Circulating microparticles from patients with valvular heart disease and cardiac surgery inhibit endothelium-dependent vasodilation

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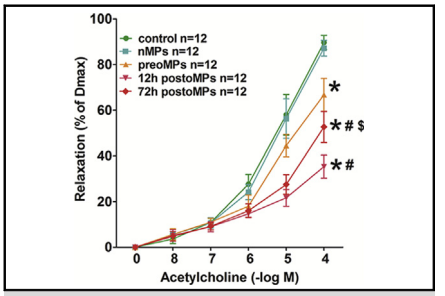
ABSTRACT

Objective: Vascular function is very important for maintaining circulation after cardiac surgery. Circulating microparticles (MPs) generated in various diseases play important roles in causing inflammation, coagulation, and vascular injury. However, the impact of MPs generated from patients who have valvular heart disease (VHD), before and after cardiac surgery, on vascular function remains unknown. This study is designed to investigate the impact of such MPs on vasodilation.

Methods: Microparticles were isolated from age-matched healthy subjects and patients who had VHD, before cardiac surgery, and at 12 hours and 72 hours afterward. The number of MPs was measured and compared. Effects evaluated were of the impact of MPs on: vasodilation of mice aorta; the phosphorylation and expression of Akt, endothelial nitric oxide synthase (eNOS), protein kinase C-βII (PKC-βII), and p70 ribosomal protein S6 kinase (p70S6K); expression of caveolin-1; the association of eNOS with heat shock protein 90 (HSP90); and generation of nitric oxide and superoxide anion of human umbilical vein endothelial cells.

Results: Compared with the healthy subjects, VHD patients had significantly higher levels of circulating MPs and those MPs before cardiac surgery can: impair endothelium-dependent vasodilation; inhibit phosphorylation of Akt and eNOS; increase activation of PKC-βII and p70S6K; enhance expression of caveolin-1; reduce the association of HSP90 with eNOS; decrease nitric oxide production, and increase superoxide anion generation. These deleterious effects were even stronger in postoperative MPs.

Conclusions: Our data demonstrate that MPs generated from VHD patients before and after cardiac surgery contributed to endothelial dysfunction, by uncoupling and inhibiting eNOS. Circulating MPs are potential therapeutic targets for the maintenance of vascular function postoperatively. (J Thorac Cardiovasc Surg 2015;150:666-72)



Microparticles from patients who had VHD and underwent cardiac surgery were found to impair endothelium-dependent vasodilation.

Central Message

Microparticles from VHD patients before and after cardiac surgery were found to impair endothelial function, and therefore, may be appropriate therapeutic targets.

Perspective

Microparticles play important roles in vascular injury. We demonstrated that MPs from VHD patients before and after cardiac surgery can impair endothelium-dependent vasodilation by uncoupling eNOS and inhibiting its activity. Our findings can partly explain the hemodynamic instability that occurs after cardiac surgery, indicating that MPs are potential therapeutic targets for increasing hemodynamic stability.

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Abbreviations and Acronyms

eNOS = endothelial nitric oxide synthase
HUVEC = human umbilical vein endothelial cell
L-NAME = NG-nitro-L-arginine methyl ester
MP = microparticle
PKC-βII = protein kinase C-βII
p70S6K = p70 ribosomal protein S6 kinase
Ser = serine
VHD = valvular heart disease

Circulating microparticles (MPs) are a group of membrane vesicles that are released by cell activation or apoptosis. Studies have demonstrated that circulating MPs, or certain subgroups of MPs, increase in various cardiovascular diseases, such as hypertension, acute coronary syndromes, and mitral valve disease.[1-3] In addition, MPs have important physiologic and pathophysiologic functions, including coagulation, inflammation, and vascular function.[4,5]

Valvular heart disease (VHD) is among the main heart problems globally. Most VHD patients need cardiac surgery to repair or replace the defective valve under cardiopulmonary bypass. During the perioperative period of such surgery, hemodynamic status is unstable. In addition, such surgery can lead to a systemic inflammatory response and cause difficulties in maintaining circulation stability.[6] Preventza and colleagues[7] additionally reported that the time to complete cardiopulmonary bypass was related to mortality after surgery.

Recently, we demonstrated that vascular function was impaired by proinflammatory high-density lipoprotein during cardiac surgery in VHD patients, via uncoupling of endothelial nitric oxide synthase (eNOS).[8] Boulanger and colleagues[9] showed that MPs from patients with myocardial infarction impaired endothelium-dependent vasodilation. Martin and colleagues[5] further demonstrated that endothelial function can be altered by T lymphocyte–derived MPs, through effects on nitric oxide and regulation of eNOS and caveolin-1. Therefore, in the present study, we investigated whether MPs from VHD patients undergoing cardiac surgery would lead to vascular dysfunction.

METHODS

Study Populations

A total of 42 patients diagnosed with VHD were recruited from the First Affiliated Hospital, Sun Yat-sen University. Patients who suffered from coronary heart disease, infectious disease, diabetes, renal failure, or severe trauma, or who had undergone surgery within the preceding 3 months, were excluded. All studied subjects were age >18 years. A total of 22 healthy subjects age >18 years were enrolled as a control group. This study was approved by the Ethics Review Board of the First Affiliated Hospital, Sun Yat-sen University. Informed consent was obtained from all subjects enrolled in this study. The clinical characteristics of the study population are described in Table 1 and operation data and postoperative drug of the VHD patients are described in Table 2.

Isolation of Blood Samples

Blood samples were drawn from the healthy subjects, and from the patients both before, and at 12 and 72 hours after, they underwent cardiac surgery with cardiopulmonary bypass. MPs were isolated as described elsewhere.[9] Briefly, the blood samples were centrifuged at 11,000 g for 2 minutes, at room temperature, to obtain platelet-poor plasma. About 50 μl of this plasma was immediately frozen and stored at −80°C for flow cytometry analysis. The rest was centrifuged at 13,000 g for 45 minutes, to yield MPs, which were resuspended in RPMI (Roswell Park Memorial Institute) medium and then frozen and stored at −80°C.

Flow Cytometry Analysis

The platelet-poor plasma was thawed on ice, and 10 μl was incubated with annexin V that had been previously diluted in 90 μl of annexin V binding buffer (2X, containing 20 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid), 0.28 M sodium chloride, and 5 mM calcium chloride; pH 7.5), for 30 minutes, at room temperature, in the dark. Platelet-poor plasma incubated with annexin V in 2X binding buffer, containing 5 mM EDTA (ethylenediaminetetraacetic acid) but no calcium chloride, was used as a negative control. Before analysis, 10 μl of flow count calibrator beads (Beckman Coulter Inc, Fullerton, Calif) with known concentration were added into the samples. The annexin V binding MPs were analyzed in MoFlo XDP (Beckman Coulter Inc). After exclusion of background noise, those positively labeled for annexin V in the gate (size = 1 μm) were considered to be MPs.

Vasodilation Study

The experimental protocol was approved by the Animal Ethics Commission of the First Affiliated Hospital, Sun Yat-sen University. All mice used were purchased from the Experimental Animal Center of Sun Yat-sen University. The vasodilation study was performed as described elsewhere.[8]

Briefly, male C57BL/6 (C57 black 6) mice were killed with pentobarbital (Sigma-Aldrich, St Louis, Mo), and aortas were extracted. Aortic rings, 3 mm wide, were obtained and connected to an isometric force transducer (DMT620M, ADInstruments, New South Wales, Australia). Aortic rings were suspended in organ chambers filled with Krebs solution and put through a 30-minute equilibration period with no tension. After that, the pretension was gradually increased to 5 mN, followed by resting for 60 minutes. Next, the rings were exposed to 60-mmol/L potassium chloride and washed out at least 3 times to achieve optimal tension. The aortic rings were treated with 4.6×10^6 per mL of MPs (the pathologic concentration of MPs in VHD patients before cardiac surgery) for 30 minutes and then preconstricted with 10^{-6} mol/L noradrenaline (Sigma-Aldrich). Endothelium-dependent vasodilation was determined by acetylcholine (10^{-8} to 10^{-4} mol/L) and NG-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L; both from Sigma-Aldrich), respectively. Endothelium-independent vasodilation was studied using the nitrovasodilator sodium nitroprusside (10^{-8} to 10^{-4} mol/L; Sigma-Aldrich). Aortic rings with no pretreatment were used as controls.

Western Blot Analysis

Passage-4 human umbilical vein endothelial cells (HUVECs) were cultured in an endothelial cell medium (both from ScienCell Research Laboratories, Carlsbad, Calif) supplemented with 5% fetal bovine serum, 1% growth factors, and 1% penicillin/streptomycin. After serum starvation with 0.5% fetal bovine serum for 24 hours, HUVECs were treated with MPs (4.6×10^6 per mL) for 1 hour. Cellular proteins were obtained, and western blotting was performed as described elsewhere.[10] Passage-4 HUVECs treated with phosphate-buffered saline were used as controls.

Antibodies of Akt, phosphorylation of Akt, phosphorylation of eNOS at Ser1177 (serine 1177 site), and caveolin-1 were from Cell Signaling Technology Inc (Danvers, Mass). Antibody of eNOS (sc-654),