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 serial centrifugation steps. PMVs were quantified and characterized by flow cytometry using annexin A5/phosphatidylserine and antibodies against CD14/CD63. 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 receptors implicated in PMV-SMC interaction were identified by blocking antibodies. PMV binding to SMCs was specifically abrogated by the integrin \( \alpha IIb\beta 3 \) inhibitor (integrilin) indicating an integrin-dependent mechanism of interaction. A proliferative effect on SMCs was measured after 4 weeks HCD (ctrl, 44592 ± 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 myelopoesis or leukocyte mobilization from bone marrow or spleen, but rather mediated by increased adhesion of myeloid cells to aortic lesions as evidenced by intravascular microscopy of carotid arteries. The live imaging revealed increased numbers of adhering CD11b+ stained myeloid cells in carotid of flutoxetine-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.

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 profile.

Figure 1

Platelets: Old Players Revisited

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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. This chronic disorder 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 (SERT) used as anti-depressive drugs 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 tissue 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 depletion resulted in significantly increased plaque size after 4 weeks HCD (ctrl, 44592 ± 5920 \( \mu m^2 \) vs fluoxetine, 68749 ± 6118 \( \mu m^2 \); n=9-10; \( P=0.01 \), in part due to an increased macrophage infiltration (ctrl, 10965 ± 1931 \( \mu m^2 \) 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 myelopoesis or leukocyte mobilization from bone marrow or spleen, but rather mediated by increased adhesion of myeloid cells to aortic lesions as evidenced by intravascular microscopy of carotid arteries. The live imaging revealed increased numbers of adhering CD11b+ stained myeloid cells in carotid of flutoxetine-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.

Figure 1

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Deletion of junctional adhesion molecule a from platelets increases early stage neointima formation after wire injury in hyperlipidemic 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. This chronic disorder 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 (SERT) used as anti-depressive drugs 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 tissue 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 depletion resulted in significantly increased plaque size after 4 weeks HCD (ctrl, 44592 ± 5920 \( \mu m^2 \) vs fluoxetine, 68749 ± 6118 \( \mu m^2 \); n=9-10; \( P=0.01 \), in part due to an increased macrophage infiltration (ctrl, 10965 ± 1931 \( \mu m^2 \) 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 myelopoesis or leukocyte mobilization from bone marrow or spleen, but rather mediated by increased adhesion of myeloid cells to aortic lesions as evidenced by intravascular microscopy of carotid arteries. The live imaging revealed increased numbers of adhering CD11b+ stained myeloid cells in carotid of flutoxetine-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.