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Dual Strategy With Oral Phosphodiesterase Type 5 Inhibition and Intracavernosal Implantation of Mesenchymal Stem Cells Is Superior to Individual Approaches in the Recovery of Erectile and Cavernosal Functions After Cavernous Nerve Injury in Rats

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ABSTRACT

Introduction: Novel effective therapeutic strategies are necessary for treating erectile dysfunction secondary to cavernous nerve injury (CNI).

Aim: To functionally evaluate the benefits of long-term oral treatment with a phosphodiesterase type 5 inhibitor on the potential capacity of intracavernosal cell therapy to recover erectile function after CNI.

Methods: Bilateral crush CNI (BCNI) was produced in anesthetized male rats. After BCNI, rats were treated with the phosphodiesterase type 5 inhibitor tadalafil (TAD; 5 mg/kg/d orally; BCNI + TAD), a single intracavernosal injection of bone marrow—derived mesenchymal stem cells (BMSCs; BCNI + BMSC), or dual therapy (BCNI + BMSC + TAD). Ex vivo function of the corpus cavernosum (CC) and in vivo intracavernosal pressure responses to CN electrical stimulation were evaluated 4 weeks after BCNI. Trichrome staining and terminal 2′-deoxyuridine-5′-triphosphate nick-end labeling assay were used for fibrosis and apoptosis determination, respectively, in the CC.

Main Outcome Measures: In vivo erectile responses in anesthetized rats, ex vivo evaluation of endothelium-dependent relaxation, neurogenic relaxation and neurogenic contraction in CC strips, and histologic evaluation of fibrosis and apoptosis in cavernosal tissue.

Results: BCNI resulted in a marked decrease of erectile responses that were partly recovered in the BCNI + TAD and BCNI + BMSC groups. Complete recovery of erectile function was achieved only in the BCNI + BMSC + TAD group. Endothelium-dependent and nitric oxide donor-induced relaxations of the CC were not altered by BCNI or the treatments. BCNI resulted in enhanced neurogenic adrenergic contractions and impaired nitrergic relaxations of the CC. The BCNI + TAD group displayed diminished neurogenic contractions, whereas the BCNI + TAD and BCNI + BMSC groups showed partly recovered nitrergic responses. In the BCNI + BMSC + TAD group, neurogenic contractions were decreased and nitrergic relaxations were normalized. Cavernosal apoptosis and fibrosis were similarly prevented in the BCNI + TAD, BCNI + BMSC, and BCNI + BMSC + TAD groups.

Conclusion: A dual strategy combining the intracavernosal injection of BMSCs and oral administration of TAD was superior to individual approaches in normalizing neurogenic control of cavernosal tone and preserving erectile function after CNI, suggesting the potential of this dual strategy in the future management of erectile dysfunction after radical prostatectomy.

Key Words: Erectile Dysfunction; Radical Prostatectomy; Cavernous Nerve Injury; Cell Therapy; Corpus Cavernosum; Endothelium-Dependent Relaxation; Nitrergic Relaxation; Phosphodiesterase Type 5

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INTRODUCTION

The treatment of reference for organ-confined prostate cancer in patients with a life expectancy of at least 10 years is radical prostatectomy (RP). Despite the adoption of surgical procedures aimed at sparing cavernous nerves (CNs), RP often causes erectile dysfunction (ED), negatively affecting quality of life of these patients. ED after RP typically results from injury to the CNs that course along the posterolateral aspects of the prostate and provide most of the autonomic input to the erectile tissue. The scientific literature has yielded discrepancies concerning ratios of spontaneous recovery of erections after surgery, ranging from 30% to 80% of patients.[1,2] Considering the high incidence of prostate cancer[3] and the increasing survival of patients,[4] the problem affects a substantial number of men. In addition, patients with ED after RP represent a group with the poorest response to the conventional treatment of ED, namely phosphodiesterase type 5 (PDE5) inhibitors. Intracavernosal injections of prostaglandin E_1 constitute the alternative therapeutic option but the rare acceptance of injections causes low adhesion rates, and last-instance treatment is penile prosthesis implantation. The search for therapeutic tools increasing the efficacy of oral treatment of ED after CN injury (CNI) represents an outstanding challenge.

PDE5 inhibitors represent first-line therapy for the treatment of ED. In animal models of ED induced by CNI, long-term administration of PDE5 inhibitors has resulted in enhanced erectile responses to CN stimulation, achieving partial recovery of erectile function[5,6] and reversing corporo-veno-occlusive dysfunction.[7,8] Results obtained in rat models of CNI have suggested that CNI causes structural and functional alterations in erectile tissue through inducing hypoxia or fibrosis, vascular insufficiency, and/or neurologic degeneration.[9–11] However, there is limited information on the endothelium-dependent, myogenic, and neurogenic functional responses of cavernosal tissue from rats with CNI.[12] The functional effects exerted by PDE5 inhibitors seem to be related mainly to the prevention of fibrosis and apoptosis associated with CNI and the preservation of endothelial cells and nitric oxide (NO) signaling, although neural regeneration of the CN also has been described.[6] In humans, PDE5 inhibitors prevent the progression of fibrosis[13] and produce a positive effect on erectile function in patients after RP, but the recovery of erectile function is obtained in a limited percentage of patients.[14–16]

Stem cell therapy also has been evaluated for the recovery of erectile function after CNI. Intracavernosal injection of neural embryonic stem cells has resulted in partial recovery of erectile responses in rats after bilateral CN crush.[17] Adult mesenchymal stem cells (MSCs) have been evaluated in ED models of CNI and proposed as an alternative for the application of cell therapy, with advantages over embryonic stem cells in greater availability and easier manipulation.[18–23] Because of the partial recovery of erectile function by stem cell application in these models, the combination of an additional therapeutic intervention directed to potentiate the efficacy of stem cells in reversing ED is a reasonable approach. A dual strategy involving cell therapy and long-term PDE5 inhibition could achieve greater efficacy in the recovery of erectile function after CNI than individual approaches. This rationale is supported by the enhancing effects exerted by PDE5 inhibition on progenitor cell function.[24,25] Bivalacqua et al[26] reported that potential activation of the NO and cyclic guanosine monophosphate (cGMP) pathway with adenoviral transfection of the endothelial NO synthase gene increased the efficacy of intracavernous injection of bone marrow–derived MSCs (BMSCs) to reverse ED in aged rats. Furthermore, although the combination of long-term low-dose sildenafil administration and skeletal muscle–derived stem cells showed no significant advantage over individual therapies in recovering erections in rats after resection of the CN,[27] implantation of human adipose-derived stem cells treated with brain-derived neurotrophic factor combined with udenafil resulted in a better outcome than separate strategies for preserving erectile responses in rats after CN crush injury.[28]

The aim of this work was to evaluate the influence of long-term oral treatment with a PDE5 inhibitor on the potential capacity of cavernosal implantation of MSCs to recover erectile function after CNI. Special attention was focused on the impact of CNI and a dual therapeutic strategy on corpus cavernosum (CC) function.

METHODS

Experimental Animals

Male 12- to 16-week-old Wistar rats (Harlan, Barcelona, Spain) maintained under 12-hour light-and-dark cycles with free access to food and water were used for the experimental procedures. Female Wistar rats (200–250 g) were used as BMSC donors. Animal studies were performed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health, and were approved by the ethics committees for animal experimentation of the Hospital Universitario Ramón y Cajal and the Hospital Universitario Puerta de Hierro (Madrid, Spain).

Nerve Crush Procedure

Animals were anesthetized with isoflurane (1%–4%) by induction in a closed chamber and then by continuous flow inhalation (2% at a flow of 2 L/h). No preanesthetic medications were used. When the appropriate depth of anesthesia was reached, animals were fastened to a pad in the supine position. Through a lower midline incision, the major pelvic ganglion on the dorsal prostate and the CN emanating from the ganglion were identified using a Zeiss operating microscope. For the CN crush injury, 5 mm distal to the major pelvic ganglion, a number 7 Dumont hemostat was applied to the CN for 30 seconds, removed for 30 seconds, and then reapplied for another