ORIGINAL ARTICLE

Erectile Dysfunction

Vacuum therapy prevents corporeal veno-occlusive dysfunction and penile shrinkage in a cavernosal nerve injured rat model

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Erectile dysfunction and penile shrinkage are the common complications after radical prostatectomy. Penile rehabilitation is widely applied after the surgery. Vacuum therapy is one of the three penile rehabilitation methods used in the clinical setting, but its mechanism is not well known. This study was designed to investigate whether vacuum erectile device (VED) can prevent corporeal veno-occlusive dysfunction and penile shrinkage in the bilateral cavernous nerve crush (BCNC) rat model. Adult male Sprague–Dawley rats were randomly assigned into three groups: sham group, BCNC group, and BCNC + VED group. After 4 weeks, penile length and intracavernosal pressure (ICP) were measured, and then the middle part of the penis was harvested after dynamic infusion cavernosometry to complete the following items: smooth muscle/collagen ratios and collagen I/III ratios; ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell; and the expression of calponin-1 and osteopontin. The penile shortening, peak ICP and ICP drop rate after alprostadil injection were significantly improved with vacuum therapy after 4-week treatment. Compared with BCNC group, VED significantly increased smooth muscle/collagen ratios, decreased collagen I/III ratios, and preserved the ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell. The data also showed that animals exposed to VED could partially reverse the expression of calponin-1 and osteopontin induced by BCNC. In conclusion, vacuum therapy is effective to prevent penile shrinkage and veno-occlusive dysfunction in penile rehabilitation, which may be associated with well-preserved structure and function of the tunica albuginea, endothelial cell, and smooth muscle cell.
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Keywords: corporeal veno-occlusive dysfunction; erectile dysfunction; penile rehabilitation; penile shrinkage; vacuum therapy

INTRODUCTION

Prostate cancer is the second leading cause of cancer death in men after lung cancer. Radical prostatectomy is the gold standard therapeutic option for patients with localized prostate cancer.[1,2] Unfortunately, previous studies have shown that many patients suffer from erectile dysfunction (ED) and penile shrinkage after radical prostatectomy.[3–5] To improve the quality of life and the acceptance of radical prostatectomy, penile rehabilitation is now widely accepted in clinical practice.[5,6] Phosphodiesterase type 5 inhibitors are considered the first-line therapy for ED patients, but lack of efficacy leads to discontinuation in >50% of postradical prostatectomy patients.[7,8]

Recent accumulating evidence has demonstrated that the vacuum erectile device (VED) has produced favorable affect for ED patients after radical prostatectomy and has become the second most commonly used modality for penile rehabilitation.[4–6] VED is an acceptable nonsurgical treatment for patients with ED secondary to corporeal veno-occlusive dysfunction (CVOD) regardless of severity. Clinical data showed that VED can also preserve penile length for patients after radical prostatectomy.[4,9–11] CVOD, also known as venous leakage,

has been proposed as one of the most common causes of venogenic ED.[9–12] Erectile tissue morphological change, which manifests as collagen deposition and smooth muscle apoptosis in postradical prostatectomy patients, may be one of the underlying mechanisms of venous leak.[13] Our previous studies suggested that daily use of VED may preserve penile size, and that the effects are related to anti-apoptosis and antihypoxia by increasing cavernous blood SO_2.[6,14] Clinical data showed the efficacy and safety of VED in ED patients caused by variety of reasons; however, there is little information from basic research to explore the efficacy and possible mechanisms of vacuum therapy preventing CVOD and penile shrinkage after radical prostatectomy.

The object of the current research was to evaluate whether VED therapy could prevent CVOD and penile shrinkage in the bilateral cavernous nerve crush (BCNC) rat model. If so, we would explore the mechanisms involved.

MATERIALS AND METHODS

Surgical preparation of animals

Because the rat penile tissue is scarce for all the tests in our research, we performed two rounds of intervention according to the same

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protocol, details as follows: thirty adult male Sprague–Dawley rats (250–300 g) were obtained from Chengdu Dashuo Biotechnology Co., Ltd (Chengdu, China). Rats were randomly assigned into three groups: (1) sham group: rats underwent the same surgical procedure but no nerve crushing; (2) BCNC group: rats underwent the surgical procedure of BCNC but no VED therapy; and (3) BCNC + VED group: rats underwent the surgical procedure of BCNC, with VED therapy beginning at 10 days after surgery, 5 min twice daily with a 1-min interval. The total treatment time was 4 weeks. The rat-specific VED device was a patent product, which was designed according to human-use VED and had been reported in our previous paper.[15] Length and diameter of the cylinder were 50.00 mm and 9.78 mm, respectively. The maximum negative pressure was around 210 mmHg. The surgical procedure of BCNC was performed according to a previous study.[6,16] The bilateral cavernous nerves were crushed using an ultra-fine straight hemostat with full tip closure for 30 s. The rats were raised in the animal experiment center of West China Hospital (Chengdu, China) and were given sufficient food and water. The animal study proposal was legally approved by the Animal Care and Welfare Committee of West China Hospital, Sichuan University (No. 2018014A).

Penile length measurement and tissue harvesting
Penile length was measured from the pubic bone to the tip of the penis before the BCNC procedure. After 4-week treatment, the rats were recorded for penile length and intracavernosal pressure (ICP).[17] Five micrograms of alprostadil was administered through a cannula into the corpora cavernosa, and the ICP was recorded. Saline was then infused through another cannula, increasing infusion rate by 0.05 ml min^{-1} every 10 s, until the ICP reached 100 mmHg. The "drop rate" was determined by recording the fall in ICP within the next 1 min after the infusion was stopped. At the completion of functional analysis, the penis was excised for histopathological analysis. The skin and the subcutaneous tissue of the penis were removed, and only the mid-shaft of the penis was harvested for examination.

Histopathology
Following routine dehydration and paraffin-embedding procedures, tissue samples, which were cut into 5-mm sections from the mid-shaft of the penis, were mounted on slides and dried. Next, the tissue slides, showing the cross section of the corpora cavernosa, were deparaffinized and rehydrated for the following studies. Collagen fibers were stained with Masson's trichrome (BA4079A, Baso Diagnostics Inc., Zhuhai, China) according to standard protocol, which was reported previously;[6] 5 μm-thick sections of corpora cavernosa were incubated with different primary antibodies to detect the presence of collagen Types I, III, and IV after antigen retrieval by 0.4% pepsase (1:250, 1:500, and 1:500, respectively). All of the primary antibodies were purchased from Abcam Company (Cambridge, MA, USA), and dilutions were done in phosphate buffer solution (PBS) containing 1% bovine serum albumin. The peroxidase/DAB EnVisionTM detection kit (DAKO, Glostrup, Denmark) was used to determine antigen antibody interaction. Negative staining controls were achieved by omitting the primary monoclonal antibody. The samples were visualized using an optical microscope (Zeiss AX10, Zeiss, Oberkochen, Germany).

Transmission electron microscopy
The samples were postfixed for 2 h with 1% osmium tetroxide (Sigma-Aldrich, St. Louis, MO, USA) and then dehydrated in a graded series of ethanol solutions. After being dried by the critical point drying method, the samples were mounted on suitable carriers and coated with gold. The samples were embedded in Epon 812 (Ted Pella, Redding, CA,

USA) before they were thinly sectioned. Thin sections were mounted on clean 200 mesh copper grids and stained with 2% uranyl acetate and 1% lead citrate. The sections were examined using a transmission electron microscope (H-7650; Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

Western blot analysis
Frozen intact corpus cavernosum tissues were isolated and prepared in the protein lysis buffer, followed by centrifugation at 12 000 *g* for 10 min at 4°C. Equal amounts of samples were separated by SDS/PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore Corporation, Billerica, MA, USA). After blocking in 5% bovine serum albumin for 1 h at room temperature, the membranes were incubated with the following antibodies at 4°C overnight: anti-calponin-1 (1:1000, Abcam, ab46794) and anti-osteopontin (1:500, Abcam, ab8448). After washing three times in PBS with 0.1% Tween 20 for 30 min, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:10000, Affinity Biosciences, Cincinnati, OH, USA) for 1 h at room temperature, followed by another 30 min of washing, and analyzed using an enhanced chemiluminescence detection system (Thermo Fisher Scientific, Waltham, MA, USA).

Real-time PCR assay
Total cellular RNA was extracted using the BIOzol total RNA extraction kit (BioFlux Co., Tokyo, Japan) according to the manufacturer's instructions. First-strand cDNAs were synthesized from total RNA using M-MLV transcriptase and oligo-dT primer. Quantitative real-time PCR was performed using duplicate SsoFast™ EvaGreen® Supermix kit (Bio-Rad, Hercules, CA, USA) in Chromo4 real-time PCR detector (Bio-Rad) following the manufacturer's instructions. The primer sequences used are presented in **Table 1**. The relative quantitation of gene expression was performed using the comparative Ct (ΔΔCt) method with glyceraldehyde-phosphate dehydrogenase (*GAPDH*) as a housekeeping gene. Experiments were repeated at least twice.

Statistical analyses
The pathological pictures (eight specimens in each group, five sections per specimen, and five fields of each slice were randomly selected under 400-fold field light microscope) were quantitatively analyzed by Image-Pro Plus version 6.0 (Media Cybernetics Inc., Rockville, MD, USA). The results were expressed as mean ± standard deviation (s.d.). The data were analyzed with Student's *t*-test using SPSS 13.0 software (SPSS, Chicago, IL, USA). The difference was considered statistically significant when $P < 0.05$.

RESULTS
Evaluation of penile length and veno-occlusive function
The results of penile length in the three groups are shown in **Figure 1**. There was no significant difference in penile length before BCNC

Table 1: The primer sequences of calponin-1, osteopontin, and glyceraldehyde-3-phosphate dehydrogenase

Gene	Primer sequence
Calponin-1	Forward: 5'-TCAATGTGGGGGTCAAATATG-3'
	Reverse: 5'-TAGGCTGTCATGCCCTGCTG-3'
Osteopontin	Forward: 5'-GAGTTTGGCAGCTCAGAGGA-3'
	Reverse: 5'-TCTGCTTCTGAGATGGGTCA-3'
GAPDH	Forward: 5'-GGACCAGGTTGTCTCCTGTG-3'
	Reverse: 5'-TGTAGGCCATGAGGTCCAC-3'

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

among the groups. After 4 weeks, penile shortening was observed in the BCNC group (mean ± s.d.: 2.98 ± 0.13 cm), compared with the sham group (mean ± s.d.: 3.24 ± 0.14 cm, $P < 0.05$) and the BCNC + VED group (mean ± s.d.: 3.16 ± 0.13 cm, $P < 0.05$). The results showed that VED therapy partially reversed BCNC-induced penile shortening.

At the end of the 4-week treatment, the rats were experimented for CVOD under alprostadil injection and saline infusion, while ICP was simultaneously recorded. As shown in **Figure 2**, the peak ICP after alprostadil was decreased for the BCNC rats (mean ± s.d.: 64.0 ± 4.0 mmHg), compared with the one in the sham group (mean ± s.d.: 76.0 ± 4.3 mmHg), which indicates the presence of ED. On saline infusion, the drop rates were very low in the sham group (mean ± s.d.: 18.0 ± 1.6 mmHg min^{-1}), confirming normal corporal veno-occlusion. However, in the BCNC rats, the drop rate (mean ± s.d.: 43.0 ± 3.0 mmHg min^{-1}) was twice more than that in the sham group, suggesting CVOD. VED therapy was found to dramatically improve the peak ICP and the drop rate after alprostadil, compared with the BCNC group ($P < 0.01$). The results showed that BCNC led to moderate CVOD, and CVOD was partially prevented by VED therapy.

Histopathology of smooth muscle and collagen fibers

Penile tissue was harvested and processed after functional measurement. Hematoxylin and eosin staining was performed to study the microscopic structure of the penile (**Figure 3**). Then, the tissue sections were stained with Masson's trichrome for the identification of the penile smooth muscle/collagen ratio. Collagen fibers are stained as blue and smooth muscle fibers are stained as purple and red (**Figure 3**). In the sham group and the VED group, the thickness of the tunica albuginea is more conforming and has more cell nucleus and regular finger-like structures than the BCNC group. After analysis, the percentage (mean ± s.d.) of smooth muscle/collagen within rat corpora cavernosa was 6.28% ± 0.69% in the sham group, 2.12% ± 0.24% in the BCNC group, and 5.74% ± 0.57% in the BCNC+VED group ($P < 0.01$ compared with the BCNC group).

The collagen subtypes I, III, and IV mainly contributed to the corporal fibrous network of the tunica albuginea. One feature of fibrosis is a change in the ratio of different collagen types. Immunohistochemistry was used for the expression of collagen subtypes. As shown in **Figure 4**, BCNC dramatically increased the collagen I/III ratios and decreased the expression of collagen IV compared with the sham group, respectively. The study showed that the collagen I/III ratio was increased by 43% after BCNC (mean ± s.d.: 1.96 ± 0.16 to 1.37 ± 0.05). However, in the BCNC + VED group (mean ± s.d.: 1.39 ± 0.05), the collagen I/III ratio approximated that, of the sham group, VED therapy prevented the
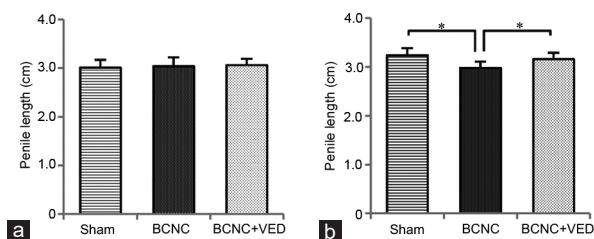
alteration of collagen ratios in the tunica albuginea ($P < 0.05$). The distribution of collagen IV was mainly located in the trabeculae of the cavernous spaces, and collagen IV of the tunica albuginea was higher positive in the BCNC group compared with the sham group and the BCNC + VED group.

The ultramicrostructure of the penis

A transmission electron microscopy was employed to demonstrate the arrangement of the collagen fibrils, endothelial cells, and smooth muscle cells in the penis. In the BCNC group, collagen fibrils of the tunica albuginea have an irregular size and spacing (**Figure 5**); cell degeneration was also noticeable in endothelial cells and smooth muscle cells. As shown in **Figure 5**, in the sham group, the intercellular tight junctions were normal; organelles in cytoplasm such as rough endoplasmic reticulum and mitochondrion appeared to be abundant. However, a loss or reduction of normal membrane contact occurred in some smooth muscle cells and endothelial cells for the BCNC group. Organelles in cytoplasm such as endoplasmic reticulum, Golgi complex, and mitochondrion appeared to be decreased. The mitochondria showed aggregation and were markedly dilated and vacuolated. The results showed that VED therapy partially reversed the ultramicrostructure. However, in the BCNC + VED group, the ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell approximated that of the sham group; VED therapy prevented the alteration of the ultramicrostructure.

The phenotype transformation of smooth muscle

Western blot analysis and real-time PCR were used to detect the expression levels of calponin-1 and osteopontin in corpus cavernosum smooth muscle. The results from Western blot analysis showed the lower protein levels of calponin-1 (mean ± s.d.: 0.53 ± 0.02 *vs* 0.76 ± 0.03, $P < 0.01$) and higher levels of osteopontin expression (mean ± s.d.: 1.14 ± 0.11 *vs* 0.80 ± 0.07, $P < 0.01$) in the BCNC group, compared with the sham group. As shown in **Figure 6**, VED therapy partially reversed the protein levels of calponin-1 (mean ± s.d.: 0.83 ± 0.03) and osteopontin (mean ± s.d.: 0.73 ± 0.07) (both $P < 0.01$ compared with the BCNC group). The results from real-time PCR also showed lower levels of calponin-1 expression (mean ± s.d.: 0.25 ± 0.09 *vs* 1.00 ± 0, $P < 0.01$) and higher levels of osteopontin expression (mean ± s.d.: 3.07 ± 0.27 *vs* 1.00 ± 0, $P < 0.01$) in the BCNC group, compared with those in the sham group. VED therapy partially reversed the expression levels of calponin-1 (mean ± s.d.: 0.53 ± 0.06) and osteopontin (mean ± s.d.: 0.86 ± 0.05) (both $P < 0.01$ compared with the BCNC group). These findings showed that VED

Figure 1: VED therapy partially reversed BCNC-induced penile shortening. Penile length was measured (**a**) before the BCNC procedure and (**b**) after 4 weeks' treatment. Compared with the baseline and the sham group, BCNC dramatically decreased penile length. However, VED treatment partially prevented penile shrinkage compared with the BCNC group. Eight rats were in each group, and statistical analysis was performed using Student's *t*-test. *$P < 0.05$. VED: vacuum erectile device; BCNC: bilateral cavernous nerve crush.
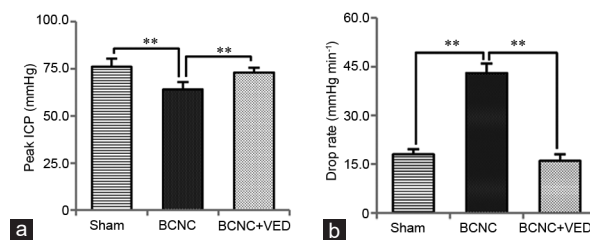

Figure 2: VED therapy partially reversed erectile function and veno-occlusive dysfunction in BCNC rats. BCNC dramatically (**a**) decreased the peak ICP after alprostadil and (**b**) increased the drop rate. VED therapy was found to significantly improve the peak ICP and the drop rate after alprostadil, compared with the BCNC group. Eight rats were in each group, and statistical analysis was performed using Student's *t*-test. **$P < 0.01$. VED: vacuum erectile device; BCNC: bilateral cavernous nerve crush; ICP: intracavernosal pressure.
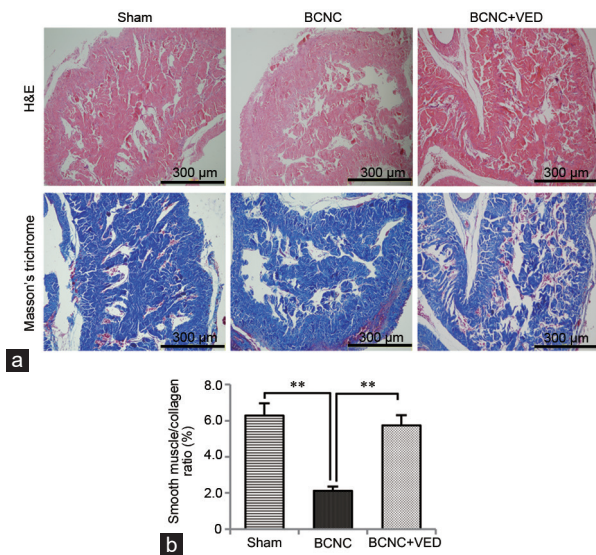
Figure 3: VED therapy partially preserved the smooth muscle/collagen ratio in BCNC rat model. (**a**) H and E staining was performed to study the microscopic structure of corpora cavernosa, and Masson's trichrome staining was used for the penile smooth muscle/collagen ratio. (**b**) Compared with the sham group, BCNC dramatically decreased the penile smooth muscle/collagen ratio. VED treatment significantly increased the penile smooth muscle/collagen ratio compared with the BCNC group. Eight specimens were in each group, and statistical analysis was performed using Student's *t*-test. **$P < 0.01$. VED: vacuum erectile device; BCNC: bilateral cavernous nerve crush; H and E: hematoxylin and eosin.
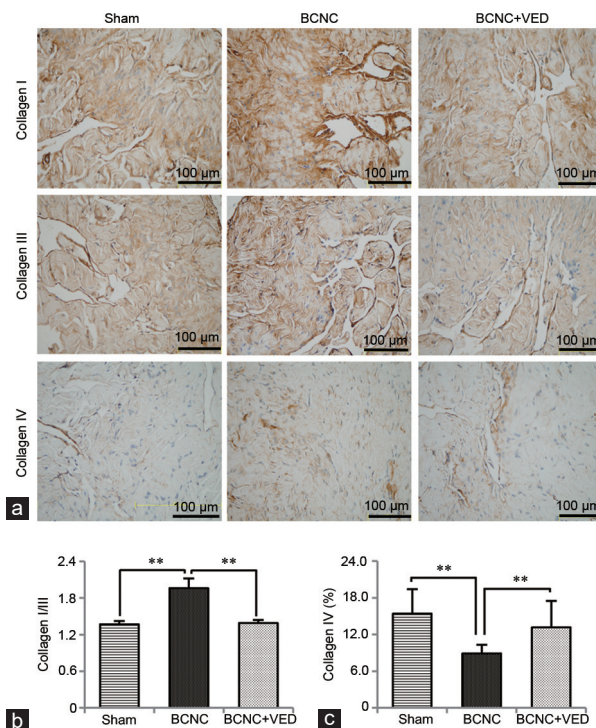

Figure 4: VED therapy partially preserved the native collagen structure of the penis to a much better degree in BCNC rat model. (**a**) Immunohistochemistry was used for the expression of collagen I, III, and IV. Statistical analysis of (**b**) collagen I/III ratio and (**c**) collagen IV were performed using Student's *t*-test. BCNC dramatically increased the collagen I/III ratios and decreased the expression of collagen IV compared with the sham group, respectively. VED therapy significantly preserved the native collagen structure in tunica albuginea. Eight specimens were in each group. **$P < 0.01$. VED: vacuum erectile device; BCNC: bilateral cavernous nerve crush.
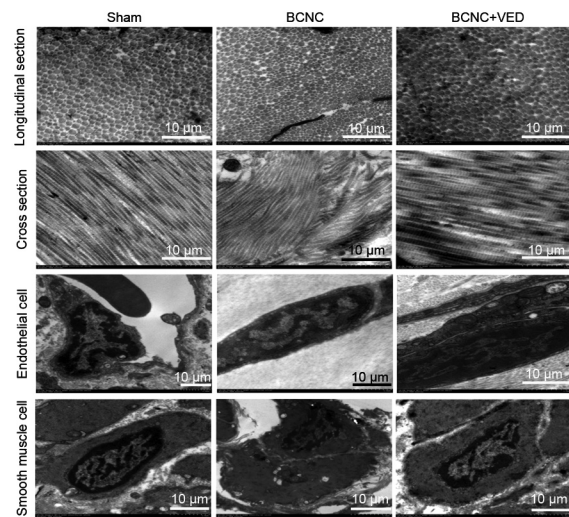

Figure 5: VED therapy partially reversed the ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell in BCNC rat model. The ultramicrostructure changes of the longitudinal and cross sections of the tunica albuginea, endothelial cell, and smooth muscle cell were observed by a transmission electron microscope. In the BCNC group, collagen fibrils of tunica albuginea had an irregular size and spacing; cell degeneration was also noticeable in endothelial cells and smooth muscle cells. VED therapy partially reversed the ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell induced by BCNC. VED: vacuum erectile device; BCNC: bilateral cavernous nerve crush.

therapy could prevent the phenotype transformation of the corpus cavernosum smooth muscle after BCNC and maintain smooth muscle systolic and diastolic function and inhibit extracellular matrix abnormal deposition.

DISCUSSION

Traditionally, radical prostatectomy is the gold standard therapeutic option for patients with localized and locally advanced prostate cancer.[18] However, this view has been challenged by recent accumulating studies documenting that no significant differences in mortality for localized prostate cancer between men who underwent surgery and those who were treated with observation only.[19,20] Moreover, surgery was also associated with a higher frequency of adverse events (ED, penile shrinkage, *etc.*) than observation.[4] The purpose of PR is to preserve normal structure and minimize damage to erectile tissue during the period of neural recovery after RP by providing adequate oxygenation to the cavernous tissues.[21] Vacuum therapy has been investigated as a treatment option for PR after RP without the constriction ring unless for sexual intercourse on demand.[5] Numerous studies indicated that the beneficial effects of VED therapy were related to antihypoxia by increasing cavernous infusion and tissue blood oxygen partial pressure (PO_2). Briefly, Broderick *et al.*[22] showed that negative pressure transiently increased central cavernous arterial blood flow velocity. Bosshardt *et al.*[23] further showed that the mean O_2 saturation of corporal blood immediately after VCD-induced erection was 79.2%, and the calculated contribution of arterial and venous blood to penile blood volume was 58% and 42%, respectively. Our previous data demonstrated that VED therapy partially reversed HIF-1α expression induced by BCNC.[6] Using the same protocol, Lin *et al.*[14] calculated that blood composition in the corpus cavernosum immediately after VED application was 62% arterial and 38% venous, which was similar with
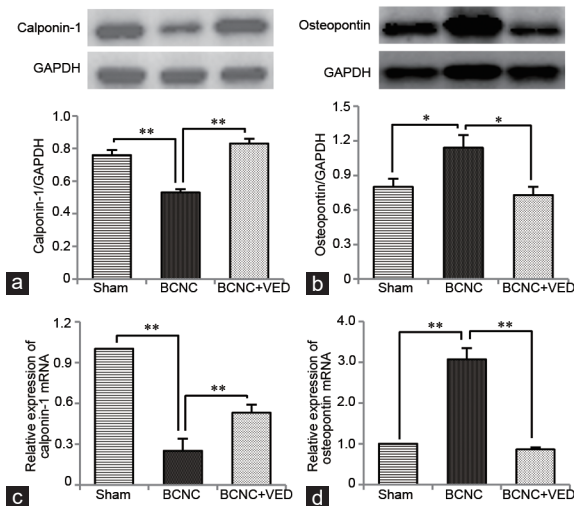
Figure 6: VED therapy partially reversed the expression levels of calponin-1 and osteopontin in BCNC rat model. Western blot analysis was used to detect the expression levels of (**a**) calponin-1 and (**b**) osteopontin in corpus cavernosum smooth muscle compared with GAPDH; PCR was used to detect the mRNA expression levels of (**c**) calponin-1 and (**d**) osteopontin in corpus cavernosum smooth muscle. Compared with the sham group, the results showed higher levels of osteopontin expression and lower levels of calponin-1 expression in the BCNC group. VED therapy partially reversed the expression levels of calponin-1 and osteopontin. Eight rats were in each group, and statistical analysis was performed using Student's *t*-test. *$P < 0.05$; **$P < 0.01$. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; VED: vacuum erectile device; BCNC: bilateral cavernous nerve crush.

Bosshardt's research.[23] In a word, vacuum passively induced penile tumescence by venous and arterial mixed blood without smooth muscle relaxation or release of neurotransmitters.

In flaccid state, the cavernous sinus space and the subtunical venous plexus are opened; the blood flows from cavernous to systemic circulation freely. When penis erects, the dilated blood sinus compresses most veins, while the larger veins are compressed between the expanded cavernosal sinus and the tunica albuginea, to maintain adequate hardness. The tunica albuginea and corpus cavernosum smooth muscle below it are believed the most important structure in corporal veno-occlusive dysfunction.

Previous reports demonstrated that VED therapy was effective in improving erectile function through antihypoxic, anti-apoptotic, and antifibrotic mechanisms.[5,6,9,10,14] However, there is little information on the mechanisms of VED therapy in preventing penile shrinkage and CVOD. The present study showed that BCNC dramatically decreased penile length (**Figure 1**), decreased the peak ICP after alprostadil, and increased the drop rate (**Figure 2**). These confirmed our previous hypothesis that CVOD is induced in BCNC rats and VED therapy obviously improved the penile length and CVOD. In the present study, we used a BCNC rat model to assess the potential mechanisms of VED therapy in penile rehabilitation. Our data demonstrated that the penile length and the ICP were improved after VED therapy in BCNC rats, and VED therapy is effective to prevent penile shrinkage and CVOD. Further studies found that VED therapy could increase smooth muscle/collagen ratios, decrease collagen I/III ratios, and preserve the ultramicrostructure of the tunica albuginea in BCNC rats. The study also showed that animals exposed to VED therapy could partially reverse the expression of calponin-1 and osteopontin induced by BCNC.

The penile corpora are a specialized vascular tissue, which are critical to the erectile process. CVOD, which is usually associated with a loss of smooth muscle cells and an increase in fibrosis within the corpora cavernosa, can be induced by an injury to the cavernosal nerves. Compared with the sham group, the BCNC group dramatically decreased penile smooth muscle/collagen ratio (**Figure 3**), and it is similar to the result of Kovanecz *et al*.[24] in bilateral cavernosal nerve resection rats. However, VED treatment partially preserves the ratio in rat penis. The presumptive mechanisms are the decrease of apoptosis of muscle cells and prevention of collagen deposition.

Along with corpus cavernous tissue, the penile length and CVOD are dependent on the structure and function of the tunica albuginea to a large extent via its compliant changes. CVOD, which occurs secondary to collagen deposition and smooth muscle apoptosis, ultimately leads to venogenic ED, and the loss of erection caused by CVOD might exacerbate the cavernous hypoxia and further increase the collagen deposition.[17,24] Eventually, those functional changes would have resulted in irreversible tissue damage.

The tunica albuginea, an important penile structure, plays a particularly determinant role in the penile veno-occlusive mechanism and penile length.[25,26] The tunica albuginea is composed of two key elements, collagen fibers (mostly Type I, but also Type III) and elastic fibers. Both collagen and elastic fibers permit increase in girth and length during tumescence. Elastic fibers can stretch to 150% of their normal length during erection. The study of Raviv *et al*.[27] on ED patients showed that Type I collagen was significantly higher in the tunica albuginea, and it is similar to the result of the present study. In our study, BCNC dramatically increased the expression of collagen I and collagen IV compared with the sham group (**Figure 4**), respectively. VED therapy preserved collagen I/III ratios in the tunica albuginea, compared with the BCNC group ($P < 0.05$). In addition, the decreased ratio of collagen Types I/III can provoke mechanical alterations of the penis, which may reduce the elasticity and compliance of the tunica albuginea.

Evidences gathered from many studies show that CVOD is resulted from degenerative and atrophic changes of the tunica albuginea collagen fibers, which could lead to the subluxation and floppiness of the tunica albuginea.[25,28] User *et al*.[29] found that damage to the subtunical smooth muscle cells prevents compression of the perforating subtunical veins, resulting in CVOD and ED. To investigate whether changes in the structure of the penis influence the veno-occlusive mechanism and penile length, the transmission electron microscopy was employed to study the ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell. VED therapy could partially reverse the ultramicrostructure of the tunica albuginea, endothelial cell, and smooth muscle cell induced by BCNC (**Figure 5**).

Different from cardiac and skeletal muscle cells that have a terminally differentiated state, smooth muscle cells can transform their phenotype from a contractile state to a proliferative state in response to the change of local environmental stimuli.[30] The overexpression of a proliferative state of smooth muscle cells would lead to more extracellular matrix secretion, causing further abnormal tissue fibrosis.[17,30] The changes in the protein expression levels of calponin-1 (contractile marker) and osteopontin (proliferative marker) have been identified as useful markers for phenotypic modulation of corpus cavernosum smooth muscle cells. A shift in phenotype from a contractile to a proliferative state in theory leads to downregulated expression of contractility-associated markers and upregulated expression of proliferative smooth muscle cell markers.[30,31] We showed

that the expression of ASMA was preserved in BCNC rats treated with VED.[6] In the present study, the expression of calponin-1 was decreased while the expression of osteopontin was increased in the BCNC group, compared with the sham group. VED therapy partially reversed the expression levels of calponin-1 and osteopontin (**Figure 6**). Therefore, VED therapy is effective to regulate the corpus smooth muscle cell phenotypic modulation in the early stages of cavernous injury model rats.

There is a major limitation in the current study – we did not optimize the regimen for the VED therapy in preventing CVOD and penile shrinkage. Therefore, further studies should be performed to determine the optimum vacuum pressure, duration and frequency, and follow-up time.

CONCLUSION
Data from the present study suggest that VED therapy is effective to prevent penile shrinkage and CVOD in penile rehabilitation, which may be associated with the well-preserved structure and function of the tunica albuginea, endothelial cells, and smooth muscle cells.

AUTHOR CONTRIBUTIONS
SQQ, FQ, and JHY designed the study. SQQ, FQ, SZ, and YY conducted the data acquisition and interpreted and analyzed the data. SQQ and FQ drafted and JHY revised the manuscript. RW and QW pointed out deficiencies and ameliorated the manuscript. JHY supervised the whole work. All authors read and approved the final manuscript.

COMPETING INTERESTS
All authors declared no competing interests.

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