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Extracorporeal shock waves improve angiogenesis after full thickness burn

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ABSTRACT

Objective: Extensive wounds of burn patients remain a challenge due to wound infection and subsequent septicemia. We wondered whether extracorporeal shock wave application (ESWA) accelerates the healing process. The aim of the study was to analyze microcirculation, angiogenesis and leukocyte endothelium interaction after burns by using ESWA with two types of low intensity.

Methods: Full-thickness burns were inflicted to the ears of hairless mice ($n = 51$; area: 1.3 mm^2). The mice were randomized into five groups: (A) low-energy shock waves after burn injury (0.04 mJ/mm^2); (B) very low-energy shock waves after burn injury (0.015 mJ/mm^2); (C) mice received burns but no ESWA (control group); (D) mice without burn were exposed to low-energy shock waves; (E) mice without burns and with no shock wave application. Intravital fluorescent microscopy was used to assess microcirculatory parameters, angiogenesis and leukocyte behavior. ESWA was performed on day 1, 3 and 7 (500 shoots, 1 Hz). Values were obtained straight after and on days 1, 3, 7 and 12 post burn.

Results: Group A showed accelerated angiogenesis (non-perfused area at day 12: 5.3% vs. 9.1% (group B) and 12.6% (group C), $p = 0.005$). Both shock wave groups showed improved blood flow after burn compared to group C. Shock waves significantly increased the number of rolling leukocytes compared to the non-ESWA-treated animals (group D: 210.8% vs. group E: 83.3%, $p = 0.017$ on day 7 and 172.3 vs. 90.9%, $p = 0.01$ on day 12).

Conclusion: Shock waves have a positive effect on several parameters of wound healing after burns, especially with regard to angiogenesis and leukocyte behaviour. In both ESWA groups, angiogenesis and blood flow outmatched the control group. Within the ESWA groups the higher intensity (0.04 mJ/mm^2) showed better results than the lower intensity group. Moreover, shock waves increased the number of rolling and sticking leukocytes as a part of an improved metabolism.

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1. Introduction

Extracorporeal shock wave application (ESWA) has cemented its position as adjuvant therapy option in many soft tissue orders, such as chronic wounds [1,2].

However, the first clinical application of extracorporeal shock waves was in the treatment of urolithiasis, where its therapy principals consist of the synergistic interaction between stress waves and cavitation [3].

Shock waves are single acoustic pulses, characterized by alternating rapid increases (ns) and decreases (μs) of positive and negative pressure. One of the main differences between the treatment for urolithiasis and the use of ESWA in soft tissue disorders is the applied energy flux density (EFD). Low doses of ESWA with an EFD under 0.15 mJ/mm^2, have significantly less energy than extracorporeal shock wave lithotripsy (ESWL).

To date little is known about the mechanisms of ESWA for impaired wound healing, even though ESWA has been shown to accelerate tissue repair in acute and chronic wounds [4]. The effect of ESWA on angiogenesis and inflammation seems to be essential for the enhanced results in skin flap tissue survival. Kuo et al. used the dorsal skin random flap model in rodents to show that shock waves can increase topical blood perfusion, in which histological staining evaluated a suppression of tumor necrosis factor alpha and reduced leukocyte infiltration [5]. These findings correspond to others, like the response to ESWA in murine isografts [6]. However, a comparative analysis of angiogenic gene expression in normal and impaired wound healing in the case of diabetic mice, showed no effect of ESWA on wound closure. Moreover, multiple doses of ESWA exacerbated wound healing [7]. Up to now, there is no consensus on the type of wounds most likely to benefit from shock waves and also on which shock wave parameters are best, regarding energy, degree of focus and frequency or number of cycles [8].

Burn wounds remain a special challenge in wound care, because of their inflammation and undersupply with O_2 and other vital nutrients caused by the microcirculatory breakdown, so the main treatment consists in the substitution of physiological saline as well as local antiseptics and symptomatic medication [9,10]. The presumption of ESWA as adjuvant, supportive therapy option is obvious.

The main purpose of the following study was two-fold: On the one hand we wanted to evaluate the influence of ESWA on angiogenesis after burn and furthermore we sought to investigate possible changes of inflammatory activities caused by ESWA.

2. Materials and methods

2.1. Animals

Male SKH-1/h mice (n = 51, bodyweight 22–24 g, age 4–6 weeks) were obtained from Charles River Laboratories (Sulzfeld, Germany). The mice were sustained in accordance to German animal care regulations, which comply with international guidelines of animal care and their use in scientific experiments. Regional authorities also approved the investigations. All mice underwent single housing in standard polycarbonate cages (21 °C, 12 h dark/light cycle). Commercial rodent food and tap water was accessed ad libitum.

2.2. Experimental design

Mice were randomized into five groups (n = 11 per group). Group A received low doses ESWA (EFD 0.04 mJ/mm^2) after full-thickness burn. Group B was treated with lower doses (EFD 0.015 mJ/mm^2) after full thickness burn. Group C was not treated with shock waves, but we inflicted burn. The animals of group D and E were not burned, but Group D received low doses of shock waves (0.04 mJ/mm^2). Group E represented the control group without treatment. The trial period lasted about 12 days, microscopic observations were performed immediately after burn injury, as well as during wound healing on days 1, 3, 7 and 12. Groups without infliction of burn wounds were also monitored. ESWA was performed on day 1, 3 and 7 *after* the microscopic observations.

The investigation area was limited to the caudal half of the right ear. We determined six regions of interest (ROI, minimum 2 arterioles and 2 venules in each region) around the burn area. In the mice without burns the corresponding areas were examined (Fig. 1). Our baseline values were derived from the recordings directly after burn injury, so the data given in percentage permitted the focus on differences in treatment and disregarded minor differences of the burn.

For the observations, we used the method of intravital microscopy. Fluorochromes were injected via the tail-veins (tube 29G, Braun, Melsungen, Germany) on every observation day. A direct visualization and quantification of microcirculatory parameters could be achieved by the injection of 25 μL FITC labeled dextran (1.0%, MW 150 kDa). Leukocyte-endothelial interaction (LEI) was displayed by staining the leukocytes *in vivo* with 25 μL rhodamine6G (0.5%, Sigma Chemicals Co., St. Louis, MO, USA).

2.3. Anesthesia/preparation

The mice were first anesthetized by inhaling spontaneous isoflurane-N_2O (FiO_2 0.35, 0.015 L/L isoflurane, Forene®, Abbott GmbH, Wiesbaden, Germany). After maintaining narcosis, the edges of the ear were fixed by pulling two microsurgical loops for extension (8/0 Surgipro®, Covidien, Neustadt/Donau, Germany). Physiological saline between ear and platform flattened the ear by adhesion. The period of narcosis lasted about 1 h. After waking from anesthesia, the mice were released in their cages.

2.4. Burn model

We used a repetitive, well-proved burn model allowing validated full-thickness burns [9,11]. The anesthetized mice were exposed to a hot air jet on the dorsal side of the left ear for 1 s without any tissue contact (117 ± 2.1 °C, Distance between ear and air jet: 1 mm). The burn area directly after infliction showed an extension of 1.47 ± 0.21 mm^2 (mean ± SE, Fig. 2).