Microparticles and type 2 diabetes

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Abstract

Cell activation or apoptosis leads to plasma membrane blebbing and microparticles (MPs) release in the extracellular space. MPs are submicron membrane vesicles, which harbour a panel of oxidized phospholipids and proteins specific to the cells they derived from. MPs are found in the circulating blood of healthy volunteers. MPs levels are increased in many diseases, including cardiovascular diseases with high thrombotic risk. Exposure of negatively charged phospholipids and tissue factor confers a procoagulant potential to MPs. Elevation of plasma MPs levels, particularly those of endothelial origin, reflects cellular injury and appears now as a surrogate marker of vascular dysfunction. Recent studies demonstrate an elevation of circulating levels of MPs in diabetes. MPs could also be involved in the development of vascular complications in diabetes for they stimulate pro-inflammatory responses in target cells and promote thrombosis, endothelial dysfunction and angiogenesis. Thus, these studies provide new insight in the pathogenesis and treatment of vascular complications of diabetes.

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Résumé

Microparticules dans le diabète de type 2

L’activation cellulaire et l’apoptose conduisent au bourgeonnement de la membrane plasmique et à la libération de microparticules (MPs) dans l’espace extracellulaire. Ces MPs sont de petites vésicules membranaires qui expriment toute une gamme de phospholipides oxydés et de protéines caractéristiques de la cellule d’origine. Les phospholipides chargés négativement et le facteur tissulaire portés par certaines MPs leur confèrent un pouvoir procoagulant. Une élévation du taux de MPs dans le sang circulant est donc le reflet d’une atteinte cellulaire et témoigne bien souvent d’une pathologie vasculaire ou thrombotique. De plus, les MPs sont de véritables vecteurs biologiques capables d’induire selon leur composition, une réponse de type pro-inflammatoire dans le compartiment vasculaire. Les études récentes menées sur les MPs et le diabète ont apporté de nouvelles données sur la pathogenèse du diabète. Le diabète de type 2 est en effet associé à une élévation des taux de MPs plasmatiques qui pourraient agir sur les étapes cruciales du diabète et de ses complications : les MPs induisent et entretiennent l’inflammation locale, la coagulation, la dysfonction endothéliale et l’angiogenèse dans le contexte du diabète et de ses complications vasculaires. Les MPs apparaissent donc comme de nouveaux éléments à prendre en considération dans les stratégies thérapeutiques employées contre le diabète.

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Cell activation or apoptosis leads to plasma membrane blebbing and the release of microparticles (MPs) in the extracellular space. MPs are submicron membrane vesicles, which express a panel of phospholipides and proteins specific to the cells they derived from. MPs are found in the circulating blood of healthy volunteers. MPs levels are

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increased in many diseases, particularly cardiovascular diseases with high thrombotic risk. Exposure of negatively charged phospholipids and tissue factor confers a procoagulant potential to MPs. Elevation of plasma MPs levels, particularly those of endothelial origin, reflects cellular injury and appears now as a surrogate marker of vascular dysfunction. MPs are also biologically active and stimulate pro-inflammatory responses in target cells. The development of vasculopathies in diabetes involves multifactorial processes including pathological activation of vascular cells. Release of microparticles by activated cells has been reported for the first time in diabetes in 2002 [1]. Consequently, MPs appears as a new prognostic potential of type 2 diabetes, particularly interesting in the early detection of vascular complications in this disease (Fig. 1).

1. Formation and characteristics of microparticles

The current knowledge on MPs formation derives mainly from experiments on isolated or cultured cells. However, the mediators and the mechanisms involved in in vivo MPs formation and shedding remain mostly unknown.

MPs formation is associated with the loss of membrane asymmetry, a characteristic of quiescent cells. This leads to exposure of phosphatidylserine on the outer leaflet as a consequence of the calcium-dependent activation of scramblase and flipase and the inhibition of flipase activities [2-4]. Phosphatidylserine exposure is not always followed by the release of MPs, which may be regulated by the level of intracellular calcium. Moreover, MPs formation and shedding necessitate modifications in cell structural architecture involving disruption of cytoskeleton proteins organization. Cell activation or apoptosis could induce these modifications. For example, platelets release MPs following activation by either thrombin, ADP plus collagen, the complement complex C5b-9, the calcium ionophore A23187, and by high shear stress [5-8]. MPs released from apoptotic cells may be different in lipid and protein composition from membrane vesicles shed following cell activation and could possibly have different patho-physiological effects [9]. Blebbing of the cellular membrane occurs rapidly after cells enter the apoptotic process. Blebs formation depends upon actin cytoskeleton and actin-myosin contraction, which is regulated by caspase3-induced Rho Kinase 1 activation [10,11]. Rho kinase activation is required for re-localization of DNA fragments from the nuclear region to membrane blebs, suggesting that MPs from apoptotic cells may contain nuclear material [11,12]. An important parameter that determines the biological effects of MPs is their protein and lipid composition, and not only their circulating numbers. The experimental evidence so far available indicates that the protein and lipid composition of MPs may vary depending upon the cell they originate from and the type of stimulus involved in their formation. Proteomics analyses have revealed that the spectrum of proteins found in MPs released in vitro from cultured cells is influenced in part by the type of stimulus used to trigger cell vesiculation [13].

2. Detection and measurements of microparticles

Plasma MPs can be detected and their cellular origin characterized using capture assays or flow cytometry. The flow cytometry analysis permit, in addition of characterization, the quantification of MPs by mean of using calibrator beads of defined concentration. A crucial point in circulating MPs analysis concerns the pre-analytical steps involved in blood sampling and platelet-free plasma preparation [14]. Both methods of MPs analysis rely on antibody detection of specific cellular markers and annexin V binding of phosphatidylserine. Although the general understanding is that MPs express phosphatidylserine, which is detected by annexin V labeling, some plasma MPs analyzed by flow cytometry express specific markers of their cellular origin, but do not bind annexin V even in the presence of high calcium concentrations. For instance, in patients with sickle cell disease, circulating endothelial MPs were either positive or negative for annexin V [15]. In a similar way, we recently observed that very few circulating platelet-derived CD41+ MP bind annexin V in some patients with end-stage renal failure [16]. These observations suggest that phosphatidylserine, even if exposed on MPs, could be already engaged in some other molecular interactions and thus unable to bind annexin V, or that other pathway(s) may be involved in their formation and release, but this remains to be demonstrated.

3. MPs: bystanders and effectors

It is now well established that, in addition to their biomarker potential in cardiovascular diseases, MPs may also transfer bioactive molecules to other cells or MPs acting as true diffusible vectors in the transcellular exchange of biological information [17]. For all these reasons, it has been suggested that they can play an important role as a messenger linking thrombosis and inflammation through interaction with other cell types. However, it is still debated whether they play a causal role in the pathogenesis of cardiovascular disease or whether they are a consequence of the disease [18].

4. MPs as markers in diabetes

Circulating levels of MPs are augmented in most cardiovascular diseases when comparing a patient population with a matched group of healthy subjects. The general consensus is that plasma levels of MPs reflect an equilibrium between their release and their removal from the circulation by phagocytes. The observation that circulating MPs are increased following acute myocardial infarction raises the question
whether these MPs might come from the ruptured plaque [19]. This possibility is highly unlikely because of the different pattern of cellular origin between plaque and plasma MPs [20]. The major MPs populations present in the atherosclerotic plaque originate from macrophages, erythrocytes and smooth muscle cells, but not from platelets while circulating MPs derived for a significant part from platelets and are not of smooth muscle cell origin. In addition, although MPs are much more abundant in atherosclerotic plaques than in plasma and account for the procoagulant activity of the lipid core, at least a dozen of large lesions (such as those found in human carotid arteries) would have to rupture simultaneously to fully account for circulating levels of MPs in these patients [20]. Consequently, MPs found in plasma reflect local injury of either vascular or circulating cells. Elevated levels of MPs originating from platelets, monocyte or endothelial cells were found in type 2 diabetes [1, 21-25], as observed also in patients with other cardiovascular diseases [26].

Several recent studies point out that circulating levels of endothelial MPs associate with impaired endothelial function in patients with cardiovascular diseases and in diabetic patients with coronary artery diseases [16, 26-27]. Moreover, endothelial MPs levels predict the presence of coronary artery disease in diabetic patients, whereas other markers of endothelial injury such as soluble ICAM have no prognostic value [27]. These data support the concept that measurement of endothelial MPs could be useful for identifying diabetic patients with increased risk of cardiovascular disease (Fig.). However, the potential mechanisms involved in increased cell vesiculation in diabetes remain unknown. No information is available regarding the effect of glucose, insulin or that of AGE-proteins. Elevated levels of endothelial and platelet MPs found in type 2 diabetes correlated with increased levels of anti-oxidized LDL antibodies in plasma of type 2 diabetic patients, suggesting that oxidized LDL could contribute to endothelial membrane vesiculation [28]. Oxidative stress in diabetes may not be the only trigger of MPs generation in diabetes. Indeed, elevated levels of remnant lipoproteins in type 2 diabetic patients are associated with plasma platelet MPs, suggesting that reducing elevated lipoproteins with lipid-lowering therapy may be an effective strategy to prevent MPs associated-thrombogenic vascular complications in type 2 diabetes. All together, these data demonstrated the clinical significance of MPs detection and characterization in type 2 diabetes.

5. MP: biological effectors in diabetes

Most of the experimental evidence available so far indicates that MPs can influence diverse biological functions. However, one should be cautious in interpreting data from studies with MPs generated in vitro or from cultured cells, as they may not be fully representative of those patients in vivo. The pattern of proteins found on MPs, as well as the level of oxidized phospholipids are likely to influence their effects on target cells [29].

Circulating MPs bear tissue factor at their surface and account for « blood borne tissue factor » [30]. They are involved in the formation of tissue factor-platelet hybrids, a critical phenomenon in thrombus propagation, following tissue factor transfer from leucocytes MPs to platelet membranes [30]. This property may not be restricted to leucocyte-derived MPs as the presence of tissue factor on platelet-erythrocyte- and hematopoietic cell-derived MPs leads also to thrombus propagation in vivo [31,32]. Type 2 diabetes is characterized by the presence of an altered platelet metabolism that may contribute to the pathogenesis of atherothrombotic complications of diabetes [33]. An increased level of circulating MPs has been suggested to be one of the procoagulant determinants in patients with type 2 diabetes [34]. Hypercoagulable state of diabetes could be initiated or maintained by elevated levels of tissue factor positive platelet MPs. Moreover, increased levels of insulin and glucose increase tissue factor procoagulant activity [35], suggesting that high concentrations of tissue factor exposed by MPs present in diabetes are highly pro-thrombogenic (Fig.). These high levels of MPs observed in patients with type 2 diabetes may be related to enhanced reactive oxygen species generation and lipid peroxidation [33, 36].

Circulating MPs also impair the release of nitric oxide from vascular endothelial cells. This was observed on isolated arteries exposed in vitro to circulating concentrations of MPs from patients with acute coronary syndromes, end stage renal failure or preeclampsia, but not with MPs from healthy subjects [16, 37, 38] (Fig. 1). The endothelial dysfunction caused by circulating human MPs appears to be mediated by MPs of endothelial origin and is associated with an impaired release of NO but no alteration in endothelial NO synthase expression. Endothelial MPs circulating in diabetic patients were also associated with vascular dysfunction in vivo [39]. Indeed, type 2 diabetic patients exhibit impaired postprandial flow-mediated dilatation, which is correlated to increases in circulating endothelial MPs. Taken together, these data suggest that consumption of high-fat meals promotes endothelial injury [39,40]. Endothelial dysfunction is a crucial step in the pathogenesis of atherosclerosis and could link diabetes, atherogenesis and hypercoagulability. This interpretation could explain in diabetic patients, the further increase of endothelial MPs when coronary artery disease is present [21,27].

It is now well established that MPs play a crucial role in inflammation. They are able to deliver arachidonic acid leading to an increased expression of endothelial cyclooxygenase type 2 [41]. Platelet MPs also stimulate endothelial cells in vitro to release cytokines and express adhesion molecules [42]. In addition, platelet MPs can directly interact with activated vascular endothelial cells by increasing leukocytes/monocytes arrest following transcellular delivery of the chemokine RANTES [43].
Finally, endothelial, platelet and tumor cell-derived MPs appear to be able to stimulate angiogenesis, an effect mediated by reactive oxygen species, metalloproteinases, growth factors such as VEGF or sphingomyelin [44-48]. In addition, Ogata and coll. showed in diabetic retinopathy that plasma levels of monocyte-derived MPs are significantly higher in patients with areas of capillary occlusion than in patients without areas of capillary occlusion, suggesting that MPs could be involved in the progression of diabetes complications such as retinopathy. Furthermore, MPs are more abundant in vitreous fluid from diabetic when compared to non-diabetic patients and induce endothelial cell proliferation, underlining the potential role of vitreous MPs in proliferative diabetic retinopathy [49] (Fig. 1).

6. Microparticles and prevention of diabetes

The recognition of a role of MPs may not only be important for our understanding of the pathogenesis of diabetes but may also have implications for the prevention and treatment of this disease. Some currently used therapies are known to affect MPs generation. For example, abciximab, a glycoprotein IIb/IIIa receptor antagonist, almost completely blocks platelet vesiculation in vitro [50,51], thus providing an alternate mechanism of MPs formation. Furthermore, MPs release from tumor necrosis factor-α-activated endothelial cells is suppressed by fluvastatin [52], whereas combination therapy with losartan and simvastatin [53], as well as antioxidative therapy such as vitamin C [36], is capable of decreasing the number of circulating monocyte-derived MPs. The major class of molecules used in diabetes, which are angiotensin II receptor blockers such as losartan or valsartan, have been shown to have beneficial effect on the angiopathy of hypertension and hyperglycemia and also on levels of microparticles in type 2 diabetic patients. For instance, angiotensin II receptor antagonists inhibit monocyte-derived MPs generation and decrease monocyte MPs levels in type 2 diabetic patients, suggesting that angiotensin II is intimately related to vascular changes that occur in type 2 diabetes mellitus [53-54]. Calcium antagonists, known to improve endothelial function in patients with hypercholesterolaemia by enhancing NO activity, and to increase endothelial NO bioavailability by antioxidant mechanisms, had also beneficial effects on MPs generation. For instance, type 2 diabetic patients treated with nifedipine showed a reduced level of platelet-, monocyte- and endothelial-cell derived MPs [55]. Benidipine administration also decreased concentrations of monocyte and endothelial MPs in hypertensive patients with type 2 diabetes [56]. Administration of probucol and triclopidine to hyperlipidemic patients with type 2 diabetes reduced monocyte and platelet-derived MPs [57]. All together, these results demonstrated the potential effectiveness of calcium antagonist therapy in type 2 diabetes. Furthermore, treatment of type 2 diabetic patients with statins reduced the exposure of glycoprotein IIb/IIIa on platelet-derived MPs by inhibiting platelet activation without affecting lipid levels [58]. These observations underlines pleiotropic effects of statins in the regulation of MPs formation in type 2 diabetes as suggest that combination of a statin and an angiotensin II receptor blocker might be valuable in reducing MPs effects in patients with type 2 diabetes.

In conclusion, recent studies on microparticles and diabetes provided new insight in the pathogenesis of diabetes. The increased levels of MPs in diabetes may be involved in crucial events leading to disease development and its complications as they promote inflammation, coagulation, endothelial dysfunction and angiogenesis. Elevated levels of endothelial MPs were found in type 2 diabetes, but mechanisms leading to their release in diabetes are unknown. Accumulation of remnant lipoproteins and oxLDL promote endothelial injury and are possible triggers.

**Fig. 1:** Microparticles and diabetes

**Legend:**
- EMPs = Endothelial MPs
- TF = Tissue Factor
- oxLDL = oxidized Low Density Lipoproteins
- Angiogenesis
- Endothelial dysfunction
- Capillary Occlusion
- Thrombosis
- Insulin+Glucose
tion in this setting. MPs could be involved in the progression of microvascular complications of diabetes. Circulating MPs could amplify endothelial dysfunction in diabetes by impairing NO release (Fig. 1). MPs express tissue factor, and tissue factor activity rises with increased levels of insulin and glucose observed in type 2 diabetes. Consequently, MPs could contribute to the hypercoagulable state of diabetes and possibly to capillary occlusion as seen in the retina. Finally, MPs could promote also angiogenesis, such as observed in diabetic retinopathy.

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References


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