Shockwave therapy - a novel modulator of macrophage function in wound healing

Ben Cooper, Susan H Berry, Aleksandra Staniszewska, Bruce M Dickson, Julie A Taylor, Paul Bachoo, Heather M Wilson

1495 UNIVERSITY OF ABERDEEN

NHS Grampian

Background

Extracorporeal shockwave therapy (ESWT) is used as a treatment to aid healing of chronic venous ulcers when conventional treatment fails. However, the mechanism(s) of how this treatment works and which patient groups would respond best is not clear. Macrophages are key players in wound healing, secreting pro-healing signal molecules, clearing apoptotic cells and debris and controlling infection.

Aim

To investigate mechanisms by which ESWT induces healing, focussing on effects on macrophages (i) from patient biopsies from chronic venous ulcers before and 14d after ESWT (ii) in isolated macrophages *in vitro.*

Methods

- Punch biopsies (3mm) were taken from non healing chronic venous ulcer wounds of consenting patients (n=10), before and 2 weeks after ESWT. Biopsies were formalin fixed for histological assessment and immunohistochemical analysis.
- Primary human blood-derived macrophages or J774 macrophages were treated with shockwave intensities similar to those administered to skin wounds (150-500 impulses/5Hz/0.1 mJ/mm^2).
- Cell viability, ability to phagocytose apoptotic thymocytes and gene expression (qPCR) of healing mediators were analysed

ESWT increases phagocytosis of apoptotic cells

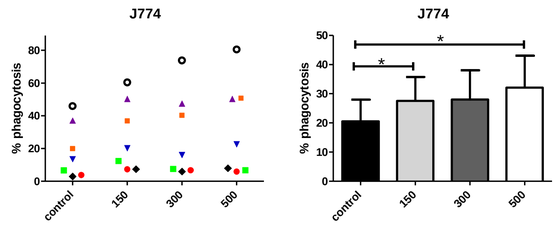
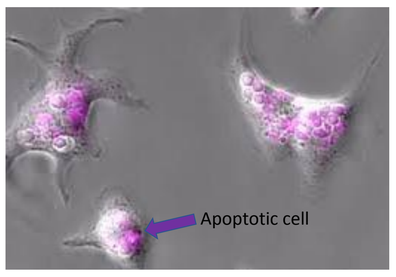
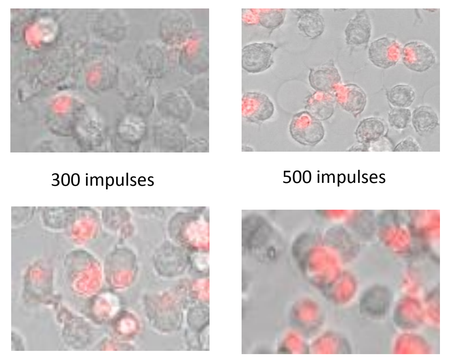
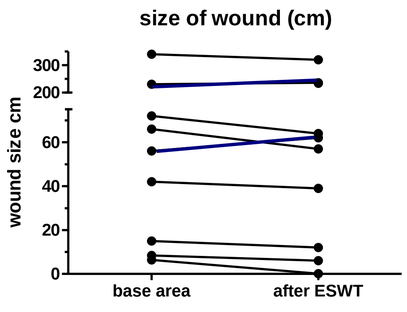


Fig. 3: Percentage phagocytosis of apoptotic cells by J774 (n=7) and human monocyte derived macrophages (n=4 individual donor preparations) were determined following ESWT. Treatment of human macrophages *in vitro* with shockwaves did not alter cell viability but significantly enhanced their phagocytic ability to uptake apoptotic cells but not polystyrene beads.
* p<0.05





ESWT improves healing and decreases number of wound macrophages



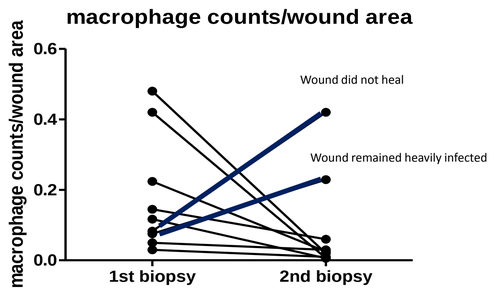


Fig. 1: The wound size (diameter, cm) decreased in 7/9 patients 14d post ESWT (mean 20% decrease) demonstrating healing is improved by ESWT in the majority of patients. Macrophage counts/area was significantly decreased in ESWT treated healing wounds (n=7) but increased in wounds showing no improvement in healing with ESWT (n=2).

Shockwaves upregulate expression of healing mediators

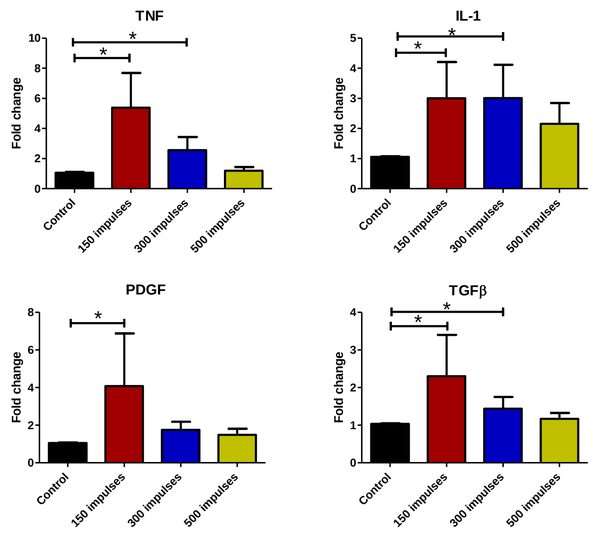


Fig. 4: Macrophages were subject to shockwave treatment and levels of cytokines measured in the culture supernatant 24h later. Shockwaves significantly upregulated expression of TNF, PDGF, IL-1 and TGF-β. n= 6, * p<0.05

ESWT increases macrophage activation

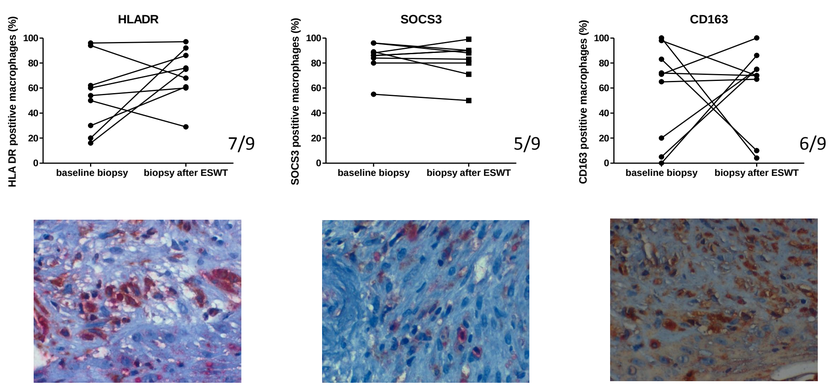


Fig. 2: Macrophages were double stained with activation markers HLADR, SOCS3 and CD168. In spite of the decrease in total number of macrophages in healing wounds after ESWT, the number of activated macrophages per wound area increased in the majority of patients.

Summary

ESWT in chronic ulcers

- Decreases macrophage number/area, increases activation, increases healing.

Shockwave application to isolated macrophages *in vitro*

- Increases phagocytosis of apoptotic cells.
- Increases production of cytokines (activates).

Conclusion. These findings provide new insights and an additional mechanism by which clinical ESWT enhances wound resolution by regulating macrophage function, suggesting ESWT might be exploited to modulate other macrophage-mediated diseases.

We acknowledge all patient volunteers at NHS Grampian vascular clinics and NHS Endowments for funding this research.