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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.

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Key Words: Low Energy Shock Wave; Erectile Dysfunction; Endogenous Progenitor Cells; Schwann Cells; Nerve Regeneration; Angiogenesis

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.[4] 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]

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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 LESW 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 LESW improves diabetic ED in an animal model by promoting nerve regeneration,[11] a finding confirmed by another group.[12] Clinically, LESW 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 LESW treatment at different energy levels. We hypothesized that LESW 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-ethynyl-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 LESW at low energy (Low), and PVNI treated with LESW at high energy (High). After 4 weeks of LESW 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 ischiocavernosus muscle (ICM) and the bulbospongiosus 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, LESW 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 (Aquasonic, 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 LESW treatment. Schwann cells received LESW 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

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