Matrix Metalloproteinase-9 in the Culprit Coronary Artery and Myocardial No-Reflow

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Abstract: *Background:* Matrix Metalloproteinases (MMPs) have been implicated in the pathogenesis of acute myocardial infarction (AMI). However, little is known about the association between MMP-9 and myocardial no-reflow. The aim of this study was to evaluate the role of MMP-9 in the culprit coronary artery as a predictor of no-reflow in patients with ST-elevation AMI. *Methods:* Ninety patients with ST-elevation AMI who underwent emergency percutaneous coronary intervention were consecutively recruited in this study. Blood samples were obtained from the extraction catheter placed distal to the culprit lesion at the beginning of percutaneous coronary intervention. No-reflow was defined as a coronary thrombolysis in myocardial infarction flow grade ≤2 after vessel reopening or thrombolysis in myocardial infarction flow 3 with a final myocardial blush grade ≤2. *Results:* No-reflow was observed in 25 patients (27.8%). Using multiple logistic regression analysis, local MMP-9 levels (odds ratio [OR] = 3.356; confidence interval [CI]: 1.441–5.881; $P = 0.007$) were found to be a significant risk factor of no-reflow together with lesion length (OR = 6.985; CI: 2.574–11.533; $P = 0.009$) and time to balloon (OR = 2.143; CI: 1.216–5.901; $P = 0.042$). *Conclusions:* Elevation of MMP-9 level in the culprit coronary artery may predict no-reflow in patients with ST-elevation AMI.

Key Indexing Terms: MMP-9; No-reflow; Inflammation; Acute myocardial infarction. **[Am J Med Sci 2015;350(5):352–356.]**

Myocardial no-reflow is an independent predictor of cardiovascular adverse outcome, which may result from multiple mechanisms, including mechanical plugging secondary to distal embolization, external compression by edematous tissue, *in situ* thrombosis, vasospasm, activation of inflammatory cascades and reperfusion injury.[1,2] However, the underlying mechanisms are still not clearly understood and the reliable predictors of no-reflow after percutaneous coronary intervention (PCI) have not been established.

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases, secreted by a variety of inflammatory or tumor cells as zymogens. Among the MMPs, MMP-9 is highly expressed in the vulnerable regions of the atherosclerotic plaque and reflects plaque vulnerability.[3,4] Plasma MMP-9 level is elevated in patients with acute myocardial infarction (AMI).[5,6] However, little is known about the association between MMP-9 and myocardial no-reflow. Therefore, the aim of this study was to evaluate the role of MMP-9 as a predictor of no-reflow in patients with AMI.

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MATERIALS AND METHODS

Patients

Ninety-five eligible AMI patients who underwent emergency PCI between October 2012 and November 2013 at Yantai Yuhuangding Hospital were consecutively recruited. AMI was defined as prolonged chest pain (>30 minutes) not responding to nitroglycerin infusion, ST-segment elevation ≥0.2 mV in 2 or more adjacent leads on standard electrocardiogram, more than double the upper normal limits of creatine kinase (CK), creatine kinase, muscle and brain (CK-MB), or relative index and successful primary PCI performed within 12 hours of the onset of chest pain. The patients included in this study were newly diagnosed AMI without history of previous coronary artery disease or heart failure. Stent implantation was successfully performed in all patients. No significant side-branch occlusion occurred during the procedure. Overall, 5 patients were excluded from the study, due to time from symptom on set ≥12 hours (n = 3) or missing of blood sample for MMP-9 measurement (n = 2). Thus, 90 patients were eventually included in the study. All the participants gave written consent for the investigation. The hospital Ethics Committee approved the study protocols, and written informed consent was obtained from all of the patients to participate in the study.

Venous peripheral blood samples for systemic MMP-9 assessment were obtained in all patients on admission. Serum levels of cardiac enzymes (CK-MB) were measured every 4 hours during the 1st day and every 24 hours in the following 3 days. Fasting peripheral venous blood samples were obtained from all of the study subjects for measuring triglycerides, total cholesterol, high-density lipoprotein and low-density lipoprotein. For other measurements, plasma samples separated by centrifugation were stored at −80°C until analysis.

Primary PCI

For each patient, 300 mg of aspirin and 300 mg of clopidogrel were administered orally in the emergency department. The patients were transferred to the cardiac catheterization laboratory and PCI was then performed. Before PCI, each patient was given 5,000 units of heparin. Glycoprotein IIb/IIIa inhibitors were used at the operator discretion. Selective coronary angiography was performed after the intracoronary administration of nitroglycerin and at least 5 standardized views of the left coronary artery and 3 views of the right coronary artery were obtained. After conventional wire crossing and Diver CE (Innovative technologies, Italy) using, direct stenting implantation was performed Brescia whenever possible, preceded by balloon predilatation if necessary.

Evaluation Coronary Blood Flow

Coronary angiography was evaluated by quantitative coronary angiography analysis.[7] For objective evaluation of coronary blood flow, thrombolysis in myocardial infarction

TABLE 1. General characteristics and demographic data of patients

	Reflow (n = 65)	No-reflow (n = 25)	*P*
Age, yr	62.9 ± 10.3	64.8 ± 15.2	0.255
Male, n (%)	40 (61.5)	14 (56.0)	0.631
BMI, kg/m^2	25.9 ± 2.8	26.3 ± 5.5	0.328
Smoking, n (%)	40 (61.5)	16 (64.0)	0.829
Hypertension, n (%)	35 (53.9)	13 (52.0)	0.875
DM, n (%)	20 (30.8)	15 (60.0)	0.011[a]
Heart rate, bpm	68.8 ± 9.6	66.2 ± 14.1	0.443
SBP, mm Hg	125.3 ± 16.7	115.3 ± 20.3	0.314
DBP, mm Hg	78.8 ± 5.6	79.3 ± 8.9	0.535
CK-MB peak, ng/mL	209.9 ± 61.55	227.9 ± 80.92	0.282
Total cholesterol, mmol/L	5.53 ± 1.17	5.49 ± 2.46	0.718
HDL cholesterol, mmol/L	1.29 ± 0.17	1.33 ± 0.25	0.348
LDL cholesterol, mmol/L	3.26 ± 0.53	3.14 ± 0.87	0.463
Triglycerides, mmol/L	2.91 ± 0.43	2.53 ± 0.88	0.287
Systemic MMP-9, pg/mL	143.2 ± 34.80	152.8 ± 45.58	0.345
Aspirin therapy, n (%)	60 (92.3)	22 (88.0)	0.520
Clopidogrel therapy, n (%)	10 (15.4)	4 (16.0)	0.942
Statin therapy, n (%)	30 (46.2)	12 (48.0)	0.875
ACE-inhibitor therapy, n (%)	20 (30.8)	8 (32.0)	0.910
Calcium antagonists, n (%)	22 (33.8)	9 (36.0)	0.847
β-blocker therapy, n (%)	31 (47.7)	11 (44.0)	0.753
Nitrates therapy, n (%)	40 (61.5)	15 (60.0)	0.893

[a] Statistically significant.

ACE, angiotensin-converting enzyme; BMI, body mass index; CK, creatine kinase; DBP, diastolic blood pressure; DM, diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MMP-9, matrix metalloproteinase-9; SBP, systolic blood pressure.

TABLE 2. Angiographic and procedural findings

	Reflow (n = 65)	No-reflow (n = 25)	*P*
Time to balloon, hr	5.60 ± 1.73	10.31 ± 3.45	0.043[a]
No. diseased vessels	1.89 ± 0.56	2.12 ± 0.78	0.785
Culprit vessel, n (%)			0.964
LAD	43 (66.2)	14 (56.0)	
LCX	8 (12.3)	2 (8.0)	
RCA	14 (21.5)	9 (36.9)	
Target lesion location, n (%)			0.494
Proximal	28 (43.1)	10 (40.0)	
Mid	37 (56.9)	15 (60.0)	
Distal	0 (50)	0 (0)	
Lesion length, mm	26.7 ± 8.3	37.1 ± 13.2	0.009[a]
Lesion length >20 mm	25 (38.5)	10 (40.0)	0.893
Reference luminal diameter, mm	4.89 ± 0.75	4.82 ± 1.95	0.058
Thrombus score ≥4, n (%)	34 (52.3)	20 (80.0)	0.016[a]
IABP use	15 (23.1)	6 (24.0)	0.926
Rentrop collaterals grading 0, n (%)	65 (100)	25 (100)	1
Local MMP-9, pg/mL	150.22 ± 35.19	186.77 ± 40.83	0.032[a]
No. stents	1.53 ± 0.52	1.83 ± 0.87	0.087
Final TIMI flow 3, n (%)	65 (100)	8 (32.0)	<0.001[a]
TIMI frame count, frames	28.62 ± 6.65	54.14 ± 16.25	<0.001[a]
Final myocardial blush grade 3, n (%)	65 (100)	0 (0)	<0.001[a]

[a] Statistically significant.

IABP, intra-aortic balloon pump; LAD, left anterior descending artery; LCX, left circumflex artery; MMP-9, matrix metalloproteinase-9; RCA, right coronary artery; TIMI, thrombolysis in myocardial infarction.

(TIMI) flow grades were assessed as previously described,[8] corrected thrombolysis in myocardial infarction frame count method developed by Gibson et al[9] was adopted. Myocardial blush grade (MBG) was done according to the criteria of van't Hof et al.[10]

Because TIMI flow grades identify primarily epicardial blood flow, MBG was used to assess microvascular perfusion. In this study, angiographic coronary no-reflow was defined as a coronary TIMI flow grade ≤2 after vessel reopening or TIMI flow 3 with a final MBG ≤2.[11] Angiographic assessment was always performed by 2 independent angiographers who were unaware of MMP-9 results, and final agreement was 90%, with discordances being resolved by consensus.

Blood Sampling and Measurements of MMP-9

Blood samples were obtained from the extraction catheter placed distal to the lesion at the beginning of PCI. The blood was immediately mixed with sodium ethylene diamine tetraacetic acid (EDTA), centrifuged at 3,000 revolutions per minute for 5 minutes, separated and stored at −80°C. Plasma levels of MMP-9 were determined in duplicate using the RayBio enzyme-linked immunosorbent assay kits (RayBiotech Inc, Norcross, GA).

Statistical Analyses

Comparisons between groups were done by *t* test for continuous variables and by Fisher's exact test for discrete variables. Variables significantly associated with no-reflow risk in the univariate analysis were considered confounders and further included in the multivariate model. All statistical analyses were performed using SAS version 8.1 (SAS Institute Inc, Cary, NC). Statistical significance was determined when $P < 0.05$.

RESULTS

Clinical Characteristics of Patients

Of the 90 patients who underwent emergency PCI, 25 (27.8%) developed no-reflow. Table 1 shows a comparison of the baseline characteristics of the patients. No difference was found between reflow group and no-reflow group regarding age, gender, frequencies of major coronary risk factors (eg,