Sexual Medicine

Molecular Mechanisms of Vacuum Therapy in Penile Rehabilitation: A Novel Animal Study

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Abstract

Background: Penile rehabilitation (PR) is widely applied after radical prostatectomy. Vacuum erectile device (VED) therapy is one of three PR methods used in the clinical setting that improve erectile function (EF) and is the only PR method which may preserve penile length. However, its unknown mechanism hampered doctors’ recommendations and patients’ compliance.

Objectives: To assess the effects of VED therapy on erectile dysfunction (ED) in a rat model of bilateral cavernous nerve crush (BCNC) and to investigate the molecular mechanism of VED in postprostatectomy ED.

Design, setting, and participants: This was an experimental study using Sprague-Dawley rats in three groups: sham, BCNC, and BCNC plus VED.

Intervention: Intervention included BCNC, electrical stimulation of the cavernous nerve (CNS), and VED therapy.

Measurements: At the end of a 4-wk period, CNS was used to assess EF by maximum intracavernosal pressure (ICP)/mean arterial pressure (MAP) ratio and duration (area under the curve [AUC]). For the structural analyses, whole rat penis was harvested. Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling assay was used for the assessment of apoptotic indices (AI). Immunohistochemistry was performed for endothelial nitric oxide synthase (eNOS), α-smooth muscle actin (ASMA), transforming growth factor beta 1 (TGF-β1), and hypoxia inducible factor-1α (HIF-1α). Staining for Masson’s trichrome was utilized to calculate the smooth muscle/collagen ratios.

Results and limitations: EF was improved with VED therapy measured by ICP/MAP ratios and AUC. VED therapy reduced HIF-1α expression and AI significantly compared with control. Animals exposed to VED therapy had decreased TGF-β1 expression, increased smooth muscle/collagen ratios, and preserved ASMA and eNOS expression.

Conclusions: To our knowledge, this is the first scientific study to suggest that VED therapy in the BCNC rat model preserves EF through antihypoxic, antiapoptotic, and antifibrotic mechanisms.

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1. Introduction

Prostate cancer is the most common solid-organ cancer in men and one of the leading causes of death [1]. With early detection and radical prostatectomy (RP), the 15-yr overall, actuarial, cancer-specific survival rate has reached 90% [2,3]. Unfortunately, RP is associated with at least transient erectile dysfunction (ED), with ED rates ranging from 20% to 90% depending upon the study reviewed [1–4]. It is postulated that the development of post-RP ED is due predominantly to a combination of temporary cavernous nerve (CN) injury and damage to the erectile tissue secondary to neuropraxia and potentially the absence of cavernosal oxygenation [5].

To improve the patients’ quality of life and the acceptance of the RP, penile rehabilitation (PR) after RP is now widely applied in clinical practice [3]. Currently, PR methods include the use of phosphodiesterase type 5 inhibitors, intracavernosal injection/intraurethral suppository, the vacuum erectile device (VED), or combination therapy [3].

Vacuum therapy utilizes negative pressure to distend the corporal sinusoids and to increase blood inflow to the penis. Clinical data indicated that vacuum therapy is the only PR method that may preserve penile length, improves patient and partner sexual satisfaction, and allows earlier return of spontaneous erection [3,6]. However, its unknown mechanism hampered doctors’ recommendation and patients’ compliance [3].

To explore the underlying mechanism of VED therapy after RP, we applied our newly designed rat-specific VED [7] to the bilateral cavernous nerve crush (BCNC) rat model. The BCNC rat model is believed to simulate the neural injury that occurs during RP and is designed to study the mechanisms of ED after RP as well as to explore ED-minimizing strategies [8].

![Fig. 1 - Erectile function assessment by intracavernous pressure tracing under cavernous nerve stimulation. Representative intracavernous pressure (ICP) tracing in response to cavernous nerve stimulation (CNS) (7.5 V for 60 s) at week 4 after (A) sham operation, (B) bilateral cavernous nerve crush (BCNC), and (C) BCNC plus VED therapy; (D) the voltage dependent erectile response to CNS represented by the ratio of maximum ICP/mean arterial pressure (MAP); (E) area under the erectile curve (AUC, mmHg s). Significant differences were found in ICP/MAP and AUC only at 5.0 V and 7.5 V, between BCNC group and BCNC plus VED group (both p < 0.05).](image-url)
2. Methods

2.1. Animal grouping, bilateral cavernous nerve crush, and vacuum erectile device therapy

Fifteen Sprague-Dawley rats (Harlan Laboratories, Houston, TX, USA), initially weighing 200–250 g, were randomly and equally divided into three groups: (1) sham (CN expose surgery only, no nerve crushing, no VED therapy); (2) control (BCNC procedure, no VED therapy); and (3) treatment group (BCNC procedure; VED therapy beginning at 2 wk after BCNC surgery, 5 min twice daily with a 1 min interval, Monday–Friday, total VED treatment time: 4 wk). The BCNC procedure was reported previously [8]. The animals were cared for and housed under strict guidelines established by the University Texas Health Science Center at Houston Institutional Animal Care and Use Committee.

2.2. Functional analysis and tissue harvesting

At the end of 4 wk of treatment, the animals were recorded for intracavernosal pressure (ICP) and the corresponding arterial pressure (AP) with CN stimulation (CNS) under pentobarbital anesthesia [9]. At the completion of functional analysis, the penis was excised for histopathology.

2.3. Histopathology

Following routine dehydration and paraffin embedding, tissue samples were cut into 5-μm sections from the midshaft of the penis mounted on slides and dried. Then the tissue slides, showing the cross-section of the corpora cavernosa (CC), were deparaffinized and rehydrated for following studies.

2.3.1. Masson’s trichrome

To evaluate the smooth muscle/collagen ratio, slides were stained for Masson’s trichrome (MT) according to standard protocol, which was reported previously [9]. Smooth muscle/collagen ratios were analyzed using ImageJ v.1.43n (US National Institutes of Health, Bethesda, MD, USA). One slide per animal (slides are from the midshaft of penis about the same level) was used to calculate the ratio of the red-staining smooth muscle to the blue-staining collagen content in the cross-section of the CC building the group average. The ratios were compared among the three groups.

2.3.2. Immunohistochemistry

The corporal tissue of the rat penis was immunohistochemically stained for endothelial nitric oxide synthase (eNOS), alpha smooth muscle actin (ASMA), hypoxia-inducible factor 1α (HIF-1α), and transforming growth factor beta 1 (TGF-β1) following the manufacturer’s instructions. The antibodies of eNOS and ASMA were from Abcam Inc. (Cambridge, MA, USA); the antibodies of HIF-1α and TGF-β1 are from R&D Systems (Minneapolis, MN, USA). The results of the tissue sample staining were compared among the three groups: sham, control, and treatment.

2.3.3. Apoptosis assessment

Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay was performed following the manufacturer’s instructions (Roche Applied Science, Mannheim, Germany) to assess apoptosis. Two slides from two different animals per group were randomly selected. Each slide was analyzed by counting cells in five nonoverlapping zones of the entire mounted CC section at × 400 magnification. The ratio of the percentage of cells stained with the TUNEL method to the total number of cells stained with 4’,6 diamidino-2-phenylindole was recorded and reported as the apoptotic index (AI).

2.4. Statistical analysis

The mean averages were built for maximum ICP/mean arterial pressure (MAP) ratios, area under the ICP curves (AUCs), smooth muscle/collagen ratios, and AIs for each group, and reported as the mean plus or minus standard error of the mean. Individual pairwise comparison between groups was analyzed with independent two-tailed t tests. Results were considered statistically significant if \( p < 0.05 \).

3. Results

3.1. Erectile function assessment

EF was assessed by tracing the ICP under the CNS, and in the meantime measuring the AP. The typical ICP tracings of
sham, BCNC, and BCNC with daily VED therapy are shown in Fig. 1A–C. The analysis is presented in ICP/MAP ratios and AUCs (Fig. 1D and E). The ICP/MAP ratios in the sham group were 0.61 ± 0.02 at 5.0 V and 0.71 ± 0.03 at 7.5 V, which were significantly higher compared with all other groups (p < 0.01). BCNC dramatically decreased the ICP/MAP ratios: 0.25 ± 0.02 at 5 V and 0.32 ± 0.03 at 7.5 V. The daily VED therapy in BCNC rats preserved the ICP/MAP ratios: 0.53 ± 0.06 at 5 V and 0.65 ± 0.03 at 7.5 V, which is significant compared with the BCNC control (p < 0.01). The AUCs demonstrated the same trend as the ICP/MAP ratios in the three groups.

### 3.2. Hypoxia assessment

On IHC, the staining intensity of HIF-1α in the BCNC group (Fig. 2B) was dramatically higher than the sham group (Fig. 2A). In the BCNC plus VED group, the HIF-1α staining (Fig. 2C) was significantly reduced compared with the BCNC group, although still higher than the sham group.

### 3.3. Apoptosis analysis

At 6 wk after BCNC, the BCNC plus VED group demonstrated a significant reduction in apoptosis within the corporal tissue (Fig. 3C) with a mean AI of 21 ± 4%, compared with an AI of 61 ± 5% in the BCNC group (p < 0.001) (Fig. 3B and D). For comparison, the AI value in the sham group was 10 ± 6% (Fig. 3A and D), which was significantly lower compared with BCNC (p < 0.001) or BCNC plus VED (p < 0.05) (Fig. 3B, C and D).

### 3.4. Penile structure molecular analysis

#### 3.4.1. Endothelial nitric oxide synthase and alpha smooth muscle actin immunohistochemistry

Immunohistochemical staining of eNOS in the BCNC group (Fig. 4B) was dramatically reduced compared with the sham group (Fig. 4A). The eNOS staining in the BCNC plus VED group (Fig. 4C) was significantly improved compared with BCNC group; however, it was still lower than sham group. ASMA expression showed the same trend (Fig. 4D–F).

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**Fig. 3 – Vacuum erectile device (VED) therapy decreased apoptosis.** Compared with the sham operation group (A), bilateral cavernous nerve crush (BCNC) (B) dramatically increased apoptosis in penile sinusoid (brown-stained nuclei; magnified ×400). VED treatment (C) significantly reduced BCNC-induced apoptosis in penile sinusoid compared with nerve crush only (B). Apoptotic indices (AI) is presented as ratio of apoptotic nuclei (brown-stained nuclei) to total number of nuclei counted. A significantly reduced AI percentage for VED therapy was found (D) compared to BCNC only (p < 0.01), although still higher than the sham group (p < 0.05).
3.4.2. TGF-β1 immunohistochemistry

Immunohistochemical staining of TGF-β1 in the BCNC group (Fig. 4H) was dramatically increased compared with the sham group (Fig. 4G). The BCNC plus VED treatment was partially reversed (Fig. 4I), although it was still higher than sham group.

3.4.3. Smooth muscle/collagen ratios

The staining with MT in the sham group revealed smooth muscle/collagen ratios of 63.8 ± 1.7%, the highest smooth muscle/collagen ratio of all animal groups (Fig. 5D). This result was significantly higher compared with 20.0 ± 1.6% for the BCNC group (Fig. 5A, B and D) (p < 0.05) and remained superior to the BCNC plus VED group as well (Fig. 5C and D) (p < 0.05). The BCNC plus VED group reached 40.4 ± 1.4%, which was significantly greater than the BCNC group (p < 0.05), and displayed a clear trend toward improvement compared with the BCNC group (20.0 ± 1.6%; p < 0.05), but not the sham group.

4. Discussion

The rat BCNC as an RP-induced ED model has been widely accepted in PR research. It was first reported by Quinlan et al in 1989 and documented in prior experiments using this animal model in the assessment of functional and structural changes of erectile tissue under various interventions [8]. Our study showed a dramatic reduction in ICP/MAP ratios and AUC in animals after BCNC when compared with the sham group. The reduced ICP/MAP ratios and AUC were associated with significantly reduced smooth muscle/collagen ratios and highly increased apoptosis rates and TGF-β1 expression in the control group compared with sham animals. Previous reports in rat CN transection models have demonstrated increased apoptosis within the corpora. Klein et al reported that apoptosis of penile erectile tissue occurs after denervation of the rat penis [10]. User et al similarly confirmed increased apoptosis, especially subjacent to the tunica albuginea involving smooth

![Fig. 4 – Vacuum erectile device (VED) therapy preserves penile endothelial nitric oxide synthase (eNOS) and alpha smooth muscle action (ASMA) expression, and ameliorated transforming growth factor beta 1 (TGF-β1) expression. Penile tissue was harvested and processed after functional measurement. Immunohistochemistry was used for the expression of eNOS, ASMA, and TGF-β1 in penile sinusoid. Bilateral cavernous nerve crush (BCNC) dramatically reduced the expression of eNOS (B) and ASMA (E) compared to the sham group (A and D), respectively. VED therapy significantly preserved the expression of (C) eNOS (BCNC plus VED therapy) and (F) ASMA (BCNC plus VED therapy) compared to (B) and (E), respectively. Compared to sham operation (G), BCNC (H) dramatically increased TGF-β1 expression in penile sinusoid. VED treatment (I) partially reversed TGF-β1 expression in penile sinusoid compared to nerve crush only (H).]
They hypothesized that damage to the subtunical smooth muscle cells prevents compression of the perforating subtunical veins, resulting in veno-occlusive dysfunction and subsequent failure of recovery of EF.

The daily use of VED therapy commencing 2 wk after BCNC improved ICP/MAP ratios and AUCs compared with the control group at 4 wk. The 4-wk time point was chosen as this is generally believed to represent the 2-yr time point in the human [9]. After RP, the period of neuropraxia may last as long as 24 mo [12] and we generally expect EF recovery by this time point if ideal nerve-sparing RP was performed. The regimen we use is exactly what is used in the VED protocol in a clinical setting [13].

VED regimen in men after RP has been documented in preserving EF and penile length and size. Raina reported 109 patients with nerve-sparing RP who were placed into early VED daily usage (group 1, n = 74) versus no erectogenic aid (group 2, n = 35) with 9-mo follow-up, and showed early use of VED facilitates early sexual intercourse and early patient/spousal sexual satisfaction [14]. Kohler et al randomized 28 patients into early VED therapy or control (control group accepted VED therapy 6 mo later) with 1-yr observation and concluded that early VED use after RP improves sexual function [15].

Our experimental findings confirmed the clinical outcome of VED application. EF becomes impaired immediately following RP secondary to damage to CN during surgery, resulting in neuropraxia [16]. A reduction in arterial inflow has also been reported due to ligation of the accessory internal pudendal arteries during RP [17,18]. The combination of nerve damage with decreased arterial inflow may cause penile tissue hypoxia, leading to apoptosis and collagen deposition, which ultimately results in venous leak. This, in turn, has been linked to the pathophysiology of ED after RP [10,19–27].

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immediately after VED-induced erection was 79.2%, which translates to 58% arterial and 42% venous flows, respectively [28]. Therefore, we believe there is increased oxygenation of the penis when VED is applied. Daily VED-induced tissue oxygenation overcomes RP-induced hypoxia in a consistently flaccid penis, which is lack of spontaneous erection and nocturnal tumescence. Nocturnal erections have been implicated in preserving normal erectile function by providing regular tissue oxygenation [29]. The partial oxygenation of VED imitates the nocturnal erection and provides the penis with regular partial oxygenation. Our data showed that with regular partial oxygenation, the apoptosis of penile tissue was partially reversed. With intermittent interruption of penile hypoxia with VED application, progressive cavernosal fibrosis produced by persistent penile hypoxia was halted. It has been shown that the progressive cavernosal fibrosis induces veno-occlusive dysfunction [30]. Our data also showed that VED therapy decreased TGF-β1 expression, and increased eNOS and ASMA, and smooth muscle/collagen ratios. Therefore, the VED regimen preserved penile EF via antihypoxic, antiapoptotic, and antifibrotic mechanisms to preserve the veno-occlusive mechanism.

The oxygen level of the VED-applied penis is a key issue; however, it could not be measured in our experiments because of the technique challenge. In addition to the tissue oxygenation mechanism, the beneficial effects of VED therapy after RP may be mediated by stretch forces, other nutrient factors, and neuroregeneration, although we did not address it in our experiments. We also did not optimize the VED regimen, such as applying optimal vacuum pressure (highest oxygen level in the penis without intolerable side effects), application duration and frequency, and follow-up time. Ongoing experiments are needed to explore these issues.

5. Conclusions

We have demonstrated that VED therapy in the BCNC model preserves EF and acts by preserving smooth muscle content and endothelial integrity via antihypoxia, antiapoptosis, and antifibrosis mechanisms. The daily VED therapy effect on EF recovery is consistent with patients’ results and without significant side effects. This scientific evidence, although from an animal model, may motivate physicians’ recommendations and improve patients’ compliance in the clinical setting.

Author contributions: Run Wang 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: Yuan, Lin, Wang.
Acquisition of data: Yuan, Lin, Li, Zhang, Luo, Berardinelli.
Analysis and interpretation of data: Yuan, Lin, Wang.
Drafting of the manuscript: Yuan.
Critical revision of the manuscript for important intellectual content: Dai, Wang.

Administrative, technical, or material support: Yuan, Lin, Li, Zhang, Luo, Dai, Wang.
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