Shock wave treatment reduces neuronal degenerationupon spinal cord ischemia via a Toll-like receptor 3 dependent mechanism

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OBJECTIVES:
Paraplegia following spinal cord ischemia represents the most severe complication of aortic surgery. Shock wave treatment (SWT) was shown to induce angiogenesis and regeneration in ischemic tissue. In pre-clinical as well as clinical studies SWT had a favorable effect on ischemic myocardium. We therefore hypothesized that SWT may have a beneficial effect on spinal cord ischemia as well.

METHODS:
Aortic cross clamp was performed between left carotid and left subclavian artery in mice. Animals were randomly divided in a treatment group (SWT, 500 shock waves at 0.1mJ/mm^2, 5Hz) and untreated controls (CTR), n=6 per group. RNA expression of angiogenic and inflammatory cytokines was measured after 24 and 48 hours. Immunofluorescence staining for degenerating neurons and macrophages was performed after 7 days. An ex-vivo spinal slice culture was performed for evaluation of Toll-like receptor (TLR) signalling. Spinal cords from wild type, TLR3 knockout and TLR4 knockout animals were cultured and set under hypoxia for 24 hours. Treatment groups (SWT) received shock wave treatment following hypoxia.

RESULTS:
Real-time PCR analysis revealed higher gene expression of angiogenic factors VEGF-A after 24h (SWT 0.21±0.06 vs. CTR 0.07±0.01, p=0.028) and 48h (SWT 0.11±0.02 vs. CTR 0.07±0.01, p>0.05) as well as HIF-1a after 24h (SWT 0.11±0.04 vs. CTR 0.04±0.01, p>0.05) and 48h (SWT 0.09±0.02 vs. CTR 0.01±0, p=0.016). Early increase of inflammatory mRNA expression was observed after 24h by TNFa (SWT 0.03±0.003 vs. CTR 0.005±0.003, p=0.007) and TGFb (SWT 0.57±0.05 vs. CTR 0.17±0.08, p=0.003). This resulted in a markedly decreased number of degenerating neurons in the treatment group 7 days after ischemia (SWT 74.50±8.14 vs. CTR 250.2±42.98, p=0.0025). Standardized coordination and motor tests performed at day 1, 3 and 7 postoperatively revealed a significantly better performance and outcome of the animals in the treatment group. In addition a Kaplan-Meier analysis revealed a survival benefit of SWT compared to normal animals. Effects of SWT were abolished in TLR3 knockout animals, whereas it was unchanged in TLR4 knockouts.

CONCLUSIONS:
Shock wave treatment induces angiogenesis and modulates inflammation in spinal cord ischemia via the activation of TLR3. This results in a marked decrease of degenerating neurons and may therefore develop as an adjunct to the treatment armentarium for paraplegia upon aortic cross clamp.