Platelet microvesicles in vascular inflammation

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Background: Microvesicles are gathering increasing attention as mediators of cell communication and as integral effectors of disease. Platelets present a major source of microvesicles and release these microvesicles either spontaneously or upon activation. Platelet microvesicles (PMVs) retain many features of their parent cells and have been shown to exert modulatory effects on vascular and immune cells.

Purpose: We hypothesize that PMVs interact with vascular smooth muscle cells (SMCs) and modulate their function in the context of vascular remodeling.

Methods: PMVs were isolated from aging human platelet concentrates by centrifugation steps. PMVs were quantified and characterized by flow cytometry using annexin A5, phosphatidylserine and antibodies against CD11b/CD18. Size-calibrated micro beads were used to quantify the absolute amount of PMVs/mL. Cell migration experiments were performed using a boyden chemotaxis chamber. Platelet receptor implications in PMV-SMC interaction were identified by blocking antibodies. Proliferation of SMCs was measured by a flow adhesion assay. Adhesion of monocytic cells to SMCs was determined by a flow adhesion assay. Relative quantification of gene expression was performed by real-time and quantitative PCR.

Results: In the presence of PMVs, SMCs showed increased migration. Under resting conditions, the PMV binding to SMCs was specifically abrogated by the integrin αIIbβ3 inhibitor (integrilin) indicating an integrin-dependent mechanism of interaction. A proliferative effect on SMCs was measured after 48 hours incubation with PMVs and this proliferation relied on interactions via integrin αIIbβ3, CD40 and P-selectin. The firm adhesion of monocytic cells to PMVs stimulated SMCs under flow conditions was significantly increased compared to untreated, resting SMCs. The adhesion mainly depended on the integrin αIIbβ3 and P-selectin but also CD40 and fractalkine. PMVs decreased gene expression of contractile proteins, i.e. αSMA and calponin.

Conclusion: Isolated PMVs have shown to exert an immunomodulatory activity on various cell types. The present data indicate a role of PMVs in inducing a phenotypic switch towards a synthetic inflammatory SMC phenotype, thus contributing to vascular atherogenesis, in particular vascular remodeling.

References:

Cardiovascular Research Supplements (2016) 111, S55

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285 Pharmacological depletion of serotonin promotes atherosclerotic plaque formation in apoe−/− mice

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Cardiovascular disease, like myocardial infarction and stroke, is the major cause of death in western countries, which mainly arise from atherosclerosis. Chronic inflammatory disease is characterized by inflammation of the vessel wall driving lesion formation. These so-called plaques contain macrophages, T cells and other immune cells as well as an accumulation of lipids. In the early phase an activation of the endothelium induces adhesion molecule expression and secretion of pro-inflammatory cytokines and chemokines leading to the recruitment of leukocytes. Platelets also contribute to the leukocyte recruitment by interacting with the endothelium and secretion of granule content including serotonin. Platelet serotonin was recently shown to promote selectin-dependent leukocyte interaction and recruitment to post-capillary venules in a mouse model of acute inflammation. Clinically available serotonin reuptake inhibitors (SSRI) are an anti-depressive drug that might be a useful strategy to impair peripheral serotonin storage in platelets and thus reduce serotonin-mediated endothelium activation in cardiovascular disease patients. We therefore hypothesized that treatment with serotonin transporter inhibitor fluoxetine (FLX) inhibits atherosclerotic plaque formation by limiting leukocyte adhesion. We fed apolipoprotein E-deficient (Apoe−/−) mice for 4 or 16 weeks with high-cholesterol diet (HCD) and treated them with FLX via the drinking water. We analyzed atherosclerotic lesion formation and composition via oil-red-O lipid staining and immunohistology of aortic root cross-sections. Leukocyte subsets in blood, bone marrow, spleen and the abdominal aorta were assessed by flow cytometry. Surprisingly, serotonin pharmacological depletion resulted in significantly increased plaque size after 4 weeks HCD (ctrl, 44592 ± 5920 μm² vs fluoxetine, 68749 ± 6118 μm²; n=9-10; P=0.01), in part due to an increased macrophage infiltration (ctrl, 10966 ± 1931 μm² vs FLX, 22424 ± 4906 n=14-15; P=0.058). After 16 weeks HCD feeding, we no longer observed a difference in lesion size. The enhanced arterial leukocyte recruitment was not due to enhanced myelopoiesis or leukocyte mobilization from bone marrow or spleen, but rather mediated by increased adhesion of myeloid cells to aortic lesions as evidenced by intravital microscopy of carotid arteries. The live imaging revealed increased numbers of adhering CD11b+ stained myeloid cells in carotids of fluoxetine-treated mice compared to control mice. Further investigations of early and advanced plaque composition, adhesion molecule expression and role of endothelial versus leukocyte-dependent effects of serotonin-mediated activation are ongoing to explain the unexpected pro-atherogenic effect of pharmacological SERT inhibition in early plaque formation. Our findings might have important clinical implications in particular for cardiovascular risk patients treated with SERT inhibitors for depression.