In Situ Activation of Penile Progenitor Cells With Low-Intensity Extracorporeal Shockwave Therapy

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ABSTRACT

Background: We previously reported that progenitor cells, or stem cells, exist within penile tissue. We hypothesized that acoustic wave stimulation by low-intensity extracorporeal shockwave therapy (Li-ESWT) would activate local stem or progenitor cells within the penis, producing regenerative effects.

Aims: To study the feasibility of in situ penile progenitor cell activation by Li-ESWT.

Methods: We performed a cohort analysis of young and middle-age male Sprague-Dawley rats treated with 5-ethynyl-2′-deoxyuridine (EdU) pulse followed by Li-ESWT. In addition, Li-ESWT was applied to cultured Schwann cells and endothelial cells to study the molecular mechanism involved in cell proliferation. Thirty minutes before Li-ESWT, each rat received an intraperitoneal injection of EdU. Li-ESWT was applied to the penis at very low (0.02 mJ/mm^2 at 3 Hz for 300 pulses) or low (0.057 mJ/mm^2 at 3 Hz for 500 pulses) energy levels. The endothelial and Schwann cells were treated with very low energy (0.02 mJ/mm^2 at 3 Hz for 300 pulses) in vitro.

Outcomes: At 48 hours or 1 week after Li-ESWT, penile tissues were harvested for histologic study to assess EdU$^+$ and Ki-67$^+$ cells, and cell proliferation, Ki-67 expression, Erk1/2 phosphorylation, translocation, and angiogenesis were examined in cultured Schwann and endothelial cells after Li-ESWT.

Results: Li-ESWT significantly increased EdU$^+$ cells within penile erectile tissues ($P < .01$) at 48 hours and 1 week. There were more cells activated in young animals than in middle-age animals, and the effect depended on dosage. Most activated cells were localized within subtunical spaces. In vitro studies indicated that Li-ESWT stimulated cell proliferation through increased phosphorylation of Erk1/2.

Clinical Translation: The present results provide a possible explanation for the clinical benefits seen with Li-ESWT.

Strengths and Limitations: The main limitation of the present project was the short period of study and the animal model used. Li-ESWT could be less effective in improving erectile function in old animals because of the decreased number and quality of penile stem or progenitor cells associated with aging.

Conclusion: Li-ESWT activation of local penile progenitor cells might be one of the mechanisms that contribute to the beneficial effects of shockwave treatment for erectile dysfunction, which represents a non-invasive alternative to exogenous stem cell therapy. **Lin G, Reed-Maldonado HB, Wang B, et al. In Situ Activation of Penile Progenitor Cells With Low-Intensity Extracorporeal Shockwave Therapy. J Sex Med 2017;XX:XXX–XXX.**

INTRODUCTION

Currently, most patients with erectile dysfunction (ED) can be satisfactorily treated with phosphodiesterase type 5 (PDE5) inhibitors. However, clinically, up to 30% to 40% of patients with ED are intolerant to the side effects of PDE5 inhibitors, are taking nitrate medication for angina, or have certain types of ED refractory to PDE5 inhibitors.[1] More importantly, PDE5 inhibitors merely treat ED symptoms without correcting 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.[2] Therefore, a novel therapeutic approach aimed at correcting the underlying pathophysiology is desperately needed.

Low-intensity extracorporeal shockwave therapy (Li-ESWT) has been used for years to treat musculoskeletal disorders,[3] ischemic heart disease,[4,5] and vasculogenic ED.[6] Since 2010, Li-ESWT has been used successfully for the treatment of ED in clinical settings, and undeniable improvements in the International Index of Erectile Function score and the Erection Hardness Score have been reported after Li-ESWT.[7] Diabetic ED[8–11] and penile neurovascular injury ED[8] animal models have been studied to explore the mechanism related to these therapeutic effects from Li-ESWT in vivo in rats. Results have indicated that Li-ESWT releases growth factors, which in turn trigger revascularization of the tissue with subsequent improvement of the blood supply[12] and promote nerve regeneration.[10] In our previous report, we found that the therapeutic effects from Li-ESWT might be related in part to the increase of local progenitor cells.[8,10] However, the underlying mechanism of Li-ESWT has not been thoroughly investigated and is not clearly understood.

Adult tissues, such as bone marrow, skin, muscle, and adipose tissue, contain stem or progenitor cells.[13] We previously identified the penile progenitor cells.[14] In general, these endogenous stem cells (SCs) possess a remarkable ability to divide and multiply and to differentiate into different cell types. These processes are induced by influences from inside and outside the body. At baseline, endogenous SCs exist in a quiescent state, which is characterized by a low metabolic rate, lower mitochondrial content, and decreased mitochondrial activity.[15,16] A strategy to commandeer the specific molecular machinery responsible for endogenous SC activation, self-renewal, and proliferation in the penile erectile tissue is strongly desired. Evidence from basic science experiments[8–10] and clinical trials[7] has suggested that the therapeutic effect of Li-ESWT in ED might be similar to the induction of stem or progenitor cell proliferation, migration, and differentiation in wound healing.[17]

The ability to therapeutically activate, proliferate, and differentiate endogenous mesenchymal SCs in a discriminant, non-invasive fashion would be a powerful treatment approach for many diseases, such as ED. We have developed a non-invasive therapeutic approach to activate endogenous penile progenitor cells in situ and thus improve penile function. We confirmed the in vivo activation of penile progenitor cells through histologic studies of the location and number of cells expressing the proliferating cell markers 5-ethynyl-2′-deoxyuridine (EdU) and Ki-67.

METHODS

Experimental Design

All animal experiments in the present study were approved by the University of California–San Francisco institutional animal care and use committee. Thirty male Sprague-Dawley rats (young = 12 weeks old, middle age = 36 weeks old; Charles River Laboratories, Wilmington, MA, USA) were used to assess the biological effects of Li-ESWT in vivo. Each group was divided into a control cohort and a Li-ESWT cohort. Animals in the Li-ESWT groups were treated with very low energy or low energy level as follows. Thirty minutes before the Li-ESWT treatment, each rat received an intraperitoneal injection of EdU 50 mg/kg (Invitrogen, Carlsbad, CA, USA) to identify penile progenitor cells. These cells incorporate EdU during the S-phase of the cell cycle. At different time points, 48 hours or 1 week, after treatment, the penile tissue was harvested for histologic study (Figure 1).

Low-Intensity Energy Shockwave Therapy

Rats in the Li-ESWT groups underwent shockwave under isoflurane anesthesia; each rat was placed in the prone position with its lower abdomen shaved. After application of ultrasound gel (Aquasonic, Parker Laboratories, Inc, Fairfield, NJ, USA), a special probe attached to a compact electromagnetic unit with a semi-focused shockwave source (LiteMed Inc, Taipei, Taiwan) was placed in contact with the pelvic region to include the penis and the major pelvic ganglia in the treatment zone. Based on our previous experiments,[8–10] we chose two levels of acoustic energy: very low (L2, 0.02 mJ/mm^2 at 3 Hz for 300 pulses) or low (L6, 0.057 mJ/mm^2 at 3 Hz for 500 pulses). In this project, the penile hemodynamic study was not conducted.

Histology and Immunofluorescence Staining

The penile tissues were harvested at the indicated time points and fixed in cold 2% formaldehyde and 0.002% saturated picric
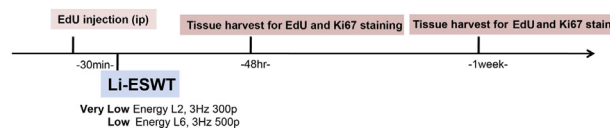

Figure 1. Experimental protocol. Animals were treated with an EdU pulse followed by Li-ESWT, and histology was performed to detect EdU$^+$ penile progenitor cells at 48 hours and 1 week after treatment. EdU = 5-ethynyl-2′-deoxyuridine; ip = intraperitoneal; Li-ESWT = low-intensity extracorporeal shockwave therapy.

acid in phosphate buffer 0.1 mol/L (pH = 8.0) for 4 hours followed by overnight immersion in buffer containing 30% sucrose. The specimens were embedded in OCT compound (Sakura Finetic USA, Torrance, CA, USA) and stored at $-70°C$ until use. Fixed frozen tissue specimens were cut at 10 μm, mounted onto SuperFrost-Plus charged slides (Fisher Scientific, Pittsburgh, PA, USA), and air dried for 5 minutes. The tissue section was subjected to EdU staining with or without immunostaining for Ki-67. For immunostaining, the slides were placed in 0.3% H_2O_2 and methanol for 10 minutes, washed twice in phosphate buffered saline (PBS) for 5 minutes, and incubated with 3% horse serum in PBS and 0.3% Triton X-100 for 30 minutes at room temperature. After draining this solution from the tissue section, the slides were incubated at room temperature with anti−Ki-67 antibody (1:500; Abcam Inc, Cambridge, MA, USA) for 1.5 hours. Control tissue sections were similarly prepared except no primary antibody was added. After rinses with PBS, the sections were incubated with fluorescein isothiocyanate−conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). After rinses with PBS, the slides were incubated with freshly made Click-iT reaction cocktail (Thermo Fisher Scientific Inc, Waltham, MA, USA) for 30 minutes at room temperature without light followed by staining with 4′,6-diamidino-2-phenylindole 1 μg/mL (for nuclear staining; Sigma-Aldrich, St Louis, MO, USA). Immunofluorescence staining with Ki-67 and pErk1/2 was conducted as previously reported.[14]

Primary Schwann Cell Isolation and Culture

Rat Schwann cells were harvested as previously described.[8,18] Briefly, sciatic nerves were harvested from Sprague-Dawley rats and enzymatically dissociated by incubation at 37°C sequentially with 1% collagenase and 0.125% trypsin for 30 and 10 minutes, respectively. The mixture was triturated, centrifuged, and resuspended in 10% fetal bovine serum in Dulbecco's Modified Eagle Medium. The cell pellets were plated on dishes pre-coated with poly-L-lysine for incubation in the same medium. On the following day, cytosine arabinoside 10 μmol/L was added and allowed to incubate for an additional 48 hours to remove fibroblasts. The cell culture was maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, forskolin 2 μmol/L (Sigma, St Louis, MO, USA), and heregulin 2 ng/mL (Sigma) to stimulate Schwann cell proliferation. For further purification, the cell culture was gently trypsinized, pelleted, and incubated with anti-Thy1 antibody (AbD Serotec, Raleigh, NC, USA) on ice for 2 hours, followed by incubation in complement (Jackson ImmunoResearch Laboratories) for an additional 2 hours.

Effect of Low-Intensity Shockwave on Activating Schwann Cells In Vitro

Schwann cells were resuspended in fresh, pre-warmed (37°C) complete medium. The Schwann cells were counted and plated on 96-well plates pre-coated with 0.01% poly-L-lysine. The cells were treated with or without Li-ESWT at 0.02 mJ/mm^2 at 3 Hz for 300 pulses. Growth curves were generated using the MTT assay to measure changes in cell number at 24, 48, 72, and 96 hours after Li-ESWT. The phosphorylation level of Erk1/2 in the cells was checked with western blot, and the location of activated Erk1/2 was checked with immunofluorescence as previously reported.[19] In addition, expression of Ki-67 in those cells was checked 48 hours after Li-ESWT.

Effect of Low-Intensity Shockwave on Activating Endothelial Cells In Vitro

Human umbilical vein endothelial cells (HUVECs) were used and maintained in endothelial cell basal medium supplemented with Bullet Kit (EBM-2, Lonza Inc, Walkersville, MD, USA) in culture flasks coated with 0.1% gelatin and maintained at 37°C with humidified 5% carbon dioxide. HUVECs cultured from passages 4 to 8 were used for this experiment. HUVECs were treated with or without Li-ESWT at the energy level of 0.02 mJ/mm^2 at 3 Hz for 300 pulses. For the tube formation assay, a total of 30,000 HUVECs with different treatments were seeded in Matrigel in serum-free medium in 24-well plates in triplicate and incubated at 37°C for 6 hours. Tubules were visualized by light microscopy at low magnification (40×). Photomicrographs from each well were captured, and total tubule length and number of tubules were analyzed using ImageJ 2.02 (National Institutes of Health, Bethesda, MD, USA).

Image and Statistical Analyses

For image analysis, five randomly selected fields per slide for each treatment group were photographed and recorded using a Retiga Q Image digital still camera and ACT-1 software (Nikon Instruments Inc, Melville, NY, USA). The images were quantified using Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA). Total EdU$^+$ cells in each section were counted double-blindly by different investigators. Data were analyzed using Prism 5 (GraphPad Software, San Diego, CA, USA) and expressed as mean ± standard error of mean. Multiple groups were compared using t-test and one-way analysis of variance followed by the Tukey-Kramer test for post hoc comparisons. Statistical significance was set at a P value less than .05.

RESULTS

Li-ESWT Activates Penile Progenitor Cells in Young and Middle-Age Rats

Previously reported results have shown that Li-ESWT alleviates ED in neurovascular and diabetic ED animal models[10,11]; however, the underlying mechanisms are not well elucidated. Therefore, to clarify the possible mechanisms of these functional improvements and histologic changes, we examined penile cell proliferation after Li-ESWT. For this purpose, EdU pulsing was used in the present project.

In the young rats, Li-ESWT significantly increased EdU$^+$ cells within penile erectile tissues ($P < .01$) at 48 hours and 1 week. This finding strongly suggests that penile progenitor cells can be "activated" by appropriate levels of Li-ESWT. Low-energy Li-ESWT activated more penile progenitor cells than very low-energy Li-ESWT in the young rats (Figure 2). The difference between the middle-age rats and young rats also was significant: Li-ESWT activated fewer penile cells in middle-age animals compared with young animals. The middle-age rats had one fifth the cellular response that the young rats had at the very low-energy setting, and the use of low-energy Li-ESWT doubled this activation. Interestingly, in the low-energy group of middle-age rats, EdU$^+$ cells activated by Li-ESWT decreased significantly by 1 week after Li-ESWT ($P < .01$; $P < .05$; Figure 2), which suggests that some activated EdU$^+$ cells proliferate and become terminally differentiated cells and thus lose the EdU marker.

Location of Penile Progenitor Cells Activated by Li-ESWT

We previously reported that penile progenitor cells exist in the subtunical and para-sinusoidal regions within the penis.[14] In the present project, the location of the EdU$^+$ cells after Li-ESWT was extensively studied in all tissue samples. As observed in Figure 2, most EdU$^+$ cells were localized in the subtunical region, and, interestingly, some cells were clustered together within the tunica (Figure 3).

Approximately 70% to 80% of EdU$^+$ cells localized in the subtunical space, 10% to 19% localized in the para-sinusoid area,

1.9% to 5.3% localized in the penile nerve, and approximately 3.8% to 6.7% localized in penile blood vessels. Interestingly, very low-energy Li-ESWT activated more progenitor cells in the penile nerves and blood vessels compared with low-energy Li-ESWT ($P < .01$). There was no significant difference between very low- and low-energy treatments in the subtunical and para-sinusoidal space distribution ($P > .05$). In addition, there were some EdU$^+$ cells within the penile dorsal nerve, which implies that Schwann cells were activated by Li-ESWT, similar to our previous report.[8] EdU$^+$ cells also were observed in small vessels, including capillaries, arterioles, and venules, which implies that the endothelium also was activated by Li-ESWT (Figure 3).

Cellular Markers of Penile Progenitor Cells Activated by Li-ESWT

To define those EdU$^+$ cells activated by Li-ESWT, we stained for antigen Ki-67, a nuclear marker of cell proliferation. Interestingly, cells expressing Ki-67 do not colocalize with EdU$^+$ cells (Figure 4), which suggests that the two markers might identify proliferating cells in different stages of the cell cycle. Only cells at the S-phase of the cell cycle incorporate EdU and thus stain strongly.

Low-Intensity Shockwave Activates Schwann Cells In Vitro

As we previously noted, Schwann cells were activated by Li-ESWT in vivo. To confirm this effect, we isolated the primary
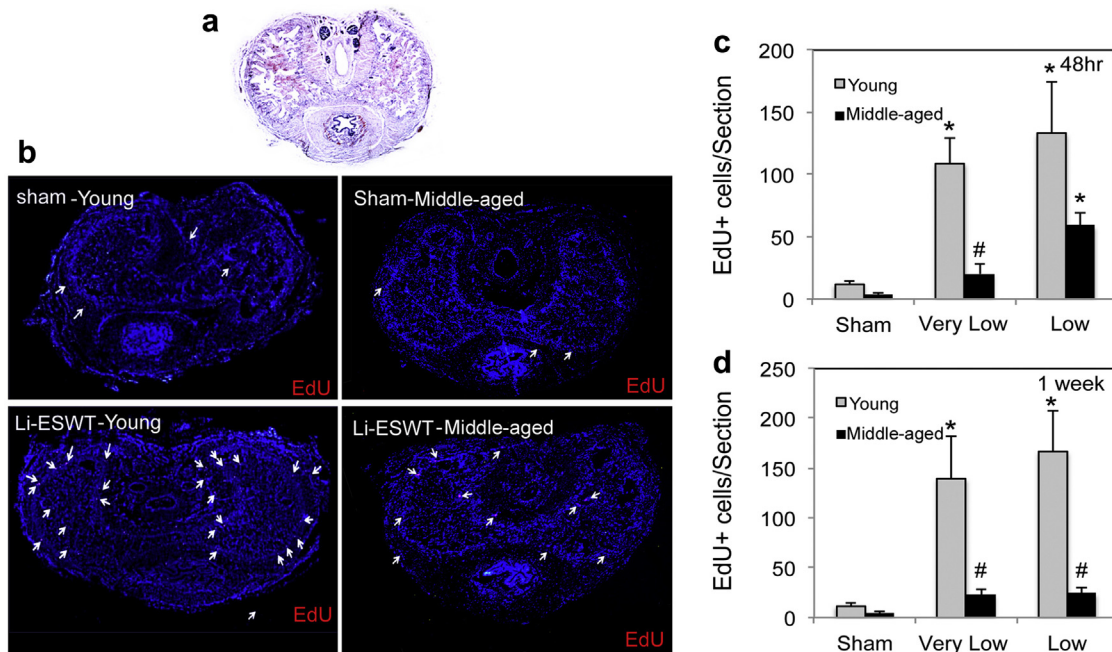

Figure 2. Representative images show actiation and proliferation of penile progenitor cells by Li-ESWT. Panel A shows a cross-section of a rat's penis. Panel B shows EdU$^+$ penile progenitor cells in young (left) and middle-age (right) rats of the sham control (top) and Li-ESWT (bottom) groups. Most EdU$^+$ cells (arrows) are localized in the subtunical region. Panel C shows a comparison of EdU$^+$ penile progenitor cells between young and middle-age rats 48 hours after Li-ESWT. Panel D shows more EdU$^+$ penile progenitor cells in young rats 1 week after Li-ESWT (*$P < .01$; #$P < .05$). EdU = 5-ethynyl-2'-deoxyuridine; Li-ESWT = low-intensity extracorporeal shockwave therapy.
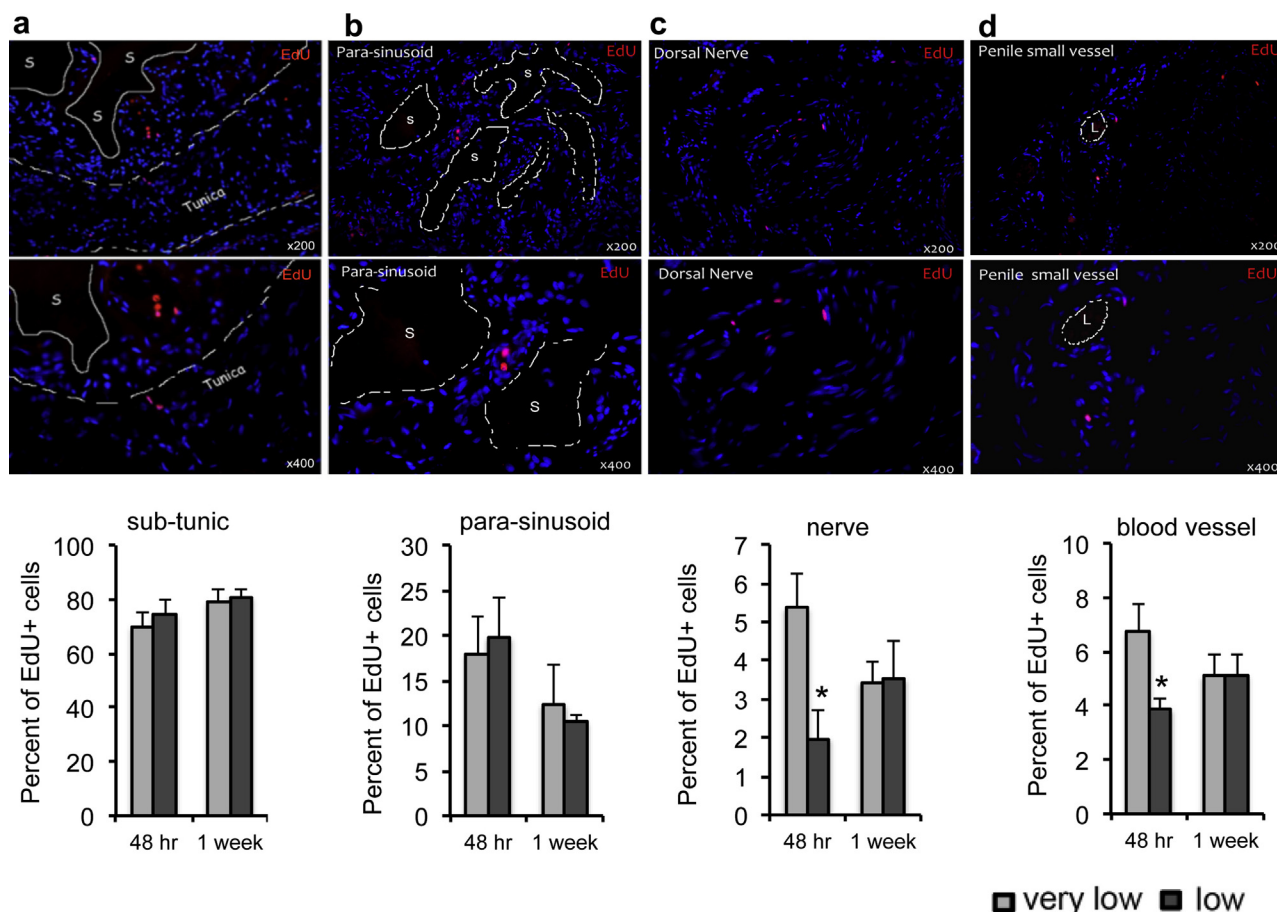
Figure 3. Location of EdU$^+$ penile progenitor cells (red areas) after low-intensity extracorporeal shockwave therapy. Panel A shows the penile subtunical region (70%–80% localized cells). Panel B shows the para-sinusoid region (10%–19% localized cells). Panel C shows the penile dorsal nerve (1.9%–5.3% localized cells). Panel D shows the penile small blood vessel (3.8%–6.7% localized cells). Upper panels are magnified ×200, and lower panels are magnified ×400. EdU = 5-ethynyl-2′-deoxyuridine; L = lumen; S = sinusoid (*$P < .01$).

Schwann cells and treated them with Li-ESWT. Li-ESWT promoted Schwann cell proliferation significantly at 48 hours after treatment ($P < .05$; Figure 5A), and this effect lasted for 96 hours. Those activated Schwann cells expressed high levels of Ki-67 ($P < .05$; Figure 5B, C). To explore the underlying mechanism of this activation, the phosphorylation level of the Erk1/2 pathway was checked in those cells. This demonstrated that low-energy shockwave increased Erk1/2 phosphorylation significantly ($P < .05$; Figure 5D) and that activated Erk1/2 translocated into the cell nucleus (Figure 5E).

Li-ESWT Activates Endothelium and Promotes Angiogenesis

It has been reported that Li-ESWT promotes angiogenesis mainly through enhanced vascular endothelial growth factor expression. In the present experiment, we assessed the effect of Li-ESWT on new blood vessel formation in vitro. HUVECs formed a robust tube network within 6 hours after seeding after Li-ESWT. The tube length and branch points increased approximately 42% and 43%, respectively, compared with that of the control groups ($P < .05$; Figure 6).

DISCUSSION

Since the first reported use of SCs for ED therapy in 2004,[20] SC-based therapies have been extensively studied in the management of ED with the goal of complete replacement of lost or damaged cells.[21] In recent years, a spate of reports related to the progress of SC-based ED therapy has been published.[22] Different therapeutic forms of SCs have been developed, including multiple sources of SCs, gene-transfected SCs, SC lysate, and SCs seeded on tissue matrices.[23] However, in recent years, tremendous limitations in the use of exogenous SCs for ED therapy have become obvious. These include the need for invasive tissue harvest, complex isolation techniques, issues related to incorporation of exogenous proteins during cell culture, and concerns about finding the few SCs remaining in the penis after transplantation. More importantly, migration of implanted SCs to existing malignant tumors, enhancing tumor growth, also has been reported in animal experiments.[24] We previously reported that there are endogenous stem or progenitor cells in penile erectile tissue.[14] Therefore, local activation of penile endogenous SCs for ED would be an ideal approach for ED to avoid many of the aforementioned limitations to the use of exogenous SCs.
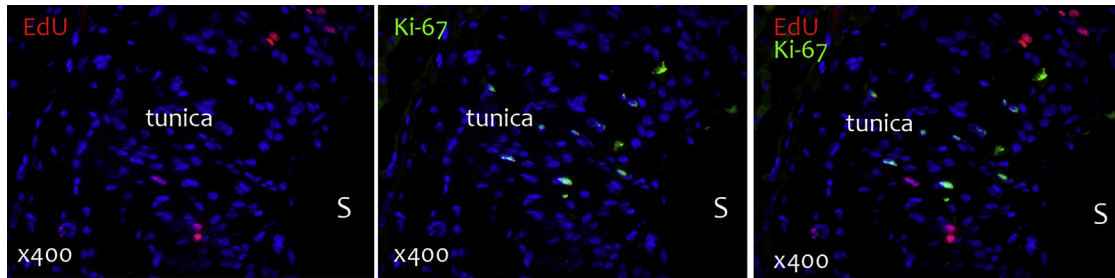
Figure 4. Expression of Ki-67 (green areas) and EdU+ (red areas) in penile progenitor cells after low-intensity extracorporeal shockwave therapy. EdU = 5-ethynyl-2′-deoxyuridine; S = sinusoid.

Of course, a well-designed comparison study will be needed to confirm this conjecture.

Signals that play critical roles in SC activation include soluble SC niche signals (growth factors and cytokines), whereas the fate of SCs is influenced by coexisting adhesive, mechanical, and topologic cues.[25] For decades, scientists have attempted to use chemistry to steer the fate of SCs, but with limited success.[26,27] Recent demonstrations of the effects of low-energy shockwave on SCs in culture have suggested the possibility of using mechanobiological methods to drive the growth and fate SCs in vivo,[28] thus avoiding the requirement for SC harvest, culture, preparation, and transplantation.[29]

It has been well demonstrated that Li-ESWT subjectively and objectively improves erectile function. However, the mechanisms underlying these beneficial effects have yet to be fully elucidated. In the penis there are many kinds of cells, including terminally differentiated cells and stem and progenitor cells, as we reported in 2015.[14] Most penile smooth muscle cells and fibroblasts are terminally differentiated and cannot be activated to proliferate. In contrast, penile progenitor cells, including subtunical penile progenitor cells, para-sinusoidal penile progenitor cells, Schwann cell progenitor cells, and endothelial progenitor cells, can be activated to re-enter the cell cycle and to proliferate and differentiate into mature penile cells. In our present study, Li-ESWT
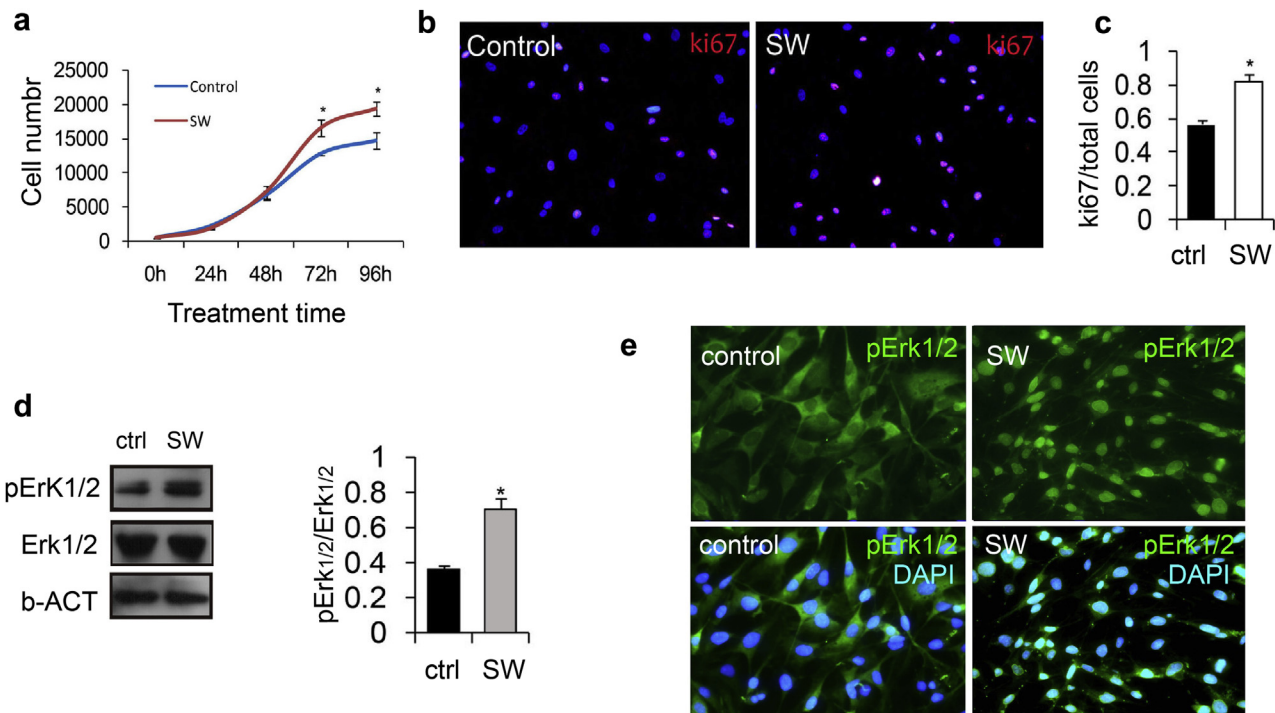

Figure 5. Low-intensity extracorporeal shockwave therapy stimulates proliferation of primary rat Schwann cells in vitro by activating the Erk pathway and increasing Ki-67 expression. Panel A shows the proliferation assay of Schwann cells in vitro. Panel B shows the expression of Ki-67 in Schwann cells after low-intensity extracorporeal shockwave therapy. Panel D shows activation of the Erk1/2 pathway in Schwann cells by low-intensity extracorporeal shockwave therapy (n = 3 each; *P < .05). Panel E shows translocation of pErk1/2 to the nucleus after low-intensity extracorporeal shockwave therapy. b-ACT = β-actin; ctrl = control; DAPI = 4′,6-diamidino-2-phenylindole; SW = shockwave.
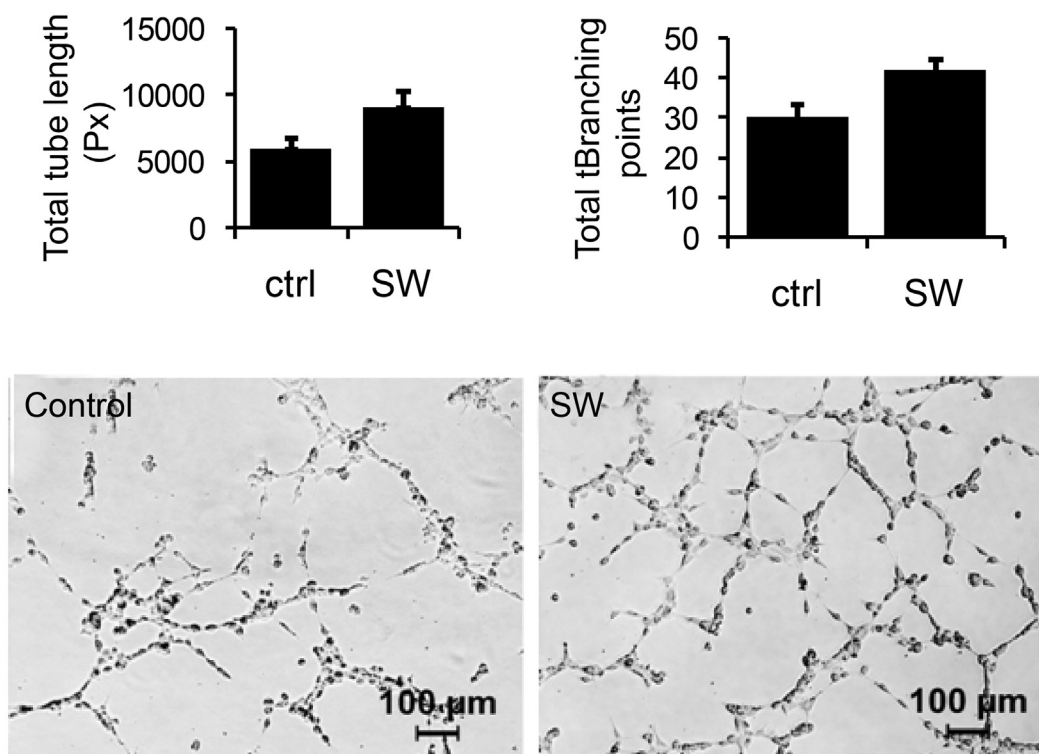
Figure 6. Low-intensity extracorporeal shockwave therapy promotes tube formation of human umbilical vein endothelial cells in cell culture. ctrl = control; DAPI = 4′,6-diamidino-2-phenylindole; SW = shockwave.

activated these cells and induced them to re-enter the cell cycle. Cells incorporate EdU during the S-phase of the cell cycle and therefore could be identified with EdU staining.

It has been reported that Li-ESWT influences cell proliferation by altering major extracellular factors and signaling pathways involved in cell proliferation. It has been hypothesized that extracellular adenosine triphosphate (ATP), released in an energy level-dependent and pulse number-dependent manner, is the trigger of the biological effects of shockwave treatment. Biologically, endogenous SCs activating out of quiescence to generate proliferating progeny require ATP to provide energy. The level of ATP in quiescent endogenous SCs might be insufficient for SC activation. Several studies have demonstrated that Li-ESWT enhances cellular ATP significantly and that the production of ATP is related to the activation of the Erk1/2 and p38 mitogen-activated protein kinase pathway.[17]

In the 1980s, ESWT was described as "mechanotherapy," with original applications for urological lithotripsy. More recently, it has been successfully applied for regenerative medicine. The molecular mechanisms of Li-ESWT are related to different pathways of biological reactions through a "mechano-transduction" process. From extensive basic science research, it has been demonstrated that Li-ESWT does not evoke a mechanical disruption of tissues and cells, but rather induces biological effects that activate a series of cellular events responsible for the therapeutic effects of Li-ESWT.[30,31] Activation of SCs by Li-ESWT is a focused treatment and therefore would

cause a minimum of off-site effects. As a non-invasive treatment approach, Li-ESWT is characterized by the absence of major side effects, repeatability, good tolerability, and excellent compliance by patients.

Many cells, including SCs, bone marrow stromal cells, osteoblasts, endothelial cells, and Schwann cells, are potential targets for mechano-transduction using Li-ESWT.[32−35] In the penis, approximately 70% to 80% of the Li-ESWT−activated cells were localized within the subtunical space, which was the same location noted in our previous report.[14] Although EdU was injected into newborn rats and immunochemical detection was performed when the rats grew to adulthood, the previous report identified the subtunical region as the "niche" of the EdU-retaining SCs. This further confirms that the Li-ESWT−activated EdU+ cells in the present study were SCs activated in situ and not cells that migrated from other locations. Moreover, approximately 1.5% to 6.7% of the activated cells were located within the penile nerve and penile vessels. This seems to confirm that Li-ESWT has the ability to activate multiple stem or progenitor cells, resulting in regeneration of blood vessels, nerves, and musclesobserved in previous clinical[7,36−38] and animal[8−11] experiments. To further study the potential of activation of endothelial and Schwann cells, the two cells were treated with low-energy shockwave in vitro. The results clearly indicate that Li-ESWT can activate endothelial and Schwann cells, and this response was related to activation of the Erk1/2 cellular signaling pathway.

As expected, the level of activation of penile progenitor cells varies with the amount of energy applied. Low-energy Li-ESWT activated more penile progenitor cells compared with very low-energy Li-ESWT. There were fewer penile cells activated by Li-ESWT in the middle-age animals compared with young animals. To maximize outcomes, further experiments are needed to identify the best treatment protocols for young and older animals and humans.

The major limitation of this study is the short timeline used and the animal models used. Li-ESWT might be less effective in improving erectile function in old animals because of the decreased number and quality of penile stem or progenitor cells associated with aging. With a longer period of study in young and old rats, we hope to better define the true benefits and limitations of this therapy.

CONCLUSION

Li-ESWT activation of local penile progenitor cells in situ might be one of the mechanisms that contribute to the beneficial effects of Li-ESWT for ED, which represents a non-invasive alternative to exogenous SC therapy.

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