Evaluation of plasma exosomal miRNA-1, miRNA-133 and miRNA-208 levels in a porcine model during acute myocardial infarction

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Background. Circulating exosomes are small (40-100nm) extracellular vesicles secreted by various different cell types, such as endothelial cells, fibroblasts, tumor cells and lymphocytes and present in almost all biological fluids. They are important carriers of stable microRNAs (miRNAs) in plasma. miRNA-1 and miRNA-133 have previously been shown to rise several hours after acute myocardial infarction (AMI). miRNA-197 and miRNA-223 have been found to predict cardiovascular death in patients with symptomatic CAD. It has therefore been hypothesized that miRNAs may be used as biomarkers in the detection, diagnosis and monitoring of cardiac diseases and therapies. In this study we assessed the change of exosomal levels of miRNA-1, miRNA-133 and miRNA-208 during the ischemic period of AMI.

Methods. In this study, we included 3 pigs which underwent a 90 minute period percutaneous occlusion of the mid left anterior descending artery (LAD) followed by reperfusion. EDTA blood samples were obtained at baseline and at 10, 30, 60 and 90 minutes of occlusion. Plasma was prepared for exosome isolation by progressive centrifugation steps of 1200 x g (10 minutes), 1800 x g (10minutes) and 10 000 x g (20 minutes) and then filtered through 0.2\textmu m syringe filters. One mL of thusly prepared plasma was suspended in 9mL of PBS and ultra-centrifuged at 100 000 x g (120 minutes), followed by a washing step and another round of centrifugation at 100 000 x g (120 minutes). Nanoparticle Tracking and Western Blot of CD63 and CD9 confirmed the isolation of exosomes. miRNAs were isolated using QIAGEN miRNeasy Serum / Plasma kits, reverse transcribed using QIAGEN miScript RT kit and qPCR was performed using miScript SYBR® Green PCR Kit. Fold changes were normalized using ce-miR-39 Spike-in-Control of the QIAGEN Serum / Plasma kit. Relative fold changes of miR-1, miR-133 and miR-208 at baseline, 10, 30, 60 and 90 minutes after begin of reperfusion were evaluated.

Results. Figure 1 shows the relative fold changes of miRNA-1, miRNA-133 and miRNA-208 during AMI and after reperfusion. All 3 types of miRNAs could be detected in the plasma exosomes. However, ultimately no significant change in the levels of miRNA-1, miRNA-133 and miRNA-208 could be shown at the different time points.

Conclusion. Even though all investigated miRNAs could be detected in plasma exosomes, no significant relative changes in miR-1, miR-133 and miR-208 levels could be shown in this study. The reason for this could potentially be that 90 minutes of ischemia are too short to significantly change levels of miRNA expression and miRNA levels in circulating plasma-exosomes.
Abstract:
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