Antimicrobial peptide LL37/ RNA complexes stimulate Toll-like receptor 3 upon shock wave therapy

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Background:
Shock wave therapy (SWT) induces angiogenesis in ischemic heart disease. It is mediated via Toll-like receptor 3 (TLR3), an endosomal receptor of the innate immune system recognizing RNA. How TLR3 is activated upon SWT remains unknown. The antimicrobial peptide LL37 has been shown to be released after mechanical stress and to form complexes with RNA.

Purpose:
We hypothesized that mechanical stimulation upon SWT leads to LL37 release, which forms complexes with RNA and leads to activation of endosomal TLR3.

Methods:
Supernatant of treated human umbilical vein endothelial cells (HUVEC) was transferred onto TLR3 reporter cells and TLR3 activation was measured. To find out whether protein/RNA complexes play a role after SWT, supernatants were treated with RNAse and proteinase. Treated HUVECs were analyzed for LL37 expression. To investigate the uptake of LL37/RNA complexes, premarked RNA was added to cells prior to treatment and uptake was tracked. C57BL/6 mice were subjected to acute myocardial infarction and subsequently treated with SWT. Echocardiography and pressure volume measurements were performed to evaluate cardiac function. Histological quantification of vessels and assessment of fibrosis was performed.

Results:
Supernatants of treated cells activated TLR3 reporter cells (CTR 7.346 ± 2,173 vs. SWT 146.005 ± 12.508; $p<0.0001$). Analysis of the supernatant revealed increased RNA levels (CTR 21 ± 2.444 vs. SWT 37 ± 1.5; $p=0.0174$). The effect could not be abolished by pre-treatment of the supernatant with RNAse, but only by a sequential digestion with proteinase and RNAse hinting strongly towards the involvement of protein/RNA complexes. Indeed, LL37 expression was significantly increased after SWT. Pre-marked RNA was added to HUVECs, followed by subsequent SWT. Cellular RNA uptake was significantly increased after SWT (CTR 31.67 ± 28,17 vs. SWT 19757 ± 1054, $p<0.0001$). Finally, SWT resulted in significantly higher numbers of capillaries (SWT 1262 vs. CTR 461, $p = 0.001$) and arterioles (SWT 461 vs. CTR 160.5, $p=0.001$), decreased fibrosis (CTR ± 2,76 vs. SWT 8,97 ± 3,08, $p=0.01$) and improved ejection fraction (CTR 35.25 ± 1.11 vs. SWT 46 ± 2.83, $p=0.01$) in treated hearts.

Conclusion:
TLR3 activation upon SWT is mediated via the release of LL37. The antimicrobial peptide forms complexes with extracellular RNA and can thus stimulate endosomal TLR3. SWT subsequently induces angiogenesis in ischemic myocardium and might therefore develop a potent regenerative treatment alternative for ischemic heart disease.