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 hypothesise 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 serial centrifugation steps. PMVs were quantified and characterized by flow cytometry using annexin A5/phosphatidylserine and CD40L receptor interacting with the endothelium and secretion of granule content including serotonin. PMVs were isolated from aging human platelet concentrates by serial centrifugation steps.

Results: In the presence of PMVs, SMCs show increased migration. Under resting conditions, the PMV binding to SMCs was specifically abrogated by the integrin IIb3 antagonist (integlin) indicating an integrin-dependent mechanism of interaction. A proliferative effect on SMCs was measured after 4 weeks. Adhesion of monocytic cells to SMCs was determined by a flow adhesion assay. Relative quantification of gene expression was determined by real time and quantitative PCR.

Conclusion: PMVs induce 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 (SERT) used as anti-depressive drugs might be a useful strategy to impair peripheral serotonin storage in platelets and thus reduce serotonin-mediated endothelial 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, pharmacological serotonin depletor treatment resulted in significantly increased plaque size after 4 weeks HCD (ctrl, 5920 ± 5920 µm2 vs. fluoxetine, 6874 ± 618 µm2; n=9-10, P=0.01), in part due to an increased macrophage infiltration (ctrl, 10965 ± 1931 µm2 vs. FLX, 22424 ± 4906 µm2; 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 adherent CD11b+ macrophages within carotid vessels treated 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.