Shock waves induce angiogenesis via exosome release

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Background:
Shock wave therapy (SWT) is developing a promising approach for the regeneration of ischemic myocardium by induction of angiogenesis. However, the mechanism of action remains unknown. Exosomes are released by mechanical shear stress and have been shown to induce angiogenic effects. We hypothesized that SWT induces exosome release and thus exerts its angiogenic effects.

Methods:
Human umbilical vein endothelial cells (HUVECs) were treated with SWT. Subsequently, exosomes were isolated from the supernatant and analyzed by transmission electron microscopy (TEM) and nanoparticle tracking analysis. In a next step, exosomes were characterized and analyzed for their angiogenic potential in vitro. Exosome content was evaluated via a sequencing array. Finally, isolated exosomes were injected into subcutaneously implanted matrigel plugs in nude mice. Perfusion of the plugs was measured via Laser Doppler perfusion imaging (LDPI). Arterioles and capillaries were quantified histologically. In vivo imaging was performed to analyze functionality of the vessels.

Results:
SWT caused exosome release in HUVECs. Supernatants of treated cells showed significantly higher concentrations of exosomes. Exosomes showed a characteristic cup-shaped morphology in TEM analysis. Treatment of HUVECs with exosomes induced phosphorylation of Akt and ERK, caused increased tube formation (CTR 19,5 ± 7,79 vs. SWT 178,5±31,14, p=0,004) and endothelial cell proliferation (CTR 0,59 ± 0,02 vs. SWT 0,77 ± 0,04, p=0,011). Pre-treatment with exosome-release inhibitor GW4869 abolished the angiogenic effects of SWT. Sequencing array showed the presence of angiogenic miRNAs in exosomes released after SWT. Injection of isolated exosomes into subcutaneously implanted matrigel plugs resulted in higher perfusion and increased number of capillaries (CTR 0,53 ± 0,19 vs. SWT 1,7 ± 0,26, p=0,0006) and arterioles (CTR 0,8 ± 0,23 vs. SWT 4,5 ± 0,54, p<0,0001). In vivo imaging of the matrigel plugs showed formation of functional vessels after exosome injection.

Conclusion:
We show for the first time how the mechanical stimulus of SWT is translated into a biological response. SWT causes exosome release. Released exosomes show a very potent angiogenic effect. SWT might develop a potent therapeutic intervention for the treatment of ischemic heart disease.