THE JOURNAL OF
SEXUAL MEDICINE

ORIGINAL RESEARCH

BASIC SCIENCE

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

Received August 15, 2015. Accepted November 30, 2015.

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http://dx.doi.org/10.1016/j.jsxm.2015.12.001

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

30 seconds. After bilateral CNI (BCNI), the abdominal incision was sutured and animals received an intramuscular injection of an analgesic (metamizol 200 mg/kg) and an antibiotic (gentamicin 10 mg/kg).

Isolation and Characterization of BMSCs

The BMSCs were obtained from the large bones of 10 donor adult female Wistar rats and expanded in vitro for 4 weeks. Bone marrow was mechanically dissociated to obtain a homogeneous cell suspension that was passed through a 70-μm nylon mesh. This sample was purified through a Ficoll-Hypaque gradient. The obtained cells were washed and seeded in 75-cm^2 flasks for tissue culture in α-minimal essential medium containing 20% fetal bovine serum. After 3 days of incubation (37°C, 5% CO_2), nonadherent cells were removed by replacing the culture medium. BMSCs were identified by their mesenchymal morphology and by cell surface expression of CD73, CD105, SH4, and vimentin, being negative for CD34, CD45, CD3, CD14, CD19, CD38, glycophorin A, and HLA II.[29] One million BMSCs were detached from confluent cultures, suspended in α-minimal essential medium (10 μL), and slowly injected into the rat CC (RCC).

Experimental Design

Sham-operated rats undergoing laparotomy and surgical access to the prostate were used as controls (SHAM group). Rats with BCNI were randomly assigned to receive intracavernosal injections of BMSCs (10^6; BCNI + BMSC group) or the same volume of cell culture medium (BCNI group) during the surgical procedure. Tadalafil (TAD) was administered to a portion of animals in each group (BCNI + TAD and BCNI + BMSC + TAD groups). Treatment started the next day after CN crush and was discontinued 3 days before the experimental procedures. This washout period is reasonable to consider the animals free of drug based on the reported half-life of TAD in rats, which is notably shorter than in humans. TAD was administered by oral gavage at a daily dose of 5 mg/kg on working days and dissolved in tap water at a 0.25-mg/mL concentration for weekend administration. The consumed volume was systematically checked to maintain the correct dosing. Blinded functional and histologic evaluations were performed 4 weeks after BCNI. This observation period was based on previous reports using the same model and on evidence showing no spontaneous recovery at this time.[30]

Evaluation of Erectile Responses

Erectile responses in rats were determined as previously described.[31–33] Animals were anesthetized with intraperitoneal ketamine (50 mg/kg) and diazepam (4 mg/kg). The left carotid artery was catheterized with a heparinized polyethylene-50 tube connected to a pressure transducer and an amplifier and data acquisition system (PowerLab, ADInstruments, Castle Hill, Australia). This allowed for the recording of mean arterial pressure (MAP) and heart rate in a computer with MacLab 3.5.6 Chart recording software (ADInstruments). At its junction with the pubic arch, the ischiocavernous muscle was divided and the tunica albuginea was visualized. Measurement of intracavernosal pressure (ICP) was achieved by inserting a 25-gauge needle into the crus of the penis. This needle was connected to an additional transducer through a heparinized polyethylene-50 tube. The original laparotomy incision was extended to the base of the exposed penis. The CN was again localized and, after careful manipulation, a stainless steel bipolar electrode with parallel hooks (1 mm apart) was placed around the nerve, just distal to the ganglion but proximal to the nerve injury area. The electrode was connected to an electrical stimulator (CS-9, Cibertec, Madrid, Spain). Stimulation parameters were a current intensity of 1.5 mA and a pulse width of 0.3 milliseconds at 1-, 3-, 10-, and 20-Hz frequencies for 60 seconds each. The maximal ICP increase (ΔICP)/MAP and area under the curve of ICP (total ΔICP)/MAP ratios were calculated.

Functional Evaluation of Cavernosal Tissues

Rats were killed by anesthetic overdose (ketamine plus diazepam) and exsanguinated by carotid artery section. The penises were immediately excised and two strips of CC from each penis were carefully dissected through the respective longitudinal incisions along the tunica albuginea. Strips of RCC were mounted on force transducers in 8-mL organ baths (37°C) containing physiologic salt solution continuously bubbled with a mixture of 95% O_2 and 5% CO_2 to maintain a pH of 7.4. Strips were subjected to 0.3 g of resting tension. After a 60-minute equilibration period, tissues were exposed to K^+ 75 mmol/L and contraction was measured. For relaxation experiments, strips were contracted with phenylephrine (PE; 1 μmol/L) and relaxation responses were evaluated by cumulative additions of carbachol (CCh; 1 nmol/L to 10 μmol/L) or sodium nitroprusside (1 nmol/L to 10 μmol/L) to the chambers. Electrical field stimulation (EFS) was applied through two platinum electrodes connected to a stimulator (Cibertec CS-9). Parameters of stimulation were a current intensity of 50 mA and pulse duration of 0.5 milliseconds in 20-second trains. Neurogenic contractions were evaluated by the application of EFS at increasing frequencies from 0.5 to 48 Hz in strips under resting tension. Neurogenic (nitrergic) relaxation was evaluated by the application of EFS (0.5–16 Hz) in strips contracted with PE and previously treated with guanethidine 30 μmol/L (for inhibiting adrenergic neurotransmission) and atropine 0.1 μmol/L (for muscarinic receptor blockade).

Determination of Cavernosal Apoptosis and Fibrosis

Histopathologic evaluation of penile tissues was performed as described previously for human erectile tissue.[34] Apoptosis was determined by terminal 2′-deoxyuridine-5′-triphosphate nick-end labeling assay in deparaffinized tissue sections (6 μm)

Table 1. Effects Of Treatments in MAP and HR

Treatment group	n	MAP (mm Hg)	HR (beats/min)
SHAM	9	96.7 ± 2.8	330 ± 8
BCNI	11	96.5 ± 2.9	328 ± 10
BCNI + TAD	6	97.4 ± 4.9	338 ± 10
BCNI + BMSC	7	100.7 ± 7.6	342 ± 10
BCNI + BMSC + TAD	6	101.4 ± 4.3	325 ± 14

Data are expressed as mean ± standard error of the mean. No significant differences among groups were obtained by one-factor analysis of variance followed by the Student-Newman-Keuls post-test.
BCNI = bilateral cavernous nerve injury; BMSC = bone marrow-derived mesenchymal stem cells; HR = heart rate; MAP, mean arterial pressure; TAD = tadalafil.

of CC. A fluorescence-based commercial kit was used according to the manufacturer's specifications (Promega Biotech Ibérica, Alcobendas, Spain). Percentage of apoptosis was calculated by counting apoptotic cell nuclei (cells positive for terminal 2′-deoxyuridine-5′-triphosphate nick-end labeling) relative to the total number of cell nuclei in six high-magnification visual fields (×400) for each sample. For determination of cavernosal fibrosis, deparaffinized tissue sections (6 μm) were stained with Masson trichrome and the percentage of the area stained in blue (fibrotic tissue) in relation to the total area of high-magnification visual fields (×200; five per sample) was calculated using morphometric software (Image J, National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

The expression pD_2 is defined as the -log mol/L of the concentration required to obtain 50% of maximal relaxation. For comparison of complete frequency- or concentration-response curves, two-factor analysis of variance was applied using StatView for Apple (SAS Institute, Cary, NC, USA). This statistical test compares frequency-response curves in their entirety, including all frequencies and concentrations in the analysis. All other data were compared by one-factor analysis of variance followed by the Student-Newman-Keuls test using InStat (GraphPad, San Diego, CA, USA). This post hoc test minimizes the risk of obtaining false positive results owing to multiple comparisons. Differences with a probability less than 0.05 were considered significant. Simple regression analyses were performed with the mean values for each group to determine the relation between cavernosal responses and erectile function. For these analyses, GraphPad Prism software was used.

RESULTS

Blood pressure and heart rate were not altered by CNI, oral TAD administration, or intracavernosal cell implantation (Table 1). Similarly, there were no significant differences among experimental groups with respect to the animals' final weight (371.6 ± 10.4, 376.7 ± 8.4, 377.2 ± 12.0, 390.2 ± 19.7, and 384.5 ± 11.9 g for SHAM, BCNI, BCNI + TAD,

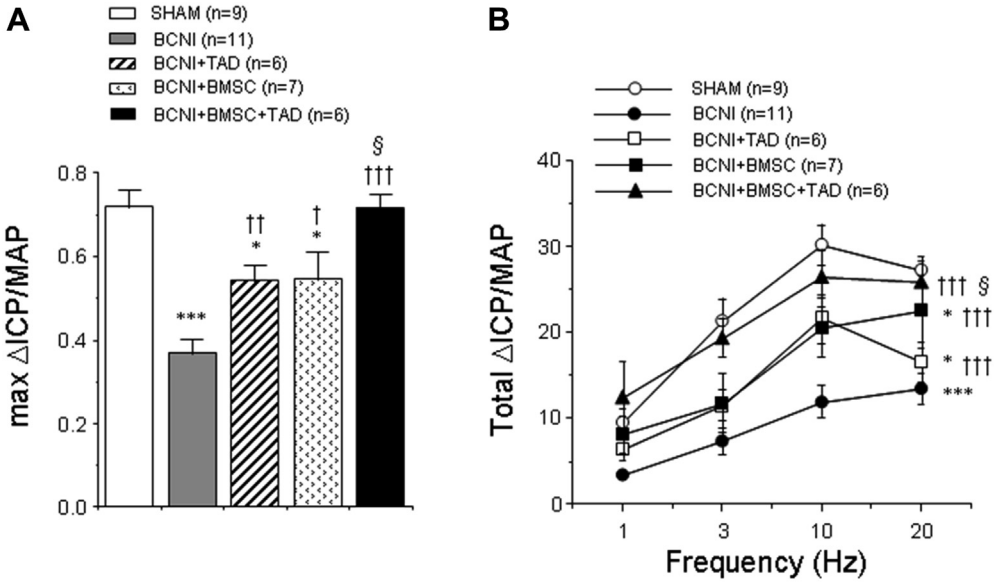

Figure 1. Erectile responses to cavernous nerve (CN) electrical stimulation (1–20 Hz) in rats 4 weeks after sham operation (SHAM) or after bilateral CN crush injury (BCNI) and the effects of treating rats with BCNI with oral tadalafil (5 mg/kg/d; BCNI + TAD), intracavernosal injection of bone marrow–derived mesenchymal stem cells (BCNI + BMSC), or dual therapy (BCNI + TAD + BMSC). (A) Mean ± standard error of the mean of maximal increase in intracavernosal pressure (ΔICP) normalized by mean arterial pressure (MAP) at the time of stimulation, irrespective of the frequency at which it was obtained. (B) Complete frequency-response curves, with erectile responses expressed as mean ± standard error of the mean of the area under the curve of the ΔICP (total ΔICP) normalized by MAP at the time of stimulation. n = number of animals used for determinations. *P < .05, ***P < 0.001 vs sham; †P < .05, ††P < .01, †††P < .001 vs BCNI; §P < .05 vs BCNI + TAD and BCNI + BMSC by (A) one-factor analysis of variance followed by the Student-Newman-Keuls test and (B) two-factor analysis of variance.

BCNI + BMSC, and BCNI + BMSC + TAD, respectively; $P > .05$ for any comparison).

Dual Therapy with Intracavernosal BMSCs and Oral TAD Administration Is Superior to Individual Therapies to Improve Erectile Responses After CNI

Four weeks after CNI, rats with BCNI displayed blunted erectile responses to CN stimulation compared with the SHAM group. This was observed when considering maximal ICP increase and when analyzing total ΔICP/MAP data in complete frequency-response curves (Figure 1). Separate long-term oral administration of the PDE5 inhibitor TAD (5 mg/kg/d) and single intracavernosal injection of BMSCs caused a significant improvement of erectile responses in rats with BCNI but did not completely prevent ED in these animals. However, the improvement achieved after combination therapy of intracavernosal BMSCs and oral TAD therapy was significantly greater than individual therapies, resulting in erectile responses that were not significantly different from those obtained in the SHAM group (Figure 1).

BCNI Does Not Result in Impairment of Endothelium-Dependent or NO Donor-Induced Relaxations of the CC

Exposure to the cholinergic agonist CCh (1 nmol/L to 10 μmol/L) caused concentration-dependent relaxations of RCC that were markedly inhibited by pretreatment with the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (100 μmol/L; maximal response (E_{max}) = 70.0 ± 4.0% vs. 22.9 ± 2.9%, respectively; n = 6, $P < 0.001$). These endothelium-dependent relaxations were preserved in rats with BCNI compared with the SHAM group. In the same way, CCh-induced relaxations of RCC were not influenced by any treatment (Figure 2A). Similarly, relaxations induced by the NO donor, sodium nitroprusside (1 nmol/L to 10 μmol/L), were not altered in rats with BCNI and were not modified by any experimental treatment (Figure 2B).

Dual Therapy With Oral TAD and Intracavernosal Injection of BMSCs Decreases Adrenergic Hypercontractility and Improves Nitrergic Relaxations in RCC After BCNI

Application of EFS (0.5—48 Hz) produced frequency-dependent contractions of RCC that were adrenergic in nature as demonstrated by the near complete abolition of EFS-induced contractions with the α_1,α_2-adrenergic antagonist, phentolamine (1 μmol/L; Figure 3A). RCC from rats with BCNI displayed significantly increased contractions to EFS compared with SHAM animals. Such potentiation of neurogenic contractions was not modified by intracavernosal injection of BMSCs but was significantly decreased by oral TAD administration alone or in combination with BMSC injection (Figure 3B). Alterations in neurogenic contractions were not due to modifications of contractile capacity because there were no significant differences in K^+-induced contractions among groups (120.6 ± 15.3, 123.8 ± 10.3, 129.7 ± 14.1, 112.3 ± 7.7, and 134.1 ± 11.6 mg for SHAM, BCNI, BCNI + TAD, BCNI + BMSC, and BCNI + BMSC + TAD, respectively; $P > .05$ for any comparison). Furthermore, there were no significant differences in contractions induced by PE (1 μmol/L) in RCC strips obtained from the different treatment groups (165.4 ± 21.7, 165.6 ± 9.3, 186.8 ± 8.5, 158.3 ± 11.9, and 183.2 ± 13.1 mg for SHAM, BCNI, BCNI + TAD, BCNI + BMSC, and BCNI + BMSC + TAD, respectively; $P > .05$ for any comparison).

In RCC strips treated with guanethidine (30 μmol/L) and atropine (0.1 μmol/L) to nullify adrenergic and cholinergic

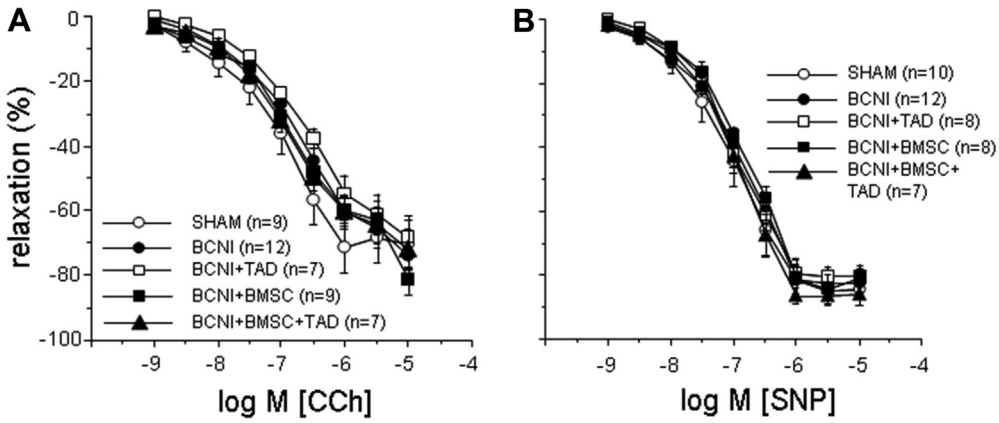

Figure 2. Endothelium-dependent relaxations in response to (A) carbachol (CCh; 1 nmol/L to 10 μmol/L) and (B) the nitric oxide donor, sodium nitroprusside (SNP; 1 nmol/L to 10 μmol/L) in corpus cavernosum strips obtained from rats 4 weeks after sham operation (SHAM) or after bilateral cavernous nerve crush injury (BCNI) and from rats with BCNI treated with oral tadalafil (5 mg/kg/d; BCNI + TAD), intracavernosal injection of bone marrow—derived mesenchymal stem cells (BCNI + BMSC), or dual therapy (BCNI + TAD + BMSC). Data are expressed as mean ± standard error of the mean of the percentage of maximal relaxation induced by papaverine (0.1 mmol/L). n = number of animals used for determinations.

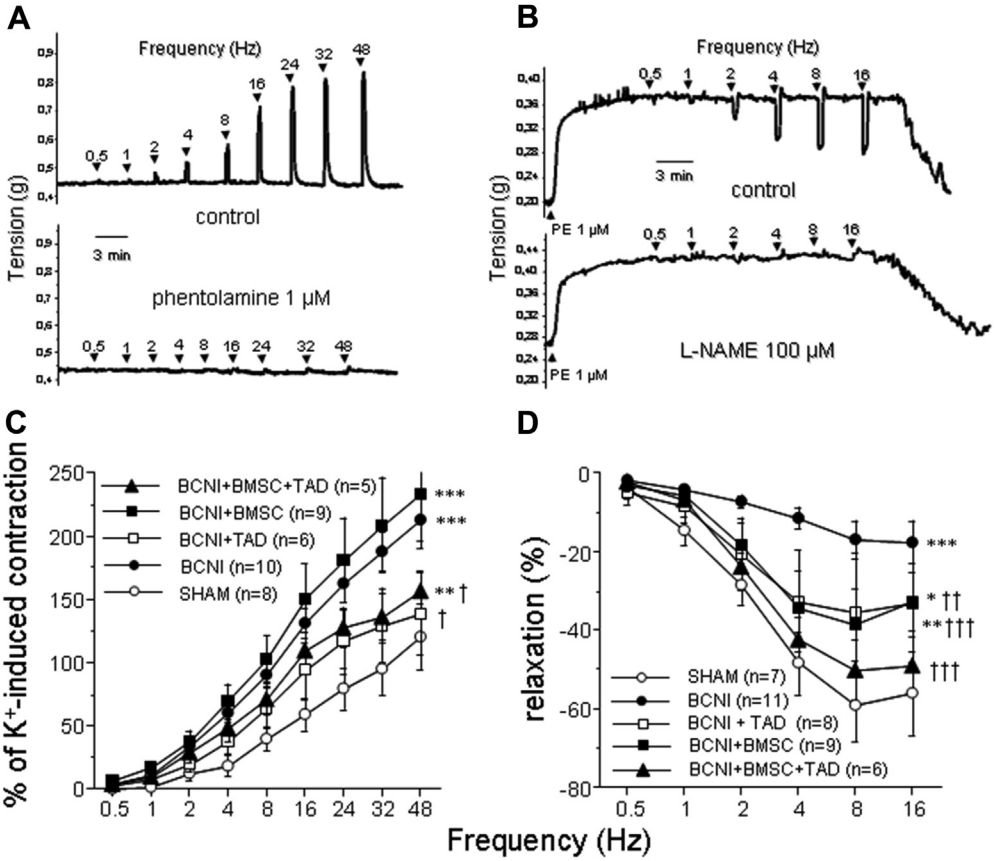

Figure 3. (A) Representative tracings showing the adrenergic nature of neurogenic contractions induced by electrical field stimulation (EFS) in rat corpus cavernosum (RCC) strips that were abolished by treatment with the α_1,α_2-adrenergic antagonist, phentolamine (1 μmol/L). (B) Representative tracings showing the nitrergic nature of neurogenic relaxations induced by EFS in RCC strips contracted with phenylephrine (PE; 1 μmol/L) and treated with guanethidine (30 μmol/L) and atropine (0.1 μmol/L) that were abolished by inhibiting nitric oxide synthase (NOS) with N^G-nitro-L-arginine methyl ester (L-NAME; 100 μmol/L). (C) Quantification of neurogenic adrenergic contractions and (D) neurogenic nitrergic relaxations in RCC strips obtained from rats 4 weeks after sham operation (SHAM) or after bilateral cavernous nerve crush injury (BCNI) and from rats with BCNI treated with oral tadalafil (5 mg/kg/d; BCNI + TAD), intracavernosal injection of bone marrow–derived mesenchymal stem cells (BCNI + BMSC), or dual therapy (BCNI + TAD + BMSC). Data are expressed as mean ± standard error of the mean of the (C) percentage of contraction elicited by K^+ (75 mmol/L) and (D) the percentage of maximal relaxation induced by papaverine (0.1 mmol/L). n = number of animals used for determinations. *P < .05, **P < .01, ***P < .001 vs SHAM; $^{\dagger}P$ < .05, $^{\dagger\dagger}P$ < .01, $^{\dagger\dagger\dagger}P$ < .001 vs BCNI by two-factor analysis of variance.

neurotransmissions, respectively, and contracted with PE (1–3 μmol/L), EFS application (0.5–16 Hz) resulted in frequency-dependent relaxations that were prevented by inhibiting NO synthase with N^G-nitro-L-arginine methyl ester (100 μmol/L; Figure 3C), confirming their nitrergic nature. Nitrergic relaxations were markedly decreased in RCC from rats with BCNI. This profound nitrergic impairment was partly prevented by separate therapy with oral TAD or BMSC intracavernosal injection. The improvement in nitrergic relaxation after dual therapy with TAD and BMSCs was superior to individual treatments and yielded near complete preservation of nitrergic responses (Figure 3D).

As shown in Figure 4, neurogenic responses were determinant for erectile function in this model. The erectile capacity in each group (measured as maximal ΔICP/MAP) inversely correlated to the maximum contractile response to neurogenic stimulation (Figure 4B) and directly correlated to the maximum relaxation response to nitrergic stimulation (Figure 4C). In contrast, no significant correlation was observed for endothelial relaxation (pD_2 for CCh; Figure 4A).

PDE5 Inhibition and Cell Therapy Prevents Penile Apoptosis and Fibrosis Associated With BCNI

An increased ratio of apoptosis was observed in cavernosal tissues from rats with BCNI. This increase was significantly attenuated by oral TAD administration or intracavernosal injection of BMSCs individually applied or given in combination (Figure 5). Apoptosis in RCC from rats with BCNI treated with BMSCs alone was similar to that in the other treatment groups but remained significantly increased compared with the SHAM group (Figure 5F). Fibrosis, as determined by the ratio of cavernosal area occupied by collagen fibers stained by Masson

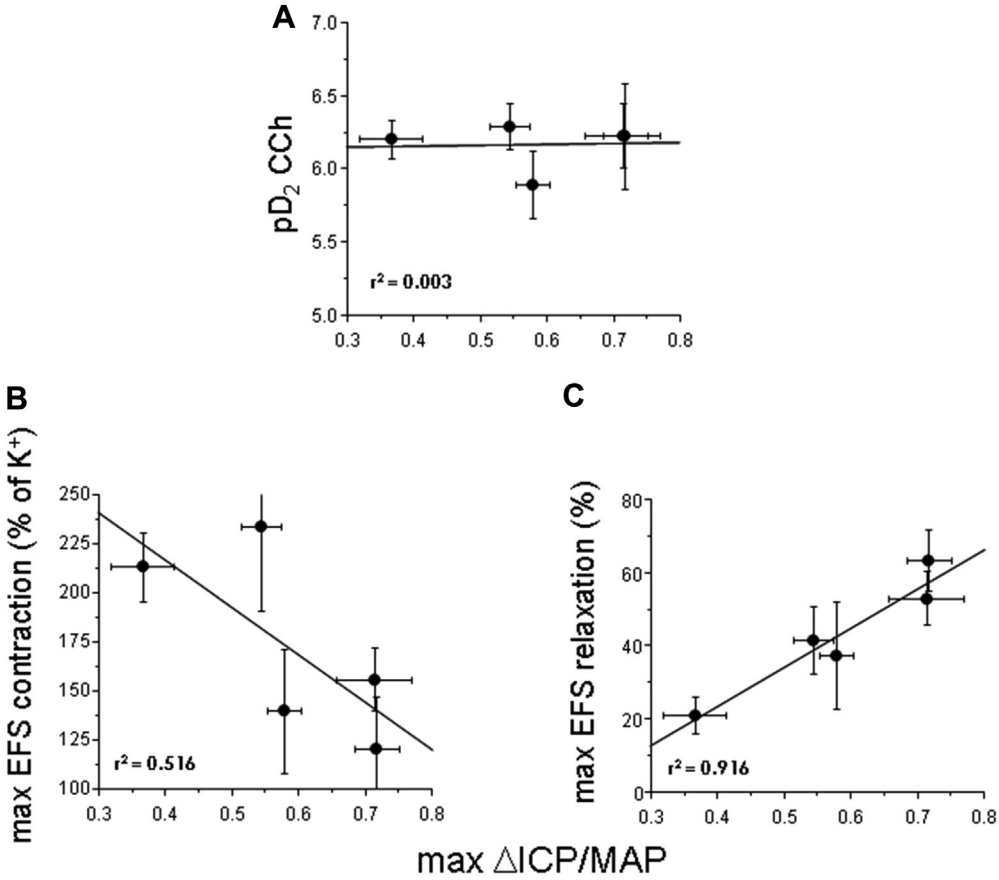

Figure 4. Correlations of maximal increases in intracavernosal pressure (ΔICP) normalized by mean arterial pressure (MAP) at the time of stimulation with (A) pD2 values for carbachol (CCh), (B) maximal contraction to electrical field stimulation (EFS), and (C) maximal EFS-induced relaxation. Data are expressed as mean ± standard error of the mean for (A) ΔICP/MAP and pD2 for CCh, (B) percentage of K^+-induced contraction, and (C) percentage of papaverine (0.1 mmol/L)-induced relaxation. Squared regression coefficients (r^2) are indicated. Significant correlations ($P < .05$) were obtained in B and C.

trichrome, also was significantly increased in RCC from rats with BCNI. Oral TAD and BMSC intracavernosal injection, individually or in combination, prevented the increment of fibrotic tissue detected in RCC after BCNI (Figure 6).

DISCUSSION

The present results suggest that a dual therapeutic strategy combining cavernosal cell therapy and oral PDE5 inhibition is superior to individual approaches in preserving erectile function after BCNI in rats. This beneficial effect on erectile function is related to the preservation of nitrergic relaxations, the limitation of the enhancement of neurogenic contractions, and the prevention of cavernosal apoptosis and fibrosis caused by BCNI.

Because of the high incidence of ED after RP and the lack of adequate rates of success in the conventional treatment of ED in these patients, the search for alternative strategies for the treatment of this type of ED is definitely justified. Important efforts have focused on the evaluation of cell therapy as a way to recover or preserve erectile function in preclinical models of CNI.[35] Therapy with different types of stem cells has been shown to be effective in improving erectile function in rats after CNI.[18–23]

Moreover, long-term administration of PDE5 inhibitors has shown a beneficial effect on erectile function. This is supported by preclinical studies performed in rats after CNI. Long-term administration of different PDE5 inhibitors to these animals right after CNI has resulted in improved erectile responses and decreased fibrosis and apoptosis in the CC.[5,7,8] However, complete recovery and preservation of erectile function after BCNI is not usually achieved.[6] Penile rehabilitation with long-term PDE5 inhibitor administration to patients undergoing radical pelvic surgery also has been evaluated in the clinical setting. This therapeutic strategy has shown positive outcomes,[14] but the recovery of adequate erectile function has been obtained in a limited number of patients.[16] Based on the positive effects of cell therapy and long-term PDE5 inhibition on erectile function after CNI, the combination of these two strategies for this condition were evaluated to potentially achieve greater efficacy in the recovery of erectile function. Supporting this idea, PDE5 inhibition augments the proliferation of neural stem cells,[25] enhances

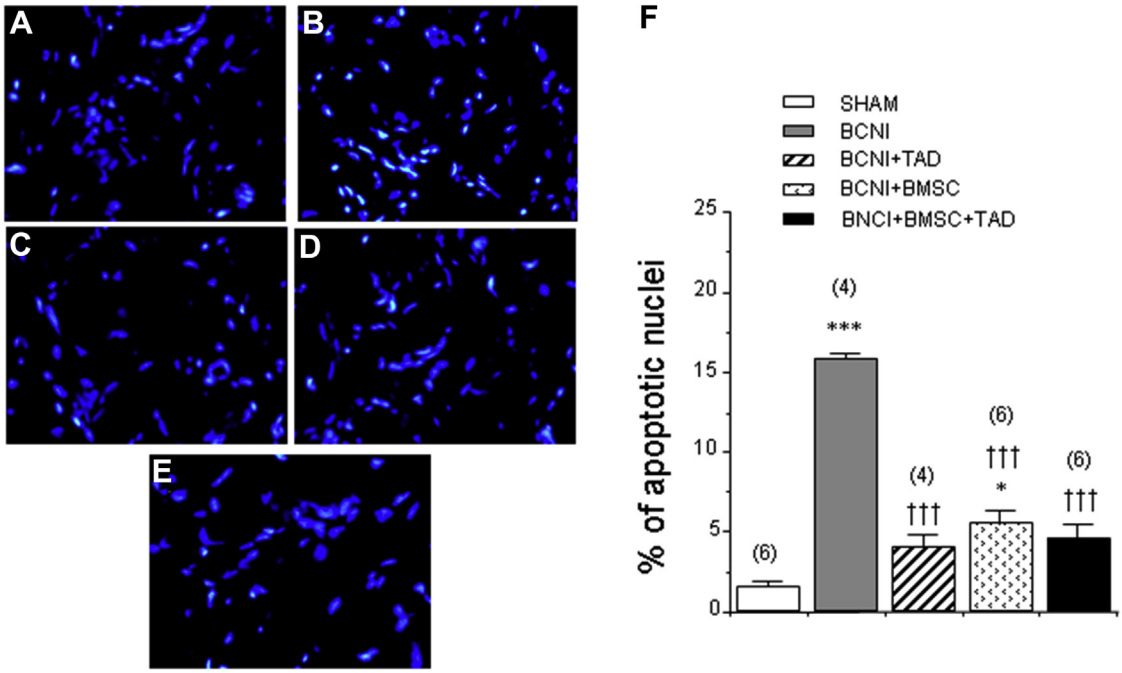

Figure 5. (A–E) Representative fluorescence images (×400) of apoptosis determination by terminal 2′-deoxyuridine-5′-triphosphate nick-end labeling assay in corpus cavernosum strips obtained from rats 4 weeks after (A) sham operation (SHAM) or (B) bilateral cavernous nerve crush injury (BCNI) and from rats with BCNI treated with (C) oral tadalafil (5 mg/kg/d; BCNI + TAD), (D) intracavernosal injection of bone marrow–derived mesenchymal stem cells (BCNI + BMSC), or (E) dual therapy (BCNI + TAD + BMSC). (F) Quantification of results expressed as mean ± standard error of the mean of the percentage of apoptotic nuclei relative to the total number of nuclei in each preparation. n = number of animals used for determinations. *$P < .05$, ***$P < .001$ vs SHAM; $^{\dagger\dagger\dagger}P < .001$ vs BCNI by one-factor analysis of variance followed by the Student-Newman-Keuls test.

neurosphere formation and neurogenesis in subventricular progenitor cells,[36] and increases the number of circulating endothelial progenitor cells in men.[24] In fact, the increase in endothelial progenitor cells induced by TAD administration has resulted in improved endothelial function in patients with ED.[37] Moreover, TAD administration has been shown to improve functional recovery in a stroke rat model by enhancing angiogenesis and neurogenesis[38] and vardenafil has been shown to enhance blood flow recovery and angiogenesis in ischemic hind limbs in rats.[39] The priming effect of cGMP increase by PDE5 inhibitors on stem cell function could be related to stimulation of hypoxia inducible factor-1 and/or Akt pathways, but cGMP-mediated protection of progenitor cells from oxidative stress also has been suggested.[40]

The present results confirm the positive effects on erectile function after BCNI by intracavernosal implantation of BMSCs or by long-term oral administration of a PDE5 inhibitor. However, these strategies showed superior efficacy when applied in combination, resulting in complete preservation of erectile responses in rats after BCNI. The improved effects of the intervention on erectile function were not related to the enhancing effects on endothelium or smooth muscle function because endothelial relaxation and NO donor-induced relaxation of RCC were not altered by BCNI and, in consequence, were not modified by any of the treatments. Preservation of endothelial and smooth muscle responses in the present BCNI model is consistent with a recent functional evaluation of human CC and penile arteries from men with ED secondary to RP that displayed preserved relaxation to acetylcholine, sodium nitroprusside, or sildenafil.[34] Moreover, consistent with observations in human CC from patients with ED after RP,[34] BCNI resulted in enhanced neurogenic contractions and markedly decreased neurogenic relaxations of RCC. The enhancement of neurogenic contractions caused by BCNI was not modified by BMSC implantation but was significantly prevented by TAD administration with or without BMSC application. In contrast, nitrergic relaxations were partly improved by BMSC implantation or TAD administration but this improvement was significantly superior when these interventions were applied simultaneously. Although the marked improvement of erectile function driven by the dual strategy of cell therapy and TAD administration was accompanied by a significant decrease of the increased fibrosis and apoptosis in RCC caused by BCNI, this effect was similar to that exerted by individual application of BMSC or TAD therapies. This suggests that histologic analyses are less sensitive than functional determinations in grading different degrees of erectile function. In fact, the erectile capacity of the rats in response to each treatment was significantly correlated only to the neurogenic contractions (inversely) and to the nitrergic relaxations (directly), highlighting the imbalanced neurogenic control of cavernosal tone as the main target in the management of ED after CNI. In a model of BCN resection in the rat, Kovanecz

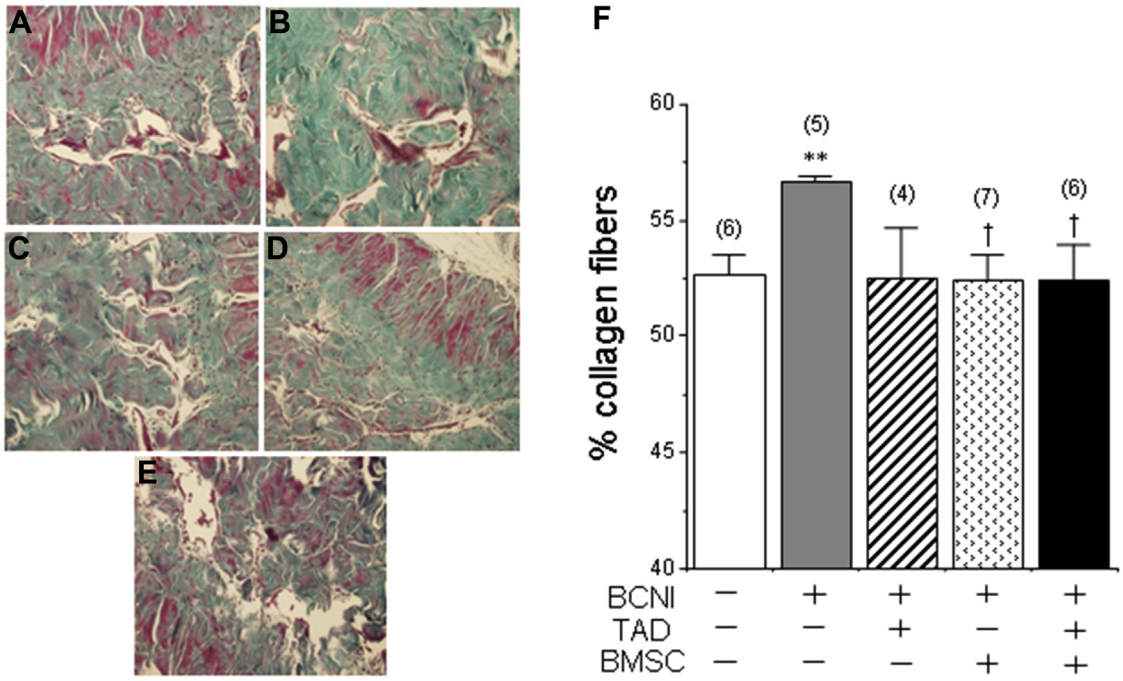

Figure 6. (A–E) Representative images (×200) of fibrosis determination by trichrome staining in corpus cavernosum strips obtained from rats 4 weeks after (A) sham operation (SHAM) or (B) bilateral cavernous nerve crush injury (BCNI) and from rats with BCNI treated with (C) oral tadalafil (5 mg/kg/d; BCNI + TAD), (D) intracavernosal injection of bone marrow–derived mesenchymal stem cells (BCNI + BMSC), or (E) dual therapy (BCNI + TAD + BMSC). (F) Quantification of results expressed as mean ± standard error of the mean of the percentage of area occupied by collagen fibers (blue and green staining) relative to the total tissue area (blue and green + red staining) in each preparation. n = number of animals used for determinations. $^{**}P < .01$ vs SHAM, $^{\dagger}P < .05$ vs BCNI by one-factor analysis of variance followed by the Student-Newman-Keuls test.

et al[27] evaluated the effects of long-term oral administration of a low dose of sildenafil in combination with intracavernosal injection of mouse skeletal muscle-derived stem cells. They did not find substantial functional improvement with the combination of these therapies compared with each therapy separately. The lack of agreement with the present results could be due to the lower dose of PDE5 inhibitor or the greater severity of injury to the CN or to the approach of calibrating the erectile function of the animals because Kovanecz et al performed cavernosometry with papaverine and determined the decreased rate in ICP, an approach resembling intracavernosal injection therapy rather than sexual stimulation. In fact, in patients with ED after RP, the outcome of intracavernosal injection therapy does not seem to be compromised.[41] Using crush CNI, evaluating erectile responses by CN electrical stimulation, and administering a high dose of udenafil (20 mg/kg/d), Jeong et al[28] found improved preservation of erectile function when combining PDE5 inhibition with application on injured the CN of brain-derived neurotrophic factor–human adipose tissue-derived stem cells compared with separate approaches.

A limitation of the present study is that the mechanism of implanted BMSCs after delivery was not elucidated. However, this work aimed to provide functional evidence of the effects of the evaluated therapies rather than to explore the interaction of implanted BMSCs with the host tissue. In fact, the relevance of this issue from a functional viewpoint weakens after considering that the improvement in erectile responses obtained in a closely related model with the implantation of intact adipose tissue-derived stem cells was comparable to that obtained with lysate-treated adipose tissue-derived stem cells.[20]

In addition, extrapolation of the present results to the clinical setting should be done with caution. According to the body surface area method for extrapolating animal to human doses.[42] the dose of TAD used in this study would be approximately equivalent to 0.8 mg/kg in humans. This dose, although not extremely high, is higher than that used for treating ED in men. In fact, most studies in animals showing regenerative or protective effects by long-term TAD used similar or higher doses than administered in the present study.[27,38] Moreover, the different regenerative capacity of humans and rats and the variability in surgical procedures and baseline health status of patients that is inherent to the clinical practice should be considered.

CONCLUSION

The present results reinforce the idea that enhancement of neurogenic adrenergic contractions and decrease of nitrergic relaxation of cavernosal tissue are the main therapeutic targets in the management of ED after CNI. The dual strategy combining BMSC

intracavernosal injection and oral long-term administration of TAD seems to be superior to individual approaches in avoiding this imbalance in neurogenic control of cavernosal tone and in preserving erectile function after CNI, suggesting the potential of this dual strategy in the future management of ED after RP.

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Conflict of Interest: The authors report no conflicts of interest.

Funding: This work was supported by a grant from the European Society for Sexual Medicine.

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