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Review

Microparticles and their role in coronary artery disease

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ABSTRACT

Despite significant advances in prevention, medical and interventional management, coronary artery disease (CAD) remains the leading cause of death worldwide. Although the number of people being diagnosed with CAD has plateaued in the western world, it is projected to increase significantly in the developing world reaching epidemic proportions, particularly in South Asia. To better stratify the risk of developing and suffering a cardiovascular event due to CAD, not only plasma biomarkers relating to disease burden but also disease activity in CAD are needed; this will allow targeting of appropriate management to high-risk patients for acute events. Over the last twenty years, data have emerged showing the role of sub-micron vesicles called microparticles (MPs) in the pathogenesis of formation and evolution of atherosclerotic plaques causing either stable angina (SA) or acute coronary syndromes (ACS). Herein we provide an overview of our current knowledge of MP formation, composition and possible mechanisms through which they could be contributing to CAD. We also reviewed currently available methods and their limitations in quantifying MPs and in determining their functional aspects. Role of various treatments ranging from dietary substitutes to oral medicines and intravenous medications to mechanistic procedures such as hemofiltration are elaborated. Although evidence implicating the role of MPs in CAD are mounting large scale prospective studies are still lacking and are the need of the hour prior to establishing the use of MPs as biomarkers for the early detection of CAD and its progression.

1. Introduction

Despite significant advances in the medical and interventional management of coronary artery disease (CAD) mortality and morbidity remain high with ischemic heart disease being the leading cause of death worldwide in the last 5 years [1]. Atherosclerotic disease, the hallmark of CAD, is now considered a chronic inflammatory process [2]. Over the last twenty years, data have emerged showing that immune cells are involved in the pathogenesis, formation and evolution of atherosclerotic plaques causing either stable angina (SA) or acute coronary syndromes (ACS) [3]. Early identification of features that define possible atherosclerotic plaque instability is vital to improve cardiovascular risk stratification and prognosis. As our understanding of CAD pathophysiology has evolved from not just a focal but ultimately a systemic disease, approaches to identify these high-risk patients may need to combine identification of local vulnerable plaques or myocardial damage but also novel plasma biomarkers relating to cumulative atherosclerosis burden.

Microparticles (MPs) are now considered key mediators of inflammation [4,5] and therefore may play a role in both the formation and progression of atherosclerosis and subsequent plaque rupture leading to ACS. MPs were referred to as “platelet dust” when first reported in 1967 [6]. The perception that MPs were merely “innocent debris” rapidly changed due to an increasing body of evidence suggesting that they have potent pro-coagulant and pro-inflammatory properties [7]. MPs are sub cellular particles (measuring <1 μm) derived from the plasma membrane of any eukaryotic cell. Although MPs are formed in response to various biological processes such as cellular activation and apoptosis, evidence for their role in pathological states comes from their presence in excess numbers in disease states such as ACS, sepsis, systemic inflammation (including vasculitis), and malignancy [8]. Over the last decade an increasing number of studies have explored the mechanisms of formation of MP, their content, and contribution to pathological states through a number of mechanisms such as angiogenesis, inflammation and coagulation. Pertinent to the pathology of ACS are MPs derived from platelets, representing 70% of total MP, but also those endothelial cells, erythrocytes and leukocytes [9].

Herein we provide an overview of our current knowledge of MP formation, composition, possible mechanisms through which they could be contributing to CAD and various treatment modalities experimented with an aim to reduce levels of MPs.

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1.1. MP formation and composition

The normal plasma membrane consists of a phospholipid bilayer [10]. The distribution of phospholipids in this bi-layer is asymmetric with the outer layer consisting of phosphatidylcholine/sphingomyelin and inner layer consisting of phosphatidylserine (PS) [11]. This pattern of distribution of phospholipids is under the control of three proteins; Flippase, Floppase and Scramblase [12]. Increased intracellular calcium following cell activation alters the function of these three proteins and results in movement of phospholipids towards the outer layer exposing intensely pro-coagulant PS (Fig.1). This reorganisation of plasma membrane lipid bi-layer is associated with loss of asymmetry of cytoskeleton thus leading to vesicle formation; these vesicles are then cleaved by Caspases into MP [10]. Caspases were further shown to play a role in the release of MPs by the cleavage of a Rho associated kinase (ROCK I) protein during apoptosis [13]. Thrombin induced endothelial cell vesiculation has also been shown to involve nuclear factor (NF)-κβ signaling and ROCK II activation [14]. The exposed PS is a potent pro-coagulant as it provides an excellent substrate for the pro-thrombinase complex [15].

1.2. MP function

The biological function of MPs depends upon the parent cell they are derived from [16] (Table 1). MP lipid and protein composition also varies according to the parent cell and the stimulus that triggered their formation [17]. Broadly they have a role in inflammation, coagulation, endothelial dysfunction, and angiogenesis. Evidence is also emerging of their role in microvascular dysfunction (MvD; see below). The pro-coagulant potential of MPs is largely secondary to PS exposure that acts as a platform for the assembly of several pro-coagulant factors along with tissue factor expression (TF) [15]. MPs also act as communicators or messengers carrying cytokines, mRNA and viruses [18]. In addition MPs also act as transporters of specific micro RNAs (miRNAs), of particular relevance to cardiovascular diseases [19]. Importantly, not all functions of MP appear to be detrimental as anticoagulant and fibrinolytic functions have also been reported [15,20]. In addition they also contain increased concentrations of oxidized phospholipids and caspases when compared to parent cell thus playing a role in cellular waste processes [21].

1.3. Quantification and phenotyping of MPs

Although there are various methods available to quantify MPs (Table 2), flow cytometry (FC) remains the most commonly used method [27]. Staining with flurochrome-conjugated Annexin V (AnV), which binds to PS, is commonly used to identify MP of mixed cellular origin with FC [28]. However some MPs don't bind with AnV. Whether this is a reflection of low PS content and/or a limitation of the technique using AnV staining to identify all MPs; or whether truly these AnV-negative MPs exist and have other functions remains to be established [29]. As MPs carry parent cell proteins and receptors, fluorochrome-conjugated antibodies directed at these components allow us to quantify the specific type of MP using FC (Table 1). The procoagulant function of MPs can be confirmed in vitro by thrombin generation assay (TGA) [30] (Table 2).

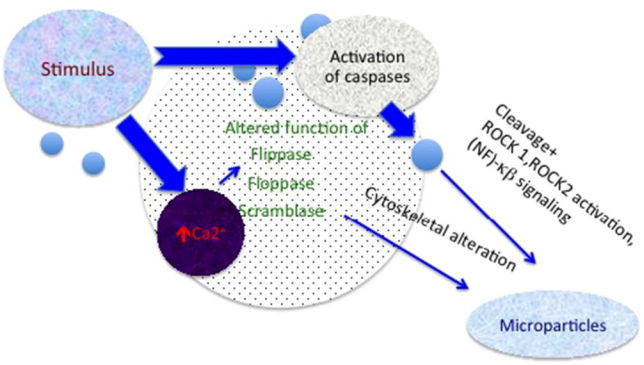

Fig. 1. Schematic diagram showing cellular activation & apoptosis leading to microparticle formation.

Table 1
Biologic function of various MPs and indicative surface markers based on cellular origin.

Cell type	Surface marker	Biological function
Platelet (PMP)	CD31,CD42a,CD42b,CD61	Inflammation, thrombogenesis, angiogenesis[a]
Endothelial cell (EMP)	CD105,CD31,CD146, CD51,CD54,CD62E, CD144, CD34,CD18	Inflammation, thrombogenesis, angiogenesis[b], endothelial dysfunction
Monocytes (MMP)	CD105,CD14,CD11a,TF+, CD40 L	Inflammation, thrombogenesis, angiogenesis
Leukocyte (LMP) [22]	CD45	Inflammation, thrombogenesis, endothelial dysfunction
Erythrocyte [23]	CD235a	Thrombogenesis
Neutrophil (NMP)	CD15, CD64,CD66b, CD66e,CD11b, MPO [24]	Inflammation, thrombogenesis, anti-inflammatory effect on macrophages

Note there may be overlap on expressed surface markers: CD146 has been found on activated T-cells; CD54 (Inter-Cellular Adhesion Molecule-1; ICAM-1) is also expressed by leukocytes; and CD51 is present on monocytes/macrophages and platelets. EMP — endothelial derived MP, LMP — leukocyte derived MP, MMP — monocyte derived MP, NMP — neutrophil derived MP, PMP — platelet derived MP.

[a] In vitro only [26].

[b] Low levels of EMP shown to promote angiogenesis where as high levels abolish angiogenesis [25].

2. Biologic function of MPs pertinent to CAD

MPs may contribute to formation and progression of atherosclerosis through a number of mechanisms such as angiogenesis, inflammation, coagulation, endothelial dysfunction, and MvD (Fig. 2). These are considered in more detail below.

2.1. Angiogenesis

Vulnerable atherosclerotic plaques (VP) have characteristic features such as increased necrotic core, increased apoptotic macrophages and vasa vasorum [37]. Atherosclerotic plaques develop their own microcirculation as they grow and this process is driven by angiogenic factors such as vascular endothelial growth factor (VEGF) [38]. These micro vessels provide an avenue so that leukocytes and erythrocytes can enter and exit the atheromatous plaque, supplying oxygen and nutrients thus promoting the growth of the plaque. These micro-vessels are not stable and can rupture easily leading to intra-plaque hemorrhage [39,40]. MPs may play role in this angiogenesis-related plaque instability as demonstrated in the in vitro and in vivo studies described below.

2.1.1. In vitro studies

Kim et al. demonstrated how PMPs could promote the proliferation and survival, migration, and tube formation in human umbilical vein endothelial cells (HUVECs) [25]. When PMPs were treated with activated charcoal, a procedure known to remove the lipid growth factors, the MP angiogenic activity was significantly reduced. These results suggest that the lipid components of the PMP may be major activating factors of protein components. In pathological states such as a growing tumour, PMPs shed from the circulating platelets may reach adequate concentrations contributing to florid neoangiogenesis [26]. In another elegant study Leroyer et al. demonstrated the potential role played by MPs in intra-plaque angiogenesis and thus plaque vulnerability. MPs were isolated from carotid endarterectomy specimens surgically obtained from 26 patients. The MPs thus isolated were further characterized by PS