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

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/mm², 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-1α 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 TNFα ( SWT 0.03±0.003 vs. CTR 0.005±0.003, p=0.007) and TGFβ ( 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 armamentarium for paraplegia upon aortic cross clamp.