BASIC SCIENCE

The Role of Long Term Label-Retaining Cells in the Treatment of Erectile Dysfunction by Vacuum Erectile Device

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ABSTRACT

Introduction: Vacuum erectile device (VED) therapy is commonly used for penile rehabilitation after radical prostatectomy, however, the underlying mechanism of this effect is not fully understood.

Aim: To evaluate the presence of label-retaining cells (LRCs), cells with long-term retention of 5-ethynyl-2-deoxyuridine (EdU) labeling and recognized as adult stem cells or progenitor-like cells, in cavernous tissue after VED treatment using a BCNC rat model.

Methods: Postnatal pups (1 day old) of Sprague Dawley (SD) rats were intraperitoneally injected with EdU (50 ug/g, BID for 3 days) and BCNC surgery was conducted at 6 weeks old (designated as natal-labeled rats). Adult SD rats underwent BCNC surgery and EdU injection (50 ug/g, once) after surgery (designated as adult-labeled rats). One week after surgery, both natal- and adult-labeled rats received daily VED treatment for 4 weeks. Intracavernous pressure (ICP) and mean arterial pressure (MAP) were measured for all rats and then the penile tissue was harvested. The ratio of ICP/MAP was calculated to represent erectile function. Penile tissue was examined by immunofluorescence staining to detect EdU positive cells.

Main Outcome Measures: The ratio of Intracavernous pressure (ICP) /MAP and the percentage of EdU positive cells were measured.

Results: The erectile function was impaired after BCNC and partially restored after VED treatment in both natal- and adult-labeled rats ($P < .05$). There was no difference in the percentage of EdU positive cells in natal-labeled rat cavernous tissue in BCNC group compared with VED group. Among the adult-labeled rats, the percentage of EdU positive cells increased in BCNC group ($P < .05$) but didn't change significantly after VED treatment ($P = .35$).

Conclusion: LRCs may play a limited role in the restoration of erectile dysfunction through VED treatment after BCNC. **Yang B, Luse D, Cao Y, et al. The Role of Long Term Label-Retaining Cells in the Treatment of Erectile Dysfunction by Vacuum Erectile Device. Sex Med 2021;9:100442.**

Key Words: Erectile Dysfunction; Bilateral Cavernous Nerve Crush; Vacuum Erectile Device; Label-Retaining Cells; Stem Cells

INTRODUCTION

Erectile dysfunction (ED) is one of the most common complications after radical prostatectomy (RP), with 60% patients experienced self-reported ED 18 months after RP.[1] Vacuum erectile device (VED) therapy has been shown to preserve penile length after radical prostatectomy.[2,3] It is commonly used for postsurgical penile rehabilitation and is the second leading modality after phosphodiesterase type 5 inhibitor among American urologists.[4] In our previous studies, VED treatment has been shown to restore erectile function in bilateral cavernous nerve crush injury (BCNC) rat models.[5,6] However, the mechanism

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by which VED preserves penile tissue and erectile capability is not fully understood.

Adult stem cells have demonstrated a role in tissue repair in multiple organs as well as the penis.[7-9] Label-retaining cells (LRCs) are recognized as adult stem cells or progenitor-like cells based on slow-cycling and infrequent replication, which allows for the retention of 5-bromo-2-deoxyuridine (BrdU) labeling, a thymidine analog that is incorporated into proliferating cells during the S phase of cell cycle.[10] Lin et al[11] demonstrated the existence of LRCs in cavernous tissue using 5-ethynyl-2-deoxyuridine (EdU) labeling, an alternative to BrdU. In this study, we hypothesize VED treatment may increase the number of LRCs in cavernous tissue after BCNC, which may play a role in preserving cavernous tissue and erectile function.

METHODS

EdU Labeling

All animal experiments in this study were approved by the Institutional Animal Care and Use Committee at University of Texas Health Science Center at Houston. Newborn male Sprague−Dawley rat pups (1 day) with dams and male adult rats (6 weeks, weight 200−250 g) were purchased from Charles River Laboratories. Cell labeling of the rat pups was conducted by intraperitoneal injection of EdU (50 μg/g, BID for 3 days)[11,12] at the time the rats were received from the vendor (natal-labeled, Figure 1A). The adult rats were intraperitoneally injected with EdU (50 μg/g, once)[13] before VED treatment. (adult-labeled, Figure 1B).

Bilateral Cavernous Nerve Crush (BCNC) Model

Eighteen natal-labeled rats were randomly selected for BCNC (n = 12) or sham surgery (n = 6) and underwent the procedure at six-weeks old (Figure 1A). Eighteen adult rats were similarly selected to undergo BCNC (n = 12) or sham (n = 6) surgery (Figure 1B). Procedure details of the BCNC surgery has been described in a previous study.[14] In brief, the rats were anesthetized, shaved and disinfected. A midline suprapubic incision was made to expose the bladder and prostate. The major pelvic ganglion (MPG) was carefully expose with cotton swabs. Cavernous nerves were then isolated and identified. At the point of 5 mm distal to the major pelvic ganglion, the bilateral cavernous nerves were crushed using an ultrafine hemostat with full tip closure for 30 seconds, removed for 30 seconds and then repeated for another 30 seconds. The incision was closed with 4−0 Vicryl sutures (Johnson & Johnson, New Brunswick, NJ, USA). Sham surgery consisted of a similar abdominal incision with exposure of the major pelvic ganglion and cavernous nerves without manipulation.

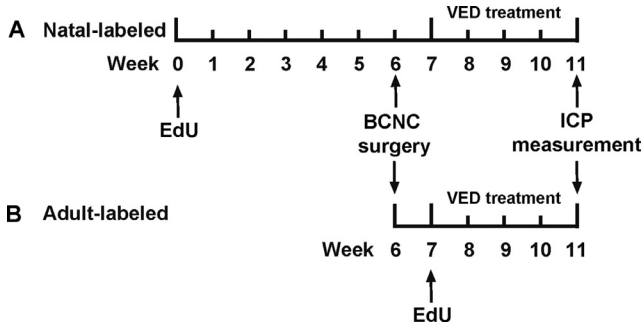

Figure 1. *Time course of EdU injection, BCNC surgery and VED treatment. (A) Natal-labeled rats.* The rat pups (1 d old) were intraperitoneally injected with EdU (50 μg/g, BID for 3 d), when received from the vender to identify label-retaining cells (LRCs). Bilateral cavernous nerve crush (BCNC) or sham surgery were conducted at six-weeks of age. *(B) Adult-labeled rats.* The 6-wk-old adult rats underwent BCNC or sham surgery followed with intraperitoneal injection of EdU (50 μg/g, once) 1 wk after surgery to identify LRCs. Vacuum erectile device (VED) treatment was conducted for part of natal- and adult-labeled rats (n = 6) one week after BCNC surgery. Intracavernous pressure (ICP) were measured after 4 weeks of VED treatment, and the penile tissue was harvested for EdU detection.

VED Treatment

A clitoral therapy device (CTD; Eros Therapy, Urometrics, St. Paul, MN, USA) and a tube with a glass funnel was employed for the rat VED treatment.[5] Rats underwent BCNC were randomly selected and conducted VED treatment daily for 4 weeks under general anesthesia with inhaled isoflurane one week after surgery (n = 6 for both natal- and adult-labeled rats) (Figure 1). The treatment included 5 vacuum-induced erections lasting 1 minute with 1 minute of spontaneous detumescence allowed between. Rats underwent sham surgery or BCNC without VED treatment also received anesthesia with same duration.

Intracavernous Pressure (ICP) and Mean Arterial Pressure (MAP) Measurement

After 4 weeks of VED treatment, the ICP and MAP of the animals was recorded (Figure 1). The procedure details of ICP and MAP measurements has been described in a previous study.[15] In brief, the rats were anesthetized with inhale isoflurane. The bilateral major pelvic ganglions and cavernous nerves were carefully exposed with cotton swabs as stated above. The corpus cavernosum was cannulated with a heparinized (200 U/mL) 27G needle connected to a pressure transducer and bio-signal collection processing system (PowerLab /4SP, ADInstruments, Dunedin, New Zealand). Electric stimulations to cavernous nerves were performed at 5 V for approximately 60 second with resting periods of 5 minute between subsequent stimulations. The maximum ICP of three stimuli per side was selected for statistical analysis in each animal. MAP was recorded using a 27G needle inserted into the aortic bifurcation. The ratio of ICP/MAP was calculated to quantify erectile function.

Immunofluorescence

Cavernous tissues were harvested immediately after ICP/MAP measurement and stabilized in cold 4% paraformaldehyde with 10% sucrose in phosphate buffer overnight. The specimens were embedded in OCT freezing medium (Sakura Finetek, Torrance, CA), sliced into 5-μm frozen sections and mounted on Select-frost Adhesion Microscope Slides (Fisher Scientific, Pittsburgh, PA, USA). EdU was detected using Click-it EdU cell proliferation kit (Thermofisher, CA, USA) according to the protocol of the kit.

Statistical Analysis

Data was presented as mean ± SEM. One-way ANOVA followed by LSD post hoc analysis was used to compare the results among the groups by GraphPad Prism 8 (GraphPad Software. La Jolla, CA, USA). $P < .05$ was considered statistically significant.

RESULTS

Erectile Function Was Impaired by BCNC Surgery, and Restored After VED Treatment

After VED treatment, ICP and MAP were measured in all rats. In both natal-labeled rats (Figure 2B) and adult-labeled rats (Figure 2C), the ratio of ICP/MAP decreased after BCNC surgery ($P < .05$) and was partially restored after VED treatment ($P < .05$).

Limited Role of LRCs in the Restoration of Erectile Function After VED Treatment

LRCs were diffusely distributed in the cavernous tissue of both natal- and adult-labeled rats. In natal-labeled rats, no significant difference of the percentage of LRCs was observed among Sham, BCNC and VED groups (Figure 3A, B). While in adult-labeled rats, the percentage of LRCs significantly increased in BCNC group ($P < .05$) but didn't change significantly after VED treatment ($P = .35$) (Figure 3C, D).

DISCUSSION

In this study, the presence and distribution of EdU-LRCs were examined in a BCNC rat model after VED treatment. While the VED group showed partial recovery of erectile function, there was no significant difference in the presence of LRCs when compared with the BCNC group and the sham group of natal-labeled rats. Conversely, LRCs increased significantly in the BCNC group compared to the sham group in adult-labeled rats. A decreased presence of LRCs was observed in the VED group compared to the BCNC group but this was not statistically different from the sham or the VED groups. These findings, coupled with the diffuse distribution of LRCs in the natal-labeled rats compared to the adult-labeled group, may indicate EdU labeling patterns of cavernous tissue are dependent on the timing of injection. Furthermore, our results show VED does not increase the number of LRCs in penile tissues and these cells may play a limited role in restoring erectile function.

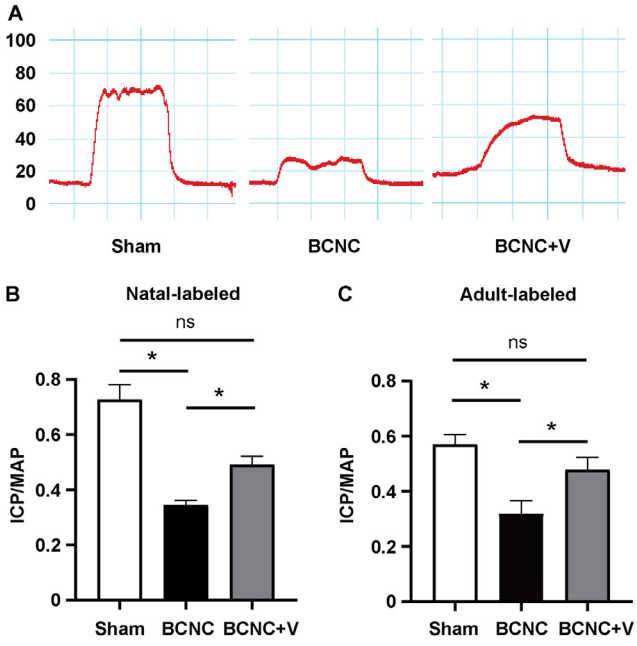

Figure 2. *Erectile function was injured after BCNC surgery and partly restored after VED treatment.* Intracavernous pressure (ICP) was measured and normalized to mean arterial pressure (MAP) to quantify erectile function. For both natal- and adult-labeled rats, each group included 6 rats. *$P < .05$.

VED therapy is a common component of penile rehabilitation after RP surgery and has demonstrated improvement of erectile function in human and animal studies. In a small randomized prospective trial by Raina et al[2], patients who underwent VED therapy post-RP had earlier return to intercourse, increased patient and spousal satisfaction, improved erectile function, better preservation of penile length and possible earlier recovery of erectile function compared to observation. Animal studies, such as Yuan et al[5], Lin et al[6] and our current study, demonstrate robust evidence that VED treatment promotes recovery of erectile function after cavernous nerve injury and prevents penile shortening and fibrosis. Further human trials are necessary to validate the findings in animal models.

BrdU, a thymidine nucleoside analogue, is incorporated into newly synthesized DNA both in vitro and in vivo and is used as a marker of cell proliferation.[16,17] BrdU labeling is thought to be a marker of stem cells given the labeling agent can be identified for an extended period of time in select cells within a tissue, indicating slow cell turnover which is characteristic of progenitor cell types.[18,19] This labeling procedure has been studied in many organs including skin[12], mammary gland,[20] muscle,[21] brain[22] and kidney.[23] In majority of those studies BrdU was applied

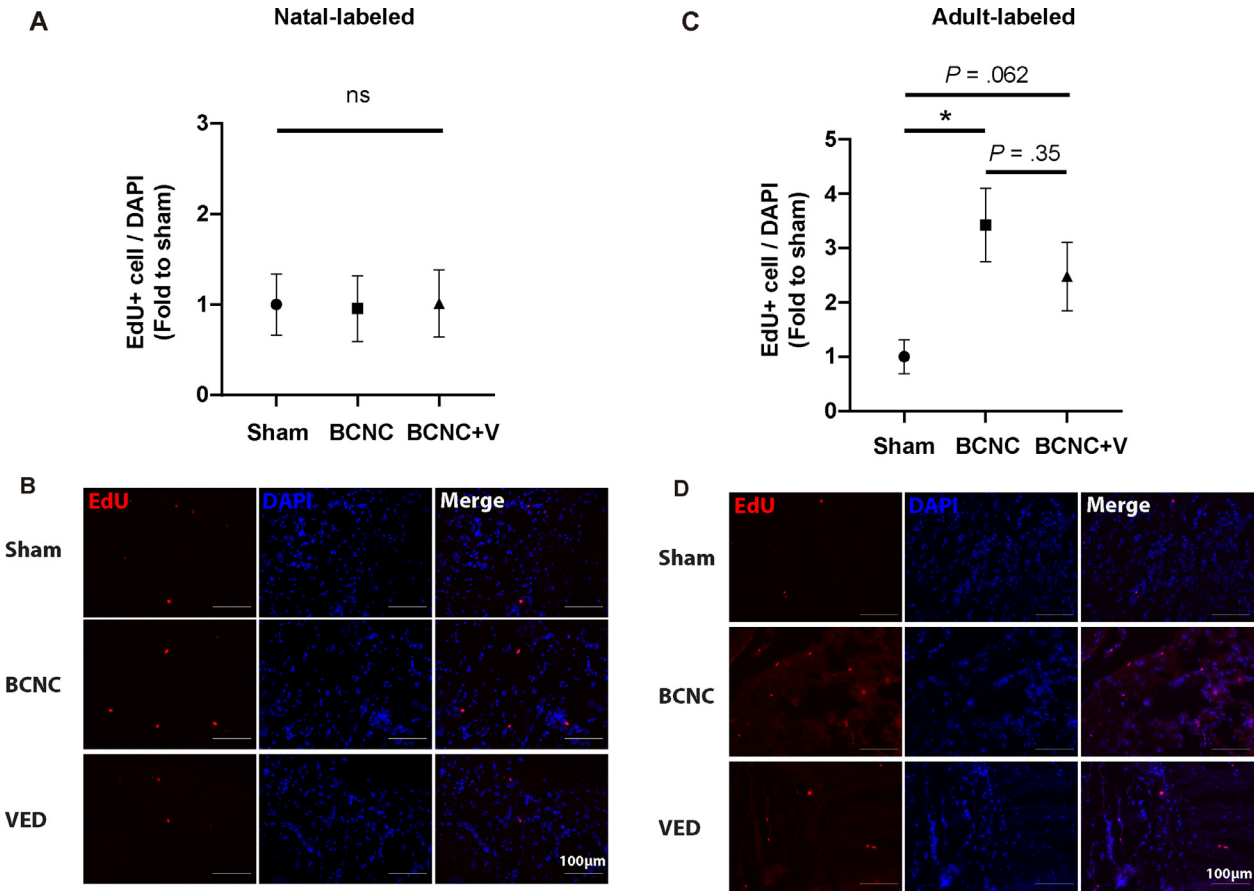

Figure 3. *Limited role of LRCs in the restoration of erectile function after VED treatment.* Immunofluorescence was performed on the penis tissue sections and quantified. (A) The percentage of EdU positive cells in natal-labeled rats (fold to sham group). No significant difference was observed among 3 groups. (B) Representative image of EdU positive cells of natal-labeled rats. (C) The percentage of EdU positive cells in adult-labeled rats (fold to sham group) increased in BCNC group ($P < .05$) but didn't change significantly in VED group ($P = .35$). (D) Representative image of EdU positive cells of adult-labeled rats. Bar = 100 μm. * $P < .05$. BCNC + V = VED treated BCNC rats.

soon after the birth of the animal. In this approach all the cells are labeled by a repeated or continuous supply of BrdU, followed by a long chase period during which the label is lost from the cycling, transit amplifying cells. Only cells with slow cycling retain the label, thus identifying stem and progenitor cells with a quiescent character.[12] EdU labeling is analogous to BrdU but does not require DNA denaturation for detection. In a study of Lin et al[13], they studied the activation of LRCs after low-intensity extracorporeal shockwave therapy by one-dose EdU injection in adult rats. In our current study, the adult labeled rats showed a different pattern of EdU staining with the natal-labeled rats. This indicated that different cells were labeled when EdU was injected in mature rat than newborn rats. Our study was limited by the lack of further characterization of the LRCs with additional cell markers in the mature and natal-injected rats. Future studies are needed to characterize cells identified within penile tissue by EdU staining.

As far as we know, this is the first study to explore the role of LRCs in the penis after VED treatment. Other limitations existed in this study. The small number of animals was used in this study. EdU positive cells were only quantified at one time point, the end of 4-week treatment. Though number of LRCs was not significantly changed after VED treatment, the potential function of LRCs, which may be not depend on the proliferation but activation, is unclear. In this study, we focus on the distribution and number of LRCs in the cavernous tissue, as the VED treatment mainly increases the arterial blood flow and the oxygen saturation in the corpus cavernosum according to our previous study.[6] Yet, the labelled cells may exist in other tissues, such as cavernous nerve, MPG and the rest of the pelvis. Future experiments are being planned to explore the labelled cells more widely to elucidate the underlying mechanisms of VED treatment.

CONCLUSION

VED treatment improved the injured erectile function in BCNC rat model. EdU positive LRCs were not shown to increase after VED treatment in natal and adult-injected rats. The mechanism by which VED improved erectile function after cavernous nerve injury remains unclear and the role of stem cells and cell proliferation in this process may be limited. More

comprehensive studies on the role of LRCs and other potential mechanisms during the development of erectile dysfunction and treatment are warranted.

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Conflict of Interest: The authors declare that they have no competing interests.

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