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Repetitive shock wave therapy improves muscular microcirculation

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ABSTRACT

Background: Extracorporeal shock wave therapy (ESWT) is mainly applied in tendon as well as bone problems based on stem-cell activation and healing acceleration. The effect of ESWT on muscle tissue is much less understood to date. However, from a clinical perspective, muscle injuries are of distinct interest especially in elite athletes such as soccer players.

Material and methods: A total of 26 rats were randomized into two groups. Group A received a single application of high-energetic focused ESWT (0.3 mJ/mm^2, 4 Hz, 1000 impulses, 10 J), whereas group B underwent the same procedure every 10 min for three sessions (3 × 0.3 mJ/mm^2, 4 Hz, 3 × 1000 impulses, totaling 30 J). Blood flow at a depth of 8 mm was measured continuously and noninvasively by a combined Laser-Doppler-Imaging and photospectrometric technique (Oxygen-to-see, O2C, LEA Medizintechnik, Germany).

Results: One minute after the application of high-energy ESWT blood flow in group A increased by 16.5% ($P = 0.007$). Thereafter, it decreased from minute 2 after application and remained significantly unchanged to baseline value until the end of the measuring period at 50 min ($P = 0.550$). Group B showed a similar significant increase in blood flow of 16.4% ($P = 0.049$) and a decrease afterward, too. After the second focused ESWT blood flow was boosted to 26.6% ($P = 0.004$), remaining significantly elevated until the third application was initiated. Muscular blood flow was increased to 29.8% after the third focused ESWT ($P < 0.001$), remaining significantly increased for another 10 min.

Conclusions: Focused ESWT enhances blood flow in the muscle of rats. Moreover, repetitive ESWT extended this beneficial effect.

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1. Introduction

1.1. *Extracorporeal shock wave therapy*

Extracorporeal shock wave therapy (ESWT) is defined as a sequence of sonic pulses characterized by high peak pressure with fast pressure rise and a short life cycle. The first description was in the 1980s for the use of lithotripsy in urolithiasis [1], but shock wave treatment was emerging more and more for soft tissue regeneration and rehabilitation in orthopedics and sports medicine.

1.2. *ESWT affects superficial and deep tissues*

Recent data indicate that ESWT has improving effects on superficial soft tissue. Enhanced re-epithelialization was found in acute and chronic wounds [2–5]. Moreover, improvement of skin quality [6,7] and a reduction of inflammatory disorders were described [8,9]. However, as the original utilization in lithotripsy also affects deeper structures, ESWT became prominent in ischemic conditions of profound tissues. Malperfused flaps in plastic surgery showed improved blood perfusion as well as myocardial and limb ischemia under shock wave treatment [10–12]. In orthopedics and trauma surgery, shock wave treatment ameliorated fracture healing in delayed union and pseudarthrosis [13]. Moreover, the use of ESWT has shown its benefits in degenerated and also acute traumatized tissue in sports medicine. Various fields of chronic musculoskeletal disorders showed improvements, e.g., chronic calcific tendinitis of the shoulder [14], medial and lateral epicondylitis [15], greater trochanteric pain syndrome and patellar tendinopathy [16,17], medial tibial stress syndrome [18,19], achilles tendinopathy [16], and plantar fasciopathy [20]. Furthermore, faster regeneration was found after shock wave treatment in the case of stress fractures [21]. Recent data indicate that ESWT might slow down muscle atrophy after an acute nerve injury [22]. Especially in the treatment of tendon disorders in elite athletes, ESWT is a good option as noninvasive treatment that is capable of shortening the time for full rehabilitation. As protocols regarding treatment energy and frequency vary greatly, data are still inconsistent [15], but the dose dependency is regularly emphasized [23].

On the other hand, ESWT application in acute muscle disorders has rarely been evaluated. Current therapy is usually limited to nonsteroidal anti-inflammatory drugs (NSAIDs), physical therapy modalities, and use of the RICE principle (rest, ice, compression, elevation). In this context, the repeated application of high-energy ESWT might have beneficial effects due to an enhanced muscle perfusion. Therefore, we compared the single application of ESWT to repetitive use of ESWT in an established rat model to evaluate additive effects on microcirculation in the muscle.

2. Methods

2.1. *Animal model and experimental protocol*

The experimental procedures were conducted in accordance with the German legislation on protection of animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council). They were approved by the Ministry of Energy, Agriculture, the Environment, and Rural Areas and are in accordance with the EU Directive 2010/63/EU for animal experiments.

Twenty-six Sprague Dawley rats (Charles River Laboratories, Sulzfeld, Germany), weighing 250 to 350 g, were used in this study. Animals were housed in 12 h per d/night cycle 2/cages at 21°C and fed ad libitum. During the experiments, the rats were under sufficient pentobarbital sodium anesthesia (55 mg/kg bw ip; Narcoren, Merial, Hallbergmoos, Germany) monitored by stable heart rate and breathing frequency to minimize microcirculatory affection, due to pain reaction. Body temperature was maintained at 36–37°C using a heating pad. Rats were randomly assigned to the groups. Group A received single high-energy extracorporeal shock wave therapy (shock waves at 0.3 mJ/mm^2 and four impulses/s with a total of 1000 impulses totaling 10J). Group B received repetitive ESWT every 10 min (shock waves at 3 $\times$ 0.3 mJ/mm^2 and four impulses/s with a total of 1000 impulses totaling 30 J). ESWT was applied to the dorsal lower leg of the left hind limb of each animal with a short-range applicator (focus 0–30 mm) using a Storz Medical Duolith SD-1 “T-Top”. Application was standard for both groups and performed by the same physician using contact gel without relevant pressure to the tissue.

2.2. *Microcirculatory analysis*

Each rat was allowed to stabilize for 10 min after anesthesia delivery before investigating the cutaneous microcirculation. Microcirculation was assessed at the lower leg of the hind limb before application as baseline measurement and 1, 2, 5, 10, 11, 12, 15, 20, 21, 22, 25, 30, 35, 40, 50 min after single ESWT application; respectively 1, 2, 5, and 10 min after the first and the second application and 1, 2, 5, 10, 15, 20, and 30 min after the third application of repetitive ESWT. Therefore, a noninvasive combined Laser-Doppler and photospectrometry system (Oxygen-to-see, 02C, LEA Medizintechnik, Giessen, Germany) was used.

The probe was fixed by a special apparatus to minimize measurement artifacts due to vibration. It was removed for shock wave therapy and then again attached to the same position on the hind limb. One minute before application of focused ESWT to the dorsal lower leg of the hind limb, baseline measurements were carried out.

The O2C system combines the determination of hemoglobin and the principle of blood flow measurement. As described before [24,25], the optical method allows measuring blood flow by Laser-Doppler-Imaging technique and hemoglobin oxygenation and hemoglobin concentration in the tissue by photospectrometric techniques. In short, an optical fiber probe, incorporating both the laser Doppler and the broadband light spectrometry technique, records the local oxygen supply parameters, the oxygen saturation of hemoglobin, the relative postcapillary venous filling pressures, and the blood flow. The probe detects changes at a depth of 8 mm with respect to capillary blood flow velocity (arbitrary units [AU]) in muscle tissue.

2.3. Statistical analysis

Data were analyzed using SigmaPlot statistical software, version 12.3 (Systat, San José). Normal distribution was tested using the Shapiro–Wilk test. In the case of parametric data, statistical analysis was carried out using ANOVA followed by Tukey test. In the case of nonparametric data, ANOVA on ranks was used followed by Tukey test. Data were expressed as mean ± SD. P-values <0.05 were regarded as statistically significant.

3. Results

3.1. Adverse effects

Focused ESWT was very well tolerated by the animals. They did not show deviant behavior or pain after anesthesia. Hematoma, seroma, and bleeding were not observed in either group.

3.2. Muscular blood flow

Baseline muscular blood flow was at 266.6 ± 72.0 arbitrary units (AU) in Group A. Immediately after the application of focused high-energy ESWT, it significantly increased by 16.5% (319.4 ± 47.0 AU, $P = 0.007$). Thereafter, blood flow decreased and remained significantly unchanged to baseline value from minute 2 after application (288.6 ± 47.6 AU; $P = 0.183$), showing a further decrease until 15 min after application to 239.4 ± 47.8 AU ($P = 0.190$). By the end of the measuring period at 50 min after application, baseline values were reached again (273.6 ± 55.3 AU, $P = 0.550$; Fig. 1).

In group B, muscular blood flow baseline was at 206.3 ± 49.8 AU. First application of repetitive ESWT (frESWT) increased blood flow to 246.7 ± 61.2 AU ($P = 0.049$) after 1 min. Afterward, muscular blood flow decreased again to 232.5 ± 53.9 AU ($P = 0.163$) and stayed slightly above baseline level, insignificantly in each case. The second application of frESWT 10 min after the first application-boosted muscular blood flow to 281.1 ± 73.9 AU ($P = 0.004$), remaining significantly elevated until the third application of frESWT. FrESWT was applied a third time 20 min after baseline measurement and increased muscular blood flow again high significantly to a level of 293.9 ± 65.4 AU ($P < 0.001$ to baseline; $P = 0.044$ to the increase after the first application). Muscular blood flow remained significantly elevated until 30 min after the first application, tending back down to baseline at the end of measuring at minute 50. The first application of frESWT increased blood flow by 16.4% and the second application by 26.6%, whereas after the third application blood flow extended by 29.8% (Fig. 2).

In comparison to group A, the increase of muscular blood flow was roughly the same 1 min after the first application. In both groups, blood flow decreased to an insignificant level compared to baseline 2 min after the first application of ESWT. Group B receiving frESWT, on the other hand, showed a significant elevation of muscular blood flow until 10 min after the second and the third application of ESWT.

4. Discussion

In this study, we showed for the first time that muscular microcirculation is significantly enhanced by the use of focused high-energy extracorporeal shock wave therapy (ESWT). Furthermore, we found that fractioned repetitive application of ESWT (frESWT) prolonged the enhancement of muscular blood flow compared to single-session ESWT. A second session of ESWT boosts microcirculatory blood flow of the muscle compared to baseline, whereas the third session even prolonged the effect.

4.1. The effects of ESWT

ESWT used in the musculoskeletal system is known to improve tissue viability and regeneration of the treated tissues. In detail, cell proliferation and enhanced synthesis of collagen were found in treated human tenocytes due to an increase of tendon-specific markers (SCX, COL1 A1, Col3 A1). Additionally, anti-inflammatory cytokines (IL-1b, IL-6, IL-10,

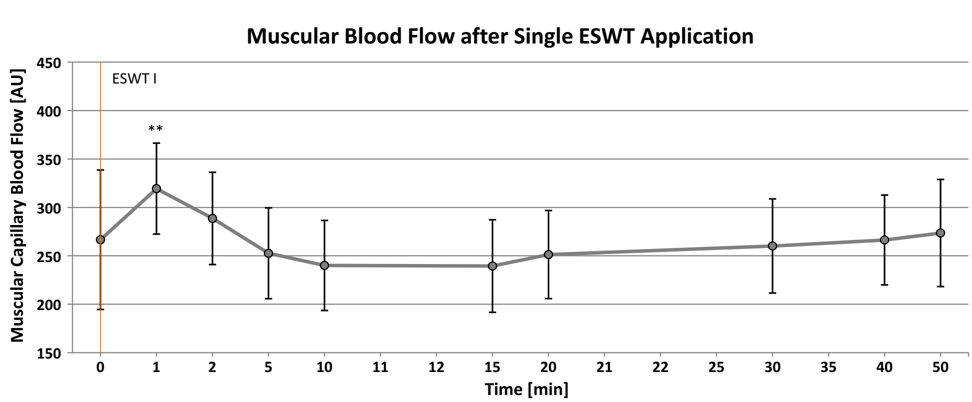

Fig. 1 – Microcirculatory effects of single-session ESWT on muscular capillary blood flow. Blood flow increased significantly 1 min after extracorporeal shockwave therapy was performed. Afterward, it decreases to baseline. AU = arbitrary units. **P < 0.01 compared to baseline. (Color version of figure is available online.)

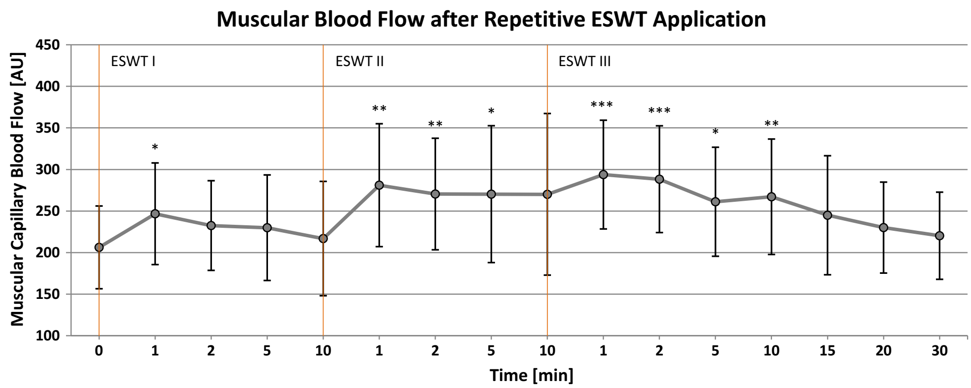

Fig. 2 – Microcirculatory effects of fractionated repetitive ESWT on muscular capillary blood flow. Blood flow was increased 1 min after the treatment, decreasing again to baseline. The second and the third treatment of extracorporeal shockwave therapy boosted the effect to an even higher extend, and muscular blood flow was significantly increased until 10 min after the third treatment. AU = arbitrary units. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to baseline. (Color version of figure is available online.)

TGFb) were increased after ESWT application [26,27]. Chondrocyte activity from the rats' knees was found increased after the transected anterior cruciate ligament was treated with ESWT [28]. Furthermore, systemic effects have been described with an enhancement of endothelial progenitor cell [9], vascular endothelial growth factor and nitric oxide release [27,29,30]. Consequently, fractionated repetitive application might boost the liberation of these regenerative elements and therefore extend their effects. In this context, prolonged shear stress is known to increase angiogenic sprouting [31]. Thus, it is thought that the mechanical stimulus of ESWT in combination with the improved blood flow might have additive effects by impacting the mechanosensory complex formation involving VEGFR-2, VE-cadherin, and PECAM-1 [12,32]. As ESWT is regularly in use for tendon disorders, and no extensive capillary ingrowth is reported, little vascularized tissues might benefit from ESWT more by the activation of specific cells and the enhancement of reparation and remodeling process.

4.2. *The use of repetitive ESWT in acute muscle disorder*

Recent studies have shown that a single application of ESWT improves microcirculatory parameters in soft tissues. Unfortunately, duration of these microcirculatory effects was limited [33]. High-energy ESWT (meaning a flux density higher than 0.15 mJ/mm^2) had an increased impact on microcirculation compared to low-energy ESWT [23]. Nevertheless, a number of clinical trials have revealed positive effects after a single application or a small number of repetitions. However, time intervals of ESWT application are often at a minimum of 2 d and might therefore have limited the positive effects as our data implicate only a short duration of microcirculatory effects after single-session ESWT. In our study, we evaluated microcirculation after a single session and fractionated repetitive application in the muscles of rats. A direct comparison in traumatized muscle tissue should not be the first step of evaluation. Therefore, we used healthy animals to analyze microcirculation in a standardized way. The results indicate that repetitive use might be beneficial in several trauma-related, orthopedic, and surgical conditions. Especially after acute soft tissue injuries, increased microcirculation might be useful to supply the tissue that is involved in the regeneration of the damaged tissue by humoral and cellular factors. Fractionated repetitive ESWT might therefore be an attractive option in acute muscle injury leading to boosted and prolonged blood perfusion compared to single-session ESWT. Furthermore, patients with indirect muscle disorders like fatigue-induced muscle disorder, delayed-onset muscle soreness, spine-related neuromuscular muscle disorder, muscle-related neuromuscular muscle disorder, and moderate-to-subtotal muscle tear might benefit from the treatment. Professional athletes in particular might profit from a faster postinjury recovery, as regeneration and rehabilitation of the muscle could be improved due to an increased microcirculation [34]. In any case, cryotherapy still remains a standard procedure in acute muscle injury to decrease severe pain and edema without reducing microvascular perfusion [35]. Hence, further studies have to be conducted to evaluate the microcirculatory effects by the combination of standard practice with single session and fractionated repetitive ESWT. Moreover, the effects of fractionated repetitive ESWT (frESWT) with short time intervals should be compared to longer intervals in acute muscle injury. Additionally, skeletal muscle protein anabolism and clinical symptoms like pain should be evaluated in the use of repetitive ESWT [36].

4.3. *Limitations*

Baseline values showed a difference between the groups. However, these values depend on muscular capillary anatomy and function. This is the reason why differences were compared to baseline and not directly to the other group. Consequently, changes over time have to be regarded as more relevant, because capillary anatomy remains relatively constant in the short measuring period and function varies due to the shock wave application. Moreover, a direct comparison of the microcirculation after the third application resulted in significantly higher values than after the first application. However, comparing changes to the baseline microcirculation of the muscle tissue is more relevant, because it describes differences from the usual perfusion.

5. Conclusion

ESWT is known to promote regeneration and rehabilitation after sports injuries by affecting humoral and cellular factors and enhancing tissue remodeling. Moreover, ESWT enhances the blood flow in muscle tissue immediately after the application. In repetitive use by frESWT, the effect is even prolonged and might therefore be of special interest in the treatment of acute muscle injury or chronic muscle disorders.

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Authors contributions: All authors have made substantial contributions to the study. T.K. was responsible for the acquisition and interpretation of data and wrote the article. W.W., V.F., E.L. and F.H.S. were responsible for acquisition and analysis of data. K.K. and P.M. were involved in the interpretation of data and in proof reading. R.K. was responsible for the study design, data analysis, and supervised the study.

Disclosure

The authors declare that they do not have a conflict of interest. Moreover, no commercial or similar relationships to products or companies mentioned in or related to the subject matter of the article are disclosed.

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