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● *Original Contribution*

IMMEDIATE DOSE–RESPONSE EFFECT OF HIGH-ENERGY VERSUS LOW-ENERGY EXTRACORPOREAL SHOCK WAVE THERAPY ON CUTANEOUS MICROCIRCULATION

Robert Kraemer,* Heiko Sorg,[†] Vinzent Forstmeier,[‡] Karsten Knobloch,[§] Eirini Liodaki,* Felix Hagen Stang,* Peter Mailaender,* and Tobias Kisch*

*Plastic and Hand Surgery, Burn Unit, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; [†]Department for Plastic, Reconstructive and Aesthetic Surgery, Hand Surgery, Alfried Krupp Krankenhaus, Essen, Germany; [‡]Department of Visceral and Thoracic Surgery, German Armed Forces Hospital Ulm, Ulm, Germany; and [§]Sportpractice Prof. Knobloch, Hannover, Germany

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Abstract—Elucidation of the precise mechanisms and therapeutic options of extracorporeal shock wave therapy (ESWT) is only at the beginning. Although immediate real-time effects of ESWT on cutaneous hemodynamics have recently been described, the dose response to different ESWT energies in cutaneous microcirculation has never been examined. Thirty-nine Sprague–Dawley rats were randomly assigned to three groups that received either focused high-energy shock waves (group A: total of 1000 impulses, 10 J) to the lower leg of the hind limb, focused low-energy shock waves (group B: total of 300 impulses, 1 J) or placebo shock wave treatment (group C: 0 impulses, 0 J) using a multimodality shock wave delivery system (Duolith SD-1 T-Top, Storz Medical, Tägerwilen, Switzerland). Immediate microcirculatory effects were assessed with the O2C (oxygen to see) system (LEA Medizintechnik, Giessen, Germany) before and for 20 min after application of ESWT. Cutaneous tissue oxygen saturation increased significantly higher after high-energy ESWT than after low-energy and placebo ESWT (A: 29.4% vs. B: 17.3% vs. C: 3.3%; $p = 0.003$). Capillary blood velocity was significantly higher after high-energy ESWT and lower after low-energy ESWT versus placebo ESWT (group A: 17.8% vs. group B: −22.1% vs. group C: −5.0%, $p = 0.045$). Post-capillary venous filling pressure was significantly enhanced in the high-energy ESWT group in contrast to the low-energy ESWT and placebo groups (group A: 25% vs. group B: 2% vs. group C: −4%, $p = 0.001$). Both high-energy and low-energy ESWT affect cutaneous hemodynamics in a standard rat model. High-energy ESWT significantly increases parameters of cutaneous microcirculation immediately after application, resulting in higher tissue oxygen saturation, venous filling pressure and blood velocity, which suggests higher tissue perfusion with enhanced oxygen saturation, in contrast to low-energy as well as placebo ESWT. Low-energy ESWT also increased tissue oxygen saturation, albeit to a lower extent, and decreases both blood velocity and venous filling pressure. Low-energy ESWT reduced tissue perfusion, but improved oxygen saturation immediately after the application. (E-mail: robert.kraemer@uksh.de)

Key Words: **Extracorporeal shock wave therapy, Cutaneous microcirculation, Dose dependency.**

INTRODUCTION

Extracorporeal shock wave therapy (ESWT) was initially established in the 1980s for urologic lithotripsy. It requires a repetitive sequence of single sonic pulses defined by a high peak pressure of 100 MPascal and a rapid rise in pressure under 10 ns with a short life cycle of approximately 10 μs. These pulses are conveyed by a generator using electrohydraulics and applied to a specific target area with a radius between 2 and 5 mm at a distinct energy density (Perez et al. 2013; Wang et al. 2007). Different protocols of ESWT have since been used in several fields of medicine, including orthopedic diseases such as fractures, non-union fractures, osteonecrosis of the femoral head, tendinopathy, calcarea of the shoulder, epicondylitis, plantar fasciitis and several inflammatory tendon diseases (Elster et al. 2010; Kuo et al. 2009a; Schaden et al. 2001; Wang et al. 2001, 2002, 2005).

Address correspondence to: Robert Kraemer, Plastic and Hand Surgery, Burn Unit, University Hospital Schleswig-Holstein, Campus Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: robert.kraemer@uksh.de

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Furthermore, ESWT has been applied in limb or myocardial ischemia and recently in the field of plastic reconstructive surgery for the treatment of acute and chronic wounds, skin flaps and burns (Kisch et al. 2015b; Omar et al. 2014; Ottomann et al. 2010, 2012; Reichenberger et al. 2012). Shock wave pre- and post-conditioning treatment has been reported to improve skin flap survival through neovascularization and early upregulation of angiogenesis-related growth factors (Mittermayr et al. 2011; Nacak et al. 2014). In the current scientific literature, there is a consensus that shock waves influence a complex spectrum of cellular and biomolecular functions. In particular, recent studies reported increased blood perfusion and microvessel number, vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase upregulation, proliferating cell nuclear antigen expression and decreased pro-inflammatory activity (Ha et al. 2013; Kisch et al. 2015a, 2016; Kuo et al. 2009a; Mittermayr et al. 2011; Yeh et al. 2012). Nevertheless, the physical and biochemical mechanisms remain unclear. There exist only a few studies that have investigated hemodynamics after shock wave treatment, including the evaluation of clinical parameters as skin flap survival, invasive measurements as *in vivo* microscopy or laboratory parameters as biomolecular proteins (Kuo et al. 2009a; Mittermayr et al. 2011; Reichenberger et al. 2012). Current trials on short-term and long-term cutaneous microcirculatory changes after the application of ESWT using different energy levels are missing. Therefore, the aim of this preliminary study was to determine the immediate effects of high-energy versus low-energy shock wave therapy on the cutaneous microcirculation in a real-time and non-invasive setting, using a rat standardized model.

METHODS

Animal model and experimental protocol

The study included 39 male Sprague–Dawley rats (250–350 g body weight, Charles River Laboratories, Sulzfeld, Germany), housed two/cage at 21°C on a natural light/dark cycle, as well as water and standard laboratory chow *ad libitum*. The experiments were conducted in accordance with the German legislation on protection of animals and the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council). Rats were randomly assigned to three groups. Group A received focused high-energy ESWT (shock waves at 0.3 mJ/mm^2 and 4 impulses/s for a total of 1000 impulses totaling 10 J). Group B received focused low-energy ESWT (shock waves at 0.1 mJ/mm^2 and 5 impulses/s for a total of 300 impulses totaling 1 J). Group C received placebo ESWT without energy application (0 impulses totaling 0 J). The hair of the left lower leg of each rat was removed with an electrical shaver. Rats were fixated with tape on a platform. The ESWT device (Duolith SD-1 T-Top, Storz Medical, Tägerwilen, Switzerland) was applied to the lower leg of the left hind limb. The device allows generation of both focused and radial shock waves. In this study, focused extracorporeal shock wave therapy was used. Applications were performed by the same experienced physician using contact gel without relevant pressure to the tissue. During the experiments, the rats were under sufficient pentobarbital sodium anesthesia (55 mg/kg body weight ip, Narcoren, Merial, Hallbergmoos, Germany) controlled by stable heart rate and breathing frequency to minimize microcirculatory affection caused by pain reaction. Body temperature was maintained at 36°C–37°C using a heating pad.

Microcirculatory analysis

After anesthesia delivery, the cutaneous microcirculation of each rat was allowed to stabilize for 10 min before investigating the cutaneous microcirculation using a non-invasive combined laser Doppler and photospectrometry system (O2C [oxygen to see], LEA Medizintechnik, Giessen, Germany). Before and continuously over 20 min after application of ESWT, the microcirculation was assessed in real time at the treated body area.

A fixation apparatus was used for placing the probe at the same location as the ESWT device, to minimize measurement artifacts caused by vibration (Fig. 1). Baseline measurements were carried out for 1 min before application of focused ESWT. After application of ESWT, microcirculation was assessed continuously in real time for 20 min.

The determination of hemoglobin and the principle of blood flow measurement are combined in the O2C system. The optical method for measuring both blood flow velocity with the laser Doppler technique and hemoglobin oxygenation and hemoglobin concentration in tissue with spectrometric techniques has been described in detail elsewhere (Frank et al. 1989; Rothenberger et al. 2014). Local oxygen supply parameters, blood flow velocity, oxygen saturation of haemoglobin and relative post-capillary venous filling pressures were recorded with an optical fiber probe. The fiber probe incorporates both the laser Doppler method and the broadband light spectrometry technique. The probe used assessed data at a 2-mm depth with respect to: cutaneous capillary blood flow velocity (arbitrary units [AU]), cutaneous tissue oxygen saturation (%) and cutaneous venous filling pressure (AU).

We recently described the use of the O2C system in evaluation of cutaneous effects of remote ischemic preconditioning and the correlation of free flap skin temperature to free flap microcirculation (Kraemer et al. 2011a, 2011b). A 5% intra-subject variability was determined