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Transpelvic Magnetic Stimulation Enhances Penile Microvascular Perfusion in a Rat Model: A Novel Interventional Strategy to Prevent Penile Fibrosis after Cavernosal Nerve Injury

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Purpose: Penile microvascular dysfunction is a known contributor to erectile dysfunction (ED) and penile fibrosis has been shown to impair microvascular perfusion (MVP). Our objectives were to: (i) determine beneficial effects of TPMS to modulate penile MVP, (ii) determine its mechanism, (iii) evaluate impact of cavernosal nerve injury (CNI) on penile MVP, and (iv) determine time-course of cavernosal tissue elastin changes after CNI in rats.

Materials and Methods: Adult male rats (n=5) were anesthetized and subjected to TPMS (13%, 15%, and 17%) and MVP changes were recorded using laser speckle contrast imaging (LSCI). Another group of male rats were subjected to either bilateral cavernosal nerve injury (CNI; n=7) or sham surgery (n=7). After recovery, animals were monitored for MVP using LSCI before and after TPMS. Rat penile tissues were harvested and analyzed for fibrosis using a marker for elastin.

Results: Rat TPMS resulted in a stimulus dependent increase in MVP; maximal perfusion was observed at 17%. L-N(G)-Nitroarginine methyl ester (L-NAME) resulted in a marked decrease in TPMS induced MVP increase (393.33 AU *vs*. 210.67 AU). CNI resulted in 40% to 50% decrease in MVP. CNI produced a remarkable increase in elastin deposits that are noticeable throughout the cavernosal tissues post injury.

Conclusions: TPMS is a novel and non-invasive intervention to improve penile MVP after CNI. Potential application includes treatment of ED and sexual function preservation following cancer treatment, possibly through improved penile hemodynamics that might help prevent penile hypoxia and fibrosis.

Keywords: Elastin; Erectile dysfunction; Fibrosis; Laser speckle contrast imaging; Microcirculation; Penis

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INTRODUCTION

To date, prostate cancer is one of the most common types of cancer in men over 50 years old in the United States [1]. Sexual complications such as erectile dysfunction (ED), or the inability to initiate or maintain adequate erection for intercourse [2-4], dramatically affect the quality of life after treatment for prostate cancer. Unfortunately, about 20% to 80% of these cancer survivors will never regain normal erectile function [5] after radical prostatectomy. Vascular damage to penile tissue or its neural innervation is recognized as a significant cause for ED after surgical therapy [6,7]. Even with nerve-sparing techniques, damage to the corpus cavernosum and related neural innervations is common and can lead to ED. Exact physiological and cellular mechanisms that contribute to ED even after nerve-sparing techniques are still unclear. Previous studies using rat models have shown that transient cavernosal nerve (CN) crush for 80 seconds can lead to cavernosal tissue fibrosis [8]. The pathophysiology of penile microcirculatory changes consequent to CN injury (CNI) and its contribution to smooth muscle fibrosis is still unclear. If microcirculation is impaired consequent to CNI, identification strategies to reverse this impaired physiological function warrant exploration.

Microcirculation refers to the smallest arteries (less than ~150 μm in diameter), arterioles, capillaries, and venules. Reports suggest that penile microvascular dysfunction is an important contributor to ED severity and an underlying mechanism linking ED to cardiovascular disease [9,10]. In addition, a pre-clinical study from Department of Urology, VA San Diego Health Care System has revealed that increased penile fibrosis has been shown to impair penile microvascular perfusion (MVP) [9] measured using a novel laser speckle contrast imaging (LSCI) system. Our studies also showed a positive correlation between penile MVP (using LSCI) and macrovascular function (measured by clinically used Doppler blood flow). These findings suggest that penile microvascular dysfunction may contribute to increased ED severity after CNI and LSCI may be employed as a non-invasive technique for the evaluation of penile MVP after CNI [11].

Repetitive magnetic stimulation (rMS) was demonstrated to potentially improve blood flow [12,13], muscle regeneration, and muscle function by minimizing post-injury inflammation, atrophy, and scar formation [14]. Repetitive transpelvic magnetic stimulation (TPMS) therapy acts by strong pulsing magnetic fields that depolarize neural elements, resulting in improved muscle contraction. Taking advantage of the reported potentials of rMS to improve blood flow [12,13], we hypothesize that TPMS intervention immediately following a CNI would be beneficial to enhance penile MVP.

Conventionally, collagen has been used as a marker of fibrosis in cavernosal tissue following CNI. Recently, elastin, another component of extra cellular matrix has been implicated to play a role in cardiac and kidney fibrosis. In a recent study focused at establishing a new marker for fibrosis to track the progression of chronic kidney diseases (CKDs), Sun et al [15] found that elastin was highly upregulated in cortical, medullar, and perivascular regions in mouse and human kidneys with CDKs in comparison to healthy controls. The role and time course of cavernosal tissue elastin changes after CNI warrants further evaluation.

Based on these reports, the aims of our study were to (i) evaluate the beneficial effects of TPMS to modulate penile MVP, (ii) identify a possible mechanism of this modulation, (iii) evaluate the impact of CNI on penile MVP, and to (iv) determine the time-course of cavernosal tissue elastin changes after CNI in a rat model.

MATERIALS AND METHODS

1. Ethics statement

The present study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (approval number: 15-006). All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). Fourteen male Sprague-Dawley rats (age 10–12 months) were subjected to the physiological studies described below. At the end of the study, the animals were sacrificed, and penile tissue was harvested for histological analysis.

2. Laser speckle contrast imaging for microvascular perfusion

Animals were anesthetized using a ketamine and xylazine anesthetic cocktail recommended for rats. Penile MVP was assessed by the PeriCam PSI system (Perimed AB, Järfälla, Sweden). This laser speckle Doppler imaging technique quantifies penile MVP and

displays a real-time image using arbitrary perfusion units (AU) [9]. The rats were prepared by using Nair hair remover in the pelvic region and were placed on a custom-made rat-restrainer lying supine, with their pelvis/penis positioned under the PeriCam (Fig. 1). An image sampling frequency of 1/s was used in between each administration of magnetic stimulation, and 2/s was used during each stimulation which lasted about 4 seconds each.

3. Effect of transpelvic magnetic stimulation in control male rats

TPMS was administered *via* the Magventure MagPro R30 with MagOption (Magventure, Alpharetta, GA, USA) using the rat coil. TPMS parameters were: 30 pulses per second, 241 pulses per train, 1 train, 4.0 seconds intervals between trains, 1.00 ramp up, and 10 ramp-up trains. Five minutes of rest were given between each wave of TPMS. After the last wave of TPMS, perfusion was measured for 3 more minutes. Each rat was anesthetized, and a baseline MVP was recorded using LSCI. After acquiring a baseline for MVP for 4 minutes, the PeriCam continuously recorded MVP as TPMS of 13%, 15%, and 17% amplitude were administered. After the trials were completed, four more Sprague-Dawley control rats were used to show a dosed response to different amplitudes of TPMS: 13%, 15%, and 17% amplitude. The rats were anesthetized, laid in the custom-made rat restrainer as shown in Fig. 1 and the pelvic region was subjected to MS and a baseline perfusion using LSCI was obtained. After 4 minutes, the first TPMS was administered (13%), and 5 minutes were given between each subsequent round of TPMS.

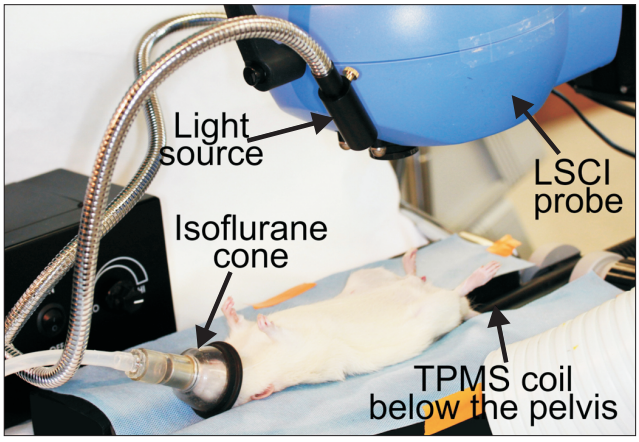

Fig. 1. Experimental setup showing LSCI probe, light source, isoflurane cone, custom-made rat restrainer, and TPMS coil. LSCI: laser speckle contrast imaging, TPMS: transpelvic magnetic stimulation.

4. The evaluation of role of nitric oxide pathway in transpelvic magnetic stimulation induced penile microvascular perfusion changes

To test the involvement of nitric oxide (NO) pathway, a few rats (n=3–4) received an intracavernosal injection of N(G)-Nitroarginine methyl ester (L-NAME; 200 μg/kg [16]). After 15 minutes, the rats were subjected to another round of TPMS stimulations at 15% amplitude while simultaneously recording MVP using LSCI.

5. Cavernosal nerve injury model

Fourteen healthy Sprague-Dawley male rats (12–14-months old) were assigned to two equal (n=7) groups: (i) control, G1 (sham surgery), (ii) experimental, G2 (CNI). All animals were anesthetized using a ketamine and xylazine anesthetic cocktail as described earlier, and baseline studies were performed first to determine the penile MVP levels (using Perimed LCSI perfusion system; Perimed AB). G2 animals were subjected to CNI, as described below. The bilateral CNI was performed under a microscope. Through a lower abdominal midline incision, the area posterolateral to the prostate was explored and the major pelvic ganglion (MPG) and CN were identified and exposed. In the sham operation group, there was no further surgical manipulation; in the remaining groups, the CN (distal to the MPG) were crushed for 90 s twice using an ultrafine hemostat. The abdomen was closed with a running Vicryl 4-O suture. After 3 weeks post-recovery, animals were monitored for MVP changes.

6. Histological evaluation for elastin as a marker for fibrosis

After these studies, the animals were sacrificed at pre-determined time points (3 weeks, 4 months, and 9 months post-CNI) for evaluation of fibrosis specifically for elastin using Verhoeff's Van Giesons (VVG) staining that shows elastin fibers in black. In addition, we also performed immunofluorescence studies using specific antibodies for collagen-1 (Abcam, Cambridge, UK; 1:200 dilution: and elastin (Abcam; 1:200 dilution) using previously reported staining protocols [17]. Images were captured and analyzed using a Zeiss AxioScan slide scanner (Zeiss, Oberkochen, Germany).

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RESULTS

1. Transpelvic magnetic stimulation-induced changes to penile microvascular perfusion

Rat TPMS resulted in a stimulus-dependent increase in MVP and maximal perfusion was observed at 17% amplitude. These MVP changes are summarized in Fig. 2. Maximal MVP (AU) at 13%, 15%, and 17% were 132, 245, and 370, respectively.

The injection of L-NAME showed a marked decrease in MVP (AU) measured via LSCI in response to TPMS stimulation at 17% amplitude. Fig. 3 shows an average MVP of 393.33 AU in response for before L-NAME injection, while the Post-L-NAME, the rats showed 210.67 AU in response to the same amplitude of stimulation (17%). The administration of L-NAME resulted in a significant (45%) decrease of MVP.

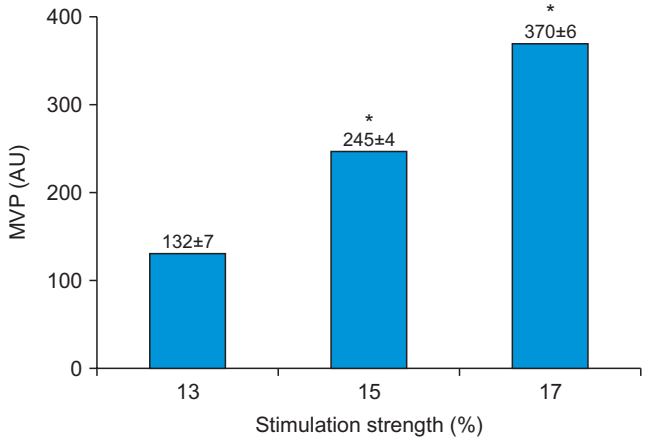

Fig. 2. Bar graph showing a stimulus-dependent increase in penile MVP in response to 13%, 15%, and 17% of TPMS in male rats (n=5). MVP: microvascular perfusion, TPMS: transpelvic magnetic stimulation. *p<0.05 compared to baseline MVP.

2. Impact of cavernosal nerve injury on microvascular perfusion

When comparing healthy adult rats to the CNI group at baseline MVP, the CNI group (97.6 AU) was 17.01% lower than the control group (81.0 AU), and these results were found to be statistically significant ($p<0.05$). At 15% amplitude, the control group showed an average perfusion of 289 AU, while the experimental rat group had an average perfusion of 160.5 AU. CNI resulted in 60% decrease in MVP in the CNI group compared to uninjured controls (Fig. 4). This result was found to be statistically significant when using a paired 2-tail t-test with unequal variances ($p=0.00128$, $\alpha=0.05$). An amplitude of 15% was chosen because it showed MVP showed an optimal response at that amplitude.

3. Histological evaluation for elastin as a marker for fibrosis

Our histological studies (Fig. 5A) showed that immediately after CNI (3-weeks), a remarkable increase in elastin deposits was noticable thorughout the cavernosal tissues. Although with time there was some reduction, the elastin deposits were seen even at 9 months post-CNI. Our immunoflorescence studies using specific

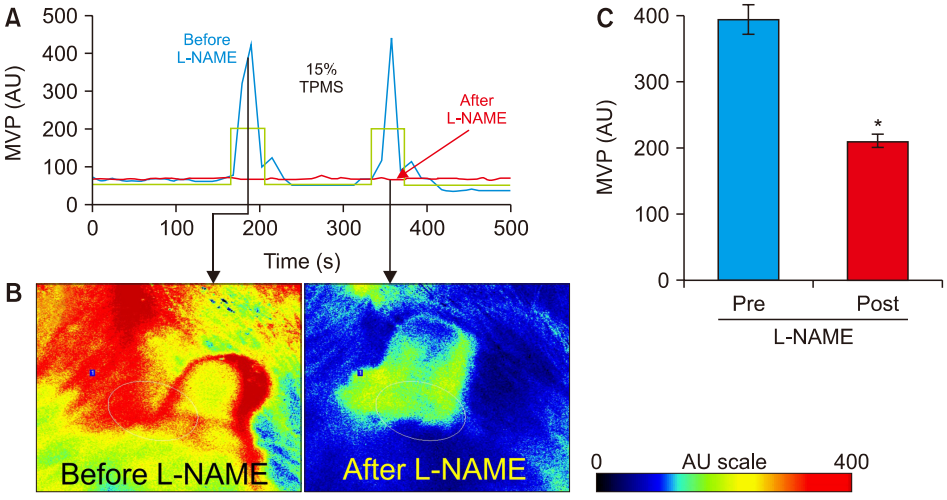

Fig. 3. (A) A representative line graph that shows MVP response to 15% amplitude before and after the injection of L-NAME into the corpus cavernosum. (B) LSCI images of the rat penis during stimulation (15%), before and after the administration of L-NAME. (C) A bar graph summary of (A), shows the average MVP (AU) in response to 15% TPMS before and after the administration of L-NAME. MVP: microvascular perfusion, L-NAME: N(G)-Nitroarginine methyl ester, LSCI: laser speckle contrast imaging. *p<0.05 compared to pre-NAME.

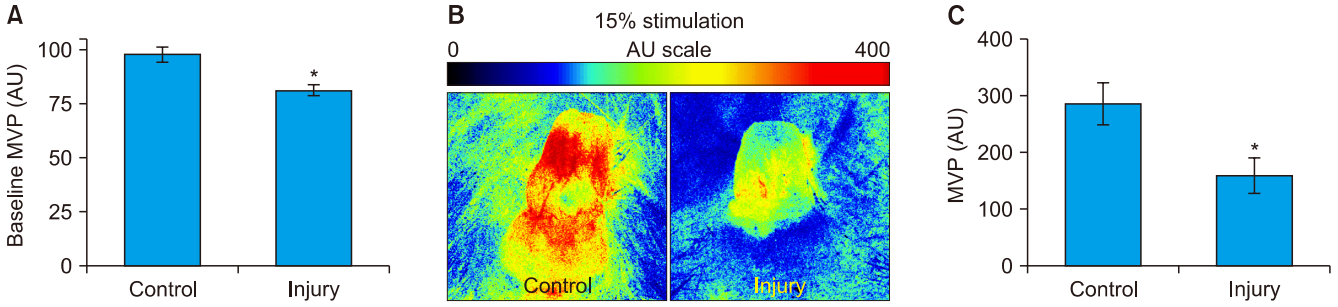

Fig. 4. Bar graph depicting MVP (AU) responses to 15% amplitude TPMS in control *vs*. injury rat groups at (A) baseline, (B) representative LSCI images, and (C) MVP changes in response to TPMS. MVP changes in the injury group was significantly lower than the control group. MVP: microvascular perfusion, TPMS: transpelvic magnetic stimulation, LSCI: laser speckle contrast imaging. *p<0.05 compared to control group.

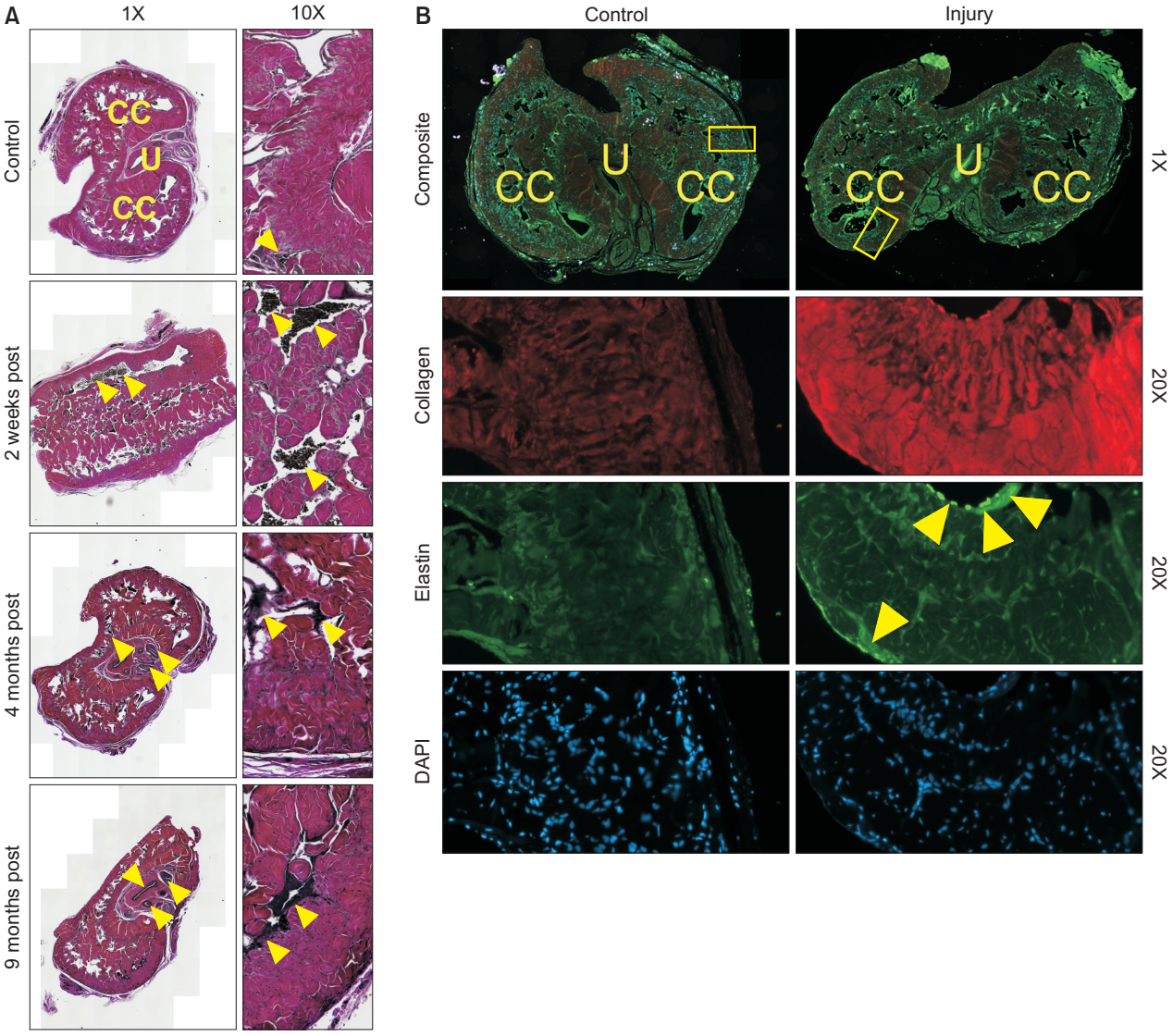

Fig. 5. (A) Photomicrographs by VVG staining showing elastin fibers (in black) in rat penile tissue samples harvested taken from control rats, as well as from injury rats 2 weeks, 4 months, and 9 months injury. Arrowheads confirm elastin localization. (B) Representative photomicrographs by VVG staining showing immunofluorescence images of elastin and collagen in control *vs*. CNI rats (at 4-months post-CNI). Arrowheads confirm elastin localization. CC: corpus cavernosum, U: urethra, CNI: cavernosal nerve injury.

The World Journal of MEN's HEALTH

antibodies provide additional confirmation of changes in elastin and collagen-1 after CNI (Fig. 5B).

DISCUSSION

Our study aims were to: (i) evaluate the beneficial effects of TPMS to modulate penile MVP, (ii) propose a possible mechanism of this modulation, (iii) evaluate the impact of CNI on penile MVP, and (iv) determine the time-course of cavernosal tissue elastin changes after CNI in a rat model. Our studies showed a significant decrease in MVP after CNI in rats. In addition, TPMS after CNI produced a significant improvement in MVP. This animal model supports our hypothesis that transient crush damage to the nerve bundles can cause impairment of MVP. In the absence of timely intervention, this MVP impairment can lead to tissue fibrosis. Our studies also suggest that elastin could serve as a useful and additional marker to monitor CNI-related cavernosal tissue fibrosis.

Recent surveys suggest that therapies for recovery of post-prostatectomy ED involve the use of phosphodiesterase type 5 inhibitors, and injection of vaso-active substances [18,19]. While these rehabilitative approaches have been shown to have some promise to recover erectile function in cancer survivors, they mostly attempt to increase cavernosal blood inflow to prevent hypoxia and alleviate ED symptoms. Use of oral PDE5-Is, which is the least invasive method that has shown promising results, has limited efficacy when dealing with ED in cancer survivors. Intracavernosal injection therapy with vasoactive agents such as prostaglandin E_1 is a well-recognized pharmacotherapy to treat ED that is due to nerve damage [20]. However, the intimidating nature of penile self-injection poses a major barrier to this therapy [21] and is associated with high dropout rates of patients undergoing intracavernosal injection (46%–80%) [20,22-24]. Major causes for dropout were patient's issue with the invasive injection and a desire for a permanent solution [25]. The development of a therapy that could prevent the onset of fibrosis and subsequent ED would address both of those issues.

Dorey et al [26] conducted a pelvic floor muscle exercise therapy in 55 men with ED due to venous leak. The results showed a significant improvement in ED symptoms after 3 months of therapy [27]. While this is a very promising approach to strengthen the pelvic floor muscles and improve venous occlusion, it would not prevent the onset of fibrosis following potential nerve damage. Due to the complex nature of injury-related denervation following radical prostatectomies, a multifactorial preventative mechanism is needed to strengthen the pelvic floor muscles while simultaneously preventing the onset of fibrosis [28]. A meta-analysis revealed that low-intensity extracorporeal shockwave therapy may be an effective way to increase blood flow to the penis and improve penile hemodynamics by promoting the expression of pro-angiogenesis markers, as well as neovascularization, which effectively remodels the affected tissue [29,30].

Although the exact mechanism of TPMS induced penile MVP is not clear. A schematic showing mechanism of TPMS induced increase in MVP and an overall summary of our hypothesis are shown in Fig. 6. Our experiments using L-NAME suggest the potential involvement of NO pathway in this enhanced blood perfusion. Previously, Bragin et al (2015) [31] demonstrated that pulsed magnetic field (PMF) stimulation of rat brain resulted in cerebral arteriolar dilation leading to an increase in microvascular blood flow and tissue oxygenation that persisted for at least 3 hours. NOS inhibition by L-NAME prevented this PMF-induced changes in arteriolar diameter, MVP, and tissue oxygenation. In another study by Funk et al (2014) [32], showed a clear increase in NO production when blood flow changes after magnetic stimulation were quantified in fingers of healthy volunteers using a fluorescence marker. These reports support our findings and inference of a potential NO-mediated MVP enhancement in rat penis.

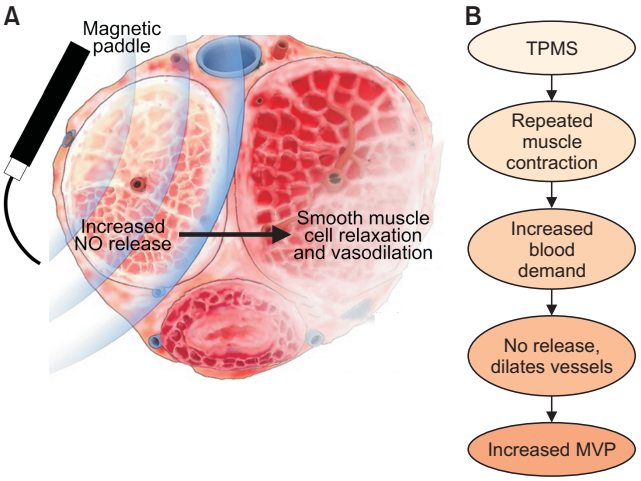

Fig. 6. (A) Schematic showing mechanism of TPMS induced increase in MVP. (B) Summary of our hypothesis. NO: nitric oxide, TMPS: transpelvic magnetic stimulation, MVP: microvascular perfusion.

Our present study with TPMS suggests that this novel approach is promising to evolve as a multifactorial preventative mechanism to improve blood flow in order to prevent fibrosis, and to simultaneously strengthen the pelvic floor muscles, preventing venous leak, a major issue in prostate cancer survivors. Our results showed that at low amplitude, TPMS can enhance blood flow and microcirculation in an animal model. TPMS acts by strong pulsing magnetic fields that depolarize neural elements, resulting in improved muscle contraction. We propose a weekly regimen of TPMS starting at two weeks after radical prostatectomy. The ability of this therapy to increase blood flow, prevent fibrosis, and prevent venous occlusion by strengthening the pelvic floor muscles make it the most ideal form of therapy for this disease state, specifically neurogenic ED consequent to prostatectomy. Additionally, the simple and non-invasive nature of the therapy makes it much more appealing. However, our study has a few limitations. Although our studies showed MVP improvement after TPMS, we did not evaluate tissue changes after TPMS. Next, we did not evaluate ICP changes after CNI or TPMS. Another limitation is that we used only a few animals to test the effect of L-NAME and in our histological studies. Our future studies would attempt to address these areas.

CONCLUSIONS

In conclusion, our preliminary pre-clinical studies investigating the effects of TPMS on penile MVP showed a significant stimulus-dependent MVP increase to varying magnitudes of TPMS. In addition, our findings also suggest LSCI is a non-invasive tool to monitor penile microvascular function after CNI. These promising findings suggest that using TPMS after radical prostate surgery, at the time of catheter removal, could be a promising novel mode of non-invasive intervention to increase blood flow, decrease hypoxia and fibrosis, and ultimately recovery erectile function in these cancer survivors. Furthermore, the significant decrease in the MVP after L-NAME, a NO synthase antagonist, suggests that this physiological response is possibly mediated by NO-mediated pathways. However, more stringent randomized controlled trials in post-radical prostatectomy patient population are recommended before this novel mode of therapy can be recommended for clinical applications.

Conflict of Interest

The authors have nothing to disclose.

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Authors Contributions

Conceptualization: MR, VB, SL. Data Curation: SS, CSC MR, VB. Formal analysis: MR, VB, SS. Funding Acquisition: MR, SL. Investigation: SS, MR, VB. Methodology: SS, MR, VB, MCC, MGP, SYC, HC. Resources: MR, SS, VB. Software: MR, SS, VB. Writing - Original Draft: MR, VB, SS. Writing - review and editing: MR, SS, TCH, VB.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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