Circulating Microparticles From Patients With Myocardial Infarction Cause Endothelial Dysfunction

Chantal M. Boulanger, PhD; Alexandra Scoazec, BSc; Talin Ebrahimian, BSc; Patrick Henry, MD, PhD; Eric Mathieu; Alain Tedgui, PhD; Ziad Mallat, MD, PhD

Background—Shed membrane microparticles circulate in the peripheral blood of nonischemic (NI) patients and patients with myocardial infarction (MI). We investigated whether or not these microparticles would affect endothelium-dependent responses.

Methods and Results—Rat aortic rings with endothelium were exposed for 24 hours to circulating microparticles isolated from 7 patients with NI syndromes and 19 patients with acute MI. Endothelium-dependent relaxations to acetylcholine were not affected by high concentrations of microparticles from NI patients (P=0.80). However, significant impairment was observed in preparations exposed to microparticles from patients with MI at low and high concentrations, corresponding to 0.7-fold and 2-fold circulating plasma levels (P=0.05 and 0.001, respectively). Impairment was not affected by diclofenac (P=0.47), nor by the cell-permeable superoxide dismutase mimetic Mn(III)tetra(4-benzoic acid) porphyrin chloride (P=0.33), but it was abolished by endothelium removal or by N’-monomethyl-L-arginine. Relaxations to the calcium ionophore ionomycin were decreased in rings exposed to microparticles from MI patients (P=0.05 and 0.009 for low and high concentrations, respectively), but microparticles from NI patients had no effect (P=0.81). Finally, high concentrations of microparticles from MI patients affected neither endothelium-independent relaxation to sodium nitroprusside (P=0.59) nor expression of the endothelial nitric oxide synthase (P=0.43).

Conclusions—Circulating microparticles from patients with MI selectively impair the endothelial nitric oxide transduction pathway and, therefore, could contribute to the general vasomotor dysfunction observed after MI, even in angiographically normal arteries. (Circulation. 2001;104:2649-2652.)

Key Words: endothelium-derived factors ■ nitric oxide synthase ■ arteries ■ vasodilation ■ myocardial infarction

Microparticles generated in vitro from activated platelets or leukocytes stimulate cultured endothelial cells to produce prostacyclin and cytokines and to express adhesion molecules.1–4 Recently, apoptotic microparticles have been identified in the circulating blood of patients with acute coronary syndromes and in nonischemic (NI) patients.5 Acute myocardial infarction (MI) is associated with substantial impairment of vasodilator function in both infarcted myocardium and myocardium perfused by normal vessels. The determinants of this vasomotor dysfunction that may contribute to the extent of ischemia and necrosis after coronary occlusion are not fully elucidated.6 In the present study, we examined whether or not microparticles circulating in the peripheral blood of NI patients and patients with acute MI affect endothelium-dependent responses in a normal blood vessels.

Methods

Patient Selection
We prospectively included 19 patients with acute MI and 7 NI patients. Patients with MI had clinical, electrocardiographic, and enzymatic changes that were diagnostic of acute MI and were subsequently documented as having significant coronary atherosclerosis by angiography (Table). Control NI patients consisted of 4 patients recruited from the same cardiology department and 3 healthy subjects. Because relaxations did not differ among them (P=0.35), these 7 subjects were pooled into one control NI group (mean age, 49±7 years). Patients with acute MI received standard antiplatelet and antithrombotic therapy before blood sampling; other treatments are listed in the Table. All patients and subjects provided informed consent.

Isolation of Circulating Microparticles
Citrated fasting venous blood samples were collected between days 0 and 4. Platelet-free plasma (2 mL; obtained after centrifugation at 11 000g for 2 minutes) was subjected to centrifugation at 13 000g for 45 minutes. The microparticle pellets were resuspended in 100 μL of RPMI medium (vehicle) and stored at −80°C until further analysis. The microparticle protein content (Biorad assay) averaged 5.0 μg/mL polymyxin-B in the presence of vehicle alone or with

Organ Chamber Experiments
Rings from rat thoracic aortas (12-week-old male Wistar rats) were incubated (24 hours; 37°C) in Dulbecco’s modified eagle medium with 10 μg/mL polymyxin-B.
microparticles from NI or MI patients to investigate the effects of these particles on endothelial function and nitric oxide (NO) synthase expression. Final microparticle concentrations corresponded to 0.7-fold and 2-fold their circulating plasma levels after 30-fold and 10-fold dilutions in vehicle and are referred to as low and high concentrations, respectively. Care and use of laboratory animals conformed to European Community standards. After 24 hours, the rings were mounted in organ chambers filled with modified Krebs-Ringer solution supplemented with 10 μg/mL polymyxin-B to study endothelium-dependent and -independent responses during contraction to norepinephrine (0.2 to 1 μmol/L, to reach 85% of its maximal response).3

Western Blot
Proteins of aortic rings were extracted,7,8 and equal amounts were loaded on SDS-polyacrylamide gels. Blots were incubated with a monoclonal endothelial NO synthase antibody9 or with a polyclonal CD31 antibody (dilution 1:200).

Statistical Analysis
Data are given as mean±SEM. Contractions are expressed in milligrams for increase in tension, and relaxations as percent inhibition of the norepinephrine contraction. Statistical analysis was performed using ANOVA for repeated measures. Differences were considered significant at P<0.05.

Results
Circulating microparticles from patients with MI significantly decreased relaxations to acetylcholine in rat aortic rings at both low and high concentrations when compared with vehicle alone (P=0.05 and P=0.001, respectively; n=9 to 10), whereas microparticles from NI patients, at high concentrations, had no effect (P=0.80; n=7; Figure 1A). The acetylcholine relaxation was significantly impaired by microparticles from MI patients when compared with NI patients (P=0.004). The inhibitory effect of microparticles from MI patients was independent of their protein concentration (r²=0.12, data not shown). Exposure to the supernatant from MI patient microparticle preparations (5% v/v; 24 hours) did not affect acetylcholine relaxations (P=0.40; n=6).

In preparations exposed to high concentrations of microparticles from MI patients, the impaired relaxation to acetylcholine was not affected by the cell-permeable superoxide dismutase mimetic Mn(III)tetra(4-benzoic acid) porphyrin chloride (Biomol-France), nor by the cyclooxygenase inhibitor diclofenac (P=0.33 and 0.47, respectively; Figure 1B). However, the acetylcholine response was abolished by endothelium removal or by the addition of the NO synthase inhibitor Nω-monomethyl-L-arginine (Figure 1B).

Relaxations to the calcium ionophore ionomycin were also significantly decreased by microparticles from patients with MI at both low and high concentrations (P=0.05 and 0.009, respectively; n=9 to 10), whereas those from NI patients had no effect (P=0.81, n=7; Figure 1C).

Microparticles from MI patients (high concentration) did not significantly affect the maximal relaxation to sodium nitroprusside (vehicle, 103±2%; high concentration, 101±2%; P=0.55; n=6) or the median effective concentration (vehicle, 8.4±3.8 μmol/L; high concentration, 14.2±5.6 μmol/L; P=0.41; n=6). In addition, microparticles from MI patients did not affect the maximal contraction to norepinephrine (vehicle, 4260±260 mg; high concentration, 4162±234 mg; P=0.76; n=9), but they did significantly shift the median effective concentration from 40.5±7.5 to 70.6±6.7 μmol/L (P=0.008; n=9).

Incubation of aortic rings with NI or MI microparticles (high concentrations) did not affect the expression of endothelial NO synthase relative to that measured in vehicle-treated preparations (P=0.28 and P=0.41, respectively; n=7). Similarly, expression of CD-31 was not affected in the 2 groups (P=0.16 and P=0.75, n=7; Figure 2).

Discussion
The present study demonstrates that circulating microparticles from patients with acute MI cause severe endothelial dysfunction in healthy blood vessels by affecting the endothelial NO transduction pathway. This inhibitory effect is specific to microparticles from patients with MI, because those obtained from NI patients, at an equivalent protein concentration, do not affect endothelium-dependent responses.

Endothelium-derived NO is the major mediator involved in relaxations to acetylcholine in the isolated rat aorta.7 The impairment of acetylcholine-induced relaxations by microparticles from patients with MI is not mediated by the concomitant release of vasoconstricting substances, as suggested by the lack of effect of the cyclooxygenase inhibitor diclofenac and the absence of an acetylcholine response in preparations without endothelium and in preparations exposed to a NO synthase inhibitor. A contribution of superox-
ide anions to the dysfunction also seems unlikely, because a cell-permeable superoxide dismutase mimetic had no effect at a concentration that improves endothelial function in an atherosclerosis model. In addition, an effect of microparticles on the mechanism of action of NO on vascular smooth muscle can be ruled out, because the relaxations to sodium nitroprusside were unaltered. Therefore, the present study suggests that microparticles from patients with MI impair the endothelial NO pathway, without changes in endothelial NO synthase expression. Although the cellular mechanism of action of microparticles remains to be investigated, our data demonstrate that microparticles can affect the post-receptor NO signal transduction pathway, because the relaxation in response to the non-receptor-mediated agonist ionomycin was impaired in a manner similar to the acetylcholine response. Our data on microparticle effects in the rat aorta does not reveal a role for other relaxing factors, such as endothelium-derived hyperpolarizing factor (EDHF).

It could be argued that the specific inhibitory effect of microparticles seen in this study is a result of different levels of circulating microparticles between NI patients and patients with MI. Indeed, the level of circulating microparticles, as estimated by their endogenous content in phosphatidylserine, was shown to be 2-fold higher in patients with MI. However, this interpretation is unlikely because a significant inhibitory effect was also observed with microparticles from patients with MI at a concentration 3 times lower than that of patients with NI syndromes. Furthermore, the different phosphatidylserine content is unlikely to explain the specific endothelial dysfunction caused by microparticles from patients with MI, because phosphatidylserine itself does not affect endothelium-dependent relaxations. In addition, background medication in the MI group may have contributed to these effects. Finally, the present results should be confirmed in a larger, age-matched population.

In conclusion, shed membrane microparticles present in the peripheral blood of patients with MI impair endothelial NO-mediated relaxation in normal blood vessels. This inhibitory effect is likely to be clinically relevant, because it was also observed in our experiments at concentrations lower than those of microparticles in circulating plasma in vivo. The severe and specific endothelial dysfunction caused by circu-
lating microparticles from patients with MI may contribute to the general impairment of vasodilator responses observed after MI, even in angiographically normal blood vessels. Thus, circulating microparticles might amplify myocardial ischemia after coronary occlusion.

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