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Review

Microparticles are vectors of paradoxical information in vascular cells including the endothelium: role in health and diseases

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Abstract:
Both inflammation and thrombosis can be orchestrated by the interactions between circulating cells, such as leukocytes and platelets, with vascular, endothelial and smooth muscle cells, which, during activation or apoptosis, can release circulating microparticles (MPs). Indeed, MPs are membrane vesicles with procoagulant and proinflammatory properties. MPs are present in blood from healthy individuals and in patients under several pathological states, for instance sepsis, preeclampsia, Crohn's disease and diabetes, strengthening the notion that MPs may play a role in these diseases. Circulating MPs or those generated *in vitro* from apoptotic T cells display deleterious effects on endothelial and/or vasomotor function. In contrast, MPs might be protective to endothelial cells. We have shown that MPs harboring the morphogen sonic hedgehog may represent a new therapeutic approach against endothelial dysfunction during acute severe endothelial injury. Indeed, these types of MPs induce NO release, decrease production of reactive oxygen species and induce angiogenesis from endothelial cells. This protective role for the endothelium was confirmed also by their *in vivo* injection in mice in which they were also able to reverse endothelial dysfunction in a model of heart ischemia/reperfusion. On the contrary, MPs from preeclamptic women compared to those from normal pregnant women showed pro-inflammatory properties in the vascular wall inducing vascular hyporeactivity in vessels from humans and mice. These effects were associated with complex interactions between NO and cyclooxygenase systems *via* endothelial cell activation. Altogether, these findings suggest that MPs can be considered as vectors of biological messages for vascular homeostasis, during immunity and inflammation.

Introduction

Microparticles (MPs), first described nearly 30 years ago and initially called "platelet dust", were reported as vesicles, smaller than 0.1 mm in diameter, which promoted coagulation [80]. Actually, MPs are considered to be membrane nano-fragments (0.05–1 μm) with procoagulant and proinflammatory properties. MPs are a disseminated storage pool of circulating ubiquitous bioeffectors, present both in healthy individuals and patients with several diseases and they take part in vascular function, strengthening the notion that they may play a role in both physiological organ functions and dysfunctions [47]. Indeed, MPs have been identified as vectors of the intercellular exchange of biologic information, such induction of endothelial modifications, angiogenesis or differentiation.

Coagulation and inflammation are an essential part of the defensive host response [24, 32]. Activation of

coagulation and generation of active proteinases are initiated by tissue factor (TF) that is expressed by cells of the innate immune system and endothelial cells after tissue disturbances and cell activation induced by cell injury [22]. These processes have several connecting points accounting for the association and/or the interaction between coagulation and inflammation pathways [46]. The first link between these processes is endothelium, which, after damage, expresses the adhesive proteins (vWF, P-selectin) and receptors, involved in both coagulation and inflammation [24, 82]. The second link are platelets which release proteins with procoagulant and proinflammatory properties after activation [15]. The third links are the serine proteinases, initiated by TF which triggers blood coagulation at sites of tissue injury by selective binding of factor VIIa and Xa. TF/VIIa/Xa can activate both the inflammatory responses of endothelial and other cells and also blood coagulation through stimulation of thrombin generation [14].

The aim of this review is to explain the physiological and pathophysiological role of MPs in regulating inflammation and thrombosis in particular, largely demonstrated in the field of experimental considerations and associated diseases such as sepsis or preeclampsia. Of note is the existence of excellent reviews dealing with this topics [40, 48, 60, 73].

Mechanisms of MP formation and intercellular communication

The MPs are generated after cell activation or apoptosis following the disturbance of membrane phospholipid asymmetry by acting on pumps responsible for phospholipid transport. Changes in phosphatidylserine (PS) and cytoskeleton degradation are followed by MP release [27].

MP composition is not yet elucidated, but it differs depending on the cell origin and stimuli of their generation [18]. An early event is, in particular, reorganization of the lipid asymmetry of the plasma membrane, with externalization of PS. Lipid rafts and their accompanying cholesterol and proteins are concentrated where the membrane buds. Increased levels of cytoplasmic calcium activate different cytosolic enzymes relevant to MP formation. Calpain is one of the most important enzyme and has several actions in MP generation including cleaving of cytoskeletal filaments, facilitating MP shedding, and activating apoptosis through procaspase-3 [13].

Different mechanisms may contribute to the processes of MPs formation in cell-specific ways and the type of MPs may change depending on a given stimulus which initiates their generation [31]. Inflammatory stimuli include cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1β) etc. Beside, chemical agents, including ionomycin, phorbol myristate acetate and concanavalin A, can also release phenotypically distinguishable MPs [17].

Recent evidence shows that MPs are important for communication between cells. It has been described that they can transfer receptors and organelles between cells and deliver mRNA and proteins into cells [61]. In this review we will focus on shedding light on these properties in two specific pathological states: preeclampsia and sepsis, characterized by inflammation and coagulopathy, representing deleterious effects of MPs on endothelial and/or vasomotor function [7, 39].

Involvement of MPs in coagulation

PS, one of the hallmarks of MPs formed during apoptosis, is considered to be a starter of coagulation cascade [16, 25] in the blood vessels. Platelet MPs (PMPs) have been extensively investigated for their participation in the normal primary hemostatic response and because they exhibit a catalytic surface for the prothrombinase enzyme complex [11]. The physiological role of PMPs in hemostasis is demonstrated by the fact that a deficiency of PMP generation leads to a bleeding disorder [10]. Indeed, the release of MPs from the platelet surface is associated with membrane exposure to factor Va and increased prothrombinase activity, with the subsequent generation of thrombin.

Extensive studies of the role of MPs in the physiological modulation of coagulation and thrombosis have been carried out on MPs generated from platelets. PMPs expose negatively charged phospholipids, which provide binding sites for activated coagulation factors, giving PMPs procoagulant properties. PMPs bind to the subendothelial matrix and adhere to thrombin-activated ECs, involving the GPIIb/IIIa receptors and provide binding sites for soluble fibrino-

gen. Most likely, PMPs could initially bind to the exposed subendothelial matrix, thus providing a substrate for further platelet adhesion *via* GPIIb/IIIa-fibrinogen bridging. Then, fibrinogen may act as a bridging molecule between platelets and GPIIb/IIIa on PMPs playing a significant role in hemostasis and atherosclerosis [48]. MPs of other cellular origin can provide TF, the initial activator of the blood coagulation pathway that culminates in the generation of a fibrin clot [23, 49]. The active form of TF requires the presence of PS which is exposed on apoptotic cell surface and MPs. Since TF is highly expressed in atherosclerotic plaques with a rich content of MPs, TF may plausibly have a significant and determinant role in thrombus formation after plaque disruption [77].

As to the surface markers, PMPs are major carriers of platelet-activating factor (PAF), a potent phospholipid mediator associated with the pathogenesis of inflammation [81]. Although PMP binding to fibrinogen and fibronectin is strongly inhibited by an inhibitor of GPIIb/IIIa, a weak inhibition of MPs binding to collagen I and III is observed using the same inhibitor, suggesting that collagen is not a major ligand for MPs and platelet GPIIb/IIIa.

Endothelial MPs (EMPs) exhibit a part of von Willebrand factor resulting in platelet aggregate formation [31]. Furthermore, during atherosclerotic plaque disruption, the principal cause of acute coronary syndromes, high levels of EMPs have been detected, suggesting a potential role for acute endothelial injury in thrombus formation [38, 74]. The EMP phenotype is similar to that displayed by activated human umbilical vein cells (HUVECs) stimulated with TNF-α, suggesting that EMPs may possess the ability to develop adhesive interactions with leukocytes in the blood. Indeed, they could bind to monocytes and modulate their procoagulant activities [67]. EMPs interact with monocytic THP-1 cells *in vitro,* stimulating TF-mediated procoagulant activity. The effect of EMPs is partially dependent on the receptor interaction between ICAM-1 on EMPs and its β2 integrins on THP-1 cells [54].

TF is also present in MPs from activated fibroblasts, monocytes and leukocytes. These vesicles may act as adhesion surfaces for coagulation factors in atherosclerotic injuries as well as EMPs. Moreover, MPs from leukocytes can transfer TF to platelets and trigger thrombosis [25, 48]. Furthermore, recently an accumulation of TF has been shown in developing thrombi *in vivo* after interaction between platelet P-selectin and its ligand P-selectin glycoprotein ligand 1 carried by MPs from leukocytes expressing TF [19]. These results emphasize the role of leukocytes-derived MPs in blood coagulation and thrombosis.

MP-derived mediators stimulate inflammation

MPs are a source of aminophospholipids and also a preferential substrate for phospholipase A2 involved in the release of lysophosphatidic acid, which, in turn, triggers platelet aggregation and the inflammatory process [26, 37].

Numerous studies henceforth showed that the MPs are real effectors of endothelial inflammation. The first works concerning the effect of the PMPs on endothelial cells in culture have shown that arachidonic acid (AA) that they transport, could lead to an increase in cyclooxygenase-2 (COX-2) and ICAM-1 expression [3, 4], COX-2 and ICAM-1 modulate the vascular and platelet functional interaction and activate a membrane-linked signaling cascade. Indeed, PMP-borne AA regulates COX-2 expression in monocytes inducing the translocation of protein kinase C (PKC) from the cytosol to the membrane with the concomitant activation of different kinases [3].

MPs can facilitate the interactions between leukocytes and endothelium. Indeed, PMPs or MPs of leukocyte origin participate in the release of several endothelial (IL-1 β, IL-6, IL-8, MCP-1) [43] or monocytic (Il-1 β, TNF-α, Il-8) cytokines. In addition, unmetabolized AA contained in PMPs is implicated in platelet aggregation and adhesive interactions of both platelets and monocytes with ECs [5]. These events could be involved in inflammatory processes and, together with subsequent endothelial dysfunction, may participate to atherosclerosis development.

Cytokines also participate in the generation of MPs including PMPs with both pro-inflammatory and procoagulant properties. Indeed, the evaluation of plasma concentrations of cytokines and PMPs in patients with arteriosclerosis provided evidence that increased levels of IL-6 were correlated with both enhanced expression of P-selectin and PMP generation under high shear stress conditions [51– 54]. Besides, PMPs could facilitate the recruitment of numerous immune cells (monocytes, T and B lymphocytes, NK cells) [56]. The

pro-inflammatory effect of MPs is probably linked to the ability of oxidized phospholipids to activate the receivers of the platelet-activating factor (PAF) present on endothelial cells and leukocytes [81].

Some evidence supports the view that interaction between platelets, leukocytes and ECs plays an important role in vascular inflammation. In the study conducted by Mesri and Altieri [42], the incubation of HUVECs with MPs derived from leukocytes induced an important *de novo* synthesis of IL-6, IL-8 as well as membrane adhesion molecules implicated in ECs-leukocytes interaction. The ability of leukocyte MPs to directly modify cellular gene expression is reflected by the up-regulation of IL-6 mRNA in stimulated ECs. This work suggests that MPs can act as agonists of inflammation.

MPs also represent an important source of substrates for the phospholipase A2, massively released during sepsis, leading to the generation of lysophosphatidic acid, a powerful pro-inflammatory mediator and platelet agonist [73].

MPs and vascular function

The effects of MPs on the vascular wall could be summarized as follows: they are able to act on both endothelial cells [39] and smooth muscle cells [59, 72], and, consequently, regulate vasomotor reactivity as well as angiogenesis [33, 71]. The endothelial responses can be immediate (release of several factors) or delayed (regulation of gene expression involved in the structural and functional regulations of the vascular wall). An increase in MPs of endothelial origin was observed in several pathologies associated with endothelial dysfunction [75]. MPs participate in the regulation of the vascular tonus, notably by decreasing the production of nitric oxide (NO), a powerful vasodilator, anti-platelet agent and a major factor of survival of endothelial cells [40]. This effect was observed in isolated arteries exposed *in vitro* to the actual concentrations of MPs isolated from blood of patients with coronary syndrome, renal failure or preeclamptic women [2, 76] while the MPs stemming from healthy volunteers had no effect.

EMPs alter NO production in rat aortas by increasing the oxidative stress (enhanced production of superoxide anion) that, in turn, reduces the bioavailability of NO [9]. MPs of lymphocytic origin isolated from diabetic patients lead to endothelial dysfunction, especially by decreasing the endothelial NO-synthase (eNOS) expression and increasing caveolin-1 expression [39].

MPs are also able to act directly on smooth muscle cells through the activation of the transcription factor κB (NF-κB), leading to enhanced expression of inducible NOS (iNOS) and COX-2 with subsequent increased NO and prostacyclin productions respectively, ending in a blunt of vascular contractility to agonists [72]. Besides, it was experimentally demonstrated that PMPs, source of thromboxane-A2, could modulate the vascular tonus in rabbit [59]. Another important contribution of the MPs to the vascular system function consists in their capacity to promote angiogenesis. PMPs isolated from healthy individuals, provoke a proliferation and migration when they are added to cultured ECs. These effects are mediated by their lipid component, notably sphingosine 1-phosphates [33]. EMPs express on their surface protease activities, of metalloproteinase (MMP) type, notably MMP-2 and 9 [71], suggesting their participation in the degradation of extracellular matrix, the stage necessary for the vascular remodeling. Besides, MPs generated *in vitro* from human T cells or from monocytes improve the synthesis of MMP-1, 3, 9 and 13 in fibroblasts [17].

From the above evidence, one can advance the hypothesis that MPs, acting on inflammation, coagulation and vascular biology play a dual role, both as a cause and, at the same time, a consequence of vascular diseases.

Deleterious biological effects of MPs in inflammatory vascular diseases: preeclampsia and sepsis

The pathophysiology of preeclampsia is associated with systemic vascular inflammation with neutrophil activation [36] and oxidative stress [63]. Previous studies concerning MPs from healthy and preeclamptic pregnant women showed that the total number of circulating MPs was not significantly altered despite an increase in the number of T-cell and granulocyte MPs. Moreover, a close relationship between endothelial dysfunction and circulating levels of EMPs

was reported in preeclamptic patients [64]. In our recent study [44], we provided evidence that women affected by preeclampsia displayed elevated levels of MPs derived from lympho-monocytes and platelets in their blood stream compared with normal pregnancy. Furthermore, we demonstrated that MPs from preeclamptic women (PrMPs) were able to induce reduced vascular responsiveness to a vasoconstrictor agent in both human omental arteries and mouse aortic rings without changes in endothelial function. These effects were observed at circulating concentrations of PrMPs, and they were associated with an up-regulation of proinflammatory protein expression, namely iNOS and COX-2, through the activation of the transcription factor NF-κB in the vascular wall. Thus, PrMPs stimulated the release of NO and COX-2 metabolites. The proinflammatory property of PrMPs was associated with oxidative and nitrosative stress in the vascular wall (Figure 1). Interestingly, when we separated and tested MPs from platelets and those of non platelet origin (most probably of leukocyte origin) for vascular reactivity, only platelet PrMPs were able to stimulate the release of NO, suggesting a positive role of this type of MPs resulting in blunting the increased blood pressure characterizing preeclampsia, whereas those of non-platelet origin induced both the release of NO and COX-2 vasoconstrictor products, especially 8-isoprostane, the circulating level of which has been reported to be increased in placenta of preeclamptic women [79].

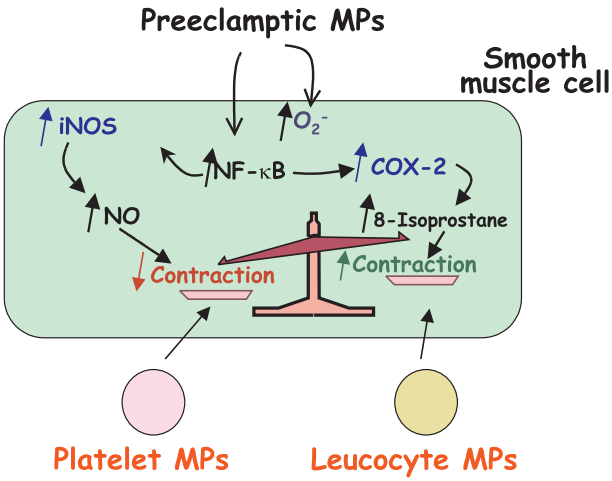

Fig. 1. The proinflammatory property of preeclamptic microparticles (PrMPs) was associated with oxidative and nitrosative stress in the vascular wall. Interestingly, platelet PrMPs were able to stimulate the release of NO from inducible NO synthase (iNOS) and decrease contraction, suggesting a positive role of this type of MPs resulting in blunting the increased blood pressure characterizing preeclampsia, whereas those of non-platelet origin most likely leucocyte MPs induced both the release of NO and cyclooxygenase-2 (COX-2) vasoconstrictor products, especially 8-isoprostane.

These studies suggest the involvement of MPs in the modulation of the vascular changes typical for preeclampsia [79]. The syncytiotrophoblast membrane fragments are an additional source of MPs during preeclampsia [62]. Placental oxidative stress destabilizes the syncytiotrophoblast cells, resulting in an increase in the release of MPs containing oxidized lipids. This type of MPs has also been shown to cause endothelial activation.

Other acute inflammatory events, such as those that occur during sepsis, lead to dysregulation of the coagulation cascade. The hemostatic imbalance in sepsis, characterized by the excessive activation of procoagulant pathways and the impairment of anticoagulant activity, leads to disseminated intravascular coagulation and results in microvascular thrombosis, tissue hypoperfusion and, ultimately, multiple organ failure and death. Furthermore, sepsis is characterized by a high cardiac output state with a low peripheral resistance hemodynamic profile that includes arterial dilatation [66]. A prolonged or excessive drop of the peripheral resistance may cause progressive hypotension that is refractory to catecholamines, and contributes to life-threatening cardiovascular failure [65]. A release of reactive oxygen species (ROS) by different pathways contributes to the failure of organs such as lung, heart, brain and liver [12]. Data collected from clinical studies and different models of endotoxemia suggest that this phenomenon is related to the activation of the NF-κB/RelA pathway, enabling the expression of several specific genes involved in the pathogenesis of septic shock, such as cytokines, adhesion molecules, COX-2 and iNOS [58]. The induction of iNOS and the overproduction of NO play a major role in endotoxemia-induced vascular hyporeactivity in different experimental models and also in the small vessels harvested from patients with septic shock [70].

Several pro-inflammatory agents are known to induce the phenotypic changes of endothelium [69]. Endothelial apoptotic cells produce IL-1β which, in turn, is capable of amplifying the mechanisms involved in endothelial cell apoptosis and to activate, *via* the activation of the transcriptional factor NF-κB, the expression of numerous products of pro-inflammatory and prothrombotic genes. Simultaneously, the endothelial surface becomes capable of recruiting and activating

the circulating platelets [6]. All these modifications constitute a prothrombotic phenotype of the endothelium [35]. During sepsis, the generation of procoagulant MPs of endothelial, platelet, erythroid and leukocyte origin, possible carriers of TF, has been reported [28, 50]. In these clinical conditions, numerous factors, such as thrombin, cytokines and oxidative stress, could contribute to procoagulant MP generation [30, 57]. MPs in this context could have a noxious role by activating the inflammatory process, the cellular apoptosis and by facilitating the development of a syndrome of multiorgan failure [34]. The MPs released by the activated endothelium or apoptotic cells participate in the global prothrombotic status improvement during sepsis by supplying supplementary lipid surfaces in thrombin generation [8] and by increasing the TF expression in monocytes. Several experimental models developed in the last years allowed to describe the cascade of events implicated in the cellular activation, the exhibition of TF, the generation of carrier MPs of TF and the release of an intravascular disseminated coagulopathy. In humans, 4 h after a single dose of endotoxin, a substantial release of MPs from monocytes or endothelial cells, carriers of TF, was observed. The activity of the TF carried by MPs remained increased in septic patients compared to normal subjects [77].

During sepsis, the increase in the actual TF activity, insufficiently counterbalanced by a greater synthesis of tissue factor pathway inhibitor (TFPI), facilitates the spread of coagulopathy, microcirculatory thrombosis, tissue hypoxia and then generation of lactates [34], which contribute to the multiorgan failure [21, 22, 50].

MPs generated during sepsis participate in the modulation of oxidative status in several tissues. During the systemic inflammatory response syndrome (SIRS) or neuromalaria [56], increased levels of MPs of endothelial origin have been detected. These MPs form microaggregates with circulating neutrophils and increase considerably the oxidizing activity [57]. NADPH oxidase subunits, implicated in oxidative stress, were identified on MPs of platelet origin associated with a greater production of ROS.

PMPs of septic individuals possess proapoptotic NAD(P)H oxidase activity, evidenced *in vitro* by a greater apoptosis of endothelial or smooth muscle cells incubated with septic MPs compared to cells incubated with MPs from healthy volunteers [29].

These studies suggest that MPs circulating in patients affected by some kind of inflammatory diseases could be considered as a novel vascular redox pathway.

Beneficial biological effects of MPs

The beneficial functions displayed by MPs in biological processes are not widely reported. MPs should play a physiological role during development, angiogenesis, wound healing, and, more generally, in tissue remodeling, under the positive or negative gradients of information delivered from neighboring cells [20, 32]. The ability to induce distinct patterns of genes by plasma membrane fragments has been considered to be relevant to development [78].

Among the candidate proteins implicated in the ligand-receptor interactions between target cells and MPs, Martinez and co-workers [41] have reported that the morphogen sonic hedgehog (Shh) is carried by MPs. Shh is involved in embryonic and adult development, and dysregulation of the Shh pathway can lead to tumors and erectile dysfunction. NO-synthase and vascular endothelial growth factor (VEGF) are downstream targets of exogenous Shh signaling, suggesting that Shh can act as a modulator of VEGF regulation and NO production [32, 55]. In a recent study [1], we provided evidence that MPs produced *in vitro* from human activated/apoptotic T-lymphocytes, expressed membrane-bound Shh. This type of MPs was able to induce NO production from endothelial cells and organs; and this effect was mediated directly by the Shh pathway, as illustrated by the reduction of NO production when the Shh pathway was silenced. Most interestingly, this type of MPs restored endothelial dysfunction in a model of mice coronary arteries subjected to ischemia/reperfusion. The endothelial dysfunction described in this model is indeed characterized by an impaired endothelium-dependent vasodilatation, exaggerated endothelium-dependent vasoconstriction and increased production of endothelin-1 and ROS, leading to increased vasoconstriction and reduction of blood flow [45].

The capacity of these MPs to generate NO was confirmed after *in vivo* injection of MPs into mice. Indeed, circulating and tissular NO generated after *in vivo* MP treatment enhanced endothelium-dependent relaxation in normal mice. Finally, MPs reverse endo-

thelial injury probably through their dual ability to increase NO and reduce ROS. These data suggest that MPs harboring Shh may represent a new therapeutic approach against endothelial dysfunction during acute severe endothelial injury.

Conclusion and perspectives for the future

The data accumulated in the literature over the last years on the beneficial or deleterious role played by MPs in physiological or pathological conditions, and, in particular, during inflammatory diseases, suggest that they could be friends or foes for the organism, for the vascular wall depending on their cellular origin and the stimuli involved in their generation in cells. It is interesting to note that, on the basis of some studies, we suggest that MPs could be considered a new therapeutic target in treatment of several pathological states, to reduce their deleterious effects linked to their procoagulant and proinflammatory properties in the vascular wall and target organs. Other, more recent studies, suggest that MPs produced *in vitro,* under particular conditions, can be considered the new therapeutic agents able to reduce endothelial dysfunction, improving or restoring normal vascular function. These two properties of MPs are not mutually exclusive. Indeed, the above reports are not in opposition to each other, taking into account that the cellular origin, contents and ways used to produce MPs are strongly variable. The common point in the literature is that MPs are small but potent biological agents implicated in exchanging biological signals, interacting with target cells and inducing both beneficial and detrimental responses due to their ability to regulate gene expression involved in inflammation, oxidative stress and regulation of the vascular function.

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