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Circulating platelet aggregates damage endothelial cells in culture

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ABSTRACT

Background: Presence of circulating endothelial cells (CECs) in systemic circulation may be an indicator of endothelial damage and/or denudation, and the body's response to repair and revascularization. Thus, we hypothesized that aggregated platelets (AgPlts) can disrupt/denude the endothelium and contribute to the presence of CEC and EC-derived particles (ECDP).

Methods: Endothelial cells were grown in glass tubes and tagged with/without 0.5 μm fluorescent beads. These glass tubes were connected to a mini-pump variable-flow system to study the effect of circulating AgPlts on the endothelium. ECs in glass tube were exposed to medium alone, nonaggregated platelets (NAgPlts), AgPlts, and 90 micron polystyrene beads at a flow rate of 20 mL/min for various intervals. Collected effluents were cultured for 72 h to analyze the growth potential of dislodged but intact ECs. Endothelial damage was assessed by real time polymerase chain reaction (RT-PCR) for inflammatory genes and Western blot analysis for von Willebrand factor.

Results and conclusion: No ECs and ECDP were observed in effluents collected after injecting medium alone and NAgPlts, whereas AgPlts and Polybeads drastically dislodged ECs, releasing ECs and ECDP in effluents as the time increased. Effluents collected when endothelial cell damage was seen showed increased presence of von Willebrand factor as compared to control effluents. Furthermore, we analyzed the presence of ECs and ECDPs in heart failure subjects, as well as animal plasma samples. Our study demonstrates that circulating AgPlts denude the endothelium and release ECs and ECDP. Direct mechanical disruption and shear stress caused by circulating AgPlts could be the underlying mechanism of the observed endothelium damage.

Experimental protocol: The Institutional Animal Care and Use Committee (IACUC) has approved the protocol for use of animals—2007A0066–4/11/2007; The Institutional Review Board approved the protocol 2007H0139–07/28/2007.

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Introduction

The measurement of circulating endothelial cells (CECs) is considered a novel and reliable method to assess endothelial damage/injury. CECs are differentiated mature endothelial cells (ECs) that are desquamated from the intima of the blood vessel in response to endothelial injury and released into blood circulation. Generally, a healthy person has a low count of CECs; therefore, an elevated count of CECs could be due to the presence of endothelial damage/injury.[1] Elevated CECs levels are well reported in many disease states including atherosclerosis,[2] acute myocardial infarction (AMI),[3] angina pectoris,[4] and hypertension.[5]

At present, the origin of CECs remains incompletely understood. However, some studies have demonstrated that CECs from patients with acute coronary syndrome and thalassemia were of macrovascular[6,7] and microvascular[8] origin, respectively. In fact, CECs are considered to be detached ECs. Proposed mechanisms responsible for the detachment of ECs include impaired adhesive properties of ECs and mechanical injury to ECs. Defective adhesive properties of ECs could be due to apoptosis[9] or the actions of proteases[10] and/or cytokines.[11] Direct mechanical damage to the endothelium can also detach ECs from the basement membrane. Recent studies demonstrated that percutaneous catheter interventions in patients with unstable angina or AMI causes desquamation of ECs in circulating blood.[6] It has also been identified that elevated levels of CECs in patients with pulmonary hypertension could be due to high pressure and shear stress present in the pulmonary vasculature.[12] However, to our knowledge, the role of circulating aggregated platelets (AgPlts) in the detachment of ECs has not been studied. In view of the aforementioned background, we hypothesize that AgPlts could damage the endothelium and contribute to the presence of CECs, EC-derived particles (ECDP), and their antibodies (auto-antibodies) in the circulation, and subsequently the damaged endothelium causes inflammation. To test this hypothesis, we developed a novel in-house mini-pump variable flow system, which enabled us to expose ECs grown in a glass tube and sheep carotid artery to circulating AgPlts at variable flow rates for different periods. We also analyzed inflammatory markers under these conditions. Furthermore, we assessed the endothelial damage by measuring a cell-specific molecule, von Willebrand factor (VWF), in the effluents and heart failure (HF) animal samples. The CECs and ECDP might be the cause for antiendothelial cell antibody (AECA), which plays a major role in the inhibition of a potent repair mechanism. To verify this, we also analyzed the presence of AECA in the HF samples of human subjects.

Materials and methods

Animal and experimental protocol

All animals in this study received humane care in compliance with The Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health. The protocol was approved by The Ohio State University Institutional Animal Care and Use Committee.

Human subjects

Following The Ohio State University School of Medicine Institutional Review Board (IRB) approval, patients (with consent) undergoing routine cardiovascular surgery at The Ohio State University Medical Center were enrolled. All patients underwent preoperative transthoracic echocardiographic assessment of ventricular function. About 5 mL of peripheral blood (PB) was collected, placed in heparinized tubes, and labeled with a unique number associated with the study. Plasma was separated immediately after collection and stored at −80°C for further analysis.

Animals (sheep)

Serum samples were collected from thrombus-embolized HF sheep animal models and stored at −80°C.[13] Briefly, the autologous platelet aggregates were injected directly into the left circumflex artery of the animals. Cardiac injury was assessed by measuring troponin I, and development of cardiac insufficiency was evaluated by echocardiography.[14] Subsequently, these sheep were humanely killed at 4, 24, 48, 72 h, and 90 d after thrombus embolization, at which time blood samples were collected, and plasma or serum was separated and stored at −80°C until use. Samples were used to determine VWF and to run the cyto-ELISA as previously described.

Chemicals

Medium M199, penicillin/streptomycin, L-glutamine, and primers were purchased from Invitrogen (Carlsbad, CA). EC growth supplements were purchased from Millipore (Temecula, CA). Fetal bovine serum (FBS) and all other chemicals were purchased from Sigma (St. Louis, MO). Then, 0.5-μ FluoSpheres and 90-μm Polybeads were purchased from Polysciences (Warrington, PA). Antibodies were purchased from Abcam (Cambridge, MA).

Cell culture

Human umbilical vein endothelial cells (HUVECs) were obtained from Prima Pure, (Genlantis Inc, San Diego, CA) and maintained in M199 medium supplemented with 20% FBS, 2-mM L-glutamine, endothelial growth supplements, and 1x penicillin-streptomycin antimycotic solution.

Sheep aortic endothelial cells (SAECs) were isolated from sheep aorta by mechanical scraping. Cells were maintained in culture in Roswell Park Memorial Institute medium (10% FBS, 100-IU/mL penicillin, and 100-ng/mL streptomycin). Cells used in this study were at confluence and between the fifth to 10th passages. The cultures were maintained in 5% CO_2 atmosphere at 37°C. FluoSpheres of 0.5μ were used for labeling of cells.

Glass tube preparation of EC (HUVECs and SAECs)

Confluent ECs (HUVECs and SAECs) either labeled/unlabeled with 0.5-μ FlouSpheres were trypsinized and used for the glass tube experiment. Cells were grown to confluence (80%-90%) in a glass tube of 7 cm length and 0.3 cm diameter. To achieve