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SHORT REPORT

Placebo-Controlled Study of Effects of Low-Energy Shockwave Therapy (LE-ESWT) on Erectile Tissue in a Diabetic Animal Model

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Objective: Low-energy extracorporeal shockwave therapy (LE-ESWT) has been shown to induce organ repair and neovascularization. The ability of LE-ESWT to improve erectile function in rodents as measured by improvements in intracavernosal pressure is well-established in various pathological situations. The underlying molecular mechanism are unclear and likely vary between different disorders, making rational drug design for synergetic effects with LE-ESWT difficult, without further research. In this placebo-controlled study, we aim to establish whether LE-ESWT can activate neovascularization biomarkers in diabetic tissues.

Material and Methods: Forty Wistar rats, aged 8 weeks, were randomly divided into 4 groups: 8 untreated controls, 12 controls that underwent LE-ESWT treatment, 8 controls with induced diabetes mellitus (DM) and 12 with DM underwent LE-ESWT treatment. DM was induced by streptozotocin. LE-ESWT treatment was performed with a Duolith SD1 machine (Storz), with a total amount of energy of 6.4 J per treatment. The rats received a total of three LE-ESWT treatments with 2-week intervals between treatments.

Results: Diabetic rats had significantly elevated blood glucose concentrations compared to control rats ($P < 0.001$) and experienced significant weight loss compared to controls ($P < 0.001$). Diabetic rats had elevated creatinine and urea and lower albumin ($P < 0.001$). Histologic analysis of penile tissue showed significant levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) expression in the LE-ESWT groups compared to controls ($P< 0.01$).

Conclusion: LE-ESWT induces neo-angiogenesis as expressed by VEGF and FGF in erectile tissue in normal and diabetic rats.

Keywords: erectile dysfunction, diabetes mellitus, extracorporeal shockwave therapy

Introduction

Over the past decade, evidence has accumulated indicating that low-energy extracorporeal shockwave therapy (LE-ESWT) can activate tissue repair mechanisms in different patient populations and animal models of various disorders, with low-energy defined as > 0.28 mJ/mm^2.[1] How LE-ESWT initiates repair and neovascularization cascades is not known but is theorized to be related to a combination of mechanotransduction and micro-trauma subsequently causing release of growth factors,[2–5] activation of associated receptors such as extracellular signal-regulated kinase (ERK) and Toll-like receptor 3,[6–8] and production of signaling molecules such as nitric oxide (NO) and interleukins.[3,5,9]

LE-ESWT has been found to be beneficial in various situations, including dermal injuries including burns, diabetic ulcers and wound healing following vein harvesting for burns,[10] coronary bypass,[11] chronic heart failure,[12,13] several musculoskeletal complaints,[14] regeneration of especially sensitive tissues such as the pancreas,[15] and urological disorders such as erectile dysfunction and Peyronie's disease.[16–21] The European Association of Urology has also published its recommendations for treating uroandrological disorders with LE-ESWT. The 2022 EAU guidelines is that LE-ESWT may be used in vasculogenic ED and for pain management in patients with Peyronie's disease.[22]

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Age- and disease-associated degeneration of penile erectile tissue and certain medical procedures reduce erectile function. The ability to stop or reverse this degenerative process with a noninvasive, drug-free procedure with little to no side effects will benefit the quality of life of affected individuals.[23,24]

In this placebo-controlled experimental animal study, we wished to demonstrate whether LE-ESWT induces tissue repair and neovascularization in normal and diabetic penile rat tissue.

Materials and Methods

Forty Wistar rats, aged 8 weeks, were randomly divided into 4 groups; 8 rats received no treatment; 12 rats underwent LE-ESWT; 8 rats had diabetes mellitus (DM) induced, and underwent no LE-ESWT treatment; finally, 12 rats with DM underwent LE-ESWT treatment. Diabetes was induced by intraperitoneal streptozotocin injection (60 mg/kg) 6 weeks before the first round of LE-ESWT. The rats were sacrificed 12 weeks after streptozotocin injection.

To minimize stress, animals were housed in a dedicated rodent facility with a single animal technician performing feeding, housing maintenance, animal health checks, tail-blood sampling, and the anesthetic procedure. The National Animal Research Inspectorate approved the study (BES40621 2010/261-1852), animals were kept in accordance with the animal welfare policy of Aarhus University.

Anesthesia

All rodents underwent a standard anesthetic procedure. A mixture of Hypnorm (fentanyl, 0.315 mg/mL; fluanisone, 10 mg/mL) and Dormicum (midazolam, 5 mg/mL) diluted with an equivalent volume of sterile water was injected subcutaneously, dose: 0.18mL final mixture per 100g body weight.

Low-Energy Extracorporeal Shockwave Therapy (LE-ESWT)

LE-ESWT was performed on the whole shaft of the penis using a handheld Duolith SD1 (Storz) (Figure 1). Treatment was performed under anesthesia and applied on the penis from the distal part to the basis with energy of 0.13 mJ/mm^2, frequency 5 Hz, and a total of 1500 shock waves representing a total amount of energy of 6.4 J per treatment. Rats received a total of three LE-ESWT treatments with 2-week intervals between them.

Sample Preparation

Blood sugar levels were measured periodically via tail-blood samples. Two weeks after the last session of LE-ESWT treatment, the rats were weighed, had a final tail-blood glucose measurement performed, and were sacrificed. Ventricular

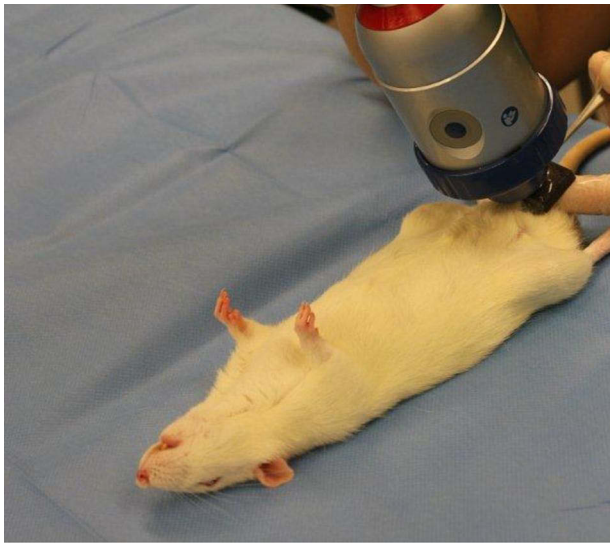

Figure 1 LE-ESWT of rat penis. The procedure is shown with the head of the probe at the penis of the anesthetized rat.

blood was extracted and stored at −80°C for later determination of creatinine, urea, albumin, and circulating vascular endothelial growth factor (VEGF) in the local department of clinical biochemistry. The penis was removed and stored in 10% formalin.

Histologic Analysis

Before shipment for histologic examination, the samples were anonymized. Tissue slices were stained with hematoxylin-eosin and the endothelium was examined by immune fluorescence staining for VEGF, mouse monoclonal antibody, (Clone sc-7269, Santa Cruz, dilution 1:75) and fibroblast growth factor (FGF), mouse monoclonal antibody, (Clone bFM-2, Millipore, dilution 1:20). Each sample was analyzed twice by an experienced pathologist who was blinded with regard to sample origin.

Data Treatment and Statistical Analysis

The VEGF and FGF staining double score is reported as low (double negative), medium (one positive, one negative), or high (double positive). Statistical Product and Service Solutions (SPSS) 15.0 (IBM, USA) was used to apply the Mann–Whitney *U*-test for LE-ESWT and non-LE-ESWT samples.

Results

Diabetic rats had a significantly elevated mean blood glucose concentration compared to control rats ($P< 0.001$) (Table 1). Their average weight loss was 37 g, while control rats had an average weight gain of 32 g over the period ($P< 0.01$) (Table 1). Diabetic rats had elevated mean serum concentrations of creatinine and urea as well as reduced albumin levels ($P< 0.001$) (Table 1).

VEGF analysis of blood samples failed because of insufficient sample material.

Blinded histologic analysis of the erectile tissue slices showed a significantly higher average VEGF score in the LE-ESWT treated groups than in the non-LE-ESWT groups (P=0.001) (Figure 2). The FGF score was also significantly higher in the LE-ESWT treated groups than in the non-LE-ESWT groups (P=0.039) (Figure 3). No statistical difference was seen between tissue VEGF and FGF staining with hematoxylin-eosin in the two LE-ESWT-treated groups, p=0.630 and P=0.266.

Discussion

Our in vitro analysis indicates that even with an ongoing diabetes-associated degenerative process affecting the penile erectile tissue, LE-ESWT can induce expression of growth factors known to activate tissue repair and neovascularization. Following LE-ESWT, both FGF and VEGF staining had similar outcome groupings: low≈27%, medium = 8%, high ≈65%. The group that did not receive LE-ESWT display similar FGF and VEGF outcome groupings.

With around 1/3 nonresponders following LE-ESWT, it would interesting to perform repeated round of treatment to establish if the treatment response rate can be increased. As Wistar rats can display trait difference intrastrain differences, a genetic or epigenetic factor cannot be excluded.[25]

Table 1 Physical Measurements Made Just Prior to Sacrifice and Serum Metabolite Measurements Based on Ventricular Blood Taken Just After Sacrifice

	Weight Before (g)	Weight After (g)	Glucose (mM)	Creatinine (μM)	Urea (mM)	Albumin (g/L)
Controls	448.5	471.5	9.4	53.4	8.5	43.0
Controls, DM	431.8	398.1	28.0	60.4	10.0	38.0
DM, LE-ESWT	413.3	371.7	34.8	62.6	11.4	35.1
Controls, LE-ESWT	427	464.5	8.8	52.3	7.7	41.8

Abbreviations: DM, diabetes mellitus; LE-ESWT, low-energy extracorporeal shockwave therapy.

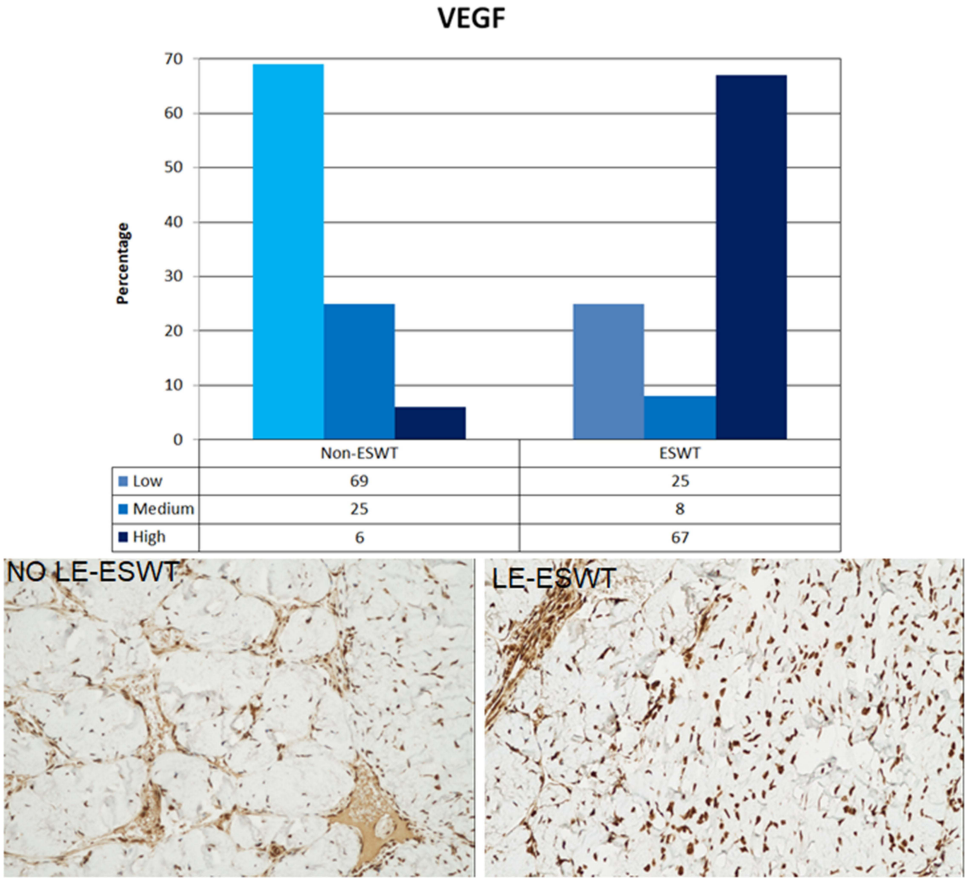

Figure 2 Vascular Endothelial Growth Factor. Graph shows scoring distribution (%) for VEGF in rats that either received LE-ESWT or anesthesia only. Bottom left image shows a representative tissue slice from the LE-ESWT-negative group. Bottom right image shows a representative tissue slice from the LE-ESWT-positive group.

The diabetic phenotype had materialized as indicated by the different blood sugar levels. The difference in weight and blood levels of creatinine and urea and the lower albumin level between the DM and non-DM groups indicates onset of severe diabetic complications, such as diabetic nephropathy. A limitation of the present paper is that we did not measure improvements in intracavernosal pressure. However, as is evident from the works cited below, this measurement has been performed in multiple studies, to the point where it can be considered well-established.

A recent similar study of growth factor levels following LE-ESWT treatment in diabetic rats found growth factor expression reduced following streptozotocin treatment and that there was a clear tendency of VEGF to normalize to control levels following LE-ESWT, but the tendency was not statistically significant[26] where we in the present study found a significant difference. However, the outcome from the above mentioned paper was measurement of mRNA detection, which may or may not correlate with protein expression patterns and final phenotype in specific situations. Correlation can vary eg due to altered post-translational regulation, different expression of binding factors, and degree of cellular excretion pathway activation.[26,27] Our results directly measure VEGF and FGF expression patterns and thus indicate that the mRNA/phenotype correlation is not completely reliable in this case, however the two studies support the same conclusion that LE-ESWT treatment can normalize growth factor expression in diabetic tissue.

Another study of type II diabetes showed that LE-ESWT also improved erectile function in Goto-Kakizaki (GK) rats, which is a validated model of DM.[28] GK rats and age-matched Wistar rats were treated with LE-ESWT twice weekly for 3 weeks, which increased erectile function. The authors reported from the same study that LE-ESWT significantly improved erectile function in GK rats to the same extent as sildenafil. However, this effect was not mediated by a NO/cyclic guanosine monophosphate-dependent mechanism. They found that co-treatment with sildenafil increased LE-ESWT efficacy. They concluded that this was a change in the preclinical paradigm regarding LE-ESWT and should be

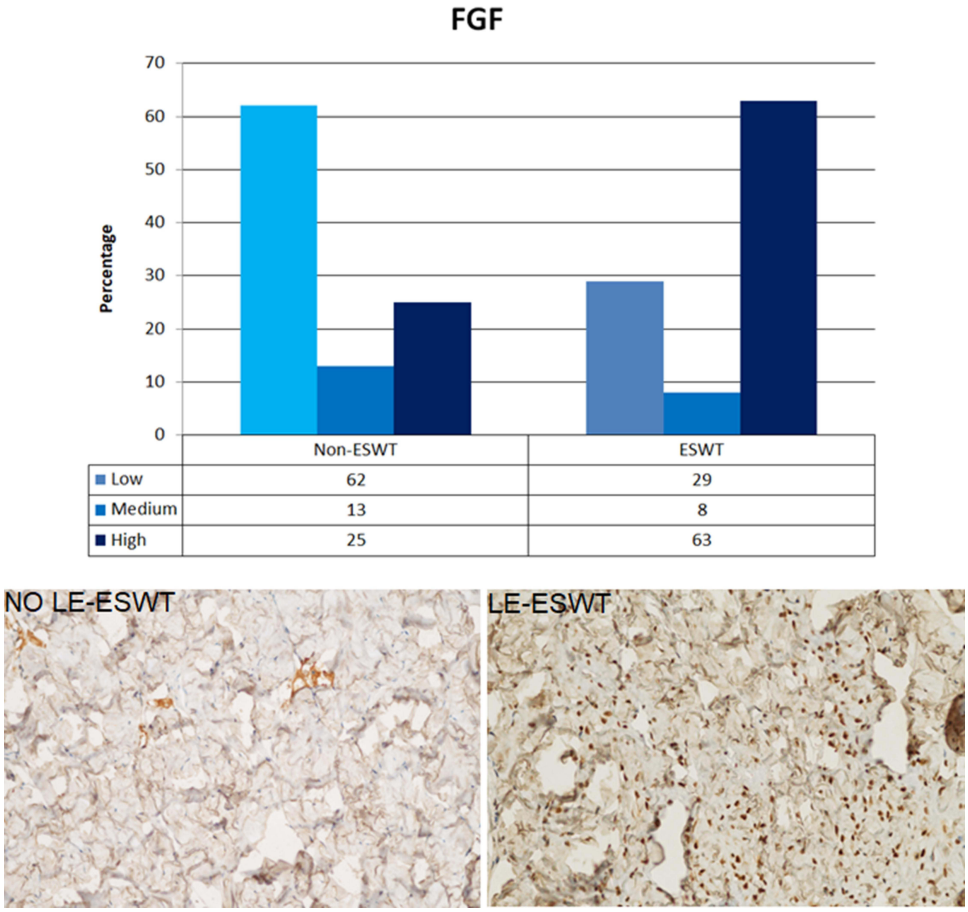

Figure 3 Fibroblast Growth Factor. Graph shows scoring distribution (%) for FGF in rats that either received LE-ESWT or anesthesia only. Bottom left image shows a representative tissue slice from the LE-ESWT-negative group. Bottom right image shows a representative tissue slice from the LE-ESWT-positive group.

further investigated as NO production has previously been proposed to be an important mediator of the beneficial effect of LE-ESWT.[3,5,9] Potentially, cotreatment could reduce the quantity of nonresponders that we see in this study.

Another study investigated the feasibility of using the Zucker fatty (ZF) rat as a model in obesity-associated erectile dysfunction.[29] They concluded that LE-ESWT restored penile hemodynamic parameters in ZF rats by reversing structural changes in the corpus cavernosum associated with obesity and diabetes-induced erectile dysfunction, such as restoration of smooth muscle and endothelium content, and by reducing lipid accumulation. The underlying LE-ESWT mechanism appears to be activation of stem/progenitor cells, which prompts cellular proliferation and accelerates penile tissue regeneration.[29] Combined, this present work and the studies discussed above support the notion of stem cell activation via release of specific growth factors as the main regenerative pathway following LE-ESWT of diabetic tissue.

Longer lifespans and increasing frequency of lifestyle-associated diseases such as DM increase the clinical need for delaying or reversing the associated degeneration of important tissue structures such as nerve endings, blood capillaries, and extracellular matrix components in sensitive organs like kidney or erectile tissue. Hand-held LE-ESWT equipment is a simple, low-cost option compared to oral or penile medication and other medical instruments In addition, LE-ESWT is non-invasive and without serious side effects, and has the ability to potentially act synergistically with other erectile dysfunction treatment modalities, including stem cell therapy and type-5 phosphodiesterase inhibitors.[17,28,30,31]

Conclusion

Our results validate and support that LE-ESWT can activate neovascularization and tissue repair mechanisms even after significant DM-associated tissue damage has occurred and that these pathways should be investigated further as potential candidates for synergistic pharmaceutical treatment.

Disclosure

Dr Milad Hanna reports paid travel and hotel accommodation during the research from STORZ Medical, during the conduct of the study; and Storz Medical sponsored my attendance EAU and WCE congresses, as I am an advisor to STORZ MEDICAL regarding training and research. The authors report no other conflicts of interest in this work.

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