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Unique effect and clinical evidence of SparkWave™ therapy in the treatment of erectile dysfunction

Introduction / scientific background

SparkWaves™ emerged as an effective and specific kind of shock wave (SW) with a unique role in tissue regeneration. The medical origin of shock waves is the urological field where high-intensity SWs are applied in order to destroy kidney stones (lithotripsy), or from regenerative orthopedics (orthotripsy) and wound healing where medium- or low-intensity SWs are utilized to induce tissue repair. The electrohydraulic shock wave machine generates acoustic waves by high voltage electrodes in an underwater explosion. SWs pass the skin and superficial tissues and produce physical shear forces that can be focused at a desired tissue depth causing microtrauma which in turn initiates beneficial cellular processes. It was shown that the extracorporeal shock wave therapy (ESWT) possesses anti-inflammatory properties and is able to increase the blood circulation and metabolism of the treated tissue, thus supporting the healing of bone fractures, tendon pathologies, chronic foot ulcers or ischemic heart diseases. Research about the underlying cellular mechanisms found out that ESWT causes stem cell migration and stimulates osteoblasts and fibroblasts during the recovery of bones and connective tissue. The SWT-induced intracellular expression of growth factors like endothelial nitric oxide synthase (eNOS), bone morphological protein (BMP-2), vessel endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA) led to angiogenesis and revascularization in the affected areas. Nowadays, due to its growth-promoting effect, ESWT is used in a wide clinical approach and has become a promising treatment perspective for vasculogenic erectile dysfunction (ED), chronic pelvic pain and Peyronie’s disease.

Current state of research and clinical evidences in the treatment of ED by shock wave therapy

ED is one of the most common disorders of middle-aged men and is defined as the inability to achieve and maintain erections adequate for sexual intercourse. Although the currently available pharmacological possibilities like phosphodiesterase type 5 inhibitors (PDE5i) are more or less effective in most of the patients, the big disadvantage of this method is obvious since this simple, limited treatment of the symptoms does not correct the underlying pathophysiology and thus cannot be considered fully satisfactory. Vascular lesions secondary to diabetes mellitus, structural lesions caused by trauma or neurologic injury in consequence of prostatectomy for example are potential causes of ED. Due to its previously reported regeneration-promoting effects, low-intensity spark wave therapy (Li-ESWT) has been introduced to the andrological field and made great progress so far. Several studies, basic research as well as preliminary clinical trials, provided encouraging evidence for a sustainable improvement of erectile function (EF) upon Li-ESWT.

In 2010, Vardi et al. published the first pilot study in patients with organic ED. They demonstrated that Li-ESWT significantly increases penile rigidity and function, an observation attributed to the positive influence of ESWT on cavernosal hemodynamics. This issue was unraveled by Qui et al., who investigated the cellular effects of Li-ESWT on EF and tissue in a diabetic rat model. They reported a significant recovery of EF upon Li-ESWT due to the regeneration of nNOS-positive nerves, endothelium, and smooth muscle in the penis supported by the recruitment of endogenous mesenchymal stem cells. In the following years, the Vardi-team and several other groups published a series of positive outcomes from cohort studies and randomized, controlled trials (RCTs) which were recently revised in a systematic meta-analysis by Tom Lue and others. The clinical standard parameters to measure the therapeutic efficacy in ED-ESWT studies are the international index of erectile function (IIEF) and the erection hardness score (EHS). The majority of the present studies showed that men experience improvements in their EF upon Li-ESWT, regardless of variations in the set-up parameters or treatment protocols, PDE5i response or constitutional predispositions. The meta-analysis of Tom Lue finally...
suggests that SWT could significantly improve the IIEF and EHS of ED patients and corresponding research which explains the underlying mechanisms of the unique SparkWave™ effect are already provided:

**MTS SparkWaves™ in basic research studies, effects and mechanisms**

1. “**Effects of low-energy shock wave therapy on erectile function and tissue of diabetic rat model**” [17]. In this study by Qiu et al., SparkWaves™ partially ameliorated diabetes mellitus-associated ED by promoting nerve regeneration of nNOS-positive nerves, endothelium and smooth muscle in the penis. Saprkwaves significantly improved erectile function and intracavernous pressure (ICP). These beneficial effects appeared to be mediated by recruitment of endogenous mesenchymal stem cells (MSCs).

2. “**Low-energy shock wave therapy ameliorates erectile dysfunction in a pelvic neurovascular injuries rat model**” [20]. Here, Li et al. showed that SparkWaves™ therapy induces endogenous progenitor cell recruitment and Schwann cell activation in the damaged area which coincided with angiogenesis, tissue and nerve regeneration and thereby improved erectile function.

**Clinical evidence for MTS SparkWaves™**

The majority of common studies engaging shock waves produce good results in case of mild to moderate ED levels. Recently, MTS together with the Fundació Pugivert analyzed a group of long-term patients suffering from severe ED and were initially intended for penile implantation surgery to prove that even severe cases of ED can benefit from SparkWave™ therapy in future.

Table of the most recent urological studies using MTS technology:

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<th>Title</th>
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<td><strong>2. Saffón et al.,</strong> published at the 23rd WAS congress in Prague 2017</td>
<td>Effectiveness of shock wave therapy: implementation of a soft wide focus applicator in patients with erectile dysfunction</td>
<td>Boston Medical Group, Colombia</td>
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<td><strong>4. Ramon et al.,</strong> published at the 20th ISMST congress in San Sebastian 2017</td>
<td>Efficacy of extracorporeal shockwave therapy (ESWT) for male chronic pelvic pain syndrome: a phase III, randomized, double blind controlled with placebo study</td>
<td>Quiron Hospital, Barcelona</td>
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</table>
1. “Penile low-intensity shock wave therapy for PDE5i non-responders suffering from vasculogenic erectile dysfunction since 2-10 years: A prospective, randomized, placebo-controlled study (2016)”

The Fundació Puigvert in Barcelona is one of the most renowned institutions for penile implants, specialized on urology and andrology. Curbelo et al. investigated the effects of penile Li-ESWT on EF in long-term patients which suffered from vasculogenic ED since 2-10 years and were refractory to PDE5i. It was a randomized, simple-blind, sham-controlled study. 22 active and 19 sham treated ED patients received Li-ESWT (1 session/week for 6 weeks; 1,500 pulses of 0.10 ml/mm² at 5 Hz, urogold100® MTS). All patients were evaluated at baseline and 1 month after the end of treatment using validated erectile dysfunction questionnaires like the IIEF-5 and the Sexual Encounter Profile (SEP). There was no significant difference between the two groups in baseline characteristics. Baseline five-item version of the IIEF-5 mean scores, in the active and sham groups, were 10.0 ± 4 and 9.9 ± 4.6, respectively (p= 0.94). At baseline, 14% of patients in the active group (3 of 22) and 10.5% of patients in the placebo group (2 of 19) had a positive answer to the SEP 3 question “Did your erection last long enough for you to have successful intercourse?” (p= 0.8). One month after treatment IIEF-5 scores mean changes from baseline, in the active and placebo group, were 2.2 ± 4.9 and 0.25 ± 4.4, respectively (p= 0.2). SEP 3 positive responders increased to 33% in the active group (7 of 22) and even decreased in the placebo group to 5% (1 of 19) (p=0.03) after LIST. Notably, erectile function (SEP 3) could be improved significantly in a substantial proportion of active-treated patients (33%), whereas the placebo effect was zero. In this prospective study, 1 month of SparkWave™ treatment led to partial recovery of erectile function.

2. “Effectiveness of shock wave therapy: implementation of a soft wide focus applicator in patients with erectile dysfunction”

The Boston Medical Group in Colombia is treating a wide spectrum of patients suffering from ED and premature ejaculation. This network of medical offices is highly experienced in dealing with shock waves for many years now. Therefore, MTS and the Boston Medical Group investigated a mixed group of patients to analyze the positive effect of the unique SparkWave™ technology. Saffón et al. performed a progress report on SparkWave™ therapy in the treatment of vascular ED using a SWFA (soft wide focused applicator) handpiece for a cohort of patients in the clinical center in Bogota. 20 patients with a mean age of 53.1 ±12.1 years were included and underwent a protocol of LI-ESWT once a week for 5 weeks, energy flux density 0.15mJ and 3000 pulses per session, with the MTS urogold100® and applicator OP155. The outcome was measured by the Erection Hardness Score (EHS), International Index of Erectile Function, 5-item version (IIEF-5) at the end of the therapy and at one month follow-up. At admission, 70% of patients had mild / moderate (n = 14), 20% (n = 4) moderate and 10% (n = 2) severe ED according to the IIEF-5 scale. After five sessions 25% (n = 5), and after one month follow-up even 45% (n = 9) of patients showed a clinical important difference (defined as an increase of ≥4 points) in the IIEF score with an average increase of 5 points (18 ±4.4, p= 0.001). Assessing the EHS, 55.5% of patients at baseline (mean EHS: 3 ±0.6) had an erection insufficient to penetrate, this proportion decreased significantly to 28% after therapy (mean EHS: 4 ±0.7, p = 0.05), a beneficial effect that was still persisting after one moth follow-up (mean EHS: 4 ±0.7, p = 0.04). The preliminary results of LI-ESWT in the treatment of ED with the MTS urogold100® and applicator OP155 are promising and indicate a clinically significant improvement in both, the IIEF and EHS by SparkWave™ technology.
3. **“Case series of weekly low intensity shock wave (LiSW) therapy for erectile dysfunction”**

The Glickman Urological and Kidney Institute of the Cleveland clinic is recognized worldwide for excellence in patient care, teaching and research, merges the urology and nephrology programs. It offers innovative treatments in urology and nephrology, including minimally invasive, scarless options for urologic procedures and medical management of kidney disease.

In order to improve patient convenience, Shoskes et al. studied the efficacy and safety of LiSW of a modified protocol of 4 weekly treatments in contrast to the established standard of twice weekly treatments for 3 weeks. 8 men (PDE5i non-responders) were enrolled yielding 3000 shocks/session at 6 treatment sites (500 shocks each) with the MTS urogold100®. Energy flux density was 0.13 mJ/mm² and frequency was 4 Hz. ED severity was measured with the Sexual Health Inventory for Men (SHIM) score at baseline and 1 month following the 4 weekly treatments. Overall, SHIM score improved from 11.0±3.6 to 17.2±5.2 (p=0.01). SHIM was unchanged in 2 patients (25%), mildly improved in 1 patient but not sufficiently for intercourse and significantly improved with erection sufficient for intercourse in 5 patients (62.5%).

Low Intensity Shockwave Lithotripsy with the urogold100® using a once a week protocol produced a similar success rate to previously published twice weekly protocols and improved erections sufficient for intercourse in 62.5% without side effects.

4. **“Efficacy of extracorporeal shockwave therapy (ESWT) for male chronic pelvic pain syndrome: a phase III, randomized, double blind controlled with placebo study”**

Quiron Barcelona Hospital is one of the leading clinics involving the latest equipment and the most experienced doctors in Spain.

Ramon et al. investigated chronic pelvic pain (CPPS) which according to the National Institute of Health (NIH) is genitourinary pain or discomfort lasting 3 or more months with undetectable uropathogenic bacteria. The primary goal was to assess the efficacy of extracorporeal shock wave therapy for treatment of males CPPS. 38 patients were evaluated. ESWT group improved their pain relief statistically significant compared to the placebo group (11 +/- 3.15 vs 6.31 +/- 2.55, p <0.05). Voiding quality also improved as measured by IPSS score (11 +/- 2 vs 7.21 +/- 1.5, p <0.05) and NIH-CPSI urinary symptoms (5 +/- 1.5 vs. 3.42 +/- 1.5, p <0.05). These results were maintained until 12 weeks. No adverse effects were observed.

At 4 and 12 weeks, patients who received ESWT experienced improvement in pain relief, quality of life, and voiding symptoms. It has been demonstrated that ESWT is an effective and safe treatment for CPPS.

**Future prospects of SparkWaves™ in the treatment of ED**

The new therapeutic concept of ED treatment by using shock waves will be the method of choice in future, since it is able to restore penile function in a gentle, non-invasive way. The key benefit is that spark wave therapy leads to a long-lasting regeneration process. The angiogenic properties increase penile blood flow and recovery of cavernosal vasculature which will enable patients to restore spontaneous and natural erections. After numerous successful clinical trials of renowned institutions, spark wave therapy becomes widely accepted and gains substantial ground in the field of ED treatment. The recent clinical studies performed by the Fundació Puigvert and the Boston Medical group further provide profound data about the effectiveness and safety of our urogold100®
MTS device in successful treatment of penile dysfunctions. Upcoming studies of MTS with other researchers are on the way and will further contribute to a better experience and knowledge of our experts in performing Li-ESWT in the treatment of ED. We intend to further optimize the SparkWave™ therapy protocol in order to establish individualized shock wave treatment concepts to get the best results for our patients.

References


Penile low intensity shock wave therapy for PDE5i non-responders suffering from vasculogenic Erectile Dysfunction since 2 to 10 years: A prospective, randomized, placebo-controlled study (2016)

Andrology Department, Fundació Puigvert / Universidad Autònoma de Barcelona, Barcelona, Spain

Curbelo, J. R. Sánchez, Álvaro Vives, Eduard Ruiz Castañé, Osvaldo Rajmil Marquenson, María Fernanda Peraza Gogoy, Daniel Moreno Mendoza, José Ignacio Vinay Barriga

**Background:** Several animal and human studies have evaluated the role of low-intensity extracorporeal shockwave therapy (LIST) in the management of multiple disorders such as chronic wounds, peripheral neuropathy and cardiac ischemic disease. LIST was reported to trigger a chain of events that releases angiogenic factors, recruits endothelial progenitor cells, induces neovascularization and enhances blood flow in treated areas. Recently, some studies with contradictory results have assessed the efficacy and safety of this therapy on patients suffering erectile dysfunction.

**Aim:** Investigate the effects of penile LIST on erectile function in long-term patients suffering from erectile dysfunction since 2-10 years and are refractory to phosphodiesterase type 5 inhibitors (PDE5i).

**Methods:** Prospective, randomized, simple-blind, sham-controlled study. In total 58 patients with vasculogenic erectile dysfunction refractory to PDE5i were randomized into two groups. 30 were treated with electrohydraulic low intensity shock waves (1 session/week for 6 weeks; 1,500 pulses of 0.10 mJ/mm² at 5 Hz, urogold100® MTS) and 28 were treated with a sham probe. Eleven patients withdrew from the study and were lost to follow-up. All patients were evaluated at baseline and 1 month after the end of treatment using validated erectile dysfunction questionnaires like the International Index of Erectile Function (IIEF-5) and the Sexual Encounter Profile (SEP). Demographic and clinical characteristics were recorded. Data analysis investigated specifically the long-term patients suffering from ED since 2-10 years, which were in total forty-one patients; 22 in the verum group, 19 in the sham group.

**Results:** 22 active-treated patients and 19 sham-treated patients, suffering from ED since 2-10 years were analyzed. There was no significant difference between the two groups in baseline characteristics. Baseline five-item version of the IIEF-5 mean scores, in the active and sham groups, were 10.0 ± 4 and 9.9 ± 4.6, respectively (p= 0.94). At baseline, 14% of patients in the active group (3 of 22) and 10.5% of patients in the placebo group (2 of 19) had a positive answer to the SEP 3 question (p= 0.8). One month after treatment IIEF-5 scores mean changes from baseline, in the active and placebo group, were 2.2 ± 4.9 and 0.25 ± 4.4, respectively (p= 0.2). SEP 3 positive responders increased by 33% in the active group (7 of 22) and even decreased in the placebo group to 5% (1 of 19) (p=0.03) after LIST.

**Conclusion:** In this prospective study, 1 month of moderate LIST treatment led to partial recovery of erectile function at one-month follow up, as the amount of positive SEP3 responders significantly
increased 4 times and the average IIEF-5 score improved 8 times in the active group compared to the sham control group which, showed no placebo-effect. More studies with larger sample size and longer follow-up, comparing different lithotripters and shock wave protocols, are imperative to define alternative protocols and the role of LIST in erectile dysfunction for long term ED patients.

### Baseline characteristics and statistical outcome of patients with 2-10 years ED

<table>
<thead>
<tr>
<th></th>
<th>Active group</th>
<th>Sham group</th>
<th>p-value (unpaired, two-tailed student’s t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>22</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Median age (years, mean ± SD)</td>
<td>62.7 ± 6.5</td>
<td>58.8 ± 7.9</td>
<td>0.1</td>
</tr>
<tr>
<td>ED duration (years, mean ± SD)</td>
<td>4.6 ± 2.9</td>
<td>5.5 ± 2.6</td>
<td>0.29</td>
</tr>
<tr>
<td>Patients with cardiovascular risk factors (%)</td>
<td>19 (86%)</td>
<td>14 (74%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Median IIEF-ED score baseline (mean ± SD)</td>
<td>10 ± 4.0</td>
<td>9.9 ± 4.6</td>
<td>0.94</td>
</tr>
<tr>
<td>Median IIEF-ED score after treatment (mean ± SD)</td>
<td>12.2 ± 6.1</td>
<td>10.2 ± 4.7</td>
<td>0.25</td>
</tr>
<tr>
<td>Median IIEF-ED score difference (after treatment - baseline) (mean ± SD)</td>
<td>2.2 ± 4.9</td>
<td>0.26 ± 4.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Positive SEP-3 (%) baseline</td>
<td>3 (14%)</td>
<td>2 (10.5%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Positive SEP-3 (%) after treatment</td>
<td>7 (32%)</td>
<td>1 (5%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Positive SEP-3 (%) difference (after treatment - baseline)</td>
<td>4 (18%)</td>
<td>-1 (0%)</td>
<td>0.94</td>
</tr>
</tbody>
</table>
Summary outcome of patients with 2-10 years ED:

1. SEP-3: Number of patients who turned from negative into positive SEP-3 response upon 1 month of LIST treatment:
   - Sham group: 0, \( n = 19 \)
   - Active group: 4, \( n = 21 \)
   → **SEP-3 increase of 400% vs. control**
   4-times more positive upon LIST compared to the control group.

2. IIEF-ED: Average improvement of the IIEF score upon 1 month of LIST:
   - Sham group: 0.26
   - Active group: 2.2
   → **IIEF-ED score increase of 829% vs. control**
   8.3-times better score in average compared to the control group.

Summary outcome of all patients that were analyzed:

1. SEP-3: Number of patients who turned from negative into positive SEP-3 response upon 1 month of LIST treatment:
   - Sham group: 1, \( n = 20 \)
   - Active group: 5, \( n = 27 \)
   → **SEP-3 increase of 500% vs. control**
   5-times more positive upon LIST compared to the control group.

2. IIEF-ED: Average improvement of the IIEF score upon 1 month of LIST:
   - Sham group: 0.5
   - Active group: 1.6
   → **IIEF-ED score increase of 319% vs. control**
   3.2-times better score in average compared to the control group.

Summary outcome of patients with cardiovascular risk factors (ischemic heart disease/hypertension/dislipemia) but NO diabetes mellitus:

1. SEP-3: Number of patients who turned from negative into positive SEP-3 response upon 1 month of LIST treatment:
   - Sham group: 1, \( n = 12 \)
   - Active group: 5, \( n = 16 \)
   → **SEP-3 increase of 500% vs. control**
   5-times more positive upon LIST compared to the control group.

2. IIEF-ED: Average improvement of the IIEF score upon 1 month of LIST:
   - Sham group: -0.7
   - Active group: 1.8
   → **IIEF-ED score increase of 180% vs. control**
   1.8-times better score in average compared to the control group.

Summary outcome of patients WITH diabetes mellitus AND cardiovascular risk factors (ischemic heart disease/hypertension/dislipemia):
1. **SEP-3**: Number of patients who turned from negative into positive SEP-3 response upon 1 month of LIST treatment:
   - Sham group: 0, (n = 8)
   - Active group: 0, (n = 11)
   → no difference between treatment and control

2. **IIEF-ED**: Average improvement of the IIEF score upon 1 month of LIST:
   - Sham group: 2.3
   - Active group: 1.3
   → SEP3 decrease of 43.3% vs. control

Patients suffering from diabetes mellitus did not respond to LIST treatment in this study.

ED: Erectile Dysfunction

IIEF-ED: International Index of Erectile Dysfunction – erectile function domain

LIST: Low-Intensity Extracorporeal Shockwave Therapy

SD: Standard Deviation

SEP: Sexual Encounter Profile question 3: *Did your erection last long enough for you to have successful intercourse?*
Effectiveness of shock wave therapy: implementation of a soft wide focus applicator in patients with erectile dysfunction (2016)

AUTHORS*: Joseph P. Saffon, Juan M. Martinez, Carolina Sandoval, Hector A. Corridor.

INTRODUCTION
Low-intensity extracorporeal shock wave therapy (LI-ESWT) is of great clinical interest for the treatment of erectile dysfunction (ED), chronic pelvic pain (CPP) and Peyronie’s disease. Extensive research in animal and human studies showed that the beneficial effect of LI-ESWT is due to its angiogenic properties. It is thought to stimulate neovascularization by inducing the expression of regeneration- and growth-related factors, like for example eNOS, VEGF and PCNA although the precise underlying mechanisms are not entirely clear yet. Thereby LI-ESWT can increase penile blood flow and endothelial function and represents a new, sustainable therapeutic strategy to restore erectile function, independent of, or supporting the conventional palliative medication. [1][2][3]

OBJECTIVE
Progress report on LI-ESWT in the treatment of vascular ED using a SWFA (soft wide focused applicator) handpiece for a cohort of 20 patients in a clinical center in Bogota, Columbia.

METHODS
Clinical records of patients treated at the medical centre were reviewed during the first half of 2016, with diagnosis of vascular ED and underwent a protocol of LI-ESWT once a week for 5 weeks, energy flux density 0.15mJ and 3000 pulses per session, with the MTS urogold100® and applicator OP155. Outcome measurements: Erection Hardness Score (EHS), International Index of Erectile Function, 5-item version (IIEF-5).

RESULTS
20 patients with a mean age of 53.1 ±12.1 years were included. At admission, 70% of patients had mild / moderate (n = 14), 20% (n = 4) moderate and 10% (n = 2) severe ED according to the IIEF-5 scale. After five sessions 25% (n = 5), and after one month follow-up even 45% (n = 9) of patients showed a clinical important difference (defined as an increase of ≥ 4 points) in the IIEF score with an average increase of 5 points (18 ±4.4, p = 0.001). Assessing the EHS, 55.5% of patients at baseline (mean EHS: 3 ±0.6) had an erection insufficient to penetrate, this proportion decreased significantly to 28% after therapy (mean EHS: 4 ±0.7, p = 0.05), a beneficial effect that was still persisting after one month follow-up (mean EHS: 4 ±0.7, p = 0.04).

CONCLUSIONS
The preliminary results of LI-ESWT in the treatment of ED with the MTS urogold100® and applicator OP155 are promising and indicate a clinically significant improvement in both, the IIEF and EHS by this technology. Studies with a larger group of patients, a longer follow-up and a comparative shock wave protocol setup are necessary to further assess the statistical, clinical significance and efficacy of this improvement in erectile function upon LI-ESWT.

(*) Boston Medical Group, Bogota, Columbia


Effectiveness of shock wave therapy: implementation of a soft wide focus applicator in patients with erectile dysfunction

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2 Epidemiologist, Universidad El Bosque, Bogota, Colombia;
3 MD, Specialist in Urology, Universidad Nacional de Colombia, Bogota, Colombia.

INTRODUCTION

Low-intensity extracorporeal shock wave therapy (LI-ESWT) is of great clinical interest for the treatment of erectile dysfunction (ED), chronic pelvic pain (CPP) and Peyronie’s disease. Extensive research in animal and human studies showed that the beneficial effect of LI-ESWT is due to its angiogenic properties. It is thought to stimulate neovascularization by inducing the expression of regeneration- and growth-related factors, like for example eNOS, VEGF and PPAR, although the precise underlying mechanisms are not entirely clear yet.

Therby LI-ESWT can increase penile blood flow and endothelial function and represents a new, sustainable, therapeutic strategy to restore erectile function, independent of, or supporting the conventional palliative medication. [1][2][3]

OBJECTIVE

Report progress on LI-ESWT in the treatment of vascualr ED using a SWFA (soft wide focused applicator) handpiece for a cohort of patients in a clinical center in Bogota, Colombia.

METHODS

Clinical records of patients treated in a Boston Medical Group centre in Bogota were reviewed during the first half of 2016, with diagnosis of vascular ED. Patients underwent a protocol of LI-ESWT once a week for 5 weeks, energy flux density 0.15 mJ and 3000 pulses per session, with the MTS urolodil100® and applicator OP155. Outcome measurements: Erection Hardness Score (EHS), International Index of Erectile Function, 5-item version (IIEF-5).

RESULTS

20 patients with a mean age of 53.1 ± 12.1 years were included. At admission, 70% of patients had mild/moderate (n=14), 20% (n=4) moderate and 10% (n=2) severe ED according to the IIEF-5 scale. After five sessions 25% (n=5), and after one month follow-up even 45% (n=9) of patients showed a clinical significant difference (defined as an increase of ≥4 points in the IIEF score with an average increase of 5 points (18 ± 4.4).

Assessing the EHS 55.5% of patients at baseline (mean EHS: 3 ±0.6) had an erection insufficient to penetrate; this proportionally decreased significantly to 20% after therapy (mean EHS: 4 ±0.7), a beneficial effect that was still persisting after one month follow-up (mean EHS: 4 ±0.7).

Conclusions:

The preliminary results of LI-ESWT in the treatment of ED with the MTS urolodil100® and applicator OP155 are promising and indicate a clinically significant improvement in both, the IIEF and EHS by this technology. Studies with a larger group of patients, a longer follow-up and a comparative shockwave protocol setup are necessary to further across the statistical, clinical significance and efficacy of this improvement in erectile function upon LI-ESWT.

REFERENCES


www.blogsaludmasculina.com
Case Series of Weekly Low Intensity Shock Wave Therapy for Erectile Dysfunction

All Cleveland Clinic, Department of Urology, Glickman Urological and Kidney Institute
Daniel A Shoskes MD, Nic Tadros MD, Brandon Mooney PA

Background & Aim: Low Intensity Shock Wave (LiSW) has emerged as a therapy for vasculogenic erectile dysfunction (ED). Mechanism may be related to angiogenesis, release of growth factors and/or recruitment of stem cells. Several sham controlled studies have shown improvement in peak arterial velocity and efficacy in the 60-65% range depending on the definition. The initial protocol of twice weekly treatments for 3 weeks with a rest period and repeat has remained the standard, although this can be very inconvenient for patients. We wished to study the efficacy and safety of LiSW using a modified protocol of 4 weekly treatments.

Methods: Men were enrolled in this IRB approved study provided they had a diagnosis of ED for at least 6 months and were able to return for weekly treatments. Low Intensity Shockwave was delivered with the Urogold 100 machine (Tissue Regeneration Technologies, Woodstock, GA) using the soft wide focused applicator probe (figure 1). There were 6 treatment sites: one at each crus of the penis and 2 on the shaft bilaterally with about 500 shocks each for a total of 3000 shocks. Energy flux was 0.13 mJ/mm² and frequency was 4 Hz yielding a biologic energy density of 1560. ED severity was measured with the Sexual Health Inventory for Men (SHIM) score at baseline and 1 month following the 4 weekly treatments. Pre and post SHIM values were compared with the paired t test with significance set at p<0.05.

Results: Eight men enrolled with a mean age of 56.8 years (range 26-70) and median duration of 36 months (range 12-120). Five had previously tried PDE5 inhibitor (PDE5i) oral medications without adequate success. One patient stopped after 3 treatments but was included for an intent to treat analysis. The treatments were painless and there were no side effects. Overall, SHIM score improved from 11.0±3.6 to 17.2±5.2 (p=0.01). SHIM was unchanged in 2 patients (25%), mildly improved in 1 patient but not sufficiently for intercourse and significantly improved with erection sufficient for intercourse in 5 patients (62.5%) (figure 2). Two of these 5 men required a PDE5i for optimal erections however both had failed PDE5i in the past.

Discussion: Low Intensity Shockwave Lithotripsy with the Urogold 100 using a once a week protocol produced a similar success rate to previously published twice weekly protocols. One of the treatment failures had psychogenic ED suggesting that inclusion criteria should focus on men with an arteriogenic etiology. Whether this once weekly therapy remains durable will await longer term follow up. Since in the United States this device is not approved by the FDA and patients will need to pay cash for therapy, a protocol that minimizes time away from work and out of pocket expense is highly desirable.

Conclusion: Once weekly low intensity shock wave lithotripsy improved erections sufficient for intercourse in 62.5% of our patients without side effects.
Introduction

Low Intensity Shock Wave (LiSW) has emerged as a therapy for vasculogenic erectile dysfunction (ED). Mechanism may be related to angiogenesis, release of growth factors and/or recruitment of stem cells.

Several sham controlled studies have shown improvement in peak arterial velocity and efficacy in the 60-65% range depending on the definition.

The initial protocol of twice weekly treatments for 3 weeks with a rest period and repeat has remained the standard, although this can be very inconvenient for patients. We wished to study the efficacy and safety of LiSW using a modified protocol of 4 weekly treatments.

Methods

Men were enrolled in this IRB approved study provided they had a diagnosis of ED for at least 6 months and were able to return for weekly treatments. Low Intensity Shockwave was delivered with the Urogold 100 machine (Tissue Regeneration Technologies, Woodstock, GA) using the soft wide focused applicator probe (figure 1). There were 6 treatment sites: one at each crus of the penis and 2 on the shaft bilaterally with about 500 shocks each for a total of 3000 shocks. Energy flux was 0.13 mJ/mm² and frequency was 4 Hz yielding a biologic energy density of 1560.

ED severity was measured with the Sexual Health Inventory for Men (SHIM) score at baseline and 1 month following the 4 weekly treatments. Pre and post SHIM values were compared with the paired t test with significance set at p<0.05.

Results

Eight men enrolled with a mean age of 56.8 years (range 26-70) and median duration of 36 months (range 12-120). Five had previously tried PDE5 inhibitor (PDE5i) oral medications without adequate success. One patient stopped after 3 treatments but was included for an intent to treat analysis. The treatments were painless and there were no side effects.

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Conclusion

Once weekly low intensity shock wave lithotripsy improved erections sufficient for intercourse in 62.5% of our patients without side effects.
Efficacy of extracorporeal shock wave therapy (ESWT) for males chronic pelvic pain syndrome: A phase III, randomized, double blind controlled with placebo study

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1 Hospital Quiron Barcelona Physical Medicine and Rehabilitation Department, Barcelona, Spain

Introduction: Chronic Prostatitis/ Chronic Pelvic Pain Syndrome (CP/CPPS) according to NIH is genitourinary pain or discomfort lasting 3 or more months with undetectable uropathogenic bacteria.

Material & Methods: Randomized, double blind, placebo controlled study has been conducted in 40 male patients who have had CPPS. Patients were randomly assigned to receive extracorporeal shock wave therapy (ESWT) or placebo. The study was conducted together by both Urology and Rehabilitation services. The primary outcome was to assess the efficacy of extracorporeal shock wave therapy for treatment of males CPPS.

Results: 38 patients were evaluated. ESWT group improved their pain relief statistically significantly compared to placebo group (11 +/- 3.15 vs 6.31 +/- 2.55, p <0.05). Also improved voiding quality as measured by IPSS score (11 +/- 2 vs 7.21 +/- 1.5, p <0.05) and NIH-CPSI urinary symptoms (5 +/- 1.5 vs. 3.42 +/- 1.5, p <0.05). These results were maintained until 12 week. No AEs.

Discussion: At 4 and 12 weeks, patients who received ESWT experienced improvement in pain relief, quality of life, and voiding symptoms. In the literature the patients experienced the maximum relief of their symptomatology after 4 weeks of treatment, according to our results patients have achieved an improvement even better at 12 weeks. The results obtained are similar to those reported in the bibliography. Several studies in orthopedics, urology and cardiology have shown very low rate of AEs derived from ESWT.

Conclusion: It has been demonstrated ESWT is an effective and safe treatment for CPPS. Due to high prevalence of CPPS and none specific treatment, ESWT should be considered an effective and safe treatment alternative.
INTRODUCTION
Chronic Prostatitis / Chronic Pelvic Pain Syndrome (CP/CPPS) is defined as chronic genitourinary pain or discomfort lasting at least 3 months according to the National Institute of Health (NIH). CPPS causes high morbidity and an important impact on patients’ quality of life (QoL). ESWT have achieved significant improvement of CPPS-related symptoms, particularly with regard to pain. The aim of our study was to study ESWT efficacy in 40 patients suffering from CPPS.

MATERIAL AND METHODS
Randomized, double-blind, placebo-controlled study has been conducted in 40 male patients diagnosed of CPPS. One month uro-drug washout period was required. Patients were randomly assigned to receive either ESWT or ESWT placebo using a perineal approach without anaesthesia. The equipment used was the Urogold 100 (MTS) op 155 soft-wade unfocused applicator. Treatment group received 1500 impulses, 0.14 ml/mm², 4 Hz. Control group received 1500 pulses, 0.01 ml/mm², 4 Hz with gel membrane on the insulation head. Both groups where treated once a week during 4 weeks. The primary endpoint was pain according to the visual analogue scale (VAS). Secondary endpoints were National Institutes of Health chronic prostatitis symptom (NIH-CPSI), International Prostate Symptom Score (IPSS), International Index of Erectile Function-5 (IIEF-5), treatment satisfaction in a Likert scale and Roles and Maudsley. Ultrasound, flowmetry and cultures were performed in all study periods. Follow-up was performed 4 and 12 weeks after ESWT.

RESULTS
From 40 CPSS patients completed outpatient treatment and follow-up, only 2 patients were lost during the follow up period so 38 patients were evaluated. Mean age was 41.9 years (25-65). ESWT Group showed statistically significant improvement of pain compared to the placebo group measured by NIH-CPSI pain (6 vs. 11). These beneficial results were maintained until 12th week (5.9 vs. 9.75). QoL measured by the NIH-CPSI improved in ESWT group compared to placebo group significantly at 4th weeks (3.35 vs. 5.81) (Table 1) and 12th weeks (3 vs. 5.69) (Table 2). ESWT patients did not show erectile dysfunction according to the IIEF-5 at any time. No significant adverse events were observed throughout the study.

<table>
<thead>
<tr>
<th>Questionnaires’s results at 4 weeks after the intervention</th>
<th>ESWT placebo</th>
<th>ESWT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSI-F</td>
<td>11 +/- 5.4</td>
<td>6 +/- 5.7</td>
<td>0.028</td>
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<tr>
<td>CPSI-U</td>
<td>4.88 +/- 3.48</td>
<td>3.29 +/- 2.68</td>
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<tr>
<td>CPSI-LF</td>
<td>5.81 +/- 3.62</td>
<td>3.35 +/- 2.12</td>
<td>0.035</td>
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<td>IPSS</td>
<td>10.3 +/- 9.7</td>
<td>6.3 +/- 6.6</td>
<td>0.18</td>
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<tr>
<td>IIEF-5</td>
<td>22.8 +/- 3.4</td>
<td>22.5 +/- 3.8</td>
<td>0.83</td>
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<tr>
<th>Questionnaires’s results at 12 weeks after the intervention</th>
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<td>CPSI-U</td>
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<td>2.41 +/- 2.42</td>
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<tr>
<td>CPSI-LF</td>
<td>5.69 +/- 3.64</td>
<td>3 +/- 2.45</td>
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<td>IPSS</td>
<td>9.44 +/- 3.8</td>
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<tr>
<td>IIEF-5</td>
<td>24.06 +/- 3.8</td>
<td>23 +/- 3.8</td>
<td>0.83</td>
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</tbody>
</table>

Fig 1. Treatment satisfaction (Likert Scale)

Fig 2. Roles and Maudsley Scale

CONCLUSIONS
• ESWT is a safe an effective treatment for patients suffering from CPSS.
• Further research is needed to confirm its effectiveness as first line treatment in CPSS and long-term effects.
The concept of SparkWave™ therapy-assisted penile tissue hyperplasia: A non-invasive alternative to augmentation phalloplasty

Zaman NM, Kharkiv National Medical University, Ukraine


The intention

Augmentation phalloplasty surgery, also known as “penis enhancement”, is therapeutically performed in patients with small penises, penile dysmorphic disorder (PDD) or microphallus (< 7.5 cm). There are multiple aetiologies, including congenital and endocrinological causes as well as pathological conditions, such as penile lichen sclerosus, trauma and genital cancer. The resulting reduction in functional penile length can lead to considerable psychosexual morbidity ¹. Therefore, phalloplasty is done in order to increase penile length and girth, but this procedure is nonstandard, still investigational and only few studies have been published so far. Due to the high risk of unwanted complications, namely infection, penile deformity, paradoxical penile shortening, disagreeable scarring, granuloma formation, migration of injected material, and sexual dysfunction, the use of cosmetic surgery to enlarge the penis remains highly controversial and should be avoided wherever possible ².

SparkWave™ therapy (SW™T) becomes established as a first line therapy for vasculogenic erectile dysfunction (ED), chronic pelvic pain (CPP) or Peyronie’s disease (PD) in several medical institutions and has proven successful in recent research studies ³⁴⁵. The angiogenic and regenerative effect of SW™T is based on the activation of stem cells and growth factors like eNOS, VEGF and PCNA, leading to tissue restoration and nerve generation ⁶⁷. Thus, SW™T represents an innovative, non-invasive treatment perspective to enlarge the penile tissue without serious impacts for the patients as in case of phalloplasty surgery. Furthermore, SW™T will not only be utilized as a real alternative to plastic surgery but will also improve the quality of erection by enhancing the blood perfusion and functionality of erectile tissue, thereby highly increasing patient satisfaction rates.
The mechanism

The mechanical expansion by the penile traction device and the hydropump stretches the penis and results in small microtears in the tissue. Cellular regeneration mechanisms are activated that will heal these small injuries via cell proliferation and lead to penile tissue hyperplasia. The duration and completion of the repair process is dependent on angiogenesis, cell recruitment and a supportive blood circulation in the affected tissue. SW™T of the expanded penis will induce vascularization and boost regeneration through induction of stem cell invasion and cell division, thereby ensuring and accelerating restoration and enlargement of the erectile tissue.

Angiogenesis and regeneration needs frequent vasodilation of the capillaries in the penile tissue in order to achieve an optimal blood supply. For this purpose, an excess of nitric oxide (NO) is provided which is produced solely from the proteinogenic amino acid L-arginine by the enzymatic action of the endothelial nitric oxide synthase (eNOS). As medical standard, L-citrulline supplementation is applied to ensure a constant bioavailability of L-arginine and NO which results in a lasting vasodilation in the regenerating tissue.

The concept

The combination of penile traction, adjunctive SW™T and L-citrulline supplementation causes angiogenesis, cell proliferation and stem cell activation in the penile tissue and leads to a permanent increase of penile length and girth without any damage to erectile function.

MD Mirza Niaz Zaman, a pioneer in the field of “therapeutic sexopathoandrology” and specialist for ED, premature ejaculation, low libido, sexual psychotherapy and non-invasive penile augmentation, conducted preliminary tests of this innovative therapeutic treatment option for patients suffering from PDD or micropenis. We provide the combinational treatment protocol to experts in the medical field and support further investigations together with the inventor MD Mirza Niaz Zaman, who conducted the research at the Kharkiv National Medical University (KNMU), Ukraine.
The perspectives

SW™T-assisted penile tissue hyperplasia can be considered as a new, non-invasive alternative for augmentation phalloplasty surgery and its potentiality, will be assessed by clinical trials in near future. Furthermore, SW™T can be utilized in order to increase the survival of autografts such as dermal fat grafts (DFG) and free fat grafts (FFG) which are widely used in penile augmentation surgery and will be analyzed in additional clinical studies as well. If you are interested this research project or want to manage or participate in a clinical trial please feel free to directly contact us for further discussion.

References

The concept of SparkWave™ therapy-assisted penile tissue hyperplasia: A non-invasive alternative to augmentation phalloplasty

Mirza Niaz Zaman MD (General Medicine, 5th course), Kharkiv National Medical University (KNMU), Ukraine

INTRODUCTION & AIM
SparkWave™ therapy (SW™T) is a special kind of shock wave therapy which is used as a first line therapy for vasculogenic erectile dysfunction (ED) in many developed countries. It works on the basis of angiogenesis and tissue regeneration.

Augmentation phalloplasty surgery, also known as penis enlargement surgery, is usually performed in patients with phallic dysmorphic disorder (PDD) and patients with microphallus. It is done to increase penile length and girth (circumference).

The aim of this research project is to assess the possibilities to induce penile tissue hyperplasia by SW™T and to establish this non-invasive procedure as an alternative for augmentation phalloplasty surgery.

MATERIALS & MECHANISM
Usage of penile traction device and penile vacuum pump causes the penile tissue to be stretched and expanded. As a result, microtears appear in the penile tissue. Due to body’s own repair mechanism, these microtears heal by the mean of cell proliferation which results in penile tissue hyperplasia. This same principle is used by some African tribes to enlarge lips and ears. The acceleration and completion of the repair process depends on several factors. One of these factors is angiogenesis which ensures the proper environment for cell proliferation by the means of improved hemodynamics.

SW™T induces angiogenesis in the local small blood vessels and the release of angiogenic growth factors such as VEGF, eNOS and PCNA. That is why SW™T can be utilized to ensure acceleration and completion of the repair process of penile tissue microtears through angiogenesis. Moreover SW™T induces migration of mesenchymal stem cells to the treated area. The "proliferation and differentiation" properties of the stem cells can be utilized to further accelerate and complete the repair process. These stem cells also induce angiogenesis because they produce cytokines and send signals to the surrounding, pre-existing cells and initiate proliferation, differentiation and the release of growth factors.

For continuation of angiogenesis, frequent vasodilatation is required which can be ensured by increased production of NO. NO is produced from the amino acid L-arginine via the enzymatic action of the endothelial nitric oxide synthase (eNOS). Using L-citrulline supplementation ensures a constant bioavailability of L-arginine thus frequent vasodilation occurs due to increased NO production which is essential for improved hemodynamics and supports the process of angiogenesis.

RESULTS & CONCLUSION
As a result of the combination of angiogenesis (inducing vascularization and improving hemodynamics) and cell proliferation (pre-existing cells and stem cells), penile tissue hyperplasia occurs which results in increased penile length and girth (circumference) permanently without any damage to erectile function. SW™T-assisted penile tissue hyperplasia can be considered as a non invasive alternative for augmentation phalloplasty surgery. To assess its potentiality, clinical trials are required.

REFERENCES

The authors declare that they have no conflict of interests.
Effects of Low-Energy Shockwave Therapy on the Erectile Function and Tissue of a Diabetic Rat Model

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¹Knuppe Molecular Urology Laboratory, Department of Urology, School of Medicine, University of California, San Francisco, CA 94143-0738, USA
²Andrology Center, Peking University First Hospital, Beijing, China

Abstract

Introduction—Low-energy shockwave therapy (LESWT) has been shown to improve erectile function in patients suffering from diabetes mellitus (DM)-associated erectile dysfunction (ED). However, the underlying mechanism remains unknown.

Aim—To investigate whether LESWT can ameliorate DM-associated ED in a rat model, and examine the associated changes in the erectile tissues.

Methods—Newborn male rats were intraperitoneally injected with 5-ethynyl-2-deoxyuridine (EdU, 50mg/kg) for the purpose of tracking endogenous mesenchymal stem cells (MSCs). Eight weeks later, 8 of these rats were randomly chosen to serve as normal control (N group). The remaining rats were injected intraperitoneally with 60 mg/kg of streptozotocin (STZ) to induce DM. Eight of these rats were randomly chosen to serve as DM control (DM group) while another 8 rats were subject to shockwave treatment (DM+SW group). Each rat in the DM+SE group received 300 shocks at energy level of 0.1mJ/mm² and frequency of 120/min. This procedure was repeated three times a week for two weeks. Another two weeks later, all 24 rats were evaluated for erectile function by intracavernous pressure (ICP) measurement. Afterward, their penile tissues were examined by histology.

Main Outcome Measures—Erectile function was measurement by ICP. Neuronal nitric oxide synthase (nNOS)-positive nerves and the endothelium were examined by immunofluorescence (IF) staining. Smooth muscle and MSCs were examined by phalloidin and EdU staining, respectively.

Results—STZ treatment caused a significant decrease in erectile function and in the number of nNOS-positive nerves and in endothelial and smooth muscle contents. These DM-associated deficits were all partially but significantly reversed by LESWT. MSCs (EdU+ cells) were significantly more numerous in DM+SW than in DM rats.

Conclusions—LESWT can partially ameliorate DM-associated ED by promoting regeneration of nNOS-positive nerves, endothelium, and smooth muscle in the penis. These beneficial effects appear to be mediated by recruitment of endogenous MSCs.

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Conflict of Interest: None
Keywords
Low-energy shockwave; Diabetes mellitus; Erectile dysfunction

Introduction
Erectile dysfunction (ED) is a prevailing health problem that seriously impacts the quality of life of men and their spouses or partners [1]. While the majority of ED patients can be satisfactorily treated with PDE5 inhibitors, a substantial population (30-40%) cannot [2]. This includes patients who are intolerant to PDE5 inhibitors’ side effects, taking nitrate medication for angina, or having certain types of ED refractory to PDE5 inhibitors. In particular, diabetes mellitus (DM) and surgery-induced cavernous nerve injuries (mainly due to radical prostatectomy) are currently the most common causes of refractory ED [2]. To treat these types of ED, one of the proposed strategies is low-energy shockwave therapy (LESWT), as seen in recently published studies [3-5] and ongoing clinical trials (NCT01317693, NCT01274923, NCT01442077, and NCT01317680 at http://clinicaltrials.gov). In one study involving 29 severe ED patients, LESWT was found to substantially increase erectile function scores with concomitant improvement of penile hemodynamics [3]. Noteworthy is that the majority of these patients (21 out of 29) were diabetic, and thus their positive response to LESWT raises the question whether LESWT is specifically effective for treating DM-associated ED. While the answer awaits further clinical studies, it should be pointed out that, despite successful demonstration in clinical trials, LESWT as an ED treatment modality has not been investigated at the basic science level and has no known mechanistic basis.

LESWT has been investigated in animal models of heart failure [6], coronary arterial disease [7], ischemic myocardial dysfunction [8], ischemic tissue necrosis [9], chronic hind limb ischemia [10], and bone defects [11]. The outcomes invariably point to induction of angiogenesis as one of the underlying mechanisms for LESWT’s therapeutic effects. In addition, one of these studies also identified recruitment of mesenchymal stem cells (MSCs) as a possible mechanism [11]. In ED field, angiogenesis is known to play important roles for the therapeutic effects of growth factors and gene therapies [12-14]. And both exogenously applied and endogenously recruited MSCs have been shown to enhance recovery of erectile function in ED animal models [15, 16]. Thus, the observed therapeutic effects of LESWT in ED patients are likely mediated by angiogenesis and MSC recruitment. In the present study we tested this hypothesis; specifically, we investigated the effects of LESWT on erectile function and related tissue structures in a streptozotocin (STZ)-induced DM rat model. We also examined whether LESWT enhanced MSC recruitment by using the label-retaining cell (LRC) strategy [17] with EdU being the thymidine analog for such labeling [18].

Materials and methods
Animals
All animal experiments in this study were approved by the Institutional Animal Care and Use Committee at our institution. Pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) for the investigation of childbirth-related urinary incontinence in separate projects. Their newborn male rats were used for this study. For the purpose of tracking endogenous MSCs, each pup received intraperitoneal injection of EdU (50 mg/kg, Invitrogen, Carlsbad, CA) immediately after birth, as described previously [18-20]. At 8 weeks of age, 8 of these rats were randomly selected to serve as normal control (N). The remaining rats were each injected intraperitoneally with 60 mg/kg of STZ (Sigma-Aldrich, St. Louis, MO), and their blood glucose level was monitored.
weekly by checking tail vein blood with Accutrend strip (Roche Diagnostics, Indianapolis, IN). Rats with fasting blood glucose of ≥200 mg/dl were designated as diabetic and selected for further tests. A total of 16 such rats were equally randomized into a diabetic group (DM) and a diabetic plus LESWT group (DM+SW).

**Shockwave treatment**

Four weeks post-STZ injection, rats in the DM+SW group were treated with shockwaves as depicted in Fig. 1 and explained in the following. Under anesthesia, each rat was placed in a supine position, its lower abdomen shaved, and its penis drawn out of the prepuce and held in place with a loop made of suture line and syringe. After application of ultrasound gel (Aquasonic; Parker Laboratories, Inc, Fairfield, NJ) on the penis, a shockwave applicator (DermaGold, MTS Europe GmbH, Konstanz, Germany) was placed in contact with the penis, and a total of 300 shocks were delivered at energy level of 0.1mJ/mm² and frequency of 120/min. This procedure was repeated three times a week for two weeks, and the entire treatment course is comparable to clinical shockwave treatment for ED patients [3-5]. Due to the fact that DermaGold is clinically approved to treat superficial wounds, its delivered shockwave is expected to penetrate a few centimeters (probably the thickness of a rat penis) in the contacted area.

**Erectile function evaluation**

Two weeks after the final shockwave treatment for rats in the DM+SW group, all 24 rats (in N, DM, and DM+SW groups) were tested for erectile function by measuring intracavernous pressure (ICP) in response to electrostimulation of cavernous nerves. Briefly, under ketamine (100mg/kg) and midazolam (5mg/kg) anesthesia, the corpus cavernosum was cannulated with a heparinized (200U/ml) 25G needle connected to a pressure transducer (Utah Medical Products, Midvale, UT). The stimulus parameters were 20Hz, pulse width of 0.2 ms, and duration of 50s with three different current settings: 0.5 mA, 1.0 mA and 1.5 mA. The maximum increase of ICP of three stimuli per side was selected for statistical analysis in each animal. ICP was normalized to mean arterial pressure (MAP), which was recorded using a 25G needle inserted into the aortic bifurcation.

**Histology**

At the conclusion of erectile function evaluation, the rats were sacrificed and their penis harvested for histology. The penis (mid-shaft portion) was fixed with 2% formaldehyde and 0.002% picric acid in 0.1M PBS for 4 hours, followed by immersion in 30% sucrose in PBS overnight at 4°C. The fixed tissue was then embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA), cut into 5 µm-thick sections, mounted on glass slides (3 sections per slide), and subjected to immunofluorescent (IF) and EdU staining. For IF staining, the slides were placed in 0.3% H2O2/methanol for 10 min, washed twice in PBS for 5 min and incubated with 3% horse serum in PBS/0.3% Triton X-100 for 30 min at room temperature. After draining this solution from the tissue section, the slides were incubated at room temperature with rabbit anti-nNOS (1:100, SC-648, Santa Cruz Biotechnology, Santa Cruz, CA) or mouse anti-rat endothelial cell antigen (RECA; 1:500; MCA-970R, AbD Serotec, Raleigh, NC) antibody overnight. Control tissue sections were similarly prepared except no primary antibody was added. After rinses with PBS, the sections were incubated with Alexa-488- or Alexa-594-conjugated secondary antibody (Invitrogen). Smooth muscle was stained by incubation with Alexa-488-conjugated phalloidin (Invitrogen) for 20 min at room temperature. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Invitrogen). For tracking EdU-positive cells, tissue sections were incubated with Click-IT reaction cocktail (Invitrogen) for 30 min at room temperature.
**Image analysis and quantification**

The stained tissues were examined with Nikon Eclipse E600 fluorescence microscope and photographed with Retiga 1300 Q-imaging camera using the ACT-1 software (Nikon Instruments, Melville, NY). For evaluation of cavernous smooth muscle and endothelial contents, two fields (both sides of the corpus cavernosum at 200× magnification) on each tissue section were photographed. For evaluation of arterial endothelial content, two arteries within the corpus cavernosum at 1000× magnification on each tissue section were photographed. For evaluation of dorsal nerve nNOS expression, two fields (the two largest dorsal nerve branches at 400× magnification) on each tissue section were photographed. For evaluation of nNOS expression around dorsal arteries, the two dorsal arteries on each tissue section at 400× were photographed. For evaluation of nNOS expression in the corpus cavernosum, two fields (both sides of the corpus cavernosum at 400× magnification) on each tissue section were photographed. In each of these photographic recordings the images were generated in green, red, and blue channels, and these single-color images were then superimposed to generate the multi-color figures. For quantification, the single-color images were analyzed with Image-Plus 5.1 software (Media Cybernetics, Bethesda, MD). To quantify cavernous endothelium, RECA-stained area (in red) was measured and expressed as pixel number. To quantify arterial endothelium, RECA-stained area (in red) was measured and expressed as a ratio (in percentage) to the phalloidin-stained area. To quantify nNOS expression, nNOS-stained dots (in red) were manually counted. To quantify cavernous smooth muscle, phalloidin-stained area (in green) was measured and expressed as a percentage of the entire corpus cavernosa.

**Statistical analysis**

Data were analyzed using Prism 5 (GraphPad Software, San Diego, CA) and expressed as mean ± standard error of mean (SEM). Multiple groups were compared using one-way analysis of variance followed by the Tukey-Kramer test for post-hoc comparisons. Statistical significance was set at p < 0.05.

**Results**

**LESWT improves erectile function in diabetic rats**

STZ treatment significantly impaired erectile function as seen in the sharp decline of the ICP/MAP value in DM rats versus normal control (Fig. 2). LESWT significantly restored erectile function to levels similar to normal control (at settings of 1.0 and 1.5 mA, Fig. 2).

**LESWT promotes nerve regeneration**

STZ treatment caused significant decreases of nNOS-containing nerves in the penis (Fig. 3). LESWT partially but significantly restored these nNOS-positive nerves in the sinusoids, around the dorsal arteries, and within the dorsal nerves (Fig. 3).

**LESWT restores endothelial and smooth muscle contents**

STZ treatment caused a significant decrease of endothelial content in both the cavernous sinusoids and small arteries, and which was partially but significantly reversed by LESWT (Fig. 4). Likewise, STZ treatment caused a significant reduction of cavernous smooth muscle content, and which was partially but significantly reversed by LESWT (Fig. 5).

**LESWT enhances recruitment of MSCs**

MSCs, recognized by their ability to retain thymidine analog EdU, were significantly more numerous in the penis of rats in the DM+SW group than in the DM group (Fig. 6).
Discussion

Despite tremendous advances in the management of ED in the past decade, DM-associated ED remains difficult to treat. To overcome this obstacle, one of the proposed therapeutic strategies is stem cell therapy, which has been actively pursued in several clinical and preclinical trials [16]. Another lesser-known strategy is LESWT, which has been tested in clinical trials, but in sharp contrast to stem cell therapy, has not been investigated at the preclinical level. Thus, the present study was designed to provide, for the first time, a mechanistic basis for LESWT’s therapeutic effects by using a well-established STZ-induced DM-ED rat model.

STZ-induced diabetic rats have been consistently shown to have poor erectile function [21, 22]. In the present study we further confirmed this observation, and more importantly, we showed that LESWT significantly improved erectile function in STZ-induced diabetic rats. It has also been known that STZ treatment caused a significant loss of nNOS-positive nerves in rat penis [22, 23], and a recent study also identified a significant reduction of nNOS-positive nerves in the penis of diabetic patients [24]. In the present study we showed that, when compared to DM rats, shockwave-treated rats displayed significantly higher numbers of nNOS-positive nerves in different compartments of the erectile tissue, including the dorsal nerves, around the dorsal arteries, and in the corpora cavernosa. This preservation of nNOS-positive nerves thus appears to be an underlying mechanism for LESWT’s therapeutic effects on diabetic patients.

Endothelial injury and dysfunction in cavernous tissue have been consistently identified in diabetic men with ED and in diabetic animal models [25]. Specifically, a reduced cavernous endothelial content is one the most consistent features of STZ-induced diabetic rats [22, 26]. In the present study we found that the endothelial contents in both the cavernous sinusoids and arteries were significantly reduced in STZ-treated rats. More importantly, we also found that LESWT was able to significantly restore the endothelial contents in both of these two tissue compartments. Thus, protection or regeneration of the endothelium represents another possible underlying mechanism for LESWT’s therapeutic efficacy in diabetic patients. In addition, it has also been shown that diabetic men and animals have reduced cavernous smooth muscle content [22, 26, 27]. In the present study we confirmed this finding in the STZ-treated rats, and more importantly, we showed that LESWT was able to significantly restore the smooth muscle content.

In all ED-related stem cell studies that have performed histological examination of the erectile tissue, restoration of nNOS-positive nerves, the endothelium, and the smooth muscle has also been consistently observed [16]. In addition, these studies also invariably pointed out that the beneficial tissue effects were likely mediated by stem cell’s paracrine capacity [16]. On the other hand, in non-ED fields, the involvement of stem cells in the therapeutic effects of LESWT has been observed in two instances. In one study of a rat model of bone defects, LESWT was found to result in the recruitment of MSCs and increased expression of TGF-β and VEGF in the defect tissues [11]. In another study of a rat model of chronic hind limb ischemia, LESWT was also found to enhance recruitment of endothelial progenitor cells in the ischemic tissue [10]. Thus, it is conceivable that the tissue effects of LESWT as observed in the present study might have a stem cell component.

LRC is a frequently employed strategy for the identification of resident or migrated stem cells in post-natal tissues [18-20]. It commonly involves the injection of thymidine analog BrdU in neonatal animals, followed by the immunohistochemical localization of BrdU in the tissue of interest weeks or months later. Because detection of BrdU is technically difficult, we recently introduced another thymidine analog EdU as a replacement for BrdU [18-20]. In
the present study we repeated this strategy for the identification of migrated stem cells in the rat penis. The results show that diabetic rats with LESWT had a significantly higher number of EdU+ cells in the penis than diabetic rats without LESWT, suggesting an increased recruitment of MSCs.

In summary, the present study showed that STZ-induced DM is associated with ED and reduced erectile components (nerves, endothelium, and smooth muscle), and LESWT was able to partially but significantly restore these function and tissues. Furthermore, we also showed that these beneficial effects of LESWT were possibly mediated by increased recruitment of MSCs into the erectile tissue. However, it should be cautioned that the present study is still preliminary and requires further validation. Specifically, at 4 weeks after STZ treatment, the nitricergic degeneration has not yet taken place [23], but in clinical situations most patients already have nitricergic degeneration and hence no response to PDE5 inhibitors. Thus, in future studies extended time points should be explored in order to better simulate the clinical situation. In addition, the effect of LESWT on the vascular supply to the penis and major pelvic ganglion should be further investigated; and Western blot analysis should be employed to more accurately quantify the nNOS, endothelial and smooth muscle contents. Finally, the identity and properties of the EdU+ cells (paracrine? differentiation?) also need to be investigated.

Conclusions

LESWT’s therapeutic efficacy for DM-associated ED is possibly mediated by increased recruitment of MSCs that promote the regeneration of DM-damaged erectile tissues.

Acknowledgments

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References


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Figure 1. Shockwave application to the rat penis
Under anesthesia, the penis was drawn out of the prepuce, held in place with a loop made of suture line and syringe (shown in inset), applied with ultrasound gel, and shockwave-treated.
Figure 2. Evaluation of erectile function
Rats in N group (n=8) were normal control. Rats in DM group (n=8) were diabetic. Rats in DM+SW group (n=8) were diabetic and treated with shockwaves. Their erectile function was evaluated as response in ICP to electrostimulation of cavernous nerves at three different amperages (0.5, 1.0, and 1.5). ICP was normalized with MAP. * denotes p<0.05 when compared to the DM group.
Figure 3. Evaluation of nNOS expression
Rats were grouped and treated as described in Fig. 1. Their penile tissues were examined by IF staining for nNOS expression. The results are shown in the representative histological images with red, green, and blue stains indicating nNOS-positive nerves, smooth muscle, and cell nuclei, respectively. For clarity, the histological images are divided into the dorsal nerves (panels A-C), the dorsal arteries (panels D-F), and the sinusoids (panels G-I). White arrows point at representative nNOS-positive dots. Quantitative data of nNOS expression in these three tissue compartments are shown in the bar chart with the asterisk denoting p<0.05 when compared to the DM group.
Figure 4. Evaluation of endothelial content
Rats were grouped and treated as described in Fig. 1. Their penile tissues were examined by IF staining for RECA expression. The results are shown in the representative histological images with red, green, and blue stains indicating the endothelium, smooth muscle, and cell nuclei, respectively. Quantitative data of RECA expression in cavernous sinusoids and arteries are shown in the left and right bar charts, respectively, with the asterisk denoting p<0.05 when compared to the DM group.
Figure 5. Evaluation of smooth muscle content
Rats were grouped and treated as described in Fig. 1. Their penile tissues were examined by fluorescent phalloidin staining for smooth muscle. The results are shown in the representative histological images with green and blue stains indicating the smooth muscle and cell nuclei, respectively. Quantitative data of cavernous smooth muscle content are shown in the bar chart with the asterisk denoting \( p<0.05 \) when compared to the DM group.
Figure 6. Evaluation of label-retaining cells
Rats were intraperitoneally injected with thymidine analog EdU immediately after birth. They were then grouped and treated as described in Fig. 1. At 14 weeks post-EdU injection, their penile tissues were examined by fluorescent chemical staining for EdU+ cells. The results are shown in the representative histological images with red, green, and blue stains indicating EdU+ cells, smooth muscle, and cell nuclei, respectively. Quantitative data of EdU+ cells are shown in the bar chart with the asterisk denoting p<0.05 when compared to the DM group. HPF: high power field (400x).
Low-energy Shock Wave Therapy Ameliorates Erectile Dysfunction in a Pelvic Neurovascular Injuries Rat Model

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ABSTRACT

Introduction: Erectile dysfunction (ED) caused by pelvic injuries is a common complication of civil and battlefield trauma with multiple neurovascular factors involved, and no effective therapeutic approach is available.

Aims: To test the effect and mechanisms of low-energy shock wave (LESW) therapy in a rat ED model induced by pelvic neurovascular injuries.

Methods: Thirty-two male Sprague-Dawley rats injected with 5-ethynyl-2'-deoxyuridine (EdU) at newborn were divided into 4 groups: sham surgery (Sham), pelvic neurovascular injury by bilateral cavernous nerve injury and internal pudendal bundle injury (PVNI), PVNI treated with LESW at low energy (Low), and PVNI treated with LESW at high energy (High). After LESW treatment, rats underwent erectile function measurement and the tissues were harvested for histologic and molecular study. To examine the effect of LESW on Schwann cells, in vitro studies were conducted.

Main Outcome Measurements: The intracavernous pressure (ICP) measurement, histological examination, and Western blot (WB) were conducted. Cell cycle, Schwann cell activation-related markers were examined in in vitro experiments.

Results: LESW treatment improves erectile function in a rat model of pelvic neurovascular injury by leading to angiogenesis, tissue restoration, and nerve generation with more endogenous EdU+ progenitor cells recruited to the damaged area and activation of Schwann cells. LESW facilitates more complete re-innervation of penile tissue with regeneration of neuronal nitric oxide synthase (nNOS)-positive nerves from the MPG to the penis. In vitro experiments demonstrated that LESW has a direct effect on Schwann cell proliferation. Schwann cell activation-related markers including p-Erk1/2 and p75 were upregulated after LESW treatment.

Conclusion: LESW-induced endogenous progenitor cell recruitment and Schwann cell activation coincides with angiogenesis, tissue, and nerve generation in a rat model of pelvic neurovascular injuries.

INTRODUCTION

Trauma-related erectile dysfunction (ED) commonly occurs in the setting of pelvic surgery or as a result of local injuries such as improvised explosive device in battlefield, and is most often associated with the damage of cavernous nerves (CN) and/or internal pudendal bundle (IPB).1,2 After injury, ischemia and neural degeneration lead to both impaired erectile capability and its lack of response to therapy.3 Current treatments include oral phosphodiesterase V inhibitors, vacuum erection devices, penile injection, transurethral therapy, and penile prosthesis, but none of these can restore normal erectile physiology.1 In addition, we lack a good animal model to study neurovascular ED. Consequently, both basic and translational researchers are continuing to search for effective strategies.5
Low-energy shockwaves (LESW) have been used for years to treat musculoskeletal disorders.\(^6\) Recently, the application of this therapy has been expanded to address ischemic heart disease\(^7\) and vasculogenic ED,\(^8\) but there are few reports concerning the effects shock waves have on nerve fibers or neurovascular ED. In 2001, Ohtori et al reported LESH stimulated reinnervation of sensory fibers,\(^9\) and in 2006 another Japanese group found that shock waves induce the expression of growth-associated protein-43 (GAP-43, a marker for axonal growth cones) in rat dorsal root ganglia (DRG).\(^10\) Shock waves have also been reported to induce DRG cells to express activating transcription factor 3 (ATF3), which promotes neurite outgrowth from the ganglion when the peripheral axon is injured.\(^10\) Also, we have reported that LESH improves diabetic ED in an animal model by promoting nerve regeneration,\(^11\) a finding confirmed by another group.\(^12\) Clinically, LESH therapy also has been proven to be a potential treatment for angiogenesis and penile rehabilitation.\(^13,14\)

Recovery of neurovascular ED is a tough task involving the vascular system and the peripheral nervous system, whereas regeneration of peripheral nerves after pelvic injury is a complex process related to neurons, Schwann cells, basal lamina, and responsiveness of end organs. Among the orchestration of these various cells, Schwann cells are often the “first responders” in this microenvironment\(^15\) and play an important guiding role,\(^16\) which could be promoted by mechanical force.\(^17\) Schwann cells play an important role in axon regeneration after injury, including CN injury that leads to ED.\(^18\) In the penile nerve system, Schwann cells have been found to be functional in Remak bundles/C fibers (mainly composed in the cavernous nerve) and A-δ fibers (mainly composed in the internal pudendal nerve).\(^15,19\) However, the effects of Schwann cells during the penile nerve regeneration have not been well elucidated though indirect evidence claims that treatments aiming to promote the growth of Schwann cells result in better erectile function recovery.\(^20,21\)

In the current study, we developed a new ED rat model of pelvic neurovascular injury (PVNI) by bilateral cavernous nerve injury and internal pudendal bundle injury, and tested the effect of LESH treatment at different energy levels. We hypothesized that LESH might improve function, angiogenesis, and innervations by activating local Schwann cells and increasing progenitor cell recruitment.

**MATERIALS AND METHODS**

**Experimental Design**

All procedures were approved by the Institutional Animal Care and Use Committee of University of California, San Francisco. A total 32 newborn male Sprague-Dawley rats were used for this study. Each pup received an intraperitoneal injection of 5-ethyl-2′-deoxyuridine (EdU, 50 mg/kg, Invitrogen, Carlsbad, CA, USA) as previously reported.\(^22\) At 12 weeks old, they were grouped into 4 (n = 8 each): sham surgery (Sham), pelvic neurovascular injury by bilateral cavernous nerve injury and internal pudendal bundle injury (PVNI), PVNI treated with LESH at low energy (Low), and PVNI treated with LESH at high energy (High). After 4 weeks of LESH treatment and 1 week of washout, all rats underwent erectile function measurement. The rats were then sacrificed and the penis (half for histology and half for Western blot), major pelvic ganglion (MPG), and urethra were harvested for histology and Western blot.

In vitro studies were conducted using primary tissue culture of rat Schwann cells. Four rats (5 weeks old) were sacrificed and the sciatic nerves were harvested for isolation of Schwann cells as previously reported.\(^23\)

**Develop Pelvic Neurovascular Injury Rat Model**

Bilateral cavernous nerve injury (CNI) was performed as previously described,\(^24\) whereas the IPB injury (IPBI) was conducted as follows: the rat was positioned into lithotomy and a horizontal perineal incision was made. The IPB was identified between the ischiocavernous muscle (ICM) and the bulbospongious muscle (BCM). Suture ligation was performed bilaterally. The sham surgery was performed exactly as the described procedure, except that no CNI or IPBI was induced.

**Primary Culture of Rat Schwann Cells**

Purified Schwann cells culture was created using methods described by Shen et al.\(^23\)

**Low-energy Shockwave Treatment**

For the in vivo experiment, LESH therapy was started 48 hours postoperatively. Shockwave was delivered to the pelvic region with a special probe that was attached to a compact electrohydraulic unit with a focused shockwave source (DermaGold, MTS Europe GmbH, Konstanz, Germany). Under anesthesia, each rat was placed in the supine position with its lower abdomen shaved and the preputial skin reduced. Standard commercial ultrasound gel (Aquisonic, Parker Laboratories Inc, Fairfield, NJ, USA) was applied between the probe and the skin of pelvic region for optimal coupling. In the low-energy group, 0.06 mJ/mm\(^2\), 300 pulses at 3 Hz was applied, while 0.09 mJ/mm\(^2\), 1000 pulses at 3 Hz was applied in the high-energy group.

For the in vitro experiment, cell cultures were used for LESH treatment. Schwann cells received LESH treatment (0.02 mJ/mm\(^2\), 200 pulses at 3 Hz) after reaching 70% confluence. The probe was handled under the cell culture dish with standard commercial ultrasound gel applied between dish and probe. The cells were treated once and then harvested or checked at corresponding time points.

**Erectile Function Evaluation**

An intracavernous pressure (ICP) test was used to evaluate erectile function as previously described.\(^24\) In brief, under
formed according to a previously described protocol. In brief, mock treatment, the cells were plated. Eight hours (for p-Erk1/2) after LESW treatment or Schwann cells were cultured on cover slips placed in 6-well plates. Eight hours at 4°C for 15 minutes. Then the cover lips were proceeded for IF staining.

The tissue sections were incubated with primary antibodies overnight at 4°C. The antibodies used in histology are listed in Table 1. The secondary antibodies used were Alexa-488 and Alexa-594 conjugated antibodies (1:500, Invitrogen, Carlsbad, CA, USA) and the incubation time for secondary antibody is 2 hours at room temperature. Smooth muscle actin (SMA) was stained by Alexa-488—conjugated phalloidin (1:400, Invitrogen) and EdU+ cells were stained with Click-IT reaction cocktail (Click-IT, Invitrogen) as manual respectively. Nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI, Invitrogen).

The stained slides were examined with a fluorescence microscope (Nikon, Eclipse, 80i). Image analysis was performed by calculating the computerized densitometry or number of positive targets using Image-Pro Plus 5.1(Media Cybernetics, Silver Spring, MD, USA). The following variables were analyzed by number calculating: number of blood vessels and nerve bundles in the dorsal section of the penis, number of EdU+ positive cells in the cavernosal section of penis, and number of nuclei for Schwann cells. The amount of von Willebrand factor (vWF), neuronal nitric oxide synthase (nNOS), S100, neurofilament (NF), and SDF-1 were analyzed with Image-Plus 5.1 software (Media Cybernetics, Bethesda, MD, USA) based on the integrated optical density of the positively stained area in high-power fields among 4 groups. All the data were calculated in a blinded fashion.

The average number of blood vessels (phalloidin stained green rings) and nerve bundles (the collections of NF stained red dots) within penile dorsal area were counted and calculated (n = 8 for each group).

**Western Blot**

Protein isolation and Western blot were conducted as previously reported and a total of 20 μg protein were loaded for each sample. The antibodies used in Western blot are listed in Table 1. After the secondary antibody incubation, the resulting images were analyzed with ChemilImage 4000 (Alpha Innotech Corp, San Leandro, CA, USA) to determine the integrated density value of each protein band.

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**Table 1. Antibodies Used in Immunofluorescence Staining (IF) and Western Blot (WB)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbrev</th>
<th>Dilution</th>
<th>Product information</th>
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<td>anti-von Willebrand factor</td>
<td>vWF</td>
<td>1:400</td>
<td>ab6994, abcam, Cambridge, MA, USA</td>
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<tr>
<td>anti-neurofilament</td>
<td>NF</td>
<td>1:400</td>
<td>MAB5262, Merk Millipore, Billerica, MA, USA</td>
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<td>anti-neuronal nitric oxide synthase</td>
<td>nNOS</td>
<td>1:200</td>
<td>SC-648, Santa Cruz Bio-technology, Santa Cruz, CA, USA</td>
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<tr>
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<td>S100</td>
<td>1:200</td>
<td>SC-47778, Santa Cruz Bio-technology, Santa Cruz, CA, USA</td>
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<tr>
<td>anti-p-Erk1/2</td>
<td>p-Erk1/2</td>
<td>1:500</td>
<td>91001, Cell Signaling Technology, Framingham, MA, USA</td>
</tr>
<tr>
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<td>β-actin</td>
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<tr>
<td>anti-neuronal nitric oxide synthase</td>
<td>SDF-1</td>
<td>1:500</td>
<td>SC-648, Santa Cruz Bio-technology, Santa Cruz, CA, USA</td>
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<tr>
<td>anti-p75</td>
<td>p75</td>
<td>1:200</td>
<td>SC-648, Santa Cruz Bio-technology, Santa Cruz, CA, USA</td>
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<td>p-Erk1/2</td>
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<td>1:200</td>
<td>91001, Cell Signaling Technology, Framingham, MA, USA</td>
</tr>
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ketamine (100 mg/kg) and midazolam (5 mg/kg) anesthesia, the MPG and CN were exposed via midline laparotomy. The corpus cavernosum was cannulated with a heparinized (200 U/mL) 25-gauge needle and connected to a pressure transducer (Utah Medical Products, Midvale, UT, USA). The stimulus parameters were 1.5 mA, 20 Hz, pulse width of 0.2 ms, and duration of 50 seconds. The maximum increase of the ICP curve of 3 stimuli per side was selected for statistical analysis in each animal. Mean arterial blood pressure (MAP) was recorded using a 25-G needle inserted into the aortic bifurcation after the ICP test. ICP/MAP was calculated as the ratio of maximum change of ICP to MAP. Area under the receiver operating characteristic curve (AUC) was recorded as manually.
Cell Cycle Assay
For cell cycle analysis, 10^4 cells were collected for each assay. Cell cycle synchronization was induced by serum starvation. The cells then underwent LESW or mock treatment. The cells were stained with the propidium iodide flow cytometry kit (ab139418, Abcam, Cambridge, MA, USA) according to the manual. The cell cycle was checked at time point 0 hours and 8, 16, and 24 hours after the LESW treatment using flow cytometry (BD, in flux, Cell Sorter, Franklin Lakes, NJ, USA) and the results were analyzed with FlowJo (Tree Star, Inc, Ashland, OR, USA).

Statistical Analysis
Results were analyzed using Prism 5 (GraphPad Software, San Diego, CA, USA) and expressed as mean ± standard deviation of the mean (SEM). Multiple groups were compared using t test (2 variables) or 1-way analysis of variance of variance followed by the Tukey-Kramer test for post-hoc comparisons (4 variables). Statistical significance was set at P < .05.

RESULTS
Low-energy Shockwave Treatment Improves Erectile Function
PVNI-impaired erectile function compared with sham procedure, whereas significant improvement was evident after LESW treatment (Figure 1A). To evaluate recovery of erectile function, we analyzed the ratio of maximum change of ICP to MAP (ICP/MAP) and AUC of the ICP results. Both treated groups showed a significant increase in ICP/MAP compared with control group (0.56 ± 0.10 and 0.82 ± 0.08 vs 0.17 ± 0.03, P < .05). Rats in the higher LESW group showed significant recovery (P < .05) compared with the control group with larger AUC (23.83 ± 2.42 vs 8.13 ± 0.55). Overall, these measures suggest partial recovery of erectile function in both treated groups, with rats in the high group demonstrating better recovery relative to the low group (Figure 1B).

LESW Treatment Enhances Penile Angiogenesis and Regains Blood Circulation in PVNI Group of Rats
To determine if the improvement in erectile function correlated to changes in tissue vascularization in the dorsal sections we counted the number of blood vessels. Post the PVNI, the penile dorsal artery collapsed and endothelium were significantly atrophied (Figures 2A and 2B). Impressively, collapsed penile dorsal arteries regain a normal-looking structure with many small blood vessels around it (Figure 2A). Also, LESW promoted the expression of vWF in the penile tissue in both the lower- and higher-energy groups (Figures 2B and 2D). In line with improvements in erectile function, the higher LESW treated group had improved outcome of tissue regeneration. These results...
indicate that while at 5 weeks after pelvic injury, the control rats could regenerate the number of blood vessels to a nearly normal level, but the erectile function was not restored in these animals. Therefore, after pelvic injury LESW treatment stimulates increased generation of blood vessel that is strongly correlated with improved function. These studies indicate that LESW is beneficial in cavernous tissue rehabilitation.

**LESW Promotes Penile Nerve Regeneration**

Within the penis, recovery of the dorsal nerve was examined with IF staining of NF. The number of nerve bundles was significantly improved in LESW-treated groups (Figures 2A and 2C). The majority of nNOS$^+$ nerve fibers originate from DCR-MPG. They form Remak bundles and join other nerve fibers to become the cavernous nerves. To tracing the regeneration process of nNOS$^+$ Remak nerve fibers, 4 levels of tissue sections were performed: at the MPG, the cavernous nerve at distal site from nerve crush along urethra (CN); dorsal penile nerve (DPN); and the penile sinusoid (Figure 3A).

Nerve injury significantly decreased nNOS$^+$ nerve fibers at all 3 nerve levels (CN, DPN, and sinusoid) except in the MPG (Figures 3A and 3B). After LESW treatment, the numbers of nNOS containing fibers increased (Figure 3B). Increased nNOS expression in the treatment groups was confirmed with Western blot using the protein lysates from penile tissue (Figures 3C and 3D). High-energy level LESW treatment might lead to enhanced regeneration of nNOS$^+$ nerve fibers compared with low energy levels of LESW; however, these differences were not significant between the 2 groups.

Figure 2. General neurovascular changes in penile tissue. A. Representative images of immunofluorescence staining for vascular smooth muscle (Pha- Alexa 488 conjugated phalloidin-green) and dorsal nerve (NF-red, original magnification is ×4) in 4 groups. Dotted lines surround the dorsal parts. Typical blood vessels were indicated with white arrows and typical nerve bundles were indicated with white triangles. B. Representative images of immunofluorescence staining for endothelium (vWF-red, original magnification is ×400) in sinusoid. C. Average number (± SEM) of nerve bundles (NF dots collections) and blood vessels (smooth muscle rings) in the dorsal neurovascular area of each sample, *P < .05 compared with control group, #P < .05 compared with sham group; D. Average IOD amount (± SEM) of vWF staining in sinusoid, *P < .05. NF = neurofilament; wWF = von Willebrand factor.
LESW Treatment Enhances Recruitment of EdU Positive Cells

Because progenitor cells or cells with stem properties are recognized by their ability to retain thymidine analog EdU for an extended period of time, we examined recruitment of EdU\(^+\) cells in animals given a single EdU injection at birth.\(^{22}\) An energy-level-dependent increase between treatment groups \((P < .05)\) in EdU\(^+\) cells in the cavernosal tissues was evident (Figures 4A and 4B). Using lysates from penile tissue, we measured the expression level of chemokine stromal derived factor 1 (SDF-1; Figures 4C and 4D), a classic chemoattractant for progenitor cell recruitment.\(^{26}\) After pelvic injury, higher expression of SDF-1 correlated with increased number of EdU\(^+\) cells, and this effect was significantly enhanced by LESW treatment, especially in the higher LESW group \((P < .05)\).

Schwann Cells During Penile Nerve Regeneration and Effects From LESW Treatment

Schwann cells are critical for nerve fiber growth and regeneration. To further examine the mechanism of nerve regeneration, we explored the process of Schwann cell activation, which is characterized by dedifferentiation, redifferentiation, proliferation, and maturation. Two kinds of Schwann cells were distributed in penile nerves: myelinated Schwann cells (mSC) and nonmyelinated Schwann cells (nmSC). In the tissue section of dorsal nerve, we quantified the relative number of DAPI\(^+\) dots in the dorsal nerve fiber (primarily Schwann cell nuclei of both mSC and nmSC),\(^{27}\) as well as mature Schwann cell marker S100 (Figures 5A and 5B). The number of Schwann cells within dorsal nerves increased in the higher LESW group compared with the other 3 groups, whereas S100 was highly expressed in both sham and higher LESW-treated groups (Figure 5B). Based on the DAPI results it appears that 5 weeks after pelvic injury, the number of cells (including Schwann and other cells) increased to normal levels spontaneously (i.e. the PVNI group) whereas LESW treatment increased the number further. The decreased expression of S100 in the PVNI group compared with the sham group possibly indicates degeneration of some Schwann cells after nerve injury. S100 staining was increased in the LESW treatment groups, suggesting that LESW may enhance proliferation of Schwann cells. Moreover, we examined 2 markers for Schwann cell dedifferentiation and proliferation after pelvic injury: p75 and p-Erk1/2. Western blot of the penile tissue indicated that both p75 and p-Erk1/2 were significantly increased after LESW therapy (Figures 5C and 5D).
Effect of LESW on Cultured Rat Schwann Cells In Vitro

Using LESW on cultured adherent Schwann cells, similar to the in vivo results, the expression of p-Erk1/2 and p75 also were significantly elevated after LESW treatment (Figures 6A and 6B). In IF staining, we observed that p-Erk1/2 tended to accumulate in Schwann cell nuclei after LESW treatment, possibly indicating that LESW therapy triggers the initiation of p-ERK1/2-mediated downstream pathways in Schwann cells (Figure 6C). Additionally, we quantified the number of cells in particular cell cycle phases. G1/G0 phase typically indicates dormancy or resting phase before proliferation, S phase indicates the DNA replication, and G2/M phase is when cell division occurs. Within 8 hours after LESW treatment, a higher percent of Schwann cells entered the S phase and the G2/M relative to untreated cells, and this increase in the percentage of cycling Schwann cells remained for 24 hours (Figures 6D and 6E). Together, these data demonstrate the growth-promoting effect of LESW on Schwann cells.

DISCUSSION

Though the standard CNI-induced ED model is consistently used to study ED, there is currently no good animal model to mimic neurovascular ED. In this study, we combined CNI with IPBI to establish an ED model in the rat that closely replicates human pelvic injury during surgery and trauma. This combined injuries model successfully impaired erectile function for a long duration, allowing for extended duration studies of ED therapies.

CNI and IPBI lead to ischemia, neurodegeneration, and impaired erectile capability. We tried to establish a new clinical approach to fix this condition. Recently, low energy shock wave (LESW) treatment is proved to be a promising therapeutic strategy for ED that has been tested in clinical trials (NCT01317693, NCT01811797, NCT01274156, NCT00901056, and others at http://clinicaltrials.gov). The mechanisms of the precise therapeutic and biological effects in LESW treatment are still not completely understood. Prior experiments found that LESW induces neovascularization by upregulating the expression of VEGF and its receptor and mobilization of progenitor cells. The biologic responses of LESW appear to be time dependent and according to a previous report, the peak response occurs 4 weeks after treatment.

In this project, rats in the treated groups showed improved functional and histologic recovery after 4 weeks of LESW treatment. We noted that angiogenesis and recirculation were...
significantly promoted after LESW treatment. The major nerve components in the penile nerve system demonstrated significantly more regeneration after LESW treatment when compared with the spontaneous regeneration in the untreated control group rats. The accelerated regeneration of nerve fibers, including nNOS$^+$ nerve fibers, is especially exciting as neural injury and lack of functional nerve recovery are believed to be the crux of why neurovascular ED is refractory to therapy.

Recruitment of sufficient progenitor cells through the vascular network and interstitial tissues is usually the first step of tissue regeneration, and is required for tissue maintenance and injury repair. However, in most studies exogenous progenitor cell application has not been highly successful for various reasons, including inefficient migration to target organs. It has been suggested that direct recruitment of endogenous progenitor cells to the target organ of interest might improve the outcome of progenitor cell treatment. In our present work we used label retaining cell technique with EdU and found more endogenous progenitor cells in penile tissue after pelvic injury, consistent with the process of tissue repair and a previous report. Additionally, we found that LESW therapy led to a significant increase in local progenitor cell numbers relative to untreated animals, in line with our previous report using a diabetic ED model, and that the expression of SDF-1, which plays a primary role in progenitor cell recruitment, is correlated with the number of EdU$^+$ cells in penile tissue. LESW might act to increase and maintain the concentration of SDF-1 in penile tissue post injury, thus potentiating and prolonging the recruitment of endogenous progenitor cells and amplifying in situ tissue regeneration. Many different types of cells can secrete SDF-1, including endothelial cells and smooth muscle cells during injury or in response to hypoxia, and progenitor cells that express CXCR4 could be recruited through SDF-1/CXCR4 axis. Progenitor cells predominantly contribute to tissue regeneration through their paracrine ability. It is currently unclear if progenitor cells in LESW-treated tissues are local cells induced to divide, recruited from other sites, or both. In addition, the biological effects of either progenitor cell division or recruitment to penile tissue in this ED model remain open questions.

In humans, peripheral nerve regeneration after injury is known to be a slow process, and may be an underlying factor in the loss of innervated tissue function. Therefore, a potential avenue for ED treatment would be a therapy that enhances the kinetics of nerve regeneration after injury. Successful peripheral nerve regeneration is promoted by Schwann cell activation. After injury, it is thought that some Schwann cells dedifferentiate into a progenitor-like state, proliferate, and then repopulate the damaged nerve. This is critical to navigate the growth of new nerve fibers, especially within the first week or 2 after injury.

Figure 5. Activation of Schwann cells by LESW in vivo. A. Representative images of immunofluorescence staining for NF (green) and S100 (red) in dorsal nerve. Original magnification is ×200. B. The number of Schwann cells by calculating the number of DAPI dots (standardized to the number of nerve fiber dots which was stained with NF) and expression of S100, which is a marker for mature Schwann cells (standardized to the densitometry of NF). C. Representative image of Western blot for p75 neurotrophin receptor (p75) and Phosphor Erk1/2 (p-Erk1/2) in penile tissue. D. Western blot analysis of p-Erk and p75. *P < .05. NF = neurofilament.
In our experiment, upregulation of the crucial activation signaling pathway mediator phosphorylated ERK (p-Erk) is significant after treatment. ERK/MAPK signaling is a classic pathway for induction of cell proliferation and high levels of p-Erk are also a crucial trigger of dedifferentiation of Schwann cells. p75 neurotrophin receptor (p75) expression is a hallmark of Schwann cell dedifferentiation. Currently, the kinetics of p75 expression after injury is unknown, but we also found that expression of p75 is significantly upregulated after LESW treatment both in vitro and in vivo. Together, increased expression of these markers indicates that LESW induces both dedifferentiation and proliferation (also verified by cell cycle analysis) of Schwann cells. Though S100 has been widely used as a cell marker for Schwann cells in vitro and in vivo, it is worth noting that S100 is not a specific marker. Downstream of dedifferentiation and proliferation, S100 is recognized as a maturation gene and represents the amount of mature Schwann cells. In our experiment, the amount of mature Schwann cells decreased after pelvic injury because of nerve degeneration. Dedifferentiation and proliferation of Schwann cells distal to the site of injury along with activation of ERK/MAPK and p75 result in more mature Schwann cells and creating and maintaining an environment amenable to nerve regrowth by LESW.

CONCLUSION

LESW treatment improves erectile function in a rat model of pelvic neurovascular injuries. Penile tissue components, especially vascular and neuronal tissue, demonstrated improved recovery after LESW therapy. The mechanism of these beneficial effects appears to be through the recruitment of endogenous progenitor cells and activation of Schwann cells.

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STATEMENT OF AUTHORSHIP

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(a) Conception and Design
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(b) Acquisition of Data
Huixi Li, Melanie P. Matheu, Fionna Sun, Lin Wang, Melissa T. Sanford, Lia Banie

Figure 6. LESW promotes the activation of Schwann cells in vitro. A. Representative image of Western blot for p75 neurotrophin receptor (p75), Phosphor Erk1/2 (p-Erk1/2) in Schwann cells 24h (p75) or 8h (p-Erk1/2) after LESW treatment (SW) or mock treatment (Control). B. Western blot analysis of p-Erk (8h) and p75 (24h). C. Representative images of immunofluorescence staining for p-Erk1/2 (green) in Schwann cells 8 hours after LESW treatment (SW) or mock treatment (Control). Original magnification is ×400. D. Representative of cell cycle image before treatment (0h) and 8, 16, 24 hours after LESW treatment (SW) or mock treatment (Control). E. Percent of cells in different cell cycle periods. *P < .05.
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Low-intensity Extracorporeal Shock Wave Treatment Improves Erectile Function: A Systematic Review and Meta-analysis

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Abstract

Context: As a novel therapeutic method for erectile dysfunction (ED), low-intensity extracorporeal shock wave treatment (LI-ESWT) has been applied recently in the clinical setting. We feel that a summary of the current literature and a systematic review to evaluate the therapeutic efficacy of LI-ESWT for ED would be helpful for physicians who are interested in using this modality to treat patients with ED.

Objective: A systematic review of the evidence regarding LI-ESWT for patients with ED was undertaken with a meta-analysis to identify the efficacy of the treatment modality.

Evidence acquisition: A comprehensive search of the PubMed and Embase databases to November 2015 was performed. Studies reporting on patients with ED treated with LI-ESWT were included. The International Index of Erectile Function (IIEF) and the Erection Hardness Score (EHS) were the most commonly used tools to evaluate the therapeutic efficacy of LI-ESWT.

Evidence synthesis: There were 14 studies including 833 patients from 2005 to 2015. Seven studies were randomized controlled trials (RCTs); however, in these studies, the setup parameters of LI-ESWT and the protocols of treatment were variable. The meta-analysis revealed that LI-ESWT could significantly improve IIEF (mean difference: 2.00; 95% confidence interval [CI], 0.99–3.00; \( p < 0.0001 \)) and EHS (risk difference: 0.16; 95% CI, 0.04–0.29; \( p = 0.01 \)). Therapeutic efficacy could last at least 3 mo. The patients with mild-moderate ED had better therapeutic efficacy after treatment than patients with more severe ED or comorbidities. Energy flux density, number of shock waves per treatment, and duration of LI-ESWT treatment were closely related to clinical outcome, especially regarding IIEF improvement.

Conclusions: The number of studies of LI-ESWT for ED have increased dramatically in recent years. Most of these studies presented encouraging results, regardless of variation in LI-ESWT setup parameters or treatment protocols. These studies suggest that LI-ESWT could significantly improve the IIEF and EHS of ED patients. The publication of robust evidence from additional RCTs and longer-term follow-up would provide more confidence regarding use of LI-ESWT for ED patients.

Patient summary: We reviewed 14 studies of men who received low-intensity extracorporeal shock wave treatment (LI-ESWT) for erectile dysfunction (ED). There was evidence that these men experienced improvements in their ED following LI-ESWT.

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1. Introduction

Phosphodiesterase type 5 inhibitors (PDE5-Is) are currently the most widely used treatments for male erectile dysfunction (ED); however, these medications merely treat ED symptoms. They do not correct the underlying penile pathophysiology, such as vascular lesions secondary to diabetes mellitus, structural lesions secondary to trauma, or neurologic injury secondary to prostatectomy, that is responsible for the ED [1]. A novel method to prevent the deterioration of erectile function due to these pathophysiologic processes is desperately needed. Based on studies generated from other clinical fields, low-intensity extracorporeal shock wave treatment (LI-ESWT) has been used to treat ED for almost 10 yr, and encouraging results have been reported.

Since the 1980s, when it was first introduced for renal lithotripsy, shock wave therapy has been rapidly adopted all over the world for different disease processes, producing either destructive effects or promoting regenerative effects. The shock wave is a kind of acoustic wave that carries energy and that, when propagating through a medium, can be targeted and focused noninvasively to affect a distant selected anatomic region. When LI-ESWT is applied to an organ, the shock waves interact with the targeted tissues and induce a cascade of biological reactions. This results in the release of growth factors, which in turn triggers neovascularization of the tissue with subsequent improvement of the blood supply [2]. LI-ESWT has been used to treat musculoskeletal disorders [3], myocardial infarction [4], nonhealing wounds [5], and ED [6].

Improvements in both International Index of Erectile Function (IIEF) and Erection Hardness Score (EHS) have been reported after using LI-ESWT for patients with ED. At the beginning of research into LI-ESWT, most studies were retrospective and included few patients. In the past 2 yr, well-designed prospective studies have been conducted and concluded that LI-ESWT is a feasible noninvasive method for improving male ED.

We performed a systematic review of the current body of literature investigating the application of LI-ESWT for ED. Our goal was to analyze the available data to determine the efficacy of LI-ESWT for ED.

2. Evidence acquisition

2.1. Search strategy

We performed a systematic search of PubMed and Embase databases for studies on LI-ESWT and ED. The search terms were shock wave AND (erectile dysfunction OR IIEF OR EHS). We investigated the current studies of LI-ESWT for patients with ED, the therapeutic efficacy of LI-ESWT for patients with ED, and the relationship of therapeutic efficacy and different setup parameters and protocols.

2.2. Inclusion and exclusion criteria

All clinical studies that investigated the efficacy of LI-ESWT for ED were included regardless of study design. Both randomized controlled trials (RCTs) and cohort studies were included. No limitation was placed on PDE5-I consumption during the LI-ESWT treatment period or on the severity of ED. The follow-up data were abstracted from these studies. If more than one study was published by a medical center, only the last report was included in our review. All literature reviews, editorial comments, background, animal models, and case reports were excluded.

2.3. Data extraction and synthesis

The abstracts were independently reviewed by three authors (Z.L., G.L., T.F.L.) to determine eligibility for inclusion. The basic details of the study, setup parameters of the LI-ESWT machine, treatment protocols, assessment tools, and p values were abstracted manually from each of the studies (G.L., Z.L.), and the data were verified (T.F.L.).

2.4. Study outcomes

Fourteen studies were included in our review. Seven studies were RCTs and were included for meta-analysis. The patients were distributed in different areas of the world, and there were no overlaps of populations among the studies. Details are shown in Table 1 and Supplementary table.

2.5. Meta-analysis

The abstracted data were analyzed with RevMan 5.3 software (Cochrane Collaboration, London, UK). The risk of bias of the included studies was assessed by the Cochrane Collaboration’s tool. The proper effect sizes and statistical analysis methods were chosen according to different data types and evaluation purposes. For continuous variables, weighted mean difference (MD) and a 95% confidence interval (CI) were used. For discontinuous variables, risk difference (RD) and a 95% CI were used. For the heterogeneity test between studies, the I^2 test was used. The data without significant heterogeneity (p > 0.05, I^2 ≤ 50%) were analyzed by the fixed-effects model. The data with heterogeneity that could not be explained were analyzed by the random-effects model. The data that could not be analyzed were described. The results of the meta-analysis are presented in forest plots. Publication bias is presented in funnel plots.

3. Evidence synthesis

A Preferred Reporting Items of Systematic Reviews and Meta-analyses (PRISMA) flow chart of screening and selection results is shown as Figure 1.

3.1. The current studies of low-intensity extracorporeal shock wave treatment for erectile dysfunction

A total of 14 studies involving 833 patients were included in this review. All of the studies were published between 2005 and 2015. These studies were performed by different medical centers in different countries. Most of these ED patients had an organic etiology, such as a vascular lesion [7,8], a nerve injury [9], or a lesion of the cavernous body of...
Table 1 – Current studies of low-intensity extracorporeal shock wave treatment for erectile dysfunction patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Year of publication</th>
<th>Country</th>
<th>Disease</th>
<th>Setup of LESW</th>
<th>Protocol of LESW treatment</th>
<th>Follow-up, mo</th>
<th>Evaluation tools for ED</th>
<th>p value of IIEF after LI-ESWT</th>
<th>Study design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olsen et al [19]</td>
<td>2015</td>
<td>Denmark</td>
<td>ED</td>
<td></td>
<td></td>
<td></td>
<td>IIEF-5, EHS</td>
<td>0.67</td>
<td>RCT</td>
</tr>
<tr>
<td>Frey A</td>
<td>2015</td>
<td>Denmark</td>
<td>ED after RP</td>
<td>NA</td>
<td></td>
<td></td>
<td>IIEF-5</td>
<td>0.0049</td>
<td>Cohort study</td>
</tr>
<tr>
<td>Bechara et al [15]</td>
<td>2015</td>
<td>Argentina</td>
<td>ED</td>
<td>0.09</td>
<td>5000</td>
<td>1, 4</td>
<td>IIEF-5, SEP2, SEP3, GAQ</td>
<td>NA</td>
<td>Cohort study</td>
</tr>
<tr>
<td>Chung and Cartmill [7]</td>
<td>2015</td>
<td>Australia</td>
<td>ED</td>
<td>0.25</td>
<td>3000</td>
<td>2, 4</td>
<td>IIEF-5, EDITS, overall satisfaction score</td>
<td>&lt; 0.05</td>
<td>Cohort study</td>
</tr>
<tr>
<td>Pelayo-Nieto et al [8]</td>
<td>2015</td>
<td>Mexico</td>
<td>ED</td>
<td>0.09</td>
<td>5000</td>
<td>1, 4</td>
<td>IIEF, SEP, GAQ</td>
<td>0.013</td>
<td>Cohort study</td>
</tr>
<tr>
<td>Hisaue</td>
<td>2015</td>
<td>Japan</td>
<td>ED</td>
<td>0.09</td>
<td>1500</td>
<td>2, 5</td>
<td>IIEF, EHS, MPCC</td>
<td>&lt; 0.05</td>
<td>Cohort study</td>
</tr>
<tr>
<td>Srin et al [16]</td>
<td>2015</td>
<td>India</td>
<td>ED</td>
<td>NA</td>
<td>NA</td>
<td>1, 3, 6, 9, 12</td>
<td>IIEF-EF, EHS, GQIC</td>
<td>0.0001</td>
<td>RCT</td>
</tr>
<tr>
<td>Yee et al [18]</td>
<td>2014</td>
<td>Hong Kong</td>
<td>ED</td>
<td>0.09</td>
<td>1500</td>
<td>2, 5</td>
<td>IIEF-ED, EHS</td>
<td>0.001</td>
<td>RCT</td>
</tr>
<tr>
<td>Palmieri et al [10]</td>
<td>2012</td>
<td>Italy</td>
<td>ED + PD</td>
<td>0.25</td>
<td>2000</td>
<td>1, 4</td>
<td>IIEF, quality of life</td>
<td>&lt; 0.05</td>
<td>Cohort study</td>
</tr>
<tr>
<td>Vardi et al [17]</td>
<td>2012</td>
<td>Israel</td>
<td>ED</td>
<td>0.09</td>
<td>1500</td>
<td>2, 5</td>
<td>IIEF, EHS, penile blood flow</td>
<td>0.0322</td>
<td>RCT</td>
</tr>
<tr>
<td>Zimmermann et al [14]</td>
<td>2009</td>
<td>Austria</td>
<td>ED + chronic pelvic pain</td>
<td>0.25</td>
<td>3000</td>
<td>1, NA</td>
<td>IIEF</td>
<td>0.034</td>
<td>RCT</td>
</tr>
<tr>
<td>Chitale et al [11]</td>
<td>2010</td>
<td>UK</td>
<td>ED + PD</td>
<td>NA</td>
<td>3000</td>
<td>1, NA</td>
<td>IIEF</td>
<td>0.249</td>
<td>RCT</td>
</tr>
<tr>
<td>Poulakis et al [12]</td>
<td>2006</td>
<td>Germany</td>
<td>ED + PD</td>
<td>0.17</td>
<td>2000</td>
<td>1, NA</td>
<td>IIEF</td>
<td>0.205</td>
<td>RCT</td>
</tr>
<tr>
<td>Skolarikos et al [13]</td>
<td>2005</td>
<td>Greece</td>
<td>ED + PD</td>
<td>NA</td>
<td>3000</td>
<td>NA</td>
<td>IIEF</td>
<td>0.06</td>
<td>Cohort study</td>
</tr>
</tbody>
</table>

CGIC = Clinical Global Impression of Change; ED = erectile dysfunction; EDITS = Erectile Dysfunction Inventory of Treatment Satisfaction; EHS = Erectile Hardness Score; GAQ = Global Assessment Questionnaire; IIEF = International Index of Erectile Function; LI-ESWT = low-intensity extracorporeal shock wave treatment; MPCC = maximal penile circumferential change; NA = not available; PD = Peyronie’s disease; RCT = randomized controlled trial; RP = radical prostatectomy; SEP = Sexual Encounter Profile.
the penis (Peyronie's disease [PD]) [10–13]. One study focused on ED patients with chronic pelvic pain [14]. Most of the studies prohibited the usage of PDE5-Is during the treatment course. Some RCTs even set a washout period for patients who had taken PDE5-I before they started LI-ESWT. Only three studies did not limit the use of PDE5-Is during the treatment [10,11,15]. One of these studies was included for meta-analysis because of its RCT design.

Of the 14 included studies, 7 were RCTs, and the remaining 7 were cohort studies (Table 1). According to the conventions of evidence-based medicine, RCTs provide level 1 evidence, the highest level of evidence. Consequently, the seven RCTs were included for meta-analysis because of its RCT design.

The setup parameters of LI-ESWT were different among studies. The energy flux density (EFD) varied from 0.09 to 0.25 mJ/mm², and the number of shock wave pulses of each treatment was between 1500 and 5000. In most of the studies, LI-ESWT directed treatment at multiple sites on the penis during each treatment. The treatment course of most studies was not longer than 6 wk, and only three studies had a longer treatment course of 9 wk.

The IIEF was the prevailing assessment tool for ED patients, and all studies in our analysis provided the IIEF before and after LI-ESWT. This made it possible to perform further meta-analysis. Another frequently used assessment tool was the EHS, which was provided by five studies. Other tools, such as the Sexual Encounter Profile, the Global Assessment Questionnaire, maximal penile circumferential change, and the Clinical Global Impression of Change, were not used consistently throughout multiple studies and so were not used for further meta-analysis.

3.2. The quality evaluation of the studies and analysis for the risk of bias

The Cochrane Collaboration's tool was used for assessing the quality of the study and the risk of bias. The RCTs reported that the patients were assigned randomly into LI-ESWT or control groups without describing the process of randomization [16,17]. Most studies did not describe how the physicians were blinded to the study participants. When the patients in the control group received the sham treatment, the LI-ESWT output energy would need to be reduced to zero, thus it would be difficult to keep the physician blinded to this change. Only the study by Yee et al [18] reported the details of how the double blinding was
Fig. 3 – Clinical outcomes. (a) Although some studies did not prove that low-intensity extracorporeal shock wave treatment (LI-ESWT) could increase International Index of Erectile Function (IIEF), the meta-analysis results showed that LI-ESWT could improve IIEF significantly (mean difference [MD]: 2.00; 95% confidence interval [CI], 0.99–3.00; \( p < 0.0001 \)). (b) Subgroup analysis: The studies that assessed the IIEF at 1 mo did not reveal a significant improvement (MD: 0.37; 95% CI, 1.45 to 2.19; \( p = 0.69 \)). However, the studies assessing IIEF at 3 mo showed significant improvement (MD: 2.71; 95% CI, 1.51–3.91; \( p < 0.0001 \)). (c) The IIEF in the group with mild erectile dysfunction (ED) increased significantly (MD: 2.86; 95% CI, 1.19–3.53; \( p < 0.0001 \)) compared with the studies recruiting ED patients with Peyronie's disease. (d) The IIEF of patients in the group with LI-ESWT plus phosphodiesterase type 5 inhibitors improved more significantly (MD: 4.20; 95% CI, 0.16–8.24; \( p = 0.04 \)).

CI = confidence interval; ED = erectile dysfunction; IIEF = International Index of Erectile Function; IV = inverse variance; LI-ESWT = low-intensity extracorporeal shock wave treatment; PD = Peyronie's disease; PDE5-I = phosphodiesterase type 5 inhibitor; RCT = randomized controlled trial; SD, standard deviation.
ensured. Figure 2 shows that 57.1% studies had an unclear risk of bias in randomization and that only 16.7% of studies had good blinding for both patients and doctors.

3.3. The evaluation of the therapeutic efficacy of low-intensity extracorporeal shock wave treatment for patients with erectile dysfunction

The IIEF, the prevailing assessment tool for ED patients, was available for abstraction from five RCTs. The data included mean value and standard deviation of the IIEF and the number of patients in the treatment and control groups. The studies by both Yee et al [18] and Poulakis et al [12] concluded that the IIEF did not increase significantly in the treatment group compared with the control group; the p values were 0.156 and 0.205, respectively. The remaining three RCTs reported that the IIEF increased significantly in the LI-ESWT group compared with the control group [11,14,17]; the p value was <0.05. The overall meta-analysis of the data revealed that LI-ESWT improved the IIEF significantly overall in the treatment groups (MD: 2.00; 95% CI, 0.99–3.00; p < 0.0001) (Fig. 3a).

Subgroup analysis was performed. Figure 3b shows that Poulakis et al [12] and Vardi et al [17] assessed IIEF at 1 mo after LI-ESWT and that the IIEF did not increase significantly (MD: 0.37; 95% CI, −1.45 to 2.19; p = 0.69). Three other studies, however, assessed IIEF at 3 mo after treatment and found that the IIEF increased significantly (MD: 2.71; 95% CI, 1.51–3.91; p < 0.0001). In Figure 3c, the studies were divided into three groups by the IIEF before LI-ESWT—≤11, 12–16, and 17–21—corresponding to severe, moderate, and mild ED, respectively. The meta-analysis showed that the IIEF of patients in the mild ED group increased significantly after LI-ESWT (MD: 2.86; 95% CI, 1.54–4.19; p < 0.0001). The patients in the severe and moderate groups did not show a significant increase in IIEF (p = 0.30 and p = 0.49). In Figure 3d, the studies were divided into two groups: the ED group and the ED with PD group. The subgroup analysis showed that the patients in the ED group improved significantly in IIEF (MD: 2.36; 95% CI, 1.19–3.53; p < 0.0001). The patients in the ED with PD group had no significant improvement in IIEF (p = 0.33). Finally, the studies were divided into two groups by usage of PDE5Is. Figure 3e shows that the IIEF increased in both groups but

![Fig. 3. (Continued).]
increased more significantly in the group with LI-ESWT combined with PDE5-I use (MD: 4.20; 95% CI, 0.16–8.24; \( p = 0.04 \)).

These results indicate that LI-ESWT increased the IIEF and improved the erectile function of ED patients. According to the results of the current studies, the patients treated by LI-ESWT developed a good therapeutic effect by 3 mo. The patients who had mild or moderate ED and the ED patients who had no comorbidities benefited more from LI-ESWT than the patients with severe ED or with comorbidities.

Different LI-ESWT setup parameters, such as EFD and number of pulses, and different treatment protocols, including treatment frequency and length of course, resulted in differences in reported efficacy. The studies were divided into three groups according to EFD. The results (Fig. 4a) showed that the studies using the highest EFD (>0.2 mJ/mm\(^2\)) reported significantly increased IIEFs (MD: 2.86; 95% CI, 1.54–4.19; \( p < 0.0001 \)). The improvement of IIEF in this ED and PD subgroup was partially due to the improvement of PD. After excluding this subgroup, we found that the improvement in IIEF was better in the group with EFD 0.09 mJ/mm\(^2\) compared with EFD 0.1–0.2 mJ/mm\(^2\), although neither group reached statistical significance. Next, the studies were divided into two groups based on the number of shock waves delivered during each treatment. The results (Fig. 4b) showed that the studies administering more shock waves reported a significant increase in IIEF (MD: 2.86; 95% CI, 1.54–4.19; \( p < 0.0001 \)) compared with the studies delivering fewer shock waves. To compare different durations of treatment, the studies were divided into two groups according to duration of treatment of LI-ESWT. Figure 4c shows that the studies with a treatment course of <6 wk reported a significant increase in the IIEF (MD: 2.11; 95% CI, 0.98–3.25; \( p = 0.0003 \)).

These results suggest that different setup parameters and different treatment protocols of LI-ESWT have substantial influence on therapeutic efficacy. In summary, within the scope of this review, lower energy density, increased number of pulses, and shorter treatment courses of <6 wk resulted in better therapeutic efficacy.

The EHS data were available for abstraction from four RCTs. In the studies by Yee et al [18] and Olsen et al [19], EHS was reported at 3 mo after LI-ESWT. In the study by Yee et al, the EHS did not increase significantly. In subgroup analysis (Fig. 5), at 1 mo after LI-ESWT, the EHS increased significantly in three studies (RD: 0.47; 95% CI, 0.38–0.56; \( p < 0.00001 \)). EHS did not improve as significantly after 3 mo as it did after 1 mo, but it still increased with statistical significance (RD: 0.16; 95% CI, 0.04–0.29; \( p = 0.01 \)). These results indicate that LI-ESWT improves the erectile hardness of the penis for ED patients, especially at 1 mo after treatment, and that this improvement lasts for at least 3 mo.

3.4. Discussion

LI-ESWT has been used as a novel therapy for ED patients for the past 10 yr. Clinical studies and reports focused on this topic have increased dramatically in past 5 yr, especially in 2015. This implies that LI-ESWT as a therapeutic method for patients with ED has been increasingly adopted by both physicians and patients.

The IIEF is a patient-reported assessment that is purely subjective. In this review, we found that in some studies, patients in the control group also reported improvement of the IIEF [12,17,18]; however, patients in the LI-ESWT group improved more significantly than those in the control group. The range of improvement in the IIEF was from 5.3 to 7.6 points for the LI-ESWT group in our analysis [14,18]. It is undeniable that some studies revealed improvement with statistical significance; however, this improvement may have no significant clinical value. The minimal clinically important difference (MCID) of IIEF better assesses the true clinical efficacy of LI-ESWT. We recommend that, in the future, investigators use the MCID of IIEF as a more accurate and meaningful tool for evaluating the effect of LI-ESWT in the treatment of patients with ED [20].

The clinical outcome of LI-ESWT is closely related to the energy delivered to the target unit area, or EFD. The EFD used varied from 0.09 to 0.25 mJ/mm\(^2\) among the studies included in our analysis. Based on this review, we could not determine the best EFD for ED therapy. Studies investigating the use of LI-ESWT for various regenerative purposes have used varying energy densities. An investigation by Goertz et al showed that an energy density of 0.04 mJ/mm\(^2\) could accelerate angiogenesis for skin burns [21]. The study by Abe et al revealed that an energy density of 0.1 mJ/mm\(^2\) for a rat model of acute myocardial infarction suppressed ventricular remodeling and had a good anti-inflammatory effect [22]. The study by Tara et al found that an energy density of 0.11–0.21 mJ/mm\(^2\) could encourage therapeutic angiogenesis for human ischemic tissues [23]. Loppolo et al reported that for some musculoskeletal disorders, energy density could be increased to 0.3 mJ/mm\(^2\) [24]. In the current review, most of the included studies used an energy density of 0.09 mJ/mm\(^2\), which Vardi et al first reported in 2010 [17]. Most subsequent studies adopted this EFD and presented encouraging results. Additional studies and a longer duration of treatment are needed to establish whether therapeutic efficacy is positively correlated with energy density.

Some studies included in our review concluded that the biological efficacy of LI-ESWT was dosage dependent [25]. It seemed that more pulses would bring better biological efficacy. With this hypothesis in mind, some studies adopted multiple treatment sites, more frequent treatments, and longer courses of treatment. Meta-analysis showed that 3000 pulses per treatment brought more improvement than 1500 or 2000 pulses per treatment; however, more frequent treatment and longer treatment course did not improve erectile function significantly. The optimal treatment protocol remains to be defined. Whether there may be a plateau stage of treatment remains uncertain and requires further investigation. In addition, based on the premise that more treatment sites would produce better results, shock waves were delivered to multiple sites, such as the dorsal surface, both sides, and both crus of the penis. It seemed that more sites treated...
Fig. 4 – Relationship of energy dosage and treatment procedures. (a) The studies using higher energy flux density (EFD; >0.2 mJ/mm²) resulted in significantly increased International Index of Erectile Function (IIEF; mean difference [MD]: 2.86; 95% confidence interval [CI], 1.54–4.19; \( p < 0.0001 \)) in the erectile dysfunction (ED) and Peyronie’s disease groups. In ED-only groups, the improvement of IIEF was better for the group with EFD 0.09 mJ/mm² compared with EFD 0.1–0.2 mJ/mm², although it did not reach statistical significance. (b) The studies delivering more shock waves per treatment resulted in an increased IIEF (MD: 2.86; 95% CI, 1.54–4.19; \( p < 0.0001 \)). (c) The studies with total course of treatment <6 wk revealed significant IIEF increase (MD: 2.11; 95% CI, 0.98–3.25; \( p = 0.0003 \)) versus studies with longer courses of treatment (9 wk). CI = confidence interval; EFD = energy flux density; IV = inverse variance; LI-ESWT = low-intensity extracorporeal shock wave treatment; SD, standard deviation.
might produce better results. It is well known that shock waves can propagate 3–5 cm in human tissue [26]. It remains to be determined if it is necessary or beneficial to deliver treatment to multiple sites. This is also an area of potential future investigation.

The underlying mechanism of action of LI-ESWT is currently under investigation. According to recent reports, the effect is primarily related to the stimulation of cell proliferation, tissue regeneration, and angiogenesis [27,28]. In 2013, Qiu et al explored the therapeutic effect of LI-ESWT on a diabetic animal model and demonstrated that LI-ESWT can partially resolve diabetes mellitus–associated ED by promoting regeneration of neuronal nitric oxide synthase (nNOS)–positive nerves, endothelium, and smooth muscle in the penis [28]. Meanwhile, Liu and colleagues reported their results after treatment of a rat model of ED with LI-ESWT. The expression of some proteins, such as α-smooth muscle actin, von Willebrand factor, nNOS, and vascular endothelial growth factor, was upregulated [29]. In 2013, Siegfried and colleagues reported that LI-ESWT could stimulate the regeneration of injured nerve fibers. They believed that the potential mechanism of LI-ESWT was enhanced by neovascularization in the regenerating nerve and that VEGF and transforming growth factor β were associated with the process [30]. Very recently, it was reported that LI-ESWT improved erectile function in a rat model of pelvic neurovascular injury. Penile tissue components, especially vascular and neuronal tissue, demonstrated improved recovery after LI-ESWT therapy [27].

Several weaknesses contributed to the quality of the data provided. As shown in Table 1, five of seven studies published in 2015 were cohort studies. It is undeniable that these cohort studies have good study designs and robust data collection; each has an appropriate sample size and clear comparison. In evidence-based medicine, however, the evidence level of cohort studies is level 2, and thus they have lower power than RCTs, which provide level 1 evidence. To evaluate the efficacy of LI-ESWT more accurately, more RCTs with good study designs are needed. In addition, even in the RCTs that were included in this review, there were still some deficiencies. The details of randomization, the implementation of double blinding, the details of the treatment protocol, and the data from long-term follow-up are fundamental factors for assessing the quality of a study. As shown in Figure 2a and 2b, we found that most of the included RCTs did not describe the details of randomization or blinding, and the potential biases involved are unclear. If bias existed, it would have a great impact on the interpretation of the meta-analysis.

Most of the studies focused on the improvement of erectile function after LI-ESWT. Nevertheless, the potential impact of factors related to ED, such as age, hypertension, diabetes, hyperlipidemia, and coronary artery disease, are not discussed. Only four RCTs in our analysis provided the age data comparing the patients in the treatment and control groups [12,17–19]. No further investigation was performed to determine the influence of age on the efficacy of LI-ESWT. Three RCTs provided the profile of patient comorbidities, such as hypertension, diabetes, hyperlipidemia, and coronary artery disease, but no further information was provided about the relationship between the clinical outcome of LI-ESWT and those comorbidities [17–19]. In the future, more RCTs with stratification of age and comorbidities will help determine the influence of these factors on the efficacy of LI-ESWT for patients with ED.

With the aim of determining the efficacy of LI-ESWT alone and to avoid confusion, most of the included studies prohibited the usage of PDE5-Is during shock wave treatment. Nevertheless, because the goal of treatment is to maximize improvement of erectile function, a combination of LI-ESWT and PDE5-Is may be the best choice. Gruenwald et al found that LI-ESWT effectively converted PDE5-I nonresponders to responders [31], and our results (Fig. 3e) support the use of LI-ESWT and PDE5-Is in...
combination. Additional clinical trials are needed to further investigate this clinical question.

4. Conclusions

In recent years, LI-ESWT as a therapy for ED has attracted extensive attention. Studies of this topic have increased sharply, and most of these studies reveal encouraging results, such as improved IIEF and EHS and an effect that lasts up to 3 mo. The setup parameters and the treatment protocols are important for the therapeutic effects of LI-ESWT for patients with ED. The mechanism of LI-ESWT is to improve or even reverse the pathologic damage of tissue that causes ED. Additional studies are needed to explore the influences of age and comorbidities on response to LI-ESWT and to define the effects of LI-ESWT in combination with PDE5-Is. From our review, it is clear that LI-ESWT may have the potential to be the first-choice noninvasive treatment for patients with ED.

Author contributions: Tom F. Lue had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Lue, Lin.

Acquisition of data: Lin, Lu, Lee, Wang.

Analysis and interpretation of data: Lu, Lee, Lin.

Drafting of the manuscript: Lu, Lin, Reed-Maldonado.

Critical revision of the manuscript for important intellectual content: Lin, Reed-Maldonado, Lue.

Statistical analysis: Lu, Lin.

Obtaining funding: Lue, Lin.

Administrative, technical, or material support: Wang, Lu.

Supervision: Lue.

Other (specify): None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.euro.2016.05.050.

References


Effects of Low-Intensity Extracorporeal Shockwave Therapy on Erectile Dysfunction: A Systematic Review and Meta-Analysis

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ABSTRACT

Introduction: Low-intensity extracorporeal shock wave therapy (Li-ESWT) has been proposed as an effective non-invasive treatment option for erectile dysfunction (ED).

Aim: To use systematic review and meta-analysis to assess the efficacy of Li-ESWT by comparing change in erectile function as assessed by the erectile function domain of the International Index of Erectile Function (IIEF-EF) in men undergoing Li-ESWT vs sham therapy for the treatment of ED.

Methods: Systematic search was conducted of MEDLINE, EMBASE, and ClinicalTrials.gov for randomized controlled trials that were published in peer-reviewed journals or presented in abstract form of Li-ESWT used for the treatment of ED from January 2010 through March 2016. Randomized controlled trials were eligible for inclusion if they were published in the peer-reviewed literature and assessed erectile function outcomes using the IIEF-EF score. Estimates were pooled using random-effects meta-analysis.

Main Outcome Measures: Change in IIEF-EF score after treatment with Li-ESWT in patients treated with active treatment vs sham Li-ESWT probes.

Results: Data were extracted from seven trials involving 602 participants. The average age was 60.7 years and the average follow-up was 19.8 weeks. There was a statistically significant improvement in pooled change in IIEF-EF score from baseline to follow-up in men undergoing Li-ESWT vs those undergoing sham therapy (6.40 points; 95% CI = 1.78–11.02; I² = 98.7%; P < .0001 vs 1.65 points; 95% CI = 0.92–2.39; I² = 64.6%; P < .0001; between-group difference, P = .047). Significant between-group differences were found for total treatment shocks received by patients (P < .0001).

Conclusion: In this meta-analysis of seven randomized controlled trials, treatment of ED with Li-ESWT resulted in a significant increase in IIEF-EF scores.

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Key Words: Erectile Dysfunction; Shock Waves; Randomized Controlled Trial; Meta-Analysis

INTRODUCTION

Erectile dysfunction (ED) is when a man is unable to achieve or maintain an erection for satisfactory sexual performance. ED is estimated to affect one in every five men and, given the aging male population and increasing prevalence of comorbid conditions, it is likely to become even more prevalent.1 Phosphodiesterase type 5 inhibitors (PDE5is) are often effective in treating patients with ED and are associated with few side effects; however, a significant proportion of men do not respond to therapy.2 In men who do not respond to PDE5is or cannot tolerate them because of side effects, options such as medicated urethral suppositories for erection, intracorporal injections, and penile prostheses are available.3 Although these treatment options can be effective, long-term usage rates are hindered by side effects and potential complications.4 Furthermore, these treatments attempt to improve erectile function without treating the underlying pathophysiology of ED.5

Low-intensity extracorporeal shockwave therapy (Li-ESWT) has been proposed as a treatment option for ED with minimal side effects. Vardi et al6 first reported on the use of Li-ESWT for ED; their rationale was extrapolated from cardiac literature reporting improvements in neovascularization. Recent studies of a diabetic rat model have recently supported the notion that Li-ESWT indeed might induce structural changes that regenerate penile tissue.7
AIMS

Given the availability of several randomized sham-treatment—controlled trials studying the effects of Li-ESWT in the treatment of ED, we performed a meta-analysis to determine whether this novel treatment improves erectile function in men with ED when assessed by the International Index of Erectile Function erectile function domain (IIEF-EF) compared with men undergoing sham therapy. In addition, from our review of the literature, we sought to provide formal recommendations for future randomized controlled trials.

METHODS

Search Strategy

Randomized controlled trials published from January 2010 (the year that SWT was first used as a treatment for ED) through March 2016 that reported on using the IIEF-EF score for men with ED receiving Li-ESWT were identified using electronic searches of MEDLINE, EMBASE, and ClinicalTrials.gov. Additional studies were identified by scanning the reference lists of articles identified, searching relevant conference abstracts, and corresponding with study investigators using the approach recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A flow diagram for study selection is presented in Figure 1. The computer-based searches combined terms: “[shockwave] OR (shock wave) AND erectile dysfunction.”

Inclusion Criteria and Trial Selection

Studies were included if they were randomized controlled trials of Li-ESWT for ED that reported on the use of the IIEF-EF, a validated six-question questionnaire that assesses erection frequency, erection firmness, penetration ability, maintenance frequency, maintenance ability, and erection confidence on a scale of 0 to 5. The most comprehensive publication was used when there were several involving the same study population. Abstracts of randomized controlled trials from relevant conferences were included in this analysis in accordance with recommendations of the Cochrane Handbook for Systematic Reviews section 6.2.2.4.

Data Extraction

The following information was extracted independently by two trained investigators using a standardized form: authors and publication year, year of study, publication type, practice setting, duration of follow-up, population, SWT regimen, IIEF-EF (six-question form), participant inclusion and exclusion criteria, sample size, geographic locale in which the study took place, mean or median participant age, and model of Li-ESWT machine. All discrepancies were resolved by discussion and adjudication of a third reviewer. Study investigators from most studies were contacted to obtain further information.

Quality Assessment

The risk of bias in the included randomized trials was assessed using the Cochrane Risk of Bias Assessment tool in the domains of randomization, sequence generation, allocation concealment, blinding, completeness of outcome data, selective outcome reporting, and other potential sources of bias. Domains were independently assessed by two trained investigators (R.I.C. and T.P.K.). All discrepancies were resolved by discussion and adjudication by a third reviewer (R.R.). A graph and a summary for risk of bias were generated with RevMan 5.2.

Data Synthesis and Analysis

The mean differences in IIEF-EF scores measured before initiating and then after treatment with Li-ESWT or placebo were calculated for each study. Overall differences were calculated by pooling the study-specific estimates using random-effects meta-analysis that included between-study heterogeneity. Between-study heterogeneity was assessed by standard χ² tests and the I² statistic (i.e., percentage of variability in prevalence estimates because of heterogeneity rather than sampling error or chance) and by comparing results from studies grouped according to prespecified study-level characteristics (total treatment shocks, mean participant age, baseline IIEF-EF score, and duration of follow up) using stratified meta-analysis and meta-regression. The influence of individual studies on the overall summary estimates was examined by serially excluding each study in a sensitivity analysis. Bias secondary to small study effects was investigated using the funnel plot and the Egger test. All analyses were performed using R 3.2.2 (R Foundation for Statistical Computing).
tests were two-sided and used a significance threshold of a \( P \) value less than .05.

**MAIN OUTCOME MEASURES**

Difference in pooled change in IIEF-EF score from baseline to follow-up in men treated with Li-ESWT was compared with that in those treated with sham therapy.

**RESULTS**

**Study Characteristics**

Seven randomized controlled trials involving 602 participants were included in this meta-analysis (Table 1). Six studies used the Omnispec ED1000 (Medispec Ltd, Yehud, Israel) and one study used an ESWT device from Richard Wolf GmbH (Knittlingen, Germany). The mean number of participants per study was 86.4 (range = 53–135), the mean age was 60.7 years, mean baseline IIEF-EF score was 9.2, and mean follow-up was 19.8 weeks (range = 13–56). All seven studies used sham therapy for the control group using shockwave probes that looked and sounded similar to the active treatment probe. All seven studies included men with vasculogenic ED and excluded men with neurogenic ED. Four studies included men with mild, mild to moderate, moderate, and severe ED. One study included only men with mild to moderate, moderate, and severe ED. One study included only men with mild ED while on PDE5i. Two studies did not specify the severity of ED for the included patients. Seven studies consisted of regiments of two treatments per week for 3 weeks, then 3 weeks without treatment, followed by 3 weeks of two treatments per week—for a total of 18,000 total treatment shocks. One study had a regimen of one treatment every 5 weeks, 4 weeks without treatment, followed by 5 weeks with one treatment per week—for a total of 6,000 total treatment shocks. All studies included in the present analysis used an energy flux density of 0.09 mJ/mm². Five studies took place in Asia, two in Europe, and one in North America. All seven trials studied IIEF-EF score as a primary outcome. Five studies were published as journal articles and two studies were published as abstracts. Further inclusion and exclusion criteria are listed in Table 1. For most studies, the risk of bias was low. However, the risk of bias was unclear for several domains of published abstracts (eFigures 1 and 2).

**Effect of Li-ESWT on Change in IIEF-EF Score**

There was a statistically significant improvement in pooled change in IIEF-EF score from baseline to follow-up in men treated with Li-ESWT compared with those receiving sham therapy (6.40 points; 95% CI = 1.78–11.02; \( \hat{\tau}^2 = 98.7\%\); \( P < .0001 \) vs 1.65 points; 95% CI = 0.92–2.39; \( \hat{\tau}^2 = 64.6\%\); \( P < .0001 \); between-group difference, \( P = .047 \); Figure 2A, B). For each study the control group was subtracted from the treatment group to determine the between-group mean difference, which was meta-analyzed (4.17 points; 95% CI = −0.5 to 8.3; \( \hat{\tau}^2 = 98.8\%\); \( P < .0001 \); Figure 2C). The sensitivity analysis demonstrated that, for the sham treatment group, no individual study affected the overall prevalence estimate by more than an absolute difference of 0.5 point. For the Li-ESWT group, two studies (Fojecki and Osterh10 and Sirini et al11) were found to affect the overall prevalence estimate by an absolute difference of 0.5 point (eTable 1).

**Effect of Li-ESWT on Change in IIEF-EF Score According to Study-Level Characteristics**

Among the seven studies, no between-group differences were noted in sub-analyses that controlled for the potential confounders of duration of follow-up, age of participant, and baseline IIEF-EF scores (\( P > .05 \) for all comparisons; Table 2). A significant between-group difference was observed for total treatment shocks when compared by stratified meta-analysis (\( P < .001 \); Figure 3).

**Assessment of Publication Bias**

Visual inspection of the funnel plot showed minimal asymmetry for the treatment group, suggesting that the pooled estimates were unlikely to be importantly biased secondary to small study effects (eFigure 3). The Egger regression asymmetry test supported this finding (treatment: \( z = 0.14 \); \( P = .89 \)). In comparison, visual inspection of the funnel plot showed significant asymmetry for the sham group; the Egger regression asymmetry test supported this (control: \( z = 2.11 \); \( P = .03 \)). This asymmetry occurs from an increased number of small studies that reported improvement during sham therapy, which is opposite any publication bias.

**DISCUSSION**

This systematic review and meta-analysis of seven randomized controlled trials involving 691 men demonstrated a statistically significant improvement in IIEF-EF score of men with ED undergoing Li-ESWT compared with men undergoing sham therapy. This positive result suggests that Li-ESWT might clinically improve erectile function in men with ED.

It has been previously determined that a change of four points in the IIEF-EF score is the minimum clinically important difference, which indicates a difference that might be clinically meaningful to patients and potentially change management. For the trials included in this study, the combined improvement in IIEF-EF score was 4.17 after treatment with Li-ESWT, which is greater than the minimum clinically important difference. Of note, one randomized controlled trial was not included in the meta-analysis because pre- and post-treatment IIEF-EF scores were not reported and were not available after attempting to contact the investigators. This study found no difference between the treatment and control groups at 5 weeks. This study used a different device than the seven included studies.
<table>
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<th>Study</th>
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<td>37</td>
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<td>—</td>
<td>6.1 2.5</td>
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<tr>
<td>Fojecki and Osther10</td>
<td>2015</td>
<td>18</td>
<td>10</td>
<td>1</td>
<td>600</td>
<td>6,000</td>
<td>63</td>
<td>63</td>
<td>10.9</td>
<td>11.5</td>
<td>0.6 1.5 65.4 63.3 Prostatectomy; radiotherapy in pelvis; hormonal therapy against prostate cancer; anatomic penis disorder; penile prosthesis; treatment with anticoagulants (except acetylsalicylic acid 75 mg); psychiatric disorder; hypogonadism; IIEF score &gt; 2% pregnant partner or delivered within past 12 mo; critical health disease; neurologic disorders</td>
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<th>Weeks of treatment</th>
<th>Treatments/ shocks per treatment</th>
<th>Total treatment shocks</th>
<th>Sample</th>
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<td>18,000</td>
<td>40</td>
<td>20</td>
<td>12.6</td>
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ED = erectile dysfunction; IIEF-EF = International Index of Erectile Function erectile function domain; PDE5i = phosphodiesterase type 5 inhibitor; SHIM = Sexual Health Inventory for Men.
(Duolith SD1, Storz, Switzerland) and had a longer follow-up time of 24 months.

The mechanism of action that leads to improvement in IIEF scores in men treated with Li-ESWT has not been elucidated completely. In vitro and animal studies have shown that SWT can promote neovascularization and expression of pro-angiogenesis markers resulting in remodeling of tissue. Studies on the effect of SWT on penile tissue in rats have shown improvement in erectile function and regeneration of endothelium, smooth muscle, and nerves expressing neuronal nitric oxide synthase. Although no histologic or gene expression studies have been carried out in human tissue, using an established protocol, several groups have reported a statistically significant improvement in flow-mediated dilatation in patients treated with Li-ESWT, indicating improvement in penile hemodynamics and endothelial function. A recent study of mice as a model of type 2 diabetes treated with Li-ESWT found that Li-ESWT improved erectile function, but not through the expected mechanism dependent on nitric oxide and cyclic guanosine monophosphate. Thus, currently, Li-ESWT is believed to be effective primarily by regenerating microvasculature and improving penile hemodynamics; this could explain why it has been studied mainly in men with vasculogenic ED and not in men with neurogenic ED.

This study is not the first meta-analysis to publish on Li-ESWT and ED. In a meta-analysis published by Lu et al, men with ED, Peyronie’s disease, and chronic pelvic pain were included. With this heterogeneous population, they found the average IIEF-EF score difference between the treatment group and the control group was 2.00. In the present study, the average IIEF-EF score difference was 4.17, a clinically significant improvement. In addition, Lu et al included randomized controlled trials and cohort studies. With the inclusion of cohort studies, Lu et al presented their meta-analytic findings at a level of evidence of 2a. Although we emphasize that we are not the first to report a systematic review and meta-analysis on the use of Li-ESWT in the treatment of ED, our study differs in that it is the first to publish on a homogenous population of men with only ED. Furthermore, our meta-analysis includes only randomized controlled trials and thus can be regarded as level 1a evidence.

Our study has important strengths and limitations. This is the first meta-analysis published on Li-ESWT that specifically reports on only men with ED, demonstrating a significant clinical and statistical improvement. All seven trials included were randomized controlled trials with sham therapy. However, most included trials had small samples; the largest study included in our meta-analysis had only 135 men. Two studies were published as abstracts. Study investigators for the abstracts were contacted for further information, and we received, for our review, a prepared report for one and a study protocol for the other. Although we are uncertain of the current publication status of these two abstracts, we are confident after thorough review of the data presented that the quality of evidence presented is similar to those presented in the peer-reviewed articles. Follow-up was limited to approximately 1 year in most studies and only one study provided follow-up data beyond 1 year. Data on the use of PDE5i during Li-ESWT treatment were available in five studies; the remainder did not report these data. The study by Kitrey et al was the only one in which patients used PDE5i during the SWT phase. Our study also had

**Table 2. Meta-regression by age and total shock energy**

<table>
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<tr>
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<th>Upper CI</th>
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<td>0.05</td>
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<tr>
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<td>0.60</td>
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<td>.53</td>
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<td>-0.05</td>
<td>-0.36</td>
<td>0.26</td>
<td>0.10</td>
<td>.75</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.41</td>
<td>-0.95</td>
<td>0.14</td>
<td>2.36</td>
<td>.14</td>
</tr>
<tr>
<td>Baseline IIEF-EF score</td>
<td>-0.37</td>
<td>-2.80</td>
<td>2.07</td>
<td>0.09</td>
<td>.77</td>
</tr>
</tbody>
</table>

IIEF-EF = International Index of Erectile Function erectile function domain.
increased heterogeneity ($I^2 = 99.4\%$), which can be attributed to two studies (Fojecki and Osher\textsuperscript{10} and Sirini et al\textsuperscript{11}) that, when systematically omitted from the sensitivity analysis, caused the overall effect to change by more than 0.5. One possible cause for this heterogeneity could be treatment regimen and subject selection. The study published by Fojecki and Osher showed minimal difference between the treatment and sham groups, which can be explained by the variation in treatment protocol. Fojecki and Osher used a total of 6,000 treatment shocks over 10 weeks, whereas all other studies used 18,000 treatment shocks over 9 weeks. Conversely, Sirini et al described a greater average treatment effect compared with all other treatment groups, which might be explained by their subject selection. The study by Sirini et al is the only one that screened men by ultrasound for vasculogenic ED; thus, they might have selected study participants who were more apt to respond to Li-ESWT. When these two trials are omitted, the heterogeneity significantly decreases ($I^2 = 0\%$) and the total treatment effect is 6.17, very similar to the original calculated treatment effect of 6.40.

Currently, it is unclear where Li-ESWT fits in the current treatment algorithm for ED. The most recent update to the European Association of Urology guidelines on male sexual dysfunction lists SWT as a potential treatment option for ED, but the association refrains from giving any recommendations at this time because of the immaturity of available data.\textsuperscript{9} The American Urological Association currently does not include SWT in its guideline on management of ED. Because no prior meta-analysis has been performed synthesizing only randomized controlled trials, this study sheds light on the effectiveness of Li-ESWT in treating ED.

However, as with many therapies, patient selection is likely to be crucial in maximizing the benefits of Li-ESWT. Results of the two randomized controlled trials in this study and the single-arm studies show that factors such as older age, several comorbidities, longer duration of ED,\textsuperscript{37,38} lower baseline IIEF-EF score, and poor initial response to PDE5i can undermine the overall effect of Li-ESWT in the improvement of the IIEF-EF score.\textsuperscript{8,13,39,40} Although our findings indicate an improvement for those undergoing Li-ESWT, more randomized controlled trials are warranted before the acceptance of this treatment becomes widespread. From our review of the literature, we put forth these recommendations for future studies: future studies should be randomized; subjects should be screened by penile Doppler ultrasound and nocturnal penile tumescence to ensure only men with vascular ED are included; the duration of follow-up should be longer than 3 months; other treatment schedules ought to be trialed to determine optimum effect; control groups should undergo sham treatment; PDE5is should be stopped completely and with appropriate washout periods; all studies should be registered on trial registry sites; and all studies should report all adverse events. It seems reasonable that future trials should start with using 18,000 shocks. Because no significant adverse effects have been reported, a more condensed protocol shorter than 6 weeks could be attempted. However, spacing out treatments could end up being more beneficial because of some yet unknown effect on penile physiology.

CONCLUSION

In this meta-analysis of randomized controlled trials evaluating the effect of Li-ESWT on ED, the improvement in IIEF-EF scores was statistically significant for men who underwent Li-ESWT compared with those who underwent sham therapy.
However, more stringent randomized controlled trials are warranted before there is widespread acceptance of this treatment.

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REFERENCES


SUPPLEMENTARY DATA

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Low-Intensity Extracorporeal Shock Wave as a Novel Treatment for Erectile Dysfunction

Michael M. Pan, MD¹, Ayman Raees, MD, FRCSC², and Jason R. Kovac, MD, PhD, FRCSC²

Abstract

The paradigm of erectile dysfunction (ED) treatment was fundamentally altered following the introduction of oral phosphodiesterase type 5 inhibitors. Unfortunately, a significant number of men exhibit a suboptimal response and require additional management strategies. One of the novel, minimally invasive strategies being developed is low-intensity extracorporeal shock wave therapy. Used in the hope of delaying placement of an inflatable penile prosthesis, the final phase of ED treatment, low-intensity extracorporeal shock wave therapy is a unique application of an established technology that may hopefully one day expand the medical options for patients with ED. This commentary will highlight the physiology underlying this technique and summarize the most recent studies.

Keywords

erectile dysfunction, shock wave therapy, growth factor, novel therapies, angiogenesis

Introduction

Erectile dysfunction (ED), a common disorder affecting men, is defined as the inability to achieve and maintain erections adequate for sexual intercourse (Garcià, Castagna, Francomano, Cerminara, & De Fazio, 2014). Pharmacological options such as phosphodiesterase type 5 inhibitors (PDE5i), while effective, do not produce satisfactory results in all men (Guay, Perez, Jacobson, & Newton, 2001). Given that recent studies suggest ~18% of patients do not respond to PDE5is (Guay et al., 2001), there has been an increased focus on developing novel therapies for management. Particularly, interest has centered on treatment methods that fundamentally alter spontaneous erectile function in a robust and enduring way.

Low-Intensity Extracorporeal Shock Wave Therapy (LI-ESWT)

LI-ESWT has been previously used in the treatment of a wide variety of conditions (Skolarikos, Alargof, Rigas, Deliveliotis, & Konstantinidis, 2005). The theories for application are based on prior experiences with acoustic waves generating pressure impulses targeted to treat kidney stones (high-intensity waves), tendinitis (medium-intensity waves), as well as Peyronie’s disease, peripheral neuropathy, cardiac, and peripheral vascular disease (low-intensity waves; Ciccone et al., 2012; Skolarikos et al., 2005; Zuoziene, Lausevicius, & Leibowitz, 2012).

Recently, LI-ESWT (administered at 7.33 MPa at 2 Hz for 300 shocks per treatment) has been considered a potential modality for the management of ED (Liu et al., 2013). Since LI-ESWT for ED is still in its infancy, the mechanisms by which LI-ESWT acts to improve ED symptoms are incompletely understood. However, it has been postulated to be related to cell membrane microtrauma and mechanical stress. The resultant release of angiogenic factors, such as vascular endothelial growth factor (VEGF), nitric oxide synthase (NOS), von Willebrand factor (vWF), fibroblast growth factor (FGF), TLR3, and the pro-inflammatory cytokines IL-6 and IL-10, result in increased angiogenesis and vascularization of target tissues (Gruenwald, Appel, Kitrey, & Vardi, 2013; Holfeld et al., 2014; Nishida et al., 2004; Wang, Yang, & Huang, 2011). Recruitment of circulating endothelial progenitor cells through chemotactant factors such as VEGF and stromal cell derived factor 1 has also been reported to

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result from LI-ESWT (Aicher et al., 2006). Based on these findings, it is suggested that LI-ESWT could increase blood flow and endothelial function in the penis thereby improving erectile function.

Based on animal studies, it is tempting to speculate that LI-ESWT may be beneficial for patients with diabetes-induced ED. This belief is founded in basic laboratory research where diabetic rats were administered LI-ESWT and noted to exhibit improvements in penile blood flow, intracavernous pressures, as well as increased VEGF, neuronal NOS-positive neurons, alpha-smooth muscle actin (α-SMA), vWF, and endogenous mesenchymal stem cells compared with controls (Liu et al., 2013; Qiu et al., 2013). Improvements were also noted in the percentage of elastic fibers within the corpus cavernosum as well as the smooth muscle to collagen ratio. Furthermore, a decreased expression of cavernosal receptors for advanced glycosylation end-products (RAGE) was identified. Given that RAGE molecules have been attributed to a component of the vascular and neurological damage seen in diabetes, the decrease in RAGE following LI-ESWT hints at a potential to reverse and/or prevent diabetes-induced ED (Liu et al., 2013). The exact mechanism regulating diabetes-induced ED is unclear. Furthermore, the results of animal data must be tempered with the fact that similar improvements have not yet been observed, or studied, in humans.

Results of LI-ESWT in initial human trials are promising but still in the investigational stage. As such, only a few studies exist and are detailed as follows. An initial pilot study examined the responses to LI-ESWT of 20 men with vasculogenic ED (Vardi, Appel, Jacob, Massarwi, & Gruenwald, 2010). Findings were promising with improvements in the International Index of Erectile Function (IIEF) scores at both 1 and 6 months. The same group of authors then followed this initial article with an open-label, prospective study on patients with severe ED who previously failed PDE5i therapy (Gruenwald, Appel, & Vardi, 2012). In these patients (n = 29), a similar protocol was followed as to the first report. Specifically, two LI-ESWT treatment sessions were administered per week for 3 weeks, and separated by a 3-week period of no treatment. This resulted in a significant mean increase of 3.5 points in ED scores within the IIEF (Gruenwald et al., 2012). Furthermore, of those treated with LI-ESWT, 65% had a ≥ 5-point greater increase in total IIEF score compared with only 20% in controls (Gruenwald et al., 2012; Gruenwald et al., 2013). Men treated with LI-ESWT also had significantly improved penile hemodynamics and increased blood flow by veno-occlusive strain gauge plethysmography. Unfortunately, both studies were limited by a very small numbers of patients, nonrandomized data, and a very short duration of follow-up (Gruenwald et al., 2012; Vardi et al., 2010).

A more recent article from the same group (Vardi, Appel, Kilchevsky, & Gruenwald, 2012) attempted to address a few of these concerns with a randomized, double-blind, sham controlled study. A total of 77 men underwent initial screening, and inclusion criteria were an IIEF-EF score of >19 while on PDE5i and a stable, heterosexual relationship for 3 months. Exclusion criteria were prior prostatectomy, pelvic radiotherapy, hormonal therapy, and current treatment for a psychiatric condition or any anatomical, neurological, or hormonal abnormalities (Vardi et al., 2010). This led to a randomization of 67 patients of which there was a 5% to 13% dropout for a final completed patient base of 20 in the sham group and 40 in the LI-ESWT group. Significant improvements were seen in multiple components of the IIEF as well as in the penile hemodynamics. A 25% sham effect was also observed, as was an unexpected (and unexplained) improvement in the Sexual Desire subdomain. Furthermore, no statistically significant improvements in the IIEF sexual satisfaction scores were noted (Vardi et al., 2010). While no patients experienced any adverse events (i.e., pain, hematoma, hematursia, and bruising) in any of the aforementioned trials, the authors did concede that the long-term risk of LI-ESWT remains unknown.

A more recent article by Yee, Chan, Hou, and Ng (2014) is the first from another group examining LI-ESWT. This study had only a 2-week washout for PDE5i (while the Vardi group discussed above chose a 4-week washout). Moreover, while Yee et al. (2014) employed a similar LI-ESWT treatment protocol based on time, intensity, and frequency as the Vardi group, follow-up was limited to 13 weeks postintervention. The Sham (n = 28 patients) and Treatment groups (n = 30 patients) did not reach statistical significance with respect to the IIEF-ED or the Erectile Hardness Score (Yee et al., 2014). When a subgroup analysis was performed, those patients with baseline "severe" ED had significantly improved IIEF-ED scores.

Conclusions

Taken together, initial reports suggest that LI-ESWT appears to be a safe and effective treatment for ED. Difficulties surrounding the treatment exist in the extensive time commitments required for the protocols as well as the cost and availability of the device. In total, 167 patients have been reported on in the literature from all of the available studies published to date (Gruenwald et al., 2012; Vardi et al., 2010; Vardi et al., 2012; Yee et al., 2014).

Improvements are postulated to be because of shockwave-induced microtrauma with subsequent angiogenic neovascularization and improvement in penile blood flow (Liu et al., 2013; Qiu et al., 2013; Vardi et al., 2012). However, further studies are needed. Randomized control trials with a greater number of patients will help determine the efficacy and safety of LI-ESWL for ED.
Measurement of other parameters may also provide useful information. Similar to animal experiments, aspiration of corporal blood for VEGF levels may provide direct evidence that neovascularization also occurs after LI-ESWT in humans. Dynamic contrast-enhanced magnetic resonance imaging can also functionally characterize tissue perfusion and a biomarker for angiogenesis in tumors and may provide a way to measure the degree of neovascularization in patients after LI-ESWT (Teo, Thng, Koh, & Ng, 2014). Sampling of penile tissue after treatment for immunohistochemistry and evaluation of smooth muscle and endothelial content, although invasive, may provide further evidence of angiogenesis. As such, while still in its infancy, LI-ESWT presents an attractive, noninvasive option for the management of ED.

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