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Local increase in microparticles from the aspirate of culprit coronary arteries in patients with ST-segment elevation myocardial infarction

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ABSTRACT

Objective: It has been reported that the levels of procoagulant microparticles (MPs) are increased in patients with acute coronary syndromes and this may contribute to the formation of intracoronary thrombi. In the current study, we investigated the presence of locally elevated MPs within the culprit coronary arteries of patients with ST-segment elevation myocardial infarction (STEMI).
Methods: The study population consisted of 45 patients with STEMI who underwent primary percutaneous coronary intervention (PCI), and 16 control patients. Before and after PCI, blood samples were collected from the femoral artery and from the culprit coronary arteries. In controls, only peripheral blood was obtained. MPs were measured by a solid-phase capture assay using a commercial kit. The cell origins of MPs were determined by antigenic capture with specific antibodies.
Results: Baseline levels of MPs in patients with STEMI were higher than in controls. Before PCI, the levels of MPs were significantly higher in culprit coronary arteries than in peripheral arteries in STEMI patients (20.7 ± 15.5 *vs.* 14.6 ± 15.4 nM phosphatidylserine (PS) equivalent, $p = 0.027$). MPs from the culprit coronary artery were significantly reduced after PCI (20.7 ± 15.5 *vs.* 14.3 ± 14.9 nM PS equivalent, $p = 0.010$). Similarly, the locally increased levels of endothelial- and platelet-derived MPs within the culprit coronary arteries were significantly decreased after PCI.
Conclusion: Locally increased levels of MPs in culprit coronary arteries and their significant reduction after successful PCI suggest a potential role in coronary atherothrombosis in the early period of STEMI.

1. Introduction

Microparticles (MP) are submicron membrane particles released by various cell types following cellular activation or apoptosis. MPs circulate in the blood and can also accumulate in atherosclerotic lesions. They promote coagulation and play a significant role in inflammatory process and vascular function [1]. Due to their complex procoagulant and proinflammatory properties, extensive research has been performed on their role in atherothrombotic diseases [2]. It has been reported that levels of procoagulant MPs are increased in patients with acute coronary syndromes (ACS) and may contribute to the formation of intracoronary thrombi [3–6]. Recently, elevation in the levels of procoagulant MPs has been reported in the occluded coronary arteries of a small number of patients with ST-segment elevation myocardial infarction (STEMI) [7]. However, no data are available comparing baseline MPs between the peripheral arteries and infarct-related arteries before percutaneous coronary intervention (PCI). Therefore, we have yet to fully elucidate whether procoagulant MPs are elevated locally within the culprit coronary arteries in patients with STEMI compared with the peripheral arteries. In the present study, we determined the levels and cellular origins of MPs in the aspirate from culprit coronary arteries of patients with STEMI who underwent primary PCI with intracoronary aspirations and compared these with levels in blood drawn from peripheral arteries. We also investigated whether the locally elevated levels of MPs were decreased by PCI with intracoronary aspirations.

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2. Materials and methods

2.1. Study population

We prospectively included 45 patients with STEMI who underwent primary PCI, and 16 controls with normal coronary arteries who underwent coronary angiography for evaluation of chest pain. STEMI was defined as resting chest pain lasting ≥30 min, together with new or presumed new ST segment elevation in ≥2 contiguous leads with the cut-off points ≥0.2 mV in leads V1, V2, or V3 and ≥0.1 mV in other leads [8]. In all patients, myocardial damage was confirmed by an elevation of CK-MB (>2 times the upper reference limit) during the hospital stay. All patients were enrolled from Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. Major criteria for exclusion were chronic kidney disease (serum creatinine > 2.0 mg/dL), apparent infectious disease, malignancy, chronic inflammatory disorders, and major trauma.

The study protocol was approved by our local Institutional Review Board (Gangnam Severance Hospital, Yonsei University College of Medicine) and all participants provided written informed consent.

2.2. Study procedure and blood sampling

All patients with STEMI were treated with a loading dose of oral aspirin (300 mg), clopidogrel (600 mg), and intravenous heparin (10,000 IU) prior to PCI. If necessary, a repeat bolus of heparin was given to maintain an activated clotting time of 250–300 s. Coronary angiography was performed using conventional methods *via* a femoral approach. A first 20 mL blood sample from peripheral artery (P1) was obtained through the vascular sheath in the femoral artery prior to PCI. The culprit coronary artery was engaged with a 6F guiding catheter and subsequently wired with a 0.014 inch guide wire. After crossing the culprit lesion, a monorail aspiration catheter (Export, Medtronic, Minneapolis, MN, USA) was advanced a few centimeters distal to the culprit lesion. A 20 mL blood aspirate from the culprit coronary artery (C1) was collected during intracoronary aspiration with the Export catheter. Patients were excluded from the study if the aspiration catheter failed to pass the culprit lesion for any reason. Balloon angioplasty and stent implantation were then performed according to standard techniques. Intravenous glycoprotein IIb/IIIa inhibitor was used at the discretion of the operator during the procedure. Post-PCI blood samples were obtained at the end of PCI from the culprit coronary artery (C2) using the aspiration catheter and from the peripheral artery (P2) through the femoral sheath. In the control group, a loading dose of intravenous heparin (2000 IU) was given prior to diagnostic coronary angiography, and only peripheral blood from the femoral artery was drawn following coronary angiography.

All blood samples were drawn into evacuated collection tubes containing sodium citrate (0.109 M). The plasma supernatant was rapidly decanted following a 15 min centrifugation at 1500 × *g* at room temperature and was then again rapidly centrifuged for 2 min at 13,000 × *g* at room temperature. The platelet-poor plasma containing circulating MPs obtained by double centrifugation was then stored frozen at −80 °C.

2.3. Microparticle assay

The frozen samples were thawed for 15 min at 37 °C just prior to analysis. MPs with procoagulant potential were measured using a solid-phase capture assay from a commercial kit (ZYMUPHEN MP-Activity kit; Hyphen BioMed, France). In brief, MPs were isolated by capture onto immobilized annexin V, and the amount of captured MPs was determined by a prothrombinase assay using their procoagulant potential [9]. The solid-phase capture assay combined with the prothrombinase assay provides a functional assessment of the procoagulant potential of isolated circulating MPs, regardless of the capture ligand [9].

The cell origins of the MPs were determined by antigenic capture with insolubilized specific antibodies instead of annexin V using similar solid-phase capture methods [9]. In the present study, the following biotinylated monoclonal antibodies were used: anti-CD11a (leukocytes), anti-CD31 (endothelial cells), anti-CD42b (endothelial cells) (Abcam, Cambridge, UK), and anti-CD146 (platelets) (Millipore, Billerica, MA, USA).

Results were expressed as phosphatidylserine equivalents (PS eq), calculated using the standard calibration curve constructed using liposomes of known concentration. All tests were performed in duplicate.

2.4. Statistical analysis

Continuous data are expressed as the mean ± S.D. and categorical data are presented as numbers and percentages. Differences in categorical variables were analyzed using the chi-square test, and continuous variables were analyzed using the Mann–Whitney *U*-test. Levels of MPs in each of the samples (P1, C1, C2, and P2) were compared using the Wilcoxon's signed rank test. A 2-tailed *p* value <0.05 was considered statistically significant. All statistical analyses were performed with PASW statistics version 18.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

Baseline characteristics are listed in Table 1. Age was not significantly different between the STEMI and control groups. In the STEMI group, primary PCI was performed within 12 h from the onset of chest pain, and door-to-balloon time was 1.9 ± 1.5 h. The culprit artery was left anterior descending artery in 29 patients (64%), right coronary artery in 12 patients (27%), and left circumflex artery in 4 patients (9%). Twenty-six patients (58%) had multivessel coronary artery disease. The initial Thrombolysis In Myocardial Infarction (TIMI) flow grade was 0 or 1 in 29 patients (64%). Optimal PCI results (TIMI flow 3 grade) were achieved in 93% of patients. In 18 patients, glycoprotein IIb/IIIa inhibitor was used as an initial bolus of abciximab 0.25 mg/kg followed by a 12 h infusion of 0.125 μg/kg/min during the PCI procedure.

The baseline level of microparticles in peripheral blood was significantly elevated in patients with STEMI compared with patients with normal coronary arteries (Table 2, Fig. 1A). In particular, circulating MPs captured with anti-CD42b or anti-CD146 were significantly elevated in STEMI patients compared with controls,

Table 1
Baseline characteristics.

	STEMI ($n = 45$)	Control ($n = 16$)	p
Age (years)	56 ± 15	62 ± 10	0.129
Male	40 (89%)	9 (56%)	0.005
Hypertension	14 (31%)	12 (75%)	0.002
Diabetes mellitus	13 (29%)	5 (31%)	0.859
Dyslipidemia	15 (33%)	4 (25%)	0.536
Smokers	31 (69%)	6 (38%)	0.027
Leukocytes (10^3/μL)	10.96 ± 4.06	6.10 ± 1.43	<0.001
Serum creatinine (mg/dL)	1.0 ± 0.2	0.9 ± 0.2	0.084
BNP (pg/mL)	143.3 ± 147.5	103.6 ± 109.2	0.506
hs-CRP (mg/L)	9.1 ± 25.8	1.0 ± 1.3	<0.001
Door-to-balloon time (hours)	1.9 ± 1.5	–	–
Peak CK-MB (μg/L)	249.1 ± 449.6	–	–
Peak Troponin T (μg/L)	4.8 ± 5.7	–	–

STEMI, ST-segment elevation myocardial infarction; BNP, brain natriuretic peptide; hs-CRP, high sensitivity C-reactive protein.