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Intratumoral Injection of Genetically Modified Adipose Tissue-Derived Stem Cells with Human Interferon α -2b for Treatment of Erectile Dysfunction in a Rat Model of Tunica Albuginea Fibrosis

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Abstract

Introduction—Peyronie's disease (PD) has frequently been associated with erectile dysfunction (ED) and may further compromise coitus.

Aim—To investigate the efficacy of intratumoral injection of genetically modified rat adipose tissue-derived stem cells (ADSCs) expressing human interferon α -2b (ADSCs-IFN) in decreasing fibrosis and restoring erectile function in a rat model of tunica albuginea fibrosis (TAF).

Methods—A total of 36 Sprague-Dawley rats (12 weeks old; 300–350 g) were randomly divided in six equal groups: (i) sham group (50 μ L saline-injected into the tunica albuginea [TA]); (ii) TAF group (transforming growth factor [TGF]- β 1 [0.5 μ g/50 μ L] injected into the TA); (iii)

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TGF- β 1 plus 5×10^5 control ADSCs injected same day; (iv) TGF- β 1 plus 5×10^5 ADSCs-IFN injected same day; (v) TGF- β 1 plus 5×10^5 control ADSCs injected after 30 days; and (vi) TGF- β 1 plus 5×10^5 ADSCs-IFN injected after 30 days. Rat allogeneic ADSCs were harvested from inguinal fat tissue.

Main Outcome Measures—Forty-five days following the TGF- β 1 injection, erectile function was assessed, and penile tissues were harvested for further evaluations.

Results—In the same-day injection groups, intratunical injection of ADSCs and ADSC-IFN improved erectile response observed upon stimulation of cavernous nerve compared with TAF group. Intratunical ADSC-IFN injection at day 30 improved erectile responses 3.1, 1.8, and 1.3 fold at voltages of 2.5, 5.0, and 7.0, respectively, when compared with TAF group. Furthermore, at voltages of 2.5 and 5.0, treatment on day 30 with ADSCs-IFN improved erectile responses 1.6- and 1.3-fold over treatment with ADSCs alone. Local injection of ADSCs or ADSCs-IFN reduced Peyronie's-like manifestations, and these effects might be associated with a decrease in the expression of tissue inhibitors of metalloproteinases.

Conclusion—This study documents that transplantation of genetically modified ADSCs, with or without human IFN α -2b, attenuated Peyronie's-like changes and enhanced erectile function in a rat model of TAF.

Keywords

Tunica Albuginea; Fibrosis; Stem Cells; Human Interferon α -2b; Gene Therapy; Erectile Dysfunction; Peyronie's Disease

Introduction

Peyronie's disease (PD) is a fibrotic wound-healing disorder of the penis characterized by plaque formation in the tunica albuginea (TA). It can be both a physically and psychologically debilitating condition for the afflicted man, and also affect his partner. Despite PD being recognized by the medical community for more than 250 years, it remains a therapeutic dilemma due to an incomplete understanding of its pathophysiology and the relative paucity of randomized, placebo-controlled trials [1]. Although there are many theories as to the etiology of PD, most authorities postulate that PD results from repetitive minor trauma to the penis during intercourse with subsequent abnormal wound healing and scar formation [2]. There is a localized disruption of the penile TA and an increase in microvascular permeability with release of various cytokines and growth factors following injury to the erect penis [3]. Current literature highlights the role of cytokine release, primarily transforming growth factor β 1 (TGF- β 1), as the predominant profibrotic factor in the development of PD [2-3]. TGF- β 1 is a cytokine that is an endogenous mediator of most fibrotic processes and abnormal wound healing. The TGF- β 1 rat model for PD was first proposed by Dr Tom Lue in the late 1990s [4]. This model has distinctive advantages in the studies of PD due to its ability to induce chronic inflammation and fibrosis in the TA over time with only a single injection of the compound. The most important disadvantages of this model relates to the inconsistencies in timing and duration of the fibrotic plaque following the local injection of TGF- β 1 [3].

The pro-fibrotic role of TGF- β 1 in the pathophysiology of PD has led to several pharmacological applications such as using interferons and phosphodiesterase 5 inhibitors. Although many nonsurgical treatments have been promoted, none offer a reliable and effective correction of the penile deformity, and for this reason, surgery remains the gold standard [5–6]. Recently, clostridium histolyticum collagenase (Xiaflex, Auxilium, Chesterbrook, PA, USA) has been approved by the Food and Drug Administration for this indication. However, since most patients present clinically in the advanced/chronic phase, the success of this modality is not foolproof.

Stem cell-based therapies have documented benefit in the prevention of ED following cavernous nerve injury in an animal model [7]. Recently, adipose tissue-derived stem cells (ADSCs) have become a valuable resource because of their abundance and ease of isolation [8]. Several investigators have reported that mesenchymal stem cell transplantation can significantly decrease fibrosis in the heart, lung, kidney, and liver [9–12]. Moreover, in a previous study, the preventative benefits of ADSCs on tunica albuginea fibrosis (TAF) and ED have been demonstrated in the acute/inflammatory phase when compared with the chronic phase in an animal model [13]. Previous studies also showed that intralesional injection therapy with IFN α -2b demonstrated beneficial effects on PD [14]. The main disadvantage of this treatment modality is the need for repeated injections.

Aims

The current study aimed to investigate the efficacy of intratunical injection of genetically modified rat ADSCs expressing human interferon α -2b (ADSCs-IFN) in decreasing fibrosis and restoring erectile function in a rat model of TAF.

Methods

Study Design

Male Sprague-Dawley rats (12 weeks old; 300–350 g) were purchased from Harlan Laboratories (Indianapolis, IN, USA) and housed in a regulated environment with a 12-hour light/dark cycle in a standard experimental laboratory of Tulane University. The animals had free access to food and water ad libitum. A total of 36 male Sprague-Dawley rats (12 weeks old; 300–350 g) were randomly divided into six equal groups: (i) sham group (50 μ L saline-injected into the TA); (ii) TAF group (0.5 μ g/50 μ L TGF- β 1 injected into the TA); (iii) TGF- β 1 plus 5×10^5 control ADSCs injected same day (ADSCs prevention group) (iv) TGF- β 1 plus 5×10^5 ADSCs-IFN injected same day (ADSCs-IFN prevention group); (v) 5×10^5 control ADSCs injected 30 days after TGF- β 1 injection (ADSCs treatment group); and (vi) 5×10^5 ADSCs-IFN injected 30 days after TGF- β 1 injection (ADSCs-IFN treatment group). Rat allogeneic ADSCs were harvested from inguinal fat tissue. Forty-five days following TGF- β 1 injection, all rats underwent evaluation of erectile function; the rats were sacrificed, and the penile tissues were harvested and stored at -80°C for further analysis. All experiments were performed according to the Guidelines for the ethical conduct in the care and use of animals and were approved by Tulane University Health Sciences Center Animal Care and Use Committee.

ADSC Isolation and Culture

The ADSC isolation and culture were extensively described in our previous study [13]. Briefly ADSCs were harvested from inguinal fat tissue of adult male Sprague–Dawley rats (350–450 g). A lower midline abdominal skin incision was made, and fat pads around the spermatic cord were excised, finely minced, and washed three times with PBS-containing penicillin (100 units/mL) and streptomycin (100 µg/mL). After centrifugation (500 g; 5 minutes), tissues samples were digested with 0.2% collagenase type 1 (Invitrogen, Carlsbad, CA, USA) by agitation at 37°C for 90 minutes. After filtration through a 60-µm nylon mesh (Millipore, Billerica, MA, USA), ADSCs were resuspended and cultured at a density of 1×10^5 cells in DMEM-F12 medium supplemented with 10% FBS, antifungal agent, and penicillin-streptomycin (Gibco-BRL, Carlsbad, CA, USA). Nonadherent cells were removed 2 days after culture, and the medium was changed every 3 days until 85–95% confluence was attained. Before harvesting, ADSCs were successfully stained with bromodeoxyuridine (BrdU, Sigma-Aldrich, St. Louis, MO, USA) in vitro as shown in our previous studies [13,15].

Characterization of the ADSCs

The ADSCs (at passage 4) were evaluated for viability and stained for flow cytometric analysis [16]. Briefly, 2×10^5 cells suspended in PBS-containing 0.2% FCS were incubated with anti-rat monoclonal antibodies for CD29, CD45, CD90, CD105 conjugated with fluorescein isothiocyanate (BD Biosciences, Franklin Lakes, NJ, USA) in 50 µL of PBS for 30 minutes in the dark at 4°C. After washing thrice, cells were analyzed on a fluorescence-activated cell sorter (FACS) (FACSCalibur; BD Biosciences). Data acquisition and analysis were performed using Cell Quest software (Becton Dickinson, Franklin Lakes, NJ, USA).

In Vitro Transduction Experiments and Measuring Human IFN-α2b in the Conditioned Medium of ADSCs

The vector expressing human IFN-α2b was a gift from Dr. Stefano Indraccolo (Department of Oncology and Surgical Sciences, University of Padova, Italy). The self-inactivating, HIV-1- derived lentivirus expressing human IFN-α2b (hIFN-α2b-LV) was generated, concentrated, and quantified for infectious particles at the Tulane Primate Center as described previously [17,18]. ADSCs were cultured overnight in six-well plates and transduced with hIFN-α2b-LV in the presence of 5 µg/mL polybrene (Santa Cruz Biotechnology, CA, USA). After overnight infection, the medium was replaced with fresh charcoal-stripped FBS medium (Gibco, NY, USA), and the conditioned media were collected after 6, 12, 24, 48, 72, and 96 hours. The medium was centrifuged and filtered to remove cellular debris and aliquoted at –80°C. The release of hIFN-α2b in the medium was measured using an ELISA kit (Mabtech, Cincinnati, OH, USA) according to the manufacturer's protocol. The efficiency and optimization of transfection were validated by hIFN-α2b-LV-GFP expressing green fluorescence protein. Each time point was collected from three wells of a 12-well plate, in addition to wells transduced with empty plasmid as a control. The results were repeated at least three times and expressed as fold change compared with the control cells.

Intratunical Injection

Each rat was anesthetized with ketamine: xylazine (10:1 ratio, i.e., 100/10 mg/kg) intraperitoneally. Using a 2-mm midline incision at the left lateral midshaft of the penis, the tunical sheath was opened up and the TA exposed. The intratunical space was then injected with either saline or TGF- β 1 using a 30-gauge needle, followed by ADSCs or ADSCs-IFN (at passage 4), based on experimental groupings. After injection, the sheath was closed with a simple surgical knot using a 5-0 vicryl suture.

Main Outcome Measures

Measurement of Erectile Function

Intracavernous pressure (ICP) response to electrical cavernous nerve stimulation (CNS) at various voltage settings was used to evaluate erectile function as previously described [13]. Briefly, rats were anesthetized with thiobutabarbital 100 mg/kg intraperitoneally. The trachea was cannulated with a short segment of PE-240 tubing to maintain a patent airway, and the left carotid artery was catheterized with PE-50 tubing for measurement of systemic arterial pressure. ICP was measured with a 25-gauge needle inserted into the left crura of the penis connected to PE-50 tubing filled with heparin. Systemic arterial pressure and ICP were measured with Namic Perceptor DT pressure transducers (Navilyst Medical, Glen Falls, NY, USA) and a data acquisition system (Biopac MP 100A-CE, Santa Barbara, CA, USA). ICP, systemic arterial pressure, and mean arterial pressure (MAP) obtained by electronic averaging were continuously recorded and were displayed and stored on a PC. The ratio between the maximal ICP and MAP obtained at the peak of erectile response after CNS was determined to control for variations in MAP. The area under the curve of the increase in ICP was measured to characterize the total erectile response. CNS was carried out with a Grass Instrument SD9 Stimulator (Grass Instruments Co., Quincy, MA, USA). A rest period of at least 5 minutes was allowed between CNS trials.

Histopathology

Histopathologic evaluation was performed on hematoxylin and eosin (H&E)-stained rat penile midshaft sections (6 μ m thick) using light microscopy (Leica Model DM 2500, Leica Microsystems CMS, Wetzlar, Germany) by a pathologist in a blinded fashion. Sections obtained from the same paraffin-embedded tissues were subjected to Masson's trichrome stain to differentiate collagen fibers, and to Verhoeff-Van Gieson (VVG) stain to detect elastic fibers.

Real-Time Polymerase Chain Reaction Analysis

The isolation of TA and the expression of mRNA in TA using qualitative polymerase chain reaction (q-PCR) were performed as previously described [13,19]. Briefly, snap frozen tissues were homogenized, and RNA was extracted using the TRIzol method according to the manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). Extracted RNA samples were measured and evaluated for their purity and integrity using Nanodrop (Thermo Scientific, Waltham, MA, USA) and by running the samples on 1% agarose gel. cDNA was synthesized from 2- μ g RNA using M-MuLV Reverse Transcriptase and oligo dT according

to a standard protocol (New England Biolabs, Inc., Ipswich, MA, USA). Gene expression analysis was performed with SYBR Green reagents (Bio-Rad, Hercules, CA, USA) and the C1000 Touch Thermal Cycler (Bio-Rad). The sequences of primer sets used in q-PCR analysis are listed in Table 1. Amplification specificity was examined using a melting curve following the manufacturer's instructions. q-PCR was quantified using the CT method for studied transcripts normalized to β -actin as a housekeeping gene. The results were expressed as a fold change $\pm$ SEM from at least three independent experiments.

Heparin-Enhanced Zymography

The enzymatic activity of MMP-1, MMP-2, or MMP-9 in penile samples was determined by gelatin or casein zymography as previously described [13,20]. Briefly, MMP9 or MMP2 were detected in 10% Zymogram (Bio-Rad), and MMP1 activity was detected in 12% Zymogram. A final concentration of 0.3 mg/mL porcine intestinal heparin (Sigma-Aldrich) was included in the ready-to-load samples. Next, 50 mg of protein extract from tissue was diluted in Zymogram sample buffer. The samples were loaded into the wells of 10% precast polyacrylamide gel. Electrophoresis was carried out in a Bio-Rad apparatus at 90 V for 2 hours, until the bromophenol blue reached the bottom of the gel. The gel was then washed at room temperature for 30 minutes with Zymogram renaturation buffer containing 2.5% Triton X-100 and subsequently incubated at 37°C for 48 hours in a Zymogram development buffer containing 50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 5 mM CaCl₂. The staining was performed for 2 hours with 0.5% Coomassie blue R-250, and destaining was conducted in 30% methanol and 10% acetic acid until clear bands over a dark background were observed. Staining and destaining were performed at room temperature on a rotatory shaker. The gels were photographed, and the intensity of gelatinolytic action (clear bands) was analyzed in the Gel-Doc XR (Bio-Rad) imaging system.

Statistical Analysis

All data were expressed as mean $\pm$ standard error of the mean (SEM) and analyzed using Prism 6.0c (GraphPad Software, San Diego, CA, USA). Differences between multiple groups were compared by one-way analysis of variance (anova) followed by Tukey's multiple comparisons test. A value of $P < 0.05$ was considered statistically significant.

Results

ADSC Characterization

FACS analysis demonstrated that rat ADSCs were positive for mesenchymal stem cell surface markers CD29 (97.98%), CD90 (90.02%), and CD105 (22.4%) (Figure 1A–C) but were negative to CD45 (1.69%), which is a recognized hematopoietic stem cell marker (Figure 1D). The unstained ADSC isotypes are shown (Figure 1E). The ADSCs were successfully stained with BrdU (Figure 1F).

Human IFN- α 2b Is Released into the Conditioned Medium of ADSCs

To test the efficiency of viral transduction and its capability to secrete IFN- α 2b into cell-conditioned medium (CM), the concentration of IFN- α 2b in CM of ADSCs was measured by ELISA in a time course manner. The CM of transduced cells showed an increase ($\sim$ 400-

fold) in the release of IFN- α 2b after 24 hours relative to time t_0 . The release of cytokine reached its maximum level at 48 hours (520-fold) and plateaued after 48 hours. The secreted protein level began to decline after 96 hours, although its level was still higher compared with control to (Figure 2).

Measurement of Erectile Responses

Following intratunical injection of TGF- β 1, erectile responses to cavernosal nerve stimulation (CNS) were significantly reduced when compared with sham animals (at 5.0 V, change in ICP: 28 ± 6 vs. 57 ± 9 mm Hg; ICP/MAP: 0.41 ± 0.06 vs. 0.59 ± 0.05 ; total ICP: $1,365 \pm 300$ vs. $2,723 \pm 384$ mm Hg $\times$ seconds, respectively) (Figure 3). The preventive activity of ADSCs injected on the same day with TGF- β 1 (control ADSCs prevention group) resulted in increases in ICP, ICP/MAP, and AUC that were also significantly greater than responses in the TAF group (5.0 V, change in ICP: 77 ± 4 vs. 28 ± 6 mm Hg; ICP/MAP: 0.76 ± 0.03 vs. 0.41 ± 0.06 ; total ICP: $4,312 \pm 135$ vs. $1,365 \pm 300$ mm Hg $\times$ seconds, respectively). In the prevention group, intratunical injection of ADSCs-IFN showed improved erectile responses compared with the TAF group (5.0 V, change in ICP: 83 ± 3 vs. 28 ± 6 mm Hg; ICP/MAP: 0.77 ± 0.01 vs. 0.41 ± 0.06 ; total ICP: $4,565 \pm 176$ vs. $1,365 \pm 300$ mm Hg $\times$ seconds, respectively). Intratunical ADSCs-IFN injection at day 30 (treatment group) improved erectile responses 3.1, 1.8, and 1.3-fold at voltages of 2.5, 5.0, and 7.0, respectively, when compared with rats in the TAF group. Furthermore, at voltages of 2.5 and 5.0, treatment on day 30 with ADSCs-IFN improved erectile responses 1.6 and 1.3-fold over treatment with ADSCs alone. There was no significant difference with regard to baseline (prestimulation) ICP between any of the groups (sham = 13 ± 1 , TAF = 13 ± 2 , ADSC treatment = 14 ± 1 , ADSC-IFN treatment = 10 ± 2 , ADSC prevention = 13 ± 2 , and ADSC-IFN prevention 12 ± 2 mm Hg).

Histopathology

Histological studies revealed regularly thickened TA with normal wavy appearance of all collagen bundles (sham and prevention groups) (Figure 4A: a, c, and e) and irregularly thickened TA with varied degree of collagen bundle disorganization and clumping with loss of the typical wavy appearance and presence of focal areas of nodulelike clumps of collagen bundles (in the TAF group) (Figure 4A: b) and tendon-like fibrous connective tissue (treatment groups) (Figure 4A: d and f), especially at the tunical-cavernosal junction. Other histopathologic changes found in most TAF sections included scattered lymphoplasmacytic and histiocytic infiltrates, which were confined to the subtunical space, and a mild increase in number of fibroblasts that were associated with the collagen, mainly dispersed between collagen fascicles. No evidence of mineralization or ossification of the tunica was identified.

In Mason's trichrome-stained sections, collagen fibers were the predominant component in the tunical tissue. They appeared as thin, well-aligned fibers with a regular undulating configuration in completely normal TA (sham group) (Figure 4B: a) and partially normal TA (prevention groups) (Figure 4B: c and e) and as disorganized and clumped collagen fibers with aberrant collagen staining, haphazard arrangement, and random orientation in the TAF and treatment groups (Figure 4B: b, d, and f).

The VVG-stained sections showed dark-stained, elongated elastic fibers running individually throughout the normal TA (sham) (Figure 4C: a), while weakly stained elastic fibers that had lost their elongated regular shape, becoming curved to coiled, fragmented, and haphazardly distributed, were encountered in the abnormal TA (TAF group) (Figure 4C: b). The other groups showed some histological improvements with abnormal elastic fibers and elongated shape in their fibers (Figure 4C).

Gene Expression of Tissue Inhibitors of Metalloproteinases (TIMPs) and Matrix Metalloproteinases (MMPs) in the TA

TGF- β 1 injection caused a significant increase in the mRNA levels of TIMP-1, TIMP-2, and TIMP-3 in the TAF group compared with the sham (Figure 5). Of interest, the expression of all TIMPs was significantly decreased in the ADSCs-IFN treatment group when compared with the TAF group. However, mRNA levels of MMP-1, MMP-2, MMP-3, and MMP-9 were increased in the TAF group when compared with the sham groups. In all treatment and prevention groups, the results showed a significant increase in the expression of MMP-1 and MMP-9 compared with the TAF group. The expression of MMP-2 and MMP-3 was increased in all treatment and prevention groups in comparison with the TAF group, but the difference was statistically significant only in the ADSCs-IFN prevention group.

Zymography of MMP Activity

MMP activities were evaluated by SDS-PAGE zymography with the use of gels containing either gelatin or casein. An increase in the activation of MMP-9 was noticed in the TAF group compared with sham animals (25%; $P < 0.01$). In the ADSCs and ADSCs-IFN prevention groups, MMP-9 gelatinolysis was upregulated by 330% and 130% ($P < 0.001$), respectively. Similarly, there was a significant increase in MMP-9 activation in the ADSCs and ADSCs-IFN treatment groups (94% and 227% respectively, $P < 0.001$ for both) (Figure 6A). A marked increase in MMP-2 activity was observed in the TAF group compared with sham (267%; $P < 0.01$). The injection of ADSCs in the prevention group increased the MMP-2 activity by 274% compared with the TAF group ($P < 0.001$). However, in the ADSCs-IFN prevention group, there was a slight increase in MMP-2 activity (12%), compared with the TAF group ($P > 0.05$). The treatment of ADSC and ADSCs-IFN showed significant increase in MMP-2 activity (140%, $P < 0.01$ and 222%, $P < 0.001$, respectively) (Figure 6B). In the casein zymogram, there was a 159% and 30% increase of MMP-1 activity in the ADSC and ADSCs-IFN prevention groups, respectively, compared with the TAF group ($P < 0.001$ and $P < 0.01$). Also, in the ADSCs-IFN treatment group, MMP-1 activity was increased approximately 82% ($P < 0.001$). However, the control ADSC in the treatment group showed only a 35% increase in the activity of MMP-1 ($P < 0.01$) (Figure 6C).

Discussion

In a previous study, the beneficial effects of local ADSC administration were investigated as a preventative modality [13]. According to the results, local injection of ADSCs was efficacious as a preventative option, as measured by assessment of retained erectile function and reduced TAF changes when compared with animals receiving ADSCs 30 days following

TGF- β 1 injection. In the current study, the objective was to develop a new combination treatment strategy that targeted the chronic phase of PD, which is more common in the clinical setting. The results of this study showed that local injection of ADSCs-IFN significantly enhanced the erectile response and reduced TAF changes, which could be associated with decreased expression of TIMPs and stimulated activity of MMPs in the chronic phase (Figure 7).

Interferons are a group of naturally occurring, low molecular weight proteins and glycoproteins that play an integral role in the immune system. These immunomodulators inhibit fibroblast proliferation, diminish collagen production, and additionally (interferon alpha-2b) stimulate collagenase activity [21]. In the only large, multicenter, placebo-controlled, clinical study, intralesional injection therapy with IFN α -2b demonstrated benefit in treating penile curvature, reducing plaque size and density, reducing pain, and improving penile vascular parameters over placebo [14]. Men underwent six bi-weekly injections of IFN- α 2b for a total of 12 weeks in this treatment protocol. This study has gained significant attention, as it was the first placebo-controlled trial of intralesional injection therapy for PD. However, multiple intralesional injections may be uncomfortable and stressful for some patients. Use of the ADSCs-IFN combination can potentially reduce the number of injections patients receive, reduce cost, and improve efficacy.

Our results using the rat model showed a higher expression of TIMPs in severe penile fibrosis. The high expression of TIMPs inhibits the degradation of collagen by MMPs and promotes the deposition of extracellular matrix. The continuous deposition of collagen fibers in the tunica leads to fibrosis and the formation of a Peyronie's plaque. Intratunical injection of ADSCs or ADSCs-IFN increased the ability to degrade this scar tissue by decreasing the expression of TIMPs and stimulating the activity of MMPs. In addition to improving the imbalance between profibrotic and antifibrotic factors, the immunomodulatory, immunosuppressive, and antioxidant effects may also contribute to the beneficial effects by stem cell therapy [22]. In a previous study, it was demonstrated that seeding syngeneic ADSCs onto porcine small intestinal submucosal (SIS) grafts resulted in significant cavernosal tissue preservation and maintenance of a normal erectile response [15]. SIS grafting alone induced transcriptional upregulation of inducible nitric oxide synthase (NOS) and downregulation of endothelial NOS, neuronal NOS, and vascular endothelial growth factor (VEGF), an effect that was restored by seeding ADSCs on the SIS graft. These findings further support the concept of regeneration of damaged TA as a treatment option for PD.

In a recent study, human xenogeneic ADSCs were injected into the TA of rats with experimentally induced TAF during the early inflammatory phase of Peyronie's-like disease [19]. Local injection of ADSCs resulted in a nearly complete prevention of elastosis and fibrosis of the TA and maintained erectile function in rats 5 weeks after injection, despite presence of only a few labeled ADSC in the penile tissues of treated rats. In another study by An et al., the authors postulated that stem cells support wound healing, particularly in the healing process of the injured corpus cavernosum [23]. They injected either muscle-derived stem cells (MDSC) or MDSC transfected with VEGF into the wound area in a rabbit penile injury model. The results showed increased numbers of smooth muscle and endothelial cells,

significantly smaller scars by magnetic resonance imaging, and better erectile function in vivo. Interestingly, they also noted that the number of stem cells in the corpus cavernosum of the VEGF combination groups were significantly higher than that in stem cell alone group at different time points after cell transplantation and hypothesized that VEGF transfection has a beneficial effect on injected stem cell survival.

This animal model produces fibrosis that extends into the cavernosal tissues and therefore does not exactly resemble the human situation where fibrosis is limited to the TA. This effect has been commented on in previous preclinical studies of PD. Nevertheless, our rat model is still the most reliable and closely resembling rat model to human PD that is currently available [19]. An additional limitation is the lack of gross penile curvature in any of the animals during CNS at any voltage. From a hemodynamic standpoint, the erectile responses did not produce a characteristic change in the ICP response in the PD group, other than being attenuated. This reduction in ICP may be caused by the tissue changes that were confirmed histologically, and supports the concept of venous leak that is more enhanced at lower voltages of CNS where there is less compression of venous outflow against the TA. A similar finding has been reported in experiments with elastin-deficient mice [24]. An incomplete understanding of the pathophysiology of PD, when present along with ED, is one of the obvious difficulties in undertaking translational research. The lack of a universally accepted animal model for PD will always make investigation of novel stem-cell therapies somewhat speculative. However, the use of stem cell therapy with gene modification offers a legitimate strategy for developing successful options for the many men who suffer from PD with or without ED [25].

The functional response, increase in ICP, is the sum total of all responding elements or components of the erectile process (nerves, endothelial cells, and smooth muscle). The histologic analysis is derived from only a small number of cells in the tissue section, where the response provides information about the overall activity of the whole organ system. It is difficult to make an absolute correlation between a histologic section and an increase in ICP/MAP.

Conclusion

This study documents that transplantation of genetically modified ADSCs, with or without human IFN α -2b, enhanced erectile function and attenuated Peyronie's-like changes in a rat model of TAF. These data support the additional benefits of using genetically modified ADSCs in the treatment of ED and PD. Transfection of ADSCs with human IFN α -2b, compared with the ADSCs alone, enhanced erectile responses and attenuated tunica albuginea fibrotic changes in both the acute and chronic phases of this PD-like animal model. Regenerative medicine using ADSCs in combination with gene modification may be an important advance for future PD/ED research and treatment.

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Figure 1.

Adipose tissue-derived stem cells (ADSCs) characterization and bromodeoxyuridine (BrdU) labeling. Flow cytometric analysis of early passage rat ADSCs depicting positive expression for CD29 (97.98%) (A), CD90 (90.02%) (B), CD105 (22.4%) (C), and negative expression for CD45 (1.69%) (D). (E) The unstained ADSC isotypes are shown. (F) ADSCs were successfully stained with BrdU, and the nuclei of ADSCs showed green fluorescence (scale bar: 100 μm).

Figure 2.

Quantification of IFN-α2b in the conditioned medium (CM) of ADSCs. ADSCs were cultured and transduced with lentiviral vector expressing human IFN-α2b. The results were repeated three times and expressed as % change in reference to control nontransduced cells (* $P < 0.001$ vs. control, # $P < 0.01$ vs. control).

Figure 3.

Measurement of erectile function. Bar graphs depicting the voltage-dependent erectile response as measured by the change in intracavernosal pressure (ICP), ICP/mean arterial pressure (MAP) ratio, and total ICP (area under the erectile curve) in response to cavernous nerve stimulation (CNS) for 1 minute at day 45. One-way ANOVA followed by the Tukey test was used for statistical comparisons.

Figure 4.

Treatment of rats with donor nascent or modified rat adipose tissue-derived stem cells (ADSCs) showing histological changes. Panel A: Photomicrograph of H&E-stained penile midshaft sections from sham (a), tunica albuginea fibrosis (TAF) (b), TAF plus ADSCs prevention (c), TAF plus ADSCs-IFN-prevention (d), TAF plus ADSCs-treatment (e), and TAF plus ADSCs-IFN-treatment (f) groups. Rats revealed regularly thickened tunica albuginea (TA) with normal wavy regular appearance of all collagen bundles in a (arrow); relatively regularly arranged collagen bundles in c (e-arrow); irregularly thickened TA with varied degrees of collagen bundle disorganization and clumping with loss of their regular wavy appearance (b,d,f); presence of focal areas of nodule-like clumps of collagen bundles (b-arrow); and tendon-like fibrous connective tissues (d, f, arrow), especially at the tunical-cavernosal junction. Panel B: Masson's trichrome-stained sections showing blue collagen fibers as the predominant component of tunical tissue. They appear as thin, well-aligned

fibers with a regular undulating configuration in completely normal TA (a, arrow); partially normal TA (c, e- arrow); and as disorganized and clumped collagen fibers with aberrant collagen staining and a haphazard arrangement and random orientation (b, d, f, arrow). Panel C: Verhoeff-Van Gieson-stained sections showing dark-stained, elongated elastic fibers running individually throughout the normal rat TA (a, arrow), whereas weakly stained elastic fibers that had lost their elongated regular shape, becoming curved to coiled, fragmented, and haphazardly distributed, were encountered in abnormal areas of TA (b, d, f, arrow) with some improvement (c, e, arrow). Scale bar is 50 μ m.

Figure 5.

Gene expression of tissue inhibitors of metalloproteinase (TIMPs) and matrix metalloproteinases (MMPs). Target genes were measured by quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Gene expression was normalized to β -actin and expressed as a fold change $\pm$ SEM from at least three independent experiments. The δ and * denote $P < 0.05$ when compared with control and TAF group, respectively.

Figure 6.

Zymography-based measurement of activity of matrix metalloproteinases (MMPs). The MMP-1 activity was examined by casein zymography (A). The MMP-2 and MMP-9 activities were examined by gelatin zymography (B, C). Intensity of the band was quantified and presented in bar graphs as mean $\pm$ SEM from three separate experiments. Statistical significance was determined by one-way ANOVA test (** $P < 0.01$, *** $P < 0.001$).

Figure 7. The relationship between ADSCs/ADSCs-IFN and TIMPs/MMPs and fibrosis/PD

Table 1
List of primer sets used in real-time polymerase chain reaction (RT-PCR) analysis

	Forward	Reverse
rMMP1	GAGGTGAAAAGGCTCAGTGC	ATGAGGCGGGGATAGTCTTT
rMMP2	CATCGCTGCACCATCGCCATCATC	CCCAGGGTCCACAGCTCATCATCAAAG
rMMP3	CCGTTTCCATCTCTCTCAAGATGA	CAGAGAGTTAGATTGGTGGGTACCA
rMMP9	GAAGACTTGCCGCGAGACCTGATCGATG	GCACCAGCGATAACCATCCGAGCGAC
rTIMP1	GGGCTACCAGAGCGATCACTT	AAGGTATTGCCAGGTGCACAA
rTIMP2	ACATCTCCTCCCCGGATGA	GGTGCCCATTGATGCTCTTC
rTIMP3	TACCCTGGCTATCAGTCCAAACA	GCTGCAGTAGCCACCCTTCT
β-Actin	GCTACAGCTTCACCACCACA	ATCGTACTCCTGCTTGCTGA