PLATELET MICROPARTICLES FROM THEIR FEATURES TO STROKE IMPLICATION

A. Bulboaca[1], Adriana Bulboaca[2]

[1]Neurology Department, "Iuliu Hatieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania

[2]Pathophysiology Department, "Iuliu Hatieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania

ABSTRACT

Since 1967 when platelet microparticles were discovered, the platelet's researchers were demonstrated the procoagulant properties of these platelet membranes nano-fragments. They are versatile bioeffectors in haemostasis because of their membrane receptors content. From these reasons, recently has been suggested that the platelet microparticles may constitute an important marker of atherothrombosis and, consequently a prognosis factor in various vascular diseases, including ischemic stroke. On the other way, the antiplatelet therapy focused on platelet microparticles activity may be beneficial.

Key words: microparticles, platelet activation, stroke, atherosclerosis markers

INTRODUCTION

Microparticles (MP) are considered to be membrane nano-fragments with 0.05-1 μm diameter with intact vesicular appearance. The smaller particles derived from endoplasmatic membranes are named exosomes, and larger particles which containing nuclear material (>1.5 μm) are known as apoptotic bodies. MPs are produced by various circulating cells and by endothelial cells after their activation or apoptosis. On their membranes exists oxidised phospholipids and specific proteins common which are common with those founded on their origin cells. MPs, including the platelet microparticles (PMP) are bioactive because of their procoagulant activity, activation of inflammation mechanism, and activation of angiogenesis (1).

Electronic microscopy studies revealed that their structural and architectural properties are wide heterogeneous their size and composition been very variable. The later methods, enzyme-linked immunosorbent assay (ELISA), and flow cytometry shows that their structural properties depend on their cells of origin and this is very useful for their identification by specific expressed antigens. The most used methods for microparticles assessment are electronic microscopy, enzyme-linked immunosorbent assay (ELISA), and flow cytometry. Other methods like measurement of total phosphate to establish phospholipids concentration or immunoelectrophoresis has been used, but the results cannot be directly compared with flow cytometry method (the most accurate for quantitative MP assessment)(2).

The composition of MPs depends also by the stimulus that triggers their formation and release. There are different composition types of MPs in the case of activation stimuli or apoptotic stimuli (3).

In 1967 Wolf described for the first time platelet's membrane fragments originated from activated platelet called "platelet dust" (4). Since then, numerous studies have reported in vitro release of vesicles from activated or apoptotic cells called later platelet microparticles (PMP). In 1985 Sandberg

Author for correspondence:
Angelo Bulboaca, "Iuliu Hatieganu" University of Medicine and Pharmacy, 8 Victor Babes Street, Cluj-Napoca, Romania
email: angelo.bulboaca@yahoo.com

revealed the procoagulant properties of PMP. He showed that the activated platelet factor 1 (PF1) and platelet factor 3 (PF3) are found both in collagen activated platelet and PMP (electron microscopy study). He also demonstrated that the activated platelet can generate two types of PMP different in size (small PMP with 80-200 nm and large PMP with 400-600 nm). Both types of PMP have been connected to procoagulant properties (5). In 1991 Bode discovered that the PMP are releasing also during the aging process. In this study membranous microparticles (MP) appearing in the supernatant plasma of stored platelet concentrates (PC) were analyzed by flow cytometry (6).

MICROPARTICLES FORMATION AND RELEASING MECHANISMS

The mechanisms of in vivo microparticles formation and release are not entirely known. The information concerning these processes are derives from in vitro studies on isolated or cultured cells which demonstrated both activation and cell's apoptosis may lead to microparticle formation and releasing. In 2007 Boulanger was made an in vivo correlation between endothelial circulating microparticles and shear stress (SS). This study demonstrates for the first time the relation between in vivo circulating endothelial MPs and mechanic hemorheological parameters in hemodialyzed patients, depicting SS as a major determinant for endothelial cell injury and vesiculation (7).

In the first step, in the generating cell the plasma membrane asymmetry occur leading to the formation of membrane vesicles. This process requires the cytoskeleton protein reorganisation as a consequence of intracellular calcium increases. The provenience of intracellular calcium is from intracellular sources (dense tubule) and by increase of intracellular calcium influx. The increase of intracellular calcium lead to calcium-dependent enzymes activations and the result is an exposure of phosphatidylserine on the outer leaflet. The level of intracellular calcium is the main factor which can lead to release or not the formatted MP. The releasing of MP is also conditioned by disruption of protein cytoskeleton organisation (8). The MP releasing from endothelial cells, monocytes, erytrocytes may be induced by proinflammatory cytokines as IL-1 and TNF alpha, by bacterial lipopolysaccharides, the complement complex C5b-9, the calcium ionophore A23187, the reactive oxygen species, the aging process (during storage). Increase of the intracellular calcium seems to be the critical step for all types MP release (9,10).

Microparticle formation in platelets and their release occurs as a result of activation by various stimuli (epinephrine, adenosine diphosphate, collagen, thrombin, A23187) and during storage (6,11). Other stimuli connected with PMP releasing were complement complex C5b-9 or high shear stress. The PMP releasing under high shear stress in related to platelet adhesion to von Willebrand factor (12-14).

Increased platelet MP formation has been documented in a variety of clinical conditions that are associated with platelet activation (15). Platelet-derived MPs that express surface glycoproteins and antibodies to CD41, CD42, and CD61 have been used to detect platelet-derived MPs (16,17).

The release of PMP in related to activation of calpain (calcium dependent enzyme which is implicated in the cellular cycle and apoptosis). Calpain induces cytoskeleton conformational changes trough activation of various intermediate enzymes. The increase of intracellular calcium is necessary for calpain activation. If the hemodynamic stress is increased the activation of protein C is mandatory for calpain activation and consequently releasing of PMP (18).

Other important enzyme implicated in PMP release is caspase-3 (an important enzyme in apoptosis process). The platelets caspase-3 activation appears to be triggered by platelets agonists like collagen, thrombin, and calcium ionophore A23187. Under the influence of these platelet agonists a platelet subpopulation generates PMP. This process begins with the exposure of the phosphatidylserine in the external membrane layer, and the next step is the moesin cleaving (a specific platelet cytoskeleton protein). The phosphatidylserine exposure was inhibited by specific antagonists of caspase-3, since the specific antagonists of calpain do not inhibits this process. Form these reasons, is considered that mechanism of PMP releasing under the calpain or caspase-3 activation is different (19).

Gelsolin is also specific for PMP releasing because gelsolin is found only in platelets. Is a specific enzyme which is induced by increased cytosolic calcium and produce the remove of the capping protein at the end of the actin filaments of the platelet cytoskeleton. Consequently platelet contraction occurs (20).

Other important enzyme for PMP formation and releasing is aminophospholipid translocase which have the role of phosphatidylserine transport from the outside to the inside of the platelets. The

intracellular calcium increasing inhibits this enzyme and activates other enzyme named scramblase. Consequently the asymmetry of membrane's phospholipids occurs and phosphatidylserine remain on the outside layer of platelets membrane (21).

The significance of MP formation and releasing is unknown, but may be assume that the cells attempt to reverse the apoptotic process by getting rid of unwanted signalling molecules. The MP release would also allow cells to escape phagocytosis by removing signal-molecules like phosphatidylserine. Alternatively was proposed that the MP release is a defence mechanism because of MP participation in intercellular communications, hemostasis, inflammation, immune process, and angiogenesis. When the MP releasing is a physiological or pathophysiological process remain under research (22).

PLATELET MICROPARTICLES INVOLVEMENT IN HEMOSTASIS

Adhesion facilitation

The GP Ib exposure on the PMP surface lead to adhesion facilitation. Like platelets the PMP may adhere to von Willebrand factor on the endothelial cells. The both P-selectin and GP Ib facilitate the PMP-neutrophil collaboration. In this way the adhesion process is associated with PMPs-leukocytes interactions. The release of arachidonic acid from PMP leads to direct activation of P-selectin and E-selectin on the endothelial cells surface. These properties supporting that the PMP are important in cell-cell communication process (23-25).

Aggregation facilitation

The platelet membrane vesiculation for PMP formation and releasing necessitate the intact GP IIb/IIIa fragment. From this reason, on the PMP surface always have an intact GP IIb/IIIa which leads to increase of PMP aggregation (intermediate by fibrinogen molecule. Even in some conditions the GP IIb/IIIa is dissociated on the MPM surface, in the presence of platelet agonists the reassociation of dissociated GPIIb and GPIIIa occurs (26-27).

Procoagulant properties

The PMP exhibits passive and active procoagulat properties. The passive procoagulant properties are revealed by the presence of factorV/Va on their surface and by ability of surface phospholipids for assembly of tenase and prothrombinase complexes. These procoagulant properties are passive because by this process the PMP may support coagulation but not themselves initiate coagulation (28).

Tissue factor exposure on the PMP surface is conected to active procoagulant properties. Tissue factor exposure occurs not only on PMP surface but on other MP type surface. The PMP tissue factor may activate the tissue factor production by various cells like endothelial cells and monocytes (29).

The procoagulant properties are important because PMP were found to be increased in association with various vascular disease as myocardial infarction, peripheral arterial disease, cardio-pulmonary bypass, and stroke (30-31).

Anticoagulant properties

In some circumstances the PMP may have anticoagulant properties by acting on protein C system. In normal subjects PMP support low levels of thrombin generation which is will bound to thrombomodulin leading to activation of protein C and thus inactivation of FVa and FVIIIa. PF4 (found on the PMP surface) is also involved in regulation of coagulation through activation of protein C system. Thus, in normal condition, PMP are involved in the control of normal hemostasis. Further studies are needed to find when the PMP are involved in accelerating or inhibiting coagulation (32-33).

PLATELET MICROPARTICLES AND ISCHEMIC CEREBROVASCULAR DISEASE

The platelet activation is an important pathogenetic process in ischemic cerebrovascular disease. Because the formation, release and level of circulating PMP reveal the platelet activation they became an interesting research subject in various ischemic vascular diseases. In 1984 Bulboaca observed (by electronic microscopy) the microvesicles released by activated platelets of patients with ischemic stroke (after in vitro activation by epinephrine) (34) (Fig. 1, 2). This phenomenon was not observed in the health subjects.

Several clinical studies were made in the attempt to find if the circulating PMP level is associated with some pathological conditions, and if they may constitute a marker for endothelial dysfunction. A potential pathophysiological link between stroke and PMP number in peripheral blood were the first time reported in patients with prosthetic heart valves (35). The research in this field, particularly

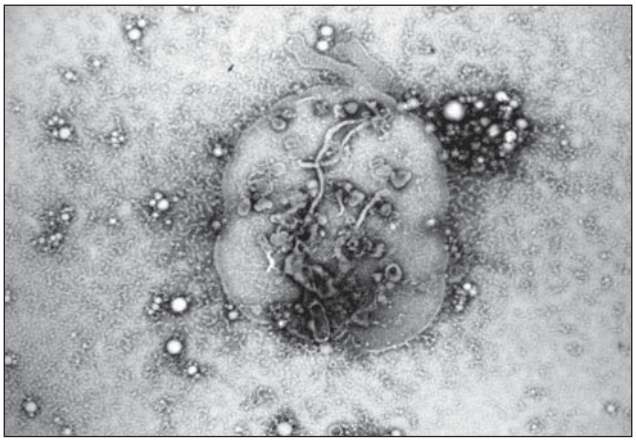

FIGURE 1. *Spread platelet (contact activation) with a lot of microparticles on the surface and around (ischemic stroke patients); electronic microscopy – 10000 magnification, negative stain with phosphotungstic acid.*

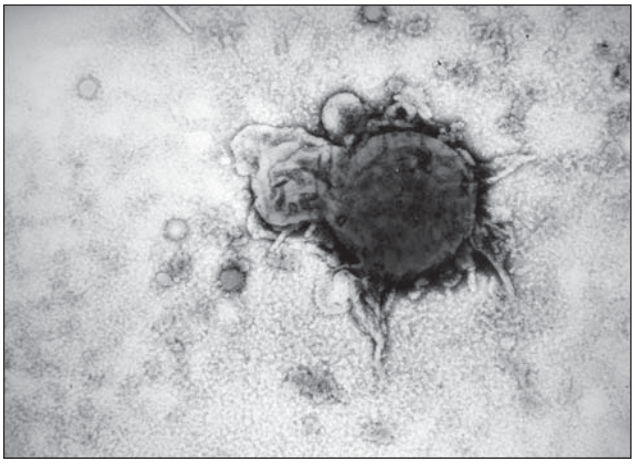

FIGURE 2. *Dendritic platelet (epinephrine activation) with pseudopodia, membrane vesiculation, and microparticles (ischemic stroke patients); electronic microscopy – 10000 magnification, negative stain with phosphotungstic acid.*

in cerebrovascular diseases is in the beginning and just a few data are available.

The PMP were founded increased in both acute and chronic phase of cerebral infarction. In 2003 Cherian and col, were founded a correlation between the elevated blood PMP and endothelial dysfunction markers like P-selectin and E-selectin in acute phase of cerebral infarction. There was a strong, graded, and independent (of age, sex, and vascular risk factors) association between increasing blood concentrations of E-selectin during the acute phase and all etiological subtypes of ischemic stroke, particularly ischemic stroke caused by large-artery atherothrombosis (36).

Kuriyama and col. revealed a high level of PMP in blood (ELISA assessment) during acute phase of cerebral infarction. The elevated PMP level was significantly correlated with concomitant intima-media thickness and with concomitant intracranial stenosis of carotid arteries. The level of PMP was also significantly elevated in the patients with cerebral infarction in anterior, posterior, middle cerebral arteries, and lacunar stroke in contrast with the group of the patients with cardioembolic stroke. This difference of the PMP number between cardioembolic and thrombotic stroke may be useful for differential diagnosis. They demonstrated also a significant direct correlation between the elevated level of PMP and the elevated levels of C reactive protein and fibrinogen. These evidences are strongly support that the level of blood PMP may be considered as an important marker for biological evaluation of atherosclerotic plaques from both points of view morphological and pathophysiological (37).

The PMP remain elevated also in chronic phase of cerebral infarction and they are not influenced by some specific antiplatelet therapy (aspirin combined with cilostazol – a phosphodisetrase inhibitor with antiplatelet and vasodilatator properties)(38). On the contrary in TIA patients the antiplatelet therapy (a combination between aspirin and Clopidogrel) produce the decrease of PMP in peripheral blood (39).

Because the levels of PMP remain unchanged in chronic phase of ischemic stroke comparative with acute phase of ischemic stroke, the PMP measurement in ischemic stroke may constitute a specific biomarker for cerebral atherosclerotic plaque assessment under the increased shear stress. From these reasons the PMP assessment in ischemic stroke reveals not only platelet activation but also an endothelial dysfunction and may be important as a prognostic parameter. Is a researchable theory if the stroke occurrence is based on previous increased PMP. Further study would be necessary for evaluation of relationship between the PMP levels, active atherosclerotic plaques and recurrence rate of ischemic stroke. More than that is researchable whether an antiplatelet therapy (which dose or combinations) can reduce the PMP levels or their procoagulant features.

REFERENCES

1. **Boulanger CM, Leroyer AS, Amabile N, Tedgui A** – Circulating endothelial microparticles: a new marker of vascular injury. *Ann Cardiol Angeiol.* 2008; 57:149-54.
2. **Horstman LL, Jy W, Jimenez JJ, Bidot C, Ahn YS** – New horizons in the analysis of circulating cell-derived microparticles. *Keio J Med.* 2004; 53:210-30.
3. **Diamant M, Tushuizen ME, Sturk A, Nieuwland R** – Cellular microparticles: new players in the field of vascular disease? *Eur J Clin Invest.* 2004; 34:392-401.
4. **Wolf P** – The nature and significance of platelet products in human plasma. *Br J Haematol.* 1967 ; 13:269-88.
5. **Sandberg H, Bode AP, Dombrose FA, Hoechli M, Lentz BR** – Expression of coagulant activity in human platelets: release of membranous vesicles providing platelet factor 1 and platelet factor 3. *Thromb Res.* 1985; 39:63-79.
6. **Bode AP, Orton SM, Frye MJ, Udis BJ** – Vesiculation of platelets during in vitro aging. *Blood.* 1991; 77:887-95.
7. **Boulanger CM, Amabile N, Guérin AP, Pannier B, Leroyer AS, Mallat Z, Nguyen C, Tedgui A; London GM** – In Vivo Shear Stress Determines Circulating Levels of Endothelial Microparticles in End-Stage Renal Disease. *Hypertension.* 2007; 49:902-908.
8. **Boulanger CM, Amabile N, Tedgui A** – Circulating microparticles, a potential prognostic marker for atherosclerotic vascular disease. *Hypertension.* 2006; 48: 180- 188.
9. **Doeuvre L, Plawinski L, Toti F, Anglés-Cano E** – Cell-derived microparticles: a new challenge in neuroscience. *J Neurochem.* 2009 ; 110: 457-68.
10. **Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH, Papassideri IS** – RBC-derived vesicles during storage: ultrastructure, protein composition, oxidation, and signaling components. *Transfusion.* 2008; 48:1943-53.
11. **Horstman LL, Ahn YS** – Platelet microparticles: a wide-angle perspective. *Crit Rev Oncol Hematol.* 1999; 30:111-42.
12. **Sims PJ, Rollins SA, Wiedmer T** – Regulatory control of complement on blood platelets. Modulation of platelet procoagulant responses by a membrane inhibitor of the C5b-9 complex. *J Biol Chem.* 1989; 264:19228-35.
13. **Reininger AJ, Heijnen HF, Schumann H, Specht HM, Schramm W, Ruggeri ZM** – Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. *Blood.* 2006; 107: 3537-45.
14. **Takano K, Asazuma N, Satoh K, Yatomi Y, Ozaki Y** – Collagen-induced generation of platelet-derived microparticles in whole blood is dependent on ADP released from red blood cells and calcium ions. *Platelets.* 2004; 15: 223-9.
15. **Solum NO** – Procoagulant expression in platelets and defects leading to clinical disorders. *Arterioscler Thromb Vasc Biol.* 1999; 19: 2841-6.
16. **Combes V, Dignat-George F, Mutin M, Sampol J** – A new flow cytometry method of platelet-derived microvesicle quantitation in plasma. *Thromb Haemost.* 1997; 77:220-5.
17. **Diamant M, Nieuwland R, Pablo RF, Sturk A, Smit JW, Radder JK** – Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes mellitus. *Circulation.* 2002; 106: 2442-7.
18. **Pasquet JM, Dachary-Prigent J, Nurden AT** – Calcium influx is a determining factor of calpain activation and microparticle formation in platelets. *Eur J Biochem.* 1996; 239: 647-54.
19. **Shcherbina A, Remold-O'Donnell E** – Role of caspase in a subset of human platelet activation responses. *Blood.* 1999; 93: 4222-31.
20. **McLaughlin PJ, Gooch JT, Mannherz HG, Weeds AG** – Structure of gelsolin segment 1-actin complex and the mechanism of filament severing. *Nature.* 1993; 364: 685-92.
21. **Zwaal RF, Comfurius P, Bevers EM** – Mechanism and function of changes in membrane-phospholipid asymmetry in platelets and erythrocytes. *Biochem Soc Trans.* 1993; 21: 248-53
22. **Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM** – A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature.* 2000; 405: 85-90.
23. **Andrews RK, Berndt MC** – Microparticles facilitate neutrophil/platelet crosstalk. *Blood.* 2008; 112: 2174-5.
24. **Lo SC, Hung CY, Lin DT, Peng HC, Huang TF** – Involvement of platelet glycoprotein Ib in platelet microparticle mediated neutrophil activation. *J Biomed Sci.* 2006; 13: 787-96.
25. **Morel O, Morel N, Freyssinet JM, Toti F** – Platelet microparticles and vascular cells interactions: a checkpoint between the haemostatic and thrombotic responses. *Platelets.* 2008; 19: 9-23.
26. **Nomura S, Suzuki M, Kido H, Yamaguchi K, Fukuroi T, Yanabu M, Soga T, Nagata H, Kokawa T, Yasunaga K** – Differences between platelet and microparticle glycoprotein IIb/IIIa. *Cytometry.* 1992; 13: 621-9.
27. **Phillips DR, Charo IF, Parise LV, Fitzgerald LA** – The platelet membrane glycoprotein IIb-IIIa complex. *Blood.* 1988; 71: 831-43.
28. **Michelson AD, Rajasekhar D, Bednarek FJ, Barnard MR** – Platelet and platelet-derived microparticle surface factor V/Va binding in whole blood: differences between neonates and adults. *Thromb Haemost.* 2000; 84: 689-94.
29. **Lechner D, Weltermann A** – Circulating tissue factor-exposing microparticles. *Thromb Res.* 2008; 122: S47-54.
30. **Blann A, Shantsila E, Shantsila A** – Microparticles and arterial disease. *Semin Thromb Hemost.* 2009; 35: 488-96.
31. **Huisse MG, Lanoy E, Tcheche D, Feldman LJ, Bezeaud A, Anglès-Cano E, Mary-Krause M, de Prost D, Guillin MC, Steg PG** – Prothrobotic markers and early spontaneous recanalization in ST-segment elevation myocardial infarction. *Thromb Haemost.* 2007; 98: 420-6.
32. **Berckmans RJ, Neiuwland R, Böing AN, Romijn FP, Hack CE, Sturk A** – Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost.* 2001; 85: 639-46.
33. **Briedé JJ, Tans G, Willems GM, Hemker HC, Lindhout T** – Regulation of platelet factor Va-dependent thrombin generation by activated protein C at the surface of collagen-adherent platelets. *J Biol Chem.* 2001; 276: 7164-8.
34. **Bulboacă A** – Evaluarea funcționalității plachetare în boala cerebro-vasculară, Teză de doctorat, UMF Cluj-Napoca, 1996.
35. **Geiser T, Sturzenegger M, Genewein U, Haeberli A, Beer JH** – Mechanisms of cerebrovascular events as assessed by procoagulant activity, cerebral microemboli, and platelet microparticles in patients with prosthetic heart valves. *Stroke.* 1998; 29: 1770-7.
36. **Cherian P, Hankey GJ, Eikelboom JW, Thom J, Baker RI, McQuillan A, Staton J, Yi Q** – Endothelial and platelet activation in acute ischemic stroke and its etiological subtypes. *Stroke.* 2003; 34: 2132-7.
37. **Kuriyama N, Nagakane Y, Hosomi A, Ohara T, Kasai T, Harada S, Takeda K, Yamada K, Ozasa K, Tokuda T, Watanabe Y, Mizuno T, Nakagawa M** – Evaluation of Factors Associated With Elevated Levels of Platelet-Derived Microparticles in the Acute Phase of Cerebral Infarction. *Clin Appl Thromb Hemost.* 2009; 4: 1076-87
38. **Shirafuji T, Hamaguchi H, Kanda F** – Measurement of platelet-derived microparticle levels in the chronic phase of cerebral infarction using an enzyme-linked immunosorbent assay. *Kobe J Med Sci.* 2008; 54: E55-61.
39. **Serebruany VL, Malinin AI, Pokov AN, Hanley DF** – Antiplatelet profiles of the fixed-dose combination of extended-release dipyridamole and low-dose aspirin compared with clopidogrel with or without aspirin in patients with type 2 diabetes and a history of transient ischemic attack: a randomized, single-blind, 30-day trial. *Clin Ther.* 2008; 30: 249-59.