Microparticles as Novel Biomarkers and Therapeutic Targets in Coronary Heart Disease

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Introduction

Microparticles (MPs) were increased in patients with coronary heart disease (CHD), with the subtypes and quantity of MPs variate in different types of CHD. There were emerging reports indicating that MPs may play important roles in the pathogenesis of CHD. Here in this review we summarized the pro-inflammation, pro-coagulation effects of MPs, as well as their impacts on endothelial function and angiogenesis. MPs have the potential of being powerful diagnostic biomarkers and therapeutic tools in CHD patients in the future.

MPs, which were first described as "cell dust,"[1] are intact vesicles derived from the outer membrane of cells during cell activation or apoptosis. MPs are mostly derived from platelets,[2] whereas MPs are also present in endothelial cells, erythrocytes, granulocytes, monocytes, lymphocytes and smooth muscle cells in lower numbers.

Microparticles are composed of a phospholipid bilayer and cytosolic components such as enzymes, transcription factors, and mRNA.[3] Under a resting state, phosphatidylserine is located in the inner monolayer. When the concentration of calcium rises in the cytosol, for example during cell activation or apoptosis, phosphatidylserine translocates to the outer layer, which ultimately leads to the escape of MPs from cytoskeleton and degradation by Ca^{2+} dependent proteolysis.[4]

Microparticles are found in low concentrations in the plasma under physiological conditions. However, the circulating levels are increased in pathological conditions such as atherosclerosis, sepsis, diabetes, chronic severe hypertension, preeclampsia, etc.[3,5] Importantly, a recent study showed significantly higher levels of endothelial MP (EMP) but not platelet MP (PMP) in the sudden cardiac death patients compared with the ST-segment elevated myocardial infarction (STEMI) patients, suggesting a crucial role of MPs in acute coronary events.[6] It was also found that the EMP level can predict major adverse cardiovascular and cerebral event risk in a sample of 200 CHD patients.[7] Another study specified that only those activated EMPs (CD62E positive) but not the apoptotic EMPs could predict cardiovascular events in 300 patients with a recent stroke.[8] MPs were increased in CHD patients comparing with non-CHD patients, with the amount of PMPs and EMPs higher in acute coronary syndrome (ACS) patients than stable angina patients.[9] A cross-sectional study of 190 healthy males found that the PMP count was significantly correlated with the 10 years Framingham CHD risk score.[10] In 488 consecutive patients with various CHD risks, plasma EMP was found to be a significant and independent predictor of future cardiovascular events during a three years follow-up, highlighting the prognostic value of EMP in CHD patients.[11]

The reports above strongly indicate that MPs may play important roles in the pathogenesis of CHD. Here, we summarize the possible pathogenic mechanisms of MPs in modulating inflammation, coagulation, endothelial function and angiogenesis. The outline of this review is as follows: The first part describes current MPs isolation and detection methods; The second part describes possible pathogenic mechanisms of MPs.

Isolation and Detection of Microparticles

The quantification of MPs is important for establishing a consistent standard of research. Unfortunately, it is not easy

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because of MPs' small size (reported diameters ranging between 100 nm and 1 μm),[12] which are below the detection range of conventional detection methods. As a consequence, the isolation protocols have not been standardized. Moreover, the results could be influenced by preanalytical factors such as venipuncture, time between blood collection and handling, the anticoagulant, centrifugation and washing procedures, the presence of lipoprotein particles and small platelets, and the viscosity of blood. Therefore, it was suggested that blood be withdrawn using a large diameter needle, and a tourniquet be only applied for locating the vein. Sodium citrate, ethylenediaminetetraacetic acid, and citrate, theophylline, adenosine, and dipyridamole anticoagulants could be used for blood collection, with blood immediately centrifuged to isolate plasma at a speed determined experimentally.[13]

Several methods have been employed to measure MPs [Table 1, adapted from van der Pol *et al*.],[13] of which fluorescent flow cytometry was most commonly used. It applied fluorescent labeled specific antibodies to identify the cellular origin of MPs. These antibodies could distinguish MPs derived from PMP, leucocytes (LMP), and EMP cells. A list of such antibody combinations has been published on the Forum "Measuring circulating cell-derived MPs" [Table 2, adapted from Jy *et al*.].[14] It has shown that differences exist in the isolation of MP, means of generic MP detection, and cell lineage-specific antigenic markers used.

For each method, the detection limit, ability to measure the size distribution and concentration, ability to provide biochemical information, and the measurement time are estimated. A method that is incapable, capable but with limitations, or capable of providing information on size distribution, concentration, or biochemical information is indicated by −, −/+, and +, respectively. The measurement times shorter than one minute and longer than one hour are indicated by + and +++, respectively.

Microparticles and Endothelial Function

Endothelium has multiple functions including antiinflammation, anticoagulation, antithrombosis, vascular tone control, and vascular wall permeability maintenance.[15] Many CHD risk factors have been found to impair endothelial functions.[16] As a result, endothelial dysfunction is thought to be an initiating process in CHD.[17] Elevated MP levels were also associated with many cardiovascular risk factors, which have been proven to impact endothelial function, such as obesity, hyperlipoproteinaemia, hypertension, and diabetes.[18] It has been found that MPs from patients with acute myocardial infarction can cause endothelial dysfunction in rat aorta through the endothelial nitric oxide synthase (eNOS) pathway while MPs from nonischemic patients had no such effect.[19,20] Another study showed that MPs from metabolic syndrome patients could reduce nitric oxide and superoxide anion production, resulting in endothelial dysfunction. *In vivo* injection of MPs from metabolic syndrome patients into mice impaired endothelium-dependent relaxation and decreased eNOS expression.[21] These results suggested a potential link between MPs and endothelial dysfunction.

In a study of 50 patients with CHD, the levels of EMP were increased in endothelial dysfunction patients defined as a loss of vascular relaxation following acetylcholine infusion during an angiographic study.[22] In a study of 84 patients with CHD, EMP levels were increased, and the EMP levels were correlated with severity and location of coronary artery stenosis.[23] Higher EMP levels were noticed in patients with ACS compared with stable angina patients.[9] Surprisingly, patients with stenosis of the left anterior descending artery

Table 1: Compare of common MP separation methods

Methods	Detection limit	Size distribution	Concentration	Biochemical information	Measurement time
Scattering flow cytometry	≥ 300 – 500 nm	−	+/−	−	+
Fluorescent flow cytometry	Single quantum dot	−	+/−	+	+
Impedance flow cytometry	≥ 300 – 500 nm	−	+/−	−	+
Electron microscopy	1 nm	+	−	+/−	+++
Capture based assay	Single MP	−	+/−	+	+

MP: Microparticle.

Table 2: Compare of common separation methods for Cell-derived MPs

Method	Isolation MP (speed, time)	Generic MP detection	Platelet MP detection	Endothelial MP detection	Leukocyte MP detection
Flow cytometry	18,000×*g*, 30 minutes	Annexin V	CD62P, CD61, CD63	CD31, CD62E or CD144	CD4, CD8, etc.
Flow cytometry	–	Annexin V	–	CD51, CD144 or CD146	CD45
Capture based assay	–	Annexin V, tissue factor	CD62P or GPIba	CD31 or CD62E	CD45
Flow cytometry	–	–	CD41 or CD42b and CD31	CD31 +/ CD42 − or CD62E	CD45
ELISA	–	–	GP IX (capture) CD62P, CD40 L	–	–
Flow cytometry	100,000×*g*, 30 minutes	Annexin V	CD41a	CD144	CD14 (monocyte)

MPs: Microparticles; ELISA: Enzyme linked immunosorbent assay.

were associated with higher EMP levels comparing with right coronary artery or triple vessel disease, EMP levels were also higher in patients with early coronary artery stenosis (20%–45%) than severe coronary artery stenosis (> 45%),[24] indicating that EMP may participate in the early pathological process of CHD. There were reports that MPs from human T lymphocytes that harbor Sonic Hedgehog (Shh) can improve endothelial function and prevent endothelial dysfunction induced by ischemia/reperfusion.[25] The injection of engineered MPs from human T lymphocytes Shh could prevent endothelial dysfunction and promote angiogenesis in animal models.[26-28] The reports above suggest that MP levels have the potential of being biomarkers as well as therapeutic targets of endothelial dysfunction in CHD patients.[29,30]

Pro-inflammatory Effects of Microparticles

Atherosclerosis is the most frequent underlying cause of cardiovascular disease, while acute thrombosis in atherosclerotic plaque with an eroded surface is the main cause of ACSs including unstable angina and acute myocardial infarction.[31] Inflammation was found to play a key role in the development of plaques, plaque rupture and thrombus formation.[32,33] There is increasing evidence indicating that the number of MPs increases during inflammation *in vivo*.[21,34,35] It is reported that MPs from leukocytes could stimulate the expression of cytokine related genes *in vitro* through tyrosine phosphorylation of c-Jun NH_2-terminal kinase-1.[36-38] These cytokines included interleukin-1 (IL-1), IL-6, IL-8, monocyte chemoattractant protein-1, tissue factor (TF), tumor necrosis factor-alpha and platelet-activating factor, which all contributed to inflammation.[38,39] Our unpublished results showed that inflammatory marker high-sensitivity C-reactive protein (CRP) was positively correlated with LMP in STEMI patients ($R^2 = 0.79$, $P < 0.01$, $n = 24$), indicating the potential role of MPs in inflammation in CHD patients.

Adhesion to and rolling of monocytes and neutrophils on the endothelium is an important step in atherosclerosis, and MPs were proven able to increase the expression of adhesion molecules.[40] It was found that high shear stress-induced activation of platelets could lead to increased PMPs, which enhanced the expression of cell adhesion molecules in endothelial cells.[41] In addition, once MPs were exposed to complement components C3 and C4, the classical complement pathway could be activated.[42] Moreover, CRP, which is a sensitive marker of inflammation,[43] was found on the surface of MPs.[44,45] PMPs were reported to induce pro-inflammatory molecules cyclooxygenase-2 and intercellular adhesion molecule-1 expression in endothelial cells,[37] while MPs from lymphocytes could activate the inflammatory nuclear factor-kappa B pathway.[46] These reports suggest that MPs are involved in multiple processes of the inflammatory response.

Pro-coagulant Potential of Microparticles

The plaque disruption and organization of thrombi contributes to the rapid progression of atherosclerosis, where the importance of blood coagulation should not be neglected.[47] It is found that the PMP surface is approximately 50–100 fold more pro-coagulant than the surface of activated platelets.[48] Moreover, MPs with pro-coagulant potential were increased in the peripheral circulating blood of patients with ACSs.[49] PMPs have been reported as a valid marker for a pro-thrombotic state through a survey of 54 stable CHD patients.[50]

Tissue factor on monocyte MPs, which is a receptor for factor VII and factor VIIa, was proven to be crucial in coagulation.[51,52] MPs correlate with artherosclerosis clinically. STEMI patients have high levels of pro-coagulant MPs, and an increased risk of fibrinolysis failure.[53] MPs were also present in atherosclerotic plaques, which are considered to promote TF-dependent coagulation, leading to thrombosis and arterial occlusion.[54,55] TF played an indispensable role in coagulation; its function was dependent on platelet P-selection receptor P-selectin glycoprotein 1, which was on the surface of monocyte MPs.[55,56] PMPs and EMPs provided binding sites for coagulation factors IXa, VIII, Va, and IIa.[57,58] EMPs also express ultra-large von Wille brand factor multimers, which can promote platelet aggregation.[59]

Microparticles and Angiogenesis

Angiogenesis is a complicated process that includes endothelial cell proliferation, migration, differentiation, and morphological change.[60] Angiogenesis processes after myocardial infarction can improve heart function.[61] In recent studies, MPs were found to be involved in angiogenic processes such as tumor neovascularization, diabetic retinopathy, wound healing, and CHD.[60] MPs derived from many types of cells are found to have angiogenic functions.[60] In a rat myocardial infarction model, ligating the left anterior descending coronary artery, PMPs injection into the peri-ischemic region resulted in a marked increase in new capillaries.[62] PMPs were found to be involved in almost all steps of angiogenesis through PI3-kinase and extracellular signal-regulated kinase pathways.[63,64] EMPs could promote vessel formation through elevating matrix metalloproteinase-2 (MMP-2) and MMP-9 activity,[65,66] which catalyze matrix degradation and angiogenesis. MPs derived from Shh, which act as an inter-cellular signal responsible for cellular fate decisions, can up-regulate angiogenic growth factors vascular endothelial growth factor (VEGF) and angiopoietins.[67] It was further confirmed that treatment of endothelial cells with MPs derived from Shh induced and accelerated the formation of capillary-like structures *in vitro* through up-regulation of pro-angiogenic factors VEGF, hepatoctye growth factor, and fms-like tyrosine kinase (FLT)-1.[68] This pro-angiogenic function could be inhibited by blocking the Shh signaling with cyclopamin.[38]

However, there are some contradictory results as well. EMPs were also reported to play an antiangiogenic role through up-regulation of antiangiogenic reactive oxygen species.[69,70] The differences may be due to different concentrations of EMPs because lower concentrations of EMPs were reported to promote angiogenesis, whereas higher concentrations could suppress angiogenesis.[38,71]

Table 3: Potential impacts of different subtypes of MPs on CHD

Subtype of MP	Aspect	Subject investigated	Impact	Reference
EMP	Clinical	23 SCD patients, 61 STEMI patients	Be increased in SCD patients comparing with STEMI patients	[6]
EMP	Clinical	200 CHD patients	Predict MACCE risk	[7]
EMP	Clinical	43 ACS patients and 33 stable angina patients	Be increased in ACS patients	[9]
EMP	Clinical	488 consecutive patients	Predict cardiovascular events	[11]
EMP	Endothelial	50 CHD patients	Be increased in endothelial dysfunction patients	[22]
EMP	Endothelial	84 CHD patients	Correlated with severity and location of coronary artery stenosis	[23]
EMP	Clinical	43 CHD patients undergoing CAG	Mild stenosis 3 - fold↑than more severe (>45%) stenosis and 5 - fold↑than those without stenosis	[24]
EMP	Angiogenesis	Endothelial cells	Promote vessel formation through MMP-2 and MMP-9	[62]
EMP (CD62E positive)	Clinical	300 patients with recent stroke	Predict cardiovascular events	[8]
PMP	Clinical	43 ACS patients and 33 stable angina patients	Be increased in ACS patients	[9]
PMP	Clinical	190 healthy male	Correlate with 10 years Framingham CHD risk score	[10]
PMP	Inflammation	High shear stress-induced activated platelets	Be increased	[39]
PMP	Inflammation	Endothelial cells	Induce COX - 2 and ICAM - 1 expression	[36]
PMP	Coagulation	54 stable CHD patients	Act as a marker for a pro-thrombotic state	[48]
PMP	Angiogenesis	Rat myocardial infarction model	Increase amount of new capillaries	[59]
PMP	Angiogenesis	Endothelial progenitor cells	Promote angiogenesis	[61]

SCD: Sudden cardiac death; STEMI: ST-segment elevated myocardial infarction; MACCE: Major adverse cerebral and cardiovascular event; ACS: Acute coronary syndrome; CAG: Coronary angiography; MPs: Microparticles; CHD: Coronary heart disease; EMP: Endothelial microparticle; PMP: Platelet microparticle; MMP: Matrix metalloproteinase; COX-2: Cyclooxygenase-2; ICAM-1: Intercellular adhesion molecule-1.

Perspectives

Given the correlation between MPs and the development of CHD, MPs have the potential of being biomarkers for CHD [Table 3]. For example, EMPs were reported as a predictor of future cardiovascular events in a population with high Framingham risk scores.[11] In ACS patients, circulating Annexin V positive MPs were strongly correlated with the occurrence of myocardial infarction or death.[3,72] In asymptomatic subjects, circulating LMPs predicted subclinical atherosclerosis as evaluated by plaque numbers in several vascular sites.[3] However, the prognostic potential of MPs has not been elucidated, additional clinical outcome studies are necessary.

In consideration of their active involvement in multiple processes of atherosclerosis, MPs have been proposed as new therapeutic targets in the treatment of CHD. First, MPs could work as vectors for gene therapy. It has been reported that MPs from lung cells contain mRNA that could be released into bone marrow cells, and modulate their phenotypes.[73-76] Moreover, engineered MPs generated *in vitro* could also incorporate mRNA into target cells and modify their phenotype.[38] Recently, it was reported that inhaled and oral MPs have been developed to deliver therapeutics.[77,78] Second, it has been reported that transfection of glioma cells with the oncogenic form of the epidermal growth factor receptor (EGFR) induces MPs over-expressing EGFR, which could be transferred to cells lacking this receptor.[76] This finding demonstrated a natural way to generate MPs overexpressing certain receptor molecules. Moreover, due to their pro-coagulation function, MPs may ameliorate platelet function in diseases such as thrombocytopenia.[79]

In addition to the molecular application of MPs mentioned above, several drugs may influence the release of MPs. Statins, for instance, could reduce the expression of GPIIIa antigen, P-selectin and TF on PMPs in patients with diabetes, dyslipidemia or peripheral arterial occlusive disease,[80-82] while statins exert controversial effects on EMP levels.[83,84] PMPs release could be reduced by ticlopidine and clopidogrel.[85,86] Aspirin could reduce the number of EMPs and PMPs in patients with CHD.[87] However, an important question remains how to control particular MPs to an ideal level, so as to achieve benefit actions and limit adverse effects. Also, the comprehensive effects of MPs need to be fully evaluated before clinical use. MPs as powerful diagnostic and therapeutic tools may benefit more CHD patients in the future.

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