					
1-day	4.6 [-7.4, 36.8]	4.0 [-10.9, 30.2]	0.64	0.14	0.67
4-week	-6.3 [-23.4, 14.3]	-0.9 [-21.7, 15.1]	0.51	0.85	>0.99
12-week	-7.9 [-38.9, 10.6]	-6.3 [-30.0, 23.2]	0.33	0.12	0.76
24-week	-4.5 [-21.5, 13.9]	-6.6 [-27.5, 25.5]	0.92	0.78	0.70
Active MMP-9/Total MMP-9 (%)					
Baseline	6.7 [2.3, 14.1]	10.6 [3.6, 19.0]	0.21	-	-
1-day	10.3 [3.9, 22.1]	13.6 [6.0, 24.9]	0.28	-	-
4-week	6.5 [3.3, 19.9]	7.9 [3.9, 15.1]	0.93	-	-
12-week	8.8 [4.9, 21.7]	8.4 [3.8, 15.9]	0.20	-	-
24-week	11.8 [5.6, 20.9]	7.9 [4.1, 17.7]	0.39	-	-
% change rate from baseline					
1-day	34.7 [-30.3, 183.4]	40.9 [-38.1, 198.8]	0.83	0.19	0.46
4-week	20.0 [-49.1, 211.7]	-9.1 [-58.3, 70.7]	0.05	0.51	>0.99
12-week	70.1 [-14.5, 214.2]	0.81 [-52.8, 60.1]	0.004	0.02	>0.99
24-week	59.9 [-12.3, 173.1]	16.3 [-40.2, 164.4]	0.14	0.01	>0.99

(Cont. Table 2)

	Low-dose group (n=57)	Appropriate-dose group (n=52)	P value intergroup	P value_Bonferroni adjustment	
				intragroup Low-dose group	intragroup Appropriate-dose group
TIMP-1, ng/mL					
Baseline	165.6 [120.3, 188.1]	151.8 [122.1, 190.0]	1.00	-	-
1-day	164.0 [133.4, 176.5]	168.9 [144.8, 197.5]	0.09	-	-
4-week	176.3 [156.4, 189.3]	171.6 [158.0, 191.5]	0.95	-	-
12-week	170.0 [150.4, 186.9]	172.2 [148.6, 188.7]	0.65	-	-
24-week	168.4 [150.5, 187.0]	170.0 [150.0, 190.8]	0.75	-	-
% change rate from baseline					
1-day	-1.0 [-10.3, 30.4]	6.1 [-7.0, 46.5]	0.14	>0.99	0.06
4-week	7.1 [-3.4, 49.8]	11.6 [-7.5, 45.8]	0.72	0.003	0.009
12-week	5.9 [-10.8, 47.4]	8.6 [-8.9, 31.3]	0.78	0.22	0.12
24-week	7.7 [-9.3, 34.6]	0.2 [-8.0, 55.1]	0.72	0.18	0.75
TIMP-2, ng/mL					
Baseline	80.2 [69.2, 91.0]	83.4 [73.2, 89.7]	0.47	-	-
1-day	72.8 [67.3, 80.8]	73.0 [63.6, 78.9]	0.65	-	-
4-week	92.7 [83.3, 101.9]	98.4 [86.3, 109.5]	0.14	-	-
12-week	94.3 [85.2, 107.0]	97.3 [87.3, 107.3]	0.42	-	-
24-week	92.2 [81.8, 104.7]	97.1 [87.1, 107.0]	0.36	-	-
% change rate from baseline					
1-day	-7.6 [-17.8, -0.1]	-12.6 [-20.4, -6.5]	0.07	<0.001	<0.001
4-week	18.5 [3.1, 30.6]	16.7 [4.3, 29.3]	0.94	<0.001	<0.001
12-week	19.2 [6.6, 33.7]	15.6 [9.7, 32.0]	0.99	<0.001	<0.001
24-week	17.2 [3.1, 27.6]	13.3 [7.5, 32.4]	0.96	<0.001	<0.001
Total MMP-2/TIMP-2					
Baseline	4.5 [3.7, 5.2]	4.5 [3.8, 4.9]	0.76	-	-
1-day	4.3 [3.7, 5.1]	4.2 [3.6, 5.0]	0.86	-	-
4-week	4.2 [3.6, 5.1]	4.5 [3.7, 5.3]	0.58	-	-
12-week	4.3 [3.6, 5.4]	4.5 [3.8, 5.2]	0.58	-	-
24-week	4.4 [3.6, 4.8]	4.5 [3.8, 5.4]	0.18	-	-
% change rate from baseline					
1-day	-5.4 [-15.2, 6.2]	-3.4 [-11.1, 7.0]	0.72	0.29	0.45
4-week	-2.1 [-13.8, 5.5]	2.0 [-10.5, 10.9]	0.18	0.56	>0.99
12-week	-2.2 [-11.1, 11.2]	4.3 [-8.1, 16.2]	0.23	>0.99	0.75
24-week	-2.4 [-13.9, 9.6]	1.7 [-7.9, 18.2]	0.06	0.27	0.92
Active MMP-2/TIMP-2					
Baseline	0.09 [0.07, 0.11]	0.08 [0.06, 0.11]	0.38	-	-
1-day	0.10 [0.07, 0.12]	0.09 [0.06, 0.12]	0.36	-	-
4-week	0.08 [0.06, 0.10]	0.08 [0.06, 0.11]	0.85	-	-
12-week	0.08 [0.05, 0.12]	0.08 [0.06, 0.11]	0.72	-	-
24-week	0.08 [0.06, 0.10]	0.08 [0.06, 0.10]	0.85	-	-
% change rate from baseline					
1-day	6.6 [-14.3, 26.4]	4.2 [-19.0, 27.7]	0.64	0.90	>0.99
4-week	-6.3 [-20.0, 8.5]	-3.8 [-20.9, 17.0]	0.48	0.26	0.77
12-week	-12.2 [-36.9, 10.5]	-2.8 [-26.1, 29.3]	0.18	0.19	>0.99
24-week	-8.2 [-26.7, 6.9]	-11.6 [-26.9, 23.4]	0.61	0.14	0.82
Total MMP-9/TIMP-1					
Baseline	0.15 [0.10, 0.36]	0.17 [0.09, 0.25]	0.61	-	-
1-day	0.10 [0.07, 0.17]	0.08 [0.05, 0.12]	0.05	-	-
4-week	0.08 [0.07, 0.13]	0.09 [0.06, 0.16]	0.61	-	-
12-week	0.09 [0.07, 0.14]	0.09 [0.07, 0.13]	0.97	-	-
24-week	0.09 [0.06, 0.13]	0.09 [0.07, 0.14]	0.41	-	-
% change rate from baseline					
1-day	-37.9 [-65.2, -3.6]	-35.1 [-67.4, -6.3]	0.61	0.001	<0.001
4-week	-49.2 [-73.3, -18.5]	-42.9 [-69.4, 25.1]	0.35	<0.001	0.007
12-week	-46.6 [-66.8, 1.1]	-36.0 [-68.5, 8.9]	0.55	<0.001	0.001
24-week	-47.8 [-74.4, -7.8]	-39.7 [-70.8, 36.5]	0.40	<0.001	0.006
Active MMP-9/TIMP-1					
Baseline	0.01 [0.00, 0.03]	0.01 [0.01, 0.03]	0.45	-	-
1-day	0.01 [0.00, 0.03]	0.01 [0.00, 0.02]	0.98	-	-
4-week	0.01 [0.00, 0.01]	0.01 [0.00, 0.02]	0.78	-	-
12-week	0.01 [0.00, 0.02]	0.01 [0.00, 0.02]	0.42	-	-
24-week	0.01 [0.00, 0.02]	0.01 [0.00, 0.02]	0.81	-	-
% change rate from baseline					
1-day	1.5 [-58.6, 93.0]	-18.4 [-73.7, 65.2]	0.27	>0.99	0.24
4-week	-30.0 [-73.0, 53.9]	-50.1 [-83.9, 37.9]	0.33	0.04	0.06
12-week	-10.3 [-60.3, 90.5]	-35.8 [-75.7, 20.6]	0.04	>0.99	0.02
24-week	-16.0 [-61.7, 68.6]	-19.9 [-70.7, 84.0]	0.54	0.59	0.37

Data are presented as median [IQR]. Within-group comparisons for continuous variables were made by Friedman test and Wilcoxon signed rank test. Multiplicity of comparisons among groups was accounted by using Bonferroni correction. MMP, matrix metalloproteinase; and TIMP, tissue inhibitor of metalloproteinase.

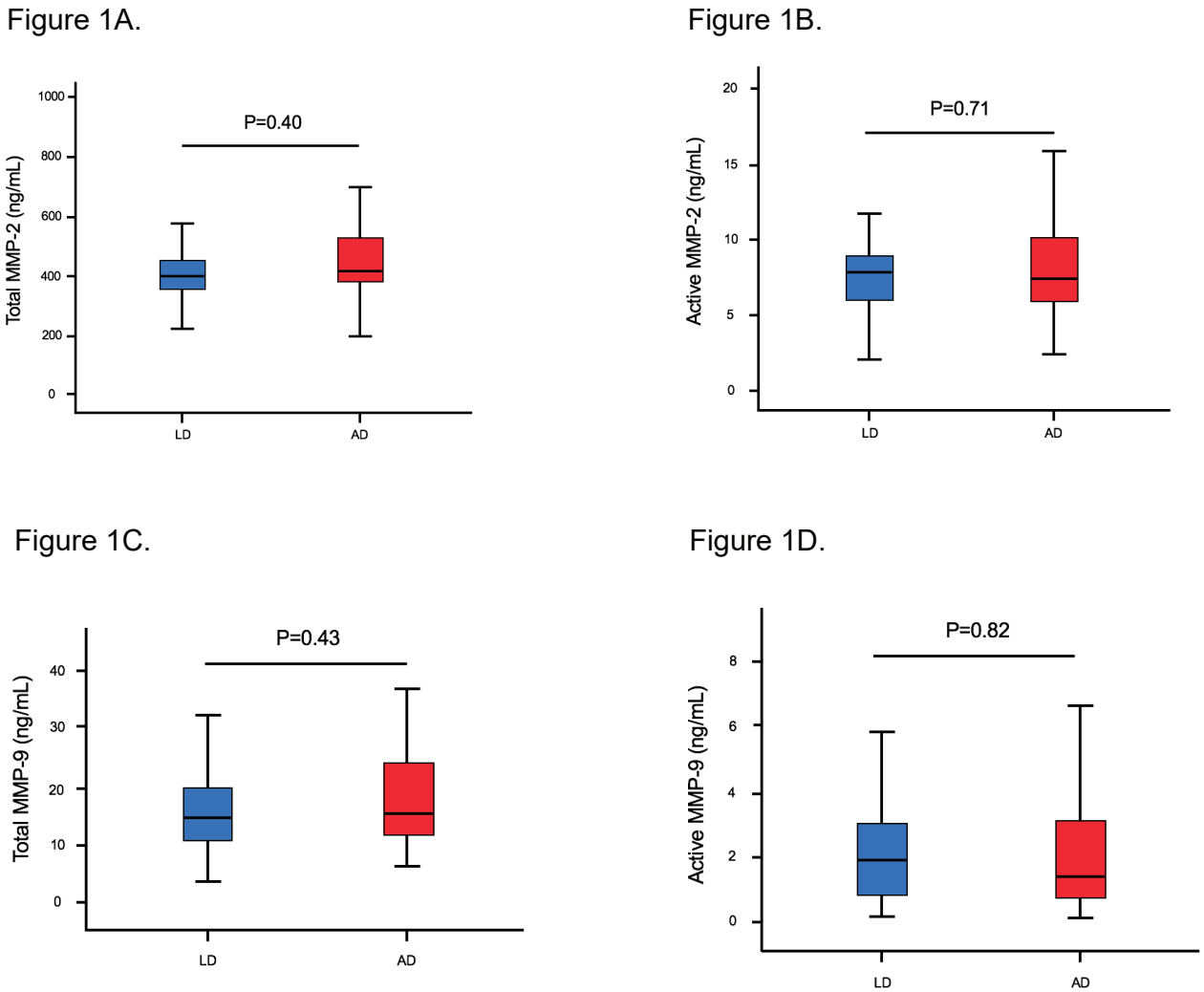


Fig. 1. Comparison the MMP levels at 24 weeks after enrollment

(A) Total MMP-2, (B) active MMP-2, (C) total MMP-9, and (D) active MMP-9.
Bars show the 95% confidence interval. LD, low-dose group; AD, appropriate-dose group; MMP, matrix metalloproteinase.

endpoint, CRP and IL-6 levels were also not different between the appropriate-dose group and the low-dose group. Because this study did not evaluate other pro-inflammatory cytokines and transcriptional factors such as NF-κB, p38 MAPK, and JNK that regulate MMP-9 [19, 20], it could not determine the detailed mechanism for MMP-9 regulation by rosuvastatin. While MMP-2 and MMP-9 are mainly secreted from macrophages, so it would be reasonable that balance between cholesterol loading by LDL-C and cholesterol efflux by HDL-C would affect focal MMPs secretion in the lesions from macrophages, in addition, this study could not demonstrate whether rosuvastatin affects the levels of MMP-9 locally or systemically. Therefore, the change of active/total MMP-9 ratio at 12 weeks might be a result of measuring any cleavage-substrate of MMPs from infarct region. In a recent OCT study, abundant local MMP-9 could cause massive red thrombosis that was mainly observed in patients with STEMI[21], revealing that local MMP-9 were significantly higher than the systemic levels. We might not be able to detect the difference in MMP levels, because our study measured only systemic levels of MMPs. In addition, the type of coronary thrombosis was not evaluated. Further basic and clinical studies are required to demonstrate the mechanism for MMP-9 regulation by statins.

Our results showed the possibility that there was a dose-dependent effect of rosuvastatin on MMP-9, while the effects on MMP-2 were not observed in this study. Yasuda *et al.* reported that MMP production in the infarcted heart was associated with left ventricular

Figure 2A.

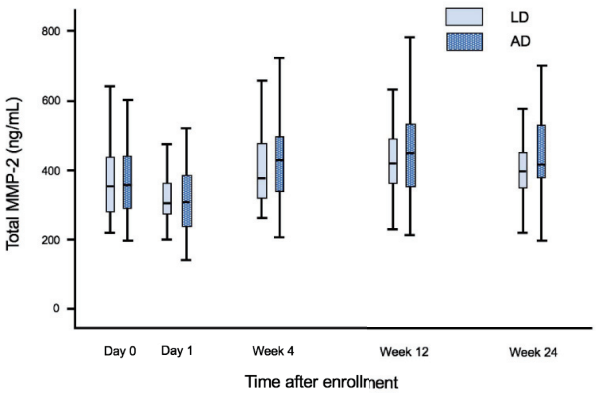


Figure 2B.

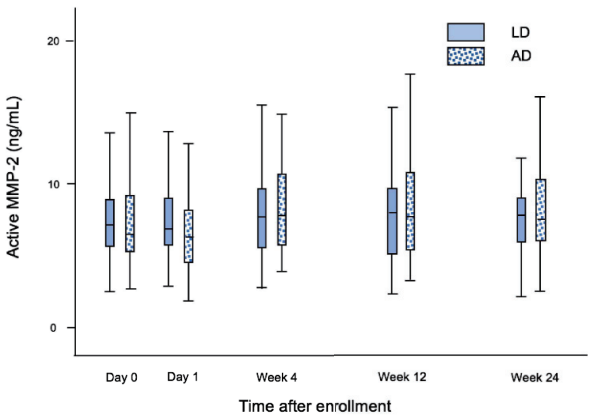


Figure 2C.

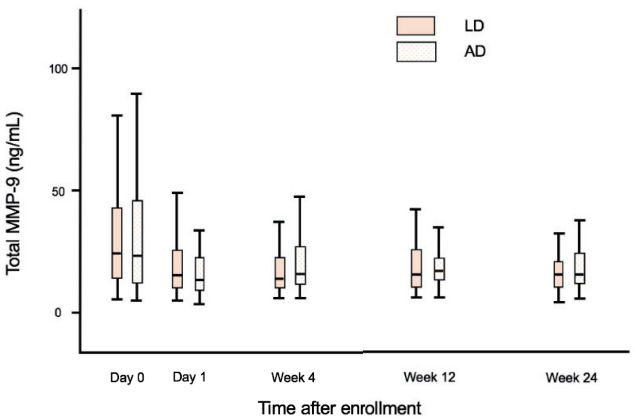
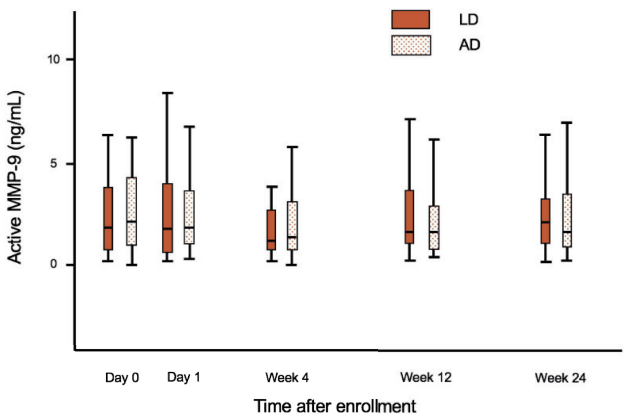


Figure 2D.



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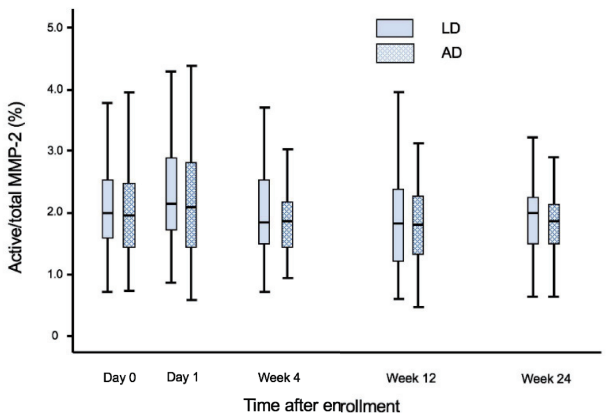

Figure 2F.

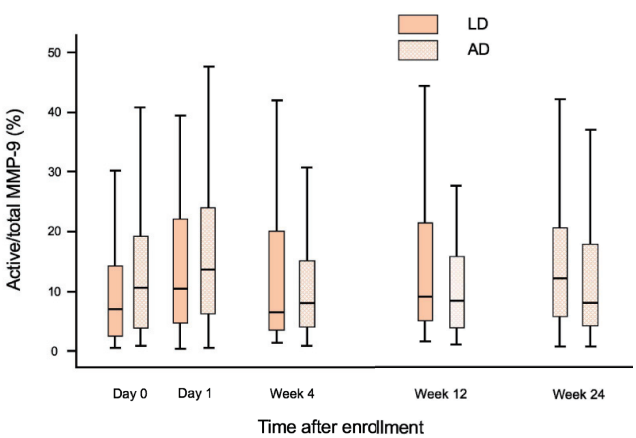

Fig. 2. Comparison the changes of MMPs during study period

(A) Total MMP-2, (B) active MMP-2, (C) total MMP-9, (D) active MMP-9, (E) Active/Total ratio of MMP-2, and (F) Active/Total ratio of MMP-9.

Figure 3A.

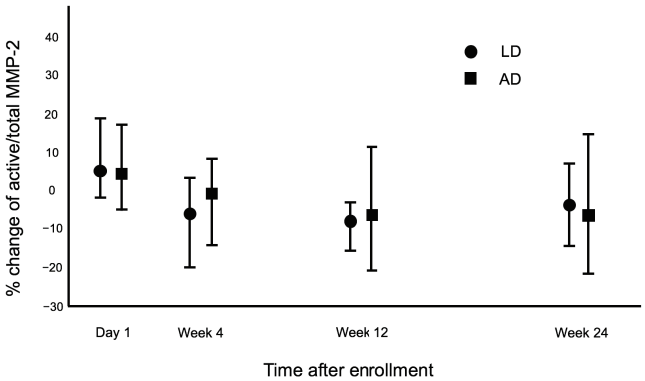

Figure 3B.

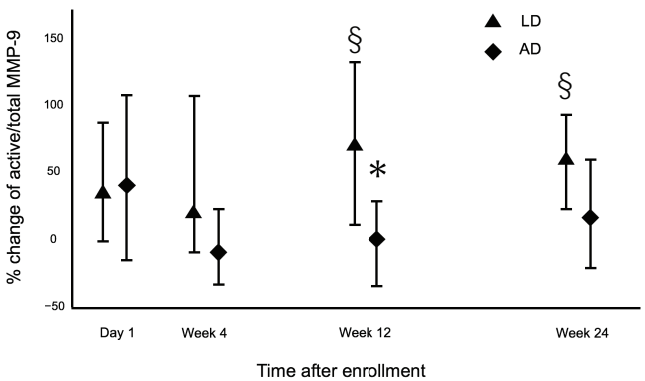

Fig. 3. Percent change from baseline of (A) the active/total ratio of MMP-2 and (B) the active/total ratio of MMP-9

Plots show the median, and bars show the 95% confidence interval. §, $P<0.05$ vs. baseline. *, $P<0.05$ vs. low-dose group. LD, low-dose group; AD, appropriate-dose group; MMP, matrix metalloproteinase.

enlargement and elevated BNP levels, and pravastatin significantly decreased cardiac MMP-2 and MMP-9 in patients with AMI, measuring the concentration gradients between the coronary sinus and aorta, and demonstrating increased cardiac MMP levels in AMI patients compared with stable CAD[7]. Although their studies were not randomized and the sample size was small (AMI, $n=20$; stable CAD, $n=10$) and involved a single dose of low-intensity statin, the results showed that pravastatin has a potentially beneficial effect in attenuating post-infarction left ventricular remodeling through both MMP-2 and MMP-9. Where our study was designed to focus on the comparison of MMPs levels by the different statin dose, so this study could not show the association between the change of MMPs and cardiovascular events, previous studies have reported that MMP-9 levels were associated with future cardiovascular events[22, 23]. Further investigation is needed to reveal the association between MMPs levels and cardiovascular events by the different statin dose. While this was an *in vitro* study that did not mimic the ischemic condition, an experimental study also demonstrated the dose-dependent effects of rosuvastatin on MMP-2 and MMP-9 [16]. Another experimental study using leukemia cells indicated that simvastatin-induced p65 instability leads to MMP-9 down-regulation, while simvastatin-induced JNK1/c-Jun/ATF-2 activation maintained the MMP-2 expression underlying p65 down-regulation[24]. Statins might have different regulation mechanisms for MMPs based on the pathogeneses and cells. Another possible reason was that the time point for MMP-2 measurement might not be relevant to detect the difference between two study groups. Hojo *et al.* evaluated the change of the MMP-2 plasma levels in patients with AMI. MMP-2 levels increased at day 14 and peaked at days 14–21 after AMI onset[25]. We measured the MMP levels at 1 and 4 weeks after enrollment, and we might not be able to detect the difference in MMP levels at these two points. Further investigation is required to demonstrate the dose-dependent effects of rosuvastatin on MMPs.

Study Limitations

This study had several limitations. Firstly, this study might not have sufficient power to detect the differences of MMPs between two study groups because of the small sample size. Secondly, and maybe limiting the generalization of our findings to the other studies, particularly those in Western countries, all patients were Japanese. Thirdly, this study did not show the possible mechanism by which the higher dose of rosuvastatin attenuated the change in the active/total ratio from baseline of MMP-9 at 12 weeks after enrollment. Several factors such as TIMPs, CRP, and IL-6, which could be associated with MMPs, did not explain the phenomenon. Fourthly, the precise dose-dependency of statins on MMPs was not confirmed, as this study examined only two different rosuvastatin doses. Fifthly, the effects on cardiac remodeling and prognosis through MMPs were not evaluated. Further, translational studies are required to confirm our results.

Conclusions

This study could not observe the superiority of higher dose of rosuvastatin in inhibiting serum MMP-2 and MMP-9 levels at 24 weeks after enrollment in patients with AMI. We need further

investigation to confirm the shorter-term benefits of the higher dose of rosuvastatin in inhibiting the change in the active/total MMP-9 ratio.

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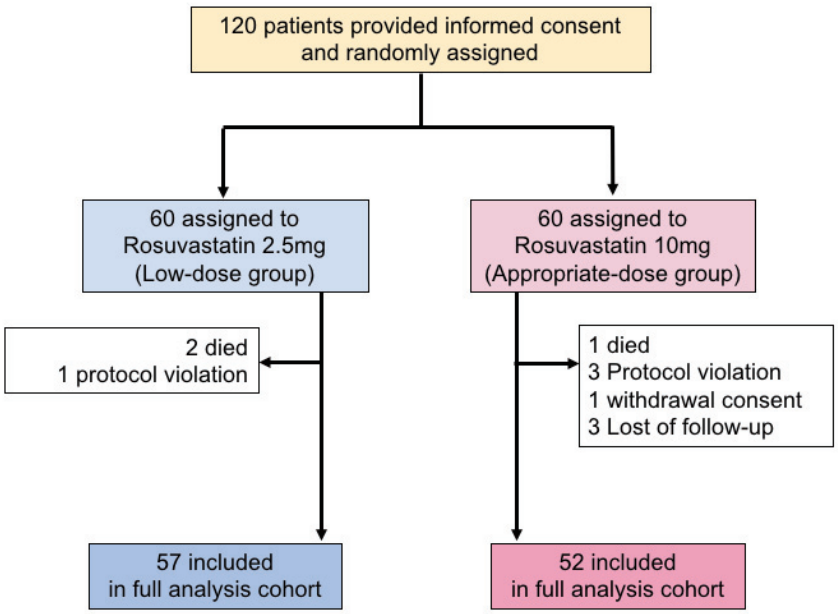
Conflict of Interest

K.S. reports significant research grant support from Daiichi Sankyo, Co., Ltd. Japan, outside the submitted work. K.K. reports significant research grant support from Bayer, Yakuhin, Ltd., Daiichi Sankyo Co., Ltd., Novartis Pharma AG., and SBI, Pharma Co., Ltd., and has received Honoraria from Bayer Yakuhin, Ltd. and Daiichi Sankyo Co., Ltd., outside the submitted work. K.T. reports significant research grant support from Astra Zeneca Kabushiki Kaisha, Sugi Yohoen Kabushiki Kaisha, and Nihon Iryo Kiki Giken Company, Ltd., and has received Honoraria from Kowa Company, Ltd., Sanofi Kabushiki Kaisha, Daiichi Sankyo Company, Ltd., Takeda Pharmaceutical Company, Ltd., Bayer Yakuhin, Ltd., and MSD Kabushiki Kaisha, outside the submitted work. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

References

1) Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM, Pravastatin or Atorvastatin E and Infection Therapy-Thrombolysis in Myocardial Infarction I: Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med, 2004; 350: 1495-1504
2) Khalifa AKM, Kubo T, Ino Y, Terada K, Emori H, Higashioka D, Katayama Y, Takahata M, Shimamura K, Shiono Y, Matsuo Y, Tanaka A, Hozumi T and Akasaka T: Optical Coherence Tomography Comparison of Percutaneous Coronary Intervention Among Plaque Rupture, Erosion, and Calcified Nodule in Acute Myocardial Infarction. Circ J, 2020; 84: 911-916
3) Galis ZS, Sukhova GK, Lark MW and Libby P: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest, 1994; 94: 2493-2503
4) Brown DL, Hibbs MS, Kearney M, Loushin C and Isner JM: Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. Circulation, 1995; 91: 2125-2131
5) Ross R: Atherosclerosis--an inflammatory disease. N Engl J Med, 1999; 340: 115-126
6) Johnson JL: Metalloproteinases in atherosclerosis. Eur J Pharmacol, 2017; 816: 93-106
7) Yasuda S, Miyazaki S, Kinoshita H, Nagaya N, Kanda M, Goto Y and Nonogi H: Enhanced cardiac production of matrix metalloproteinase-2 and -9 and its attenuation associated with pravastatin treatment in patients with acute myocardial infarction. Clin Sci (Lond), 2007; 112: 43-49
8) Matsunaga T, Abe N, Kameda K, Hagii J, Fujita N, Onodera H, Kamata T, Ishizaka H, Hanada H, Osanai T and Okumura K: Circulating level of gelatinase activity predicts ventricular remodeling in patients with acute myocardial infarction. Int J Cardiol, 2005; 105: 203-208
9) Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P and Glynn RJ: Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med, 2017; 377: 1119-1131
10) Nakaya R, Uzui H, Shimizu H, Nakano A, Mitsuke Y, Yamazaki T, Ueda T and Lee JD: Pravastatin suppresses the increase in matrix metalloproteinase-2 levels after acute myocardial infarction. Int J Cardiol, 2005; 105: 67-73
11) Zhang X, Wu D, Choi JC, Minard CG, Hou X, Coselli JS, Shen YH and LeMaire SA: Matrix metalloproteinase levels in chronic thoracic aortic dissection. J Surg Res, 2014; 189: 348-358
12) Kunz P, Sähr H, Lehner B, Fischer C, Seebach E and Fellenberg J: Elevated ratio of MMP2/MMP9 activity is

associated with poor response to chemotherapy in osteosarcoma. BMC Cancer, 2016; 16: 223
13) Fujisue K, Shirakawa T, Nakamura S, Yamamoto N, Oshima S, Matsumura T, Tsunoda R, Hirai N, Tayama S, Nakamura N, Hirose T, Maruyama H, Fujimoto K, Kajiwara I, Sakamoto T, Nakao K, Sakaino N, Hokimoto S, Nagayoshi Y, Hokamaki J, Shimomura H, Sakamoto K, Yamamoto E, Izumiya Y, Kaikita K, Ogawa H, Tsujita K and investigators Is: Dose-dependent INhibitory effect of rosuVastatin In Japanese patienTs with Acute myocardial infarcTION on serum concentration of matrix metalloproteinases - INVITATION trial. J Cardiol, 2018; 72: 350-355
14) Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Thygesen K, Alpert JS, White HD, Jaffe AS, Katus HA, Apple FS, Lindahl B, Morrow DA, Chaitman BR, Clemmensen PM, Johanson P, Hod H, Underwood R, Bax JJ, Bonow JJ, Pinto F, Gibbons RJ, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasche P, Ravkilde J, Ohman EM, Antman EM, Wallentin LC, Armstrong PW, Simoons ML, Januzzi JL, Nieminen MS, Gheorghiade M, Filippatos G, Luepker RV, Fortmann SP, Rosamond WD, Levy D, Wood D, Smith SC, Hu D, Lopez-Sendon JL, Robertson RM, Weaver D, Tendera M, Bove AA, Parkhomenko AN, Vasilieva EJ, Mendis S, Bax JJ, Baumgartner H, Ceconi C, Dean V, Deaton C, Fagard R, Funck-Brentano C, Hasdai D, Hoes A, Kirchhof P, Knuuti J, Kolh P, McDonagh T, Moulin C, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Torbicki A, Vahanian A, Windecker S, Morais J, Aguiar C, Almahmeed W, Arnar DO, Barili F, Bloch KD, Bolger AF, Botker HE, Bozkurt B, Bugiardini R, Cannon C, de Lemos J, Eberli FR, Escobar E, Hlatky M, James S, Kern KB, Moliterno DJ, Mueller C, Neskovic AN, Pieske BM, Schulman SP, Storey RF, Taubert KA, Vranckx P and Wagner DR: Third universal definition of myocardial infarction. J Am Coll Cardiol, 2012; 60: 1581-1598
15) Tousoulis D, Andreou I, Tentolouris C, Antoniades C, Papageorgiou N, Gounari P, Kotrogiannis I, Miliou A, Charakida M, Trikas A and Stefanadis C: Comparative effects of rosuvastatin and allopurinol on circulating levels of matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with chronic heart failure. Int J Cardiol, 2010; 145: 438-443
16) Sapienza P, Borrelli V, Sterpetti AV, Dinicola S, Tartaglia E and di Marzo L: Dose-dependent effect of rosuvastatin in the regulation of metalloproteinase expression. Ann Vasc Surg, 2011; 25: 823-829
17) Guo C, Zhang S, Zhang J, Liu H, Li P, Liu H and Wang Y: Correlation between the severity of coronary artery lesions and levels of estrogen, hs-CRP and MMP-9. Exp Ther Med, 2014; 7: 1177-1180
18) Noji Y, Kajinami K, Kawashiri MA, Todo Y, Horita T, Nohara A, Higashikata T, Inazu A, Koizumi J, Takegoshi T and Mabuchi H: Circulating matrix metalloproteinases and their inhibitors in premature coronary atherosclerosis. Clin Chem Lab Med, 2001; 39: 380-384
19) Iyer RP, de Castro Brás LE, Jin YF and Lindsey ML: Translating Koch's postulates to identify matrix metalloproteinase roles in postmyocardial infarction remodeling: cardiac metalloproteinase actions (CarMA) postulates. Circ Res, 2014; 114: 860-871
20) Wang T, Jin X, Liao Y, Sun Q, Luo C, Wang G, Zhao F and Jin Y: Association of NF-κB and AP-1 with MMP-9 Overexpression in 2-Chloroethanol Exposed Rat Astrocytes. Cells, 2018; 7:
21) Nishiguchi T, Tanaka A, Taruya A, Emori H, Ozaki Y, Orii M, Shiono Y, Shimamura K, Kameyama T, Yamano T, Yamaguchi T, Matsuo Y, Ino Y, Kubo T, Hozumi T, Hayashi Y and Akasaka T: Local Matrix Metalloproteinase 9 Level Determines Early Clinical Presentation of ST-Segment-Elevation Myocardial Infarction. Arterioscler Thromb Vasc Biol, 2016; 36: 2460-2467
22) Renko J, Kalela A, Jaakkola O, Laine S, Höyhtyä M, Alho H and Nikkari ST: Serum matrix metalloproteinase-9 is elevated in men with a history of myocardial infarction. Scand J Clin Lab Invest, 2004; 64: 255-261
23) Wagner DR, Delagardelle C, Ernens I, Rouy D, Vaillant M and Beissel J: Matrix metalloproteinase-9 is a marker of heart failure after acute myocardial infarction. J Card Fail, 2006; 12: 66-72
24) Chen YJ and Chang LS: Simvastatin induces NFκB/p65 down-regulation and JNK1/c-Jun/ATF-2 activation, leading to matrix metalloproteinase-9 (MMP-9) but not MMP-2 down-regulation in human leukemia cells. Biochem Pharmacol, 2014; 92: 530-543
25) Hojo Y, Ikeda U, Ueno S, Arakawa H and Shimada K: Expression of matrix metalloproteinases in patients with acute myocardial infarction. Jpn Circ J, 2001; 65: 71-75



Supplementary Fig. 1. Study flow diagram