Poster Session S05 – EVs in Cardiovascular Disease
Chairs: TBD 5:15–6:30 p.m.

PS05.01

Proteomic profiling reveal Src as a novel microvesicle-associated biomarker for myocardial infarction

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Please see OPT02.05

PS05.02

Quantification of the circulating vesicle-bound pools of adipocytokines reveals that MFG-E8 and MIF are conveyed by plasmatic EVs

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Introduction: Obesity-associated metabolic diseases are linked to dysregulated production of many factors secreted by adipose tissue, known as adipocytokines. Accumulating evidences suggest a role for circulating extracellular vesicles (EVs), significantly increased in obesity, in obesity-associated metabolic dysfunctions. Since EVs may convey hormones and metabolites, we aimed to evaluate their contribution in the secretion of adipocytokines.
Methods: EV subsets, including microvesicles (MV) and exosomes (EXO), were isolated from plasma samples collected from patients suffering of metabolic syndrome (MS) and quantified by NTA and flow cytometry. Patients were classified according to their body mass index (BMI): control (BMI < 27), overweight (27 < BMI < 30) and obese (BMI > 30). 22 adipocytokines circulating concentrations were successively measured on total, MV- and EV-depleted plasma samples by multiplex immunoassays.
We first showed that circulating MV and EXO populations were significantly increased with BMI supporting a role of these vesicles as metabolic relays in the context of obesity. Multiplex analysis of plasmatic adipocytokines confirms dysregulated production of these factors with increased BMI. Sequential depletion of MV and EXO from all plasma patients did not modify adipocytokine circulating levels, at the exception of MFG-E8 (Milk Fat Globule-EGF-Factor VIII) and MIF (macrophage migration inhibitory factor), which were decreased. Of interest, 37.3% of circulating MFG-E8 and 57.3% of circulating MIF were associated to EVs. Notably, MFGE-E8 preferentially associated with EXO (24%) whereas MV carried more than half of circulating MIF (50.6%). Nonetheless, EV-associated proportions of these two adipokines were unchanged with obesity suggesting that MFG-E8 and MIF constitutively used EVs as secretory pathways.
Results: Our results highlight that a significant proportion of MFG-E8 and MIF associate with EXO and MV, respectively, in plasma. Thus, this study emphasises the importance to consider EV secretory pathways in the metabolic actions of adipocytokines.
This study was approved by Angers University hospital ethical committee (NCT: 00997165) and received written consent from patients.

Funding: This work was funded by a research national grant (ANR MilkChEST n°ANR-12-BSV6-0013-04), by GIS APIS-GENE and the French Society of Diabetes.

PS05.03

Adipocyte extracellular vesicles increase leucocyte attachment to vascular endothelial cells

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Introduction: The hypoxic and chronic low grade inflammatory state of adipose tissue in obesity is associated with an overall increased risk of cardiovascular disease. The aim of this study was to determine if extracellular vesicles (EVs) derived from adipocytes cultured in a manner that mimics that of obese adipose tissue, play a functional role in the progression of cardiovascular disease, by promoting leucocyte attachment to vascular endothelial cells.
Methods: Differentiated 3T3-L1 adipocytes were incubated for 24 h in one of four conditions: "control" (serum-free medium (SFM), 95% air/5% CO_2), "TNFα" (SFM plus 30 ng/ml TNFα, 95% air/5% CO_2), "hypoxia" (SFM, 1% O_2) or "TNFα & hypoxia" (SFM plus 30 ng/ml TNFα, 1% O_2). EV were isolated from media by ultracentrifugation. Freshly isolated primary human umbilical vein endothelial cells (HUVEC) were treated with (and without) EV for 6 h before either being lysed for western blotting, or used in leucocyte attachment assays.
Results: EV from "TNFα" and "TNFα & hypoxia" treated 3T3-L1 cells, increased vascular cell adhesion molecule (VCAM) protein expression in HUVEC, an effect that could be blocked in a dose-dependent fashion using an anti-TNFα neutralising antibody. No changes in the expression of other HUVEC adhesion molecules (E-selectin, P-selectin, platelet endothelial cell adhesion molecule (PECAM) and VE-Cadherin) were observed in any treatment group. Pre-incubation of HUVEC with "TNFα" EV and "TNFα & hypoxia" EV increased the subsequent attachment of freshly isolated leukocytes.
Conclusion: EV derived from adipocytes cultured in conditions mimicking that of obese adipose tissue, induce VCAM expression in vascular endothelial cells in a TNFα-dependant manner. This predisposes endothelial cells to the subsequent attachment of leukocytes. Preventing the adipocyte EV-induced upregulation of endothelial VCAM may offer a novel therapeutic window for minimising vascular disease in obese patients.

PS05.04

Diabetes affects extracellular vesicle content and function

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Introduction: The role of circulating extracellular vesicles in cardiovascular diseases remains incompletely understood. We previously demonstrated that extracellular vesicles circulating in plasma of healthy mice can suppress monocyte activation through transfer of anti-inflammatory microRNAs. Here we set out to determine the effect of diabetes on the function of plasma extracellular vesicles since diabetes is known to negatively affect vascular function, playing a contributory role in cardiovascular diseases.
Methods: Circulating plasma extracellular vesicles were isolated from mouse and rat models of type 2 diabetes. Extracellular vesicles were characterised with nanoparticle tracking analysis. Furthermore, qPCR and RNA-sequencing approaches were used to characterise vesicle content and function.

Results: We found that vesicle abundance and size were increased in mouse and rat models of type 2 diabetes. MicroRNAs in plasma extracellular vesicles were dysregulated during the progression of diabetes in these models. Finally, we demonstrate that vesicles isolated from diabetic plasma can activate inflammatory pathways in endothelial cells. Current studies are seeking to determine the contribution of microRNA transfer to endothelial dysfunction.
Conclusions: These studies suggest that the microRNA content and function of extracellular vesicles are dysregulated during diabetes. Advancements in this area could facilitate the development of more effective non-invasive diagnostics, prognostics, and therapeutics.
Funding: Supported by funding from the Canadian Vascular Network and the Canadian Institutes of Health Research.

PS05.05

Intra-cardiac release of extracellular vesicles governs infiltrating monocyte activation following myocardial infarction

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Introduction: A rapid and massive influx of inflammatory cells occurs into ischemic areas following myocardial infarction (MI). This results in the local release of cytokines and growth factors, but the mechanisms regulating their production are not fully explored in the ischemic myocardium. Extracellular vesicle (EV) release in the interstitial space curbs important biological functions, including inflammation. So far, there is no evidence of EVs in situ release in the heart following MI. The present study tested the hypothesis that local generation of EVs in the infarcted heart coordinates cardiac inflammation following MI.
Methods: MI was induced by permanent left anterior descending artery ligation in C57BL/6 mice. Sham-operated mice were used as controls. Sham and MI mice were sacrificed between 0 and 3 days after the onset of ischemia. EVs from ischemic and sham left ventricles were isolated by sequential centrifugations, and separated into microvesicle-enriched (MVs) and exosome-enriched (Exos) fractions. Both fractions were analysed by TRPS (qNANO). In addition, MVs cellular origin and phosphatidylserine exposure were determined by flow cytometry. FACS-sorted Ly6 C+ monocytes were isolated from ischemic myocardium 24 h post-ligation and were exposed *in vitro* for 24 hours to either MVs or Exos isolated from MI hearts 24 h post ligation. ELISA assessed subsequent cytokine release from cardiac-derived monocytes.
Results: Coronary artery ligation in mice transiently increases EV levels in the left ventricle when compared to sham animals. EVs from infarcted hearts were characterised as MVs and Exos based on their size (Exos mean diameter 118 ± 4 nm vs MVs 252 ± 18 nm). Exos fraction was enriched in the exosomal markers CD63 and CD9. MVs were transiently release at 15 and 24 h post ischemia and then returned to shams levels. Exosomes follow similar time-dependent pattern. MVs mostly originated from cardiomyocytes and increased the release of IL-6, chemokines CCL2, CCL7 and interleukin-10 from FACS-sorted Ly6C+ cardiac monocytes. Exos stimulated the release of anti-inflammatory IL-10 only.
Conclusion: These results highlight the paracrine crosstalk between cardiac inflammatory cells and endogenously released extracellular vesicles following myocardial infarction.

PS05.06

Acoustic trapping of microparticles and its application in measuring the effect of bilberry powder consumption on plasma microparticles in patients with myocardial infarction

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Introduction: Microparticles (MPs) are submicron particles released under several physiological and pathological states. Isolation, enumeration and characterisation of MPs from human plasma are promising approaches to understand their role in pathophysiology of cardiovascular diseases (CVD). Current methods are time consuming, and require large sample volumes, which is a limitation when analysing clinical samples. Here we have developed an alternative method based on a microscale acoustic standing wave technology to retain particles in a microfluidic channel. Bilberries (*Vaccinium myrtillum*) are known to improve endothelial function and the antioxidant status. In order to understand the effect of bilberry powder consumption on circulating MPs, we analysed plasma samples from myocardial infarction (MI) patients before and 8 weeks after bilberry powder consumption.
Methods: All participants provided informed consent and the study was approved by the regional ethics review committee in Uppsala, Sweden. MPs were isolated from diluted cell free plasma using acoustic trapping. Briefly, exciting a capillary with a piezoelectric transducer creates a standing wave that traps MPs together with 12 mm polystyrene seed particles through acoustic radiation forces. We used an internal control of pooled frozen plasma from healthy volunteers to validate device performance. MPs originating from endothelial cells (EMPs) and platelets (PMPs) were enumerated using flow cytometry.
Results: The acoustic trapping method achieved ±52% recovery of CD42a+ MPs using the internal control. Isolation and enumeration of EMPs and PMPs in the patient samples showed that bilberry powder consumption for 8 weeks decreased the levels of both types of MPs.
Summary: The acoustic trapping is a fast and efficient method for plasma MP isolation. It uses small sample volumes, making it a promising solution in clinical practice. Bilberry extract consumption decreases EMP and PMP levels in patients with myocardial infarction.
Financial support: This work was supported by Swedish Foundation for Strategic Research, Knut and Alice Wallenberg Foundation and Örebro University Hospital Research Foundation.

PS05.07

The signature of apoptotic endothelial cell-derived microparticles in patients with different phenotypes of chronic heart failure

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Introduction: Chronic heart failure (HF) remains a leading cause of cardiovascular (CV) mortality and morbidity worldwide. The aim of the study was to investigate whether the pattern of endothelial progenitor cells (EPCs) with angiogenic capacity and apoptotic endothelial cell-derived microparticles (EMPs) would be able to differentiate HF with reduced (HFrEF) and preserved (HFpEF) ejection fraction.
Methods: One hundred and sixty-four chronic HF subjects met inclusion criteria. Patients with global left ventricular ejection fraction 50–59% were categorised as the HFpEF group (n = 79) and those with ≤45% as the HFrEF group (n = 85). The flow cytometric technique was used for predictably distinguishing circulating cell subsets depending on expression of CD45, CD34, CD14, Tie-2 and CD309 antigens and determining endothelial cell-derived microparticles. CD31+/annexin V + was defined as apoptotic endothelial cell-derived MPs, MPs labelled for CD105+ or CD62E+ were determined as MPs produced due to activation of endothelial cells.
Results: In multivariate logistic regression model T2DM (R^2 = 0.26, p = 0.001), obesity (R^2 = 0.22, p = 0.001), previous MI (R^2 = 0.17, p = 0.012), galectin-3 (R^2 = 0.67, p = 0.012), CD31+/annexin V+ EMPs to $CD14^+CD309^+$ cells ratio (R^2 = 0.16, p = 0.001), $CD31^+$/annexin V^+ EMPs (R^2 = 0.11, p = 0.001), NT-proBNP (R^2 = 0.11, p = 0.046), CD31+/annexin V+ EMPs to $CD14^+CD309^+Tie\text{-}2^+$ cells