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Review Article

The Current Status of Stem-Cell Therapy in Erectile Dysfunction: A Review

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Stem cells are undifferentiated cells that are capable of renewal and repair of tissue due to their capacity for division and differentiation. The purpose of this review is to describe recent advances in the use of stem cell (SC) therapy for male erectile dysfunction (ED). We performed a MEDLINE database search of all relevant articles regarding the use of SCs for ED. We present a concise summary of the scientific principles behind the usage of SC for ED. We discuss the different types of SCs, delivery methods, current pre-clinical literature, and published clinical trials. Four clinical trials employing SC for ED have been published. These articles are summarized in this review. All four report improvements in ED after SC therapy. SC therapy remains under investigation for the treatment of ED. It is reassuring that clinical trials thus far have reported positive effects on erectile function and few adverse events. Safety and methodical concerns about SC acquisition, preparation and delivery remain and require continued investigation prior to wide-spread application of these methods.

Key Words: Erectile dysfunction; Stem cells

INTRODUCTION

Erectile dysfunction (ED) is defined as the inability to attain or maintain a penile erection satisfactory for sexual intercourse [1]. ED is a prevalent health problem that seriously impacts the quality of life of men and their partners [2]. Ultimately, approximately 50% of men between the ages of 40 and 70 years have some degree of ED [3]. The majority of ED patients can now be treated satisfactorily with phosphodiesterase type-5 inhibitors (PDE5is), such as sildenafil, vardenafil, tadalafil, and avanafil [4]. However, PDE5is can cause a variety of side effects that make them unsuitable for some patients, and they are contraindicated in patients who also take nitrates because of the danger of synergistic hypotensive effects [5]. Several other management options exist for ED, including lifestyle modifications and pharmacotherapeutic strategies such as intraurethral alprostadil, intracorporal injection therapy, vacuum erection devices, and surgery, including penile revascularization and penile prosthesis implantation. Despite the efficacy of these modalities, limitations to their use exist, such as intolerance to side effects, cost limitations, and unsatisfactory outcomes [6]. With the exceptions of lifestyle modification and revascularization procedures, these

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methods merely treat the manifestations of ED, offering symptomatic relief rather than a cure for the underlying disease process. The pressing need to develop a curative treatment for ED has stimulated interest in utilizing stem-cell therapy in ED patients [7].

RATIONALE FOR USING STEM-CELL THERAPY

The corpora cavernosa are composed of a lattice of sinusoids lined by a single layer of endothelial cells (ECs) and are surrounded by multiple layers of circular and longitudinal cavernous smooth muscle cells (CSMCs). In the flaccid state, CSMCs are contracted and maintain a small amount of blood flow into and out of the sinusoids. With stimulation, nitric oxide (NO) is released from terminal fibers of the cavernous nerves (CNs) and enters the CSMCs, elevating the level of cyclic guanosine monophosphate (cGMP) and resulting in CSMC relaxation, which allows blood to rush in and engorge the sinusoids. Maintenance of erection is thought to result from additional NO release from sinusoidal ECs. Further sinusoidal engorgement leads to compression of the venules between the trabeculae and the tunica albuginea, resulting in occlusion of venous outflow and full erection [8]. Detumescence occurs when cGMP is degraded by type 5 phosphodiesterase, leading to contraction of the CSMCs with subsequently decreased inflow and a reduction in the size of the sinusoidal spaces, allowing more venous outflow through the subtunical venules [9].

Mechanically, the key components of erection are the ECs, the CSMCs, and the neuronal NO synthase-positive nerves of the CN [10]. In various disease states, these components or interactions of these components are altered, leading to ED. After radical prostatectomy, the CN can be damaged [11]. The short-term consequence is neurogenic ED, which may be reversible. The long-term consequences include diminished NO production, atrophy of CSMCs, and CSMC apoptosis, leading to penile fibrosis and permanent ED [12,13]. Radiation-based therapies are thought to cause ED via the same type of mechanism [14]. In diabetes mellitus, high blood glucose can cause dysfunction and reduction in the CN, EC, and CSMC content [15,16]. In hyperlipidemia, cavernous NO levels are decreased, and the subsequent impairment of CN and EC function is well documented [17], but whether or not structural changes take place is not as certain [16].

Stem cells are believed to be able to differentiate into various cell types, including ECs, smooth muscle cells (SMCs), Schwann cells, and neurons [18]. Consequently, the concept of employing stem-cell therapy for ED was initially based on the hypothesis that transplantation of stem cells into the penis might replenish depleted EC or CSMC content [10]. An additional hypothesis was that transplanted stem cells might encourage the regeneration of the host's own ECs and ESMCs or might restore proper interactions between ECs and SMCs through a paracrine effect. Based on studies of ED and other conditions, this seems to be the main mechanism of stem cell action [19].

ROLE OF STEM CELLS IN THE TREATMENT OF ERECTILE DYSFUNCTION

Over the past few years, considerable excitement has developed about the potential use of stem cell-based therapies in medicine in general and in the specialty of urology in particular. The treatment of ED has received a tremendous amount of publicity, as it is becoming an increasingly recognized global public health problem [20]. Nonetheless, stem-cell therapy has been in use since the 1990s in other fields for conditions such as acute and chronic graft-versus-host-disease to enhance engraftment in patients receiving allogeneic hematopoietic stem cell transplantation [21]. The anti-inflammatory, restorative, and immunomodulatory qualities of stem cells are currently being exploited clinically to treat hematologic diseases [22-24]; malignancies [25]; cardiovascular disease [26]; neurologic diseases such as amyotrophic lateral sclerosis [27-29], Parkinson disease [30], and stroke [31]; autoimmune diseases such as multiple sclerosis [31,32] and systemic lupus erythematosus [33]; as well as refractory wounds [34], spinal cord injuries [35], and cartilage defects [36]. It makes sense that the field of urology would also benefit from the utilization of this type of approach for acute and chronic urologic illnesses.

Many preclinical studies have explored the utility of stem cells, often referred to in studies as regenerative cells, for ED in animal models. A recent article by Soebadi et al

[7] published in 2016 summarized all of these studies. These authors described the many preclinical trials that have provided data on the utilization of both bone marrow stem cells and adipose-derived stem cells (ADSCs) for ED. Human data on stem-cell therapy for ED are finally emerging approximately 10 years after the first reports on animal models. So far, 4 clinical trials in patients with ED have been published, and these trials are summarized below.

Most authors have utilized an animal model simulating either an acute iatrogenic trauma to the neurovascular bundle, as in the result of radical prostatectomy, or a chronic disease state such as aging, diabetes mellitus, or hyperlipidemia. In acute ED models, the mechanism of action of stem cells is presumed to be by paracrine action [37-39]. In contrast, in chronic ED, the theoretical method of stem cell action is postulated to be both engraftment and cellular differentiation [7]. However, the exact mechanism of action of stem cells in chronic ED remains uncertain [40].

CLASSIFICATION OF STEM CELLS

Stem cells are undifferentiated cells that are capable of self-renewal and differentiation [18]. Stem cells are classified according to their potential for differentiation [6]. Their capacity for division, differentiation, and tissue regeneration is highly dependent on the surrounding environment, or niche [41]. The niche is a microenvironment that supports stem cells in their quiescent state and promotes stem cell self-renewal or tissue regeneration when tissue damage occurs [41]. Niche cells are classified as epithelial or stromal depending on their location and proximity to the stem cells. Niche properties, including proximity to the bloodstream, the presence of certain cytokines and growth factors, low oxygen tension, and other physiochemical properties allow optimal interaction among stem cells, their neighboring stromal or epithelial cells, and the extracellular matrix [41]. The stem cell niche is often present in the perivascular space, allowing direct access to the bloodstream when damaged tissues need stem cells for regeneration.

Totipotent stem cells have the highest potential for differentiation and can differentiate into any tissue type originating from the ectoderm, mesoderm, or endoderm [42]. The zygote and morula daughter cells are examples of totipotent stem cells [43]. Pluripotent stem cells can differentiate into cells from the 3 different germ cell layers and gonadal ridge, but not into extraembryonic tissues. Embryonic stem cells (ESCs), which are derived from the inner cell mass of the blastocyst, are an example of pluripotent stem cells [44,45].

Multipotent stem cells, such as hematopoietic, mesenchymal, and neural stem cells, are capable of self-renewal and can differentiate into organ-specific cell types; they are isolated from the developing germ layer or from developed adult organs [42]. Unipotent stem cells can give rise to only one distinct cell type, such as epithelial cells; these cells have a limited capacity for self-renewal and labeling then as stem cells has been debated [46]. Ethical concerns regarding the use of ESCs have arisen due to the need to destroy embryos to isolate such cells. This has shifted interest to other cell types, such as adult stem cells, as an alternative stem cell source [47]. Induced pluripotent stem cells are cells that have been manipulated in the laboratory to express genes that are normally present in ESCs. As such, these cells can differentiate into cells of all organs and tissues [6]. They can be generated from somatic cells by overexpression of ESC-specific transcription factors [48,49]. These cells share characteristics with ESCs, although the differences between them have not been fully elucidated [50].

TYPES OF STEM CELLS USED IN ERECTILE DYSFUNCTION

The use of pluripotent ESCs in ED research has been limited due to ethical concerns. Only the study of Bochinski et al [37] investigated the outcome of intracavernosal and major pelvic ganglion injection of neural ESCs in a rat model of neurogenic ED induced by cavernosal nerve injury. These authors demonstrated improved erectile function with significantly higher intracavernosal pressures than were found in the control group. Immunohistochemical staining also revealed differences in the quality of the nitric oxide synthase-containing nerve fibers. Neurofilament staining was significantly better in the experimental groups injected with neural ESCs. No further studies utilizing ESCs have been published.

METHODS OF DELIVERY

Different routes have been suggested for the delivery of stem cells, and research continues to assess the most effective route of instillation. Some studies have involved the direct injection of cells into the organ of concern [51-53]. Other studies have investigated intraperitoneal or intravenous injections of stem cells [21]. Studies have shown that less than 1% of stem cells infused via the intravenous route actually reach the target tissue, and those that do reach the target tissue dissipate after a few days [54]. In spite of the low level of actual engraftment, stem cells exert clinically relevant influence through paracrine effects, triggering endogenous mechanisms of regeneration, rather than transdifferentiation into different cell types [21]. Stem cells promote the propagation and differentiation of resident progenitor cells and encourage the recovery of injured tissue via the production of antiapoptotic and proangiogenic factors [55-57]. A developing body of evidence also indicates that stem cells have immunomodulatory characteristics and immunoprivileged properties [58], and can modulate a wide range of target cells within the immune system [54].

In preclinical studies, the intravenous injection of ADSCs has been shown to lead to improvements in erectile function [59]. The intracorporal injection of stem cells for ED treatment has also been common in preclinical studies, as it is both straightforward and logical [6]. The regenerative effect of stem cells is achieved by either secreting growth factors locally via a paracrine mechanism or by migration to the major pelvic ganglia [60]. Direct injection into the major pelvic ganglia has not been methodically studied due to the inherent challenges involved in the injection process [37,61]. Periprostatic injection, with or without a concurrent intracorporal injection, has also been attempted [62-64]. Intraperitoneal infusion of stem cells was less efficacious than intracorporal injection in improving erectile function in a CN injury mouse model, though both groups did show improvement from baseline values [65,66].

PRECLINICAL TRIALS

Many preclinical trials have been performed to investigate the safety, efficacy, and mechanisms of stem-cell therapy for ED in animal models. A recent article by Soebadi et al [7] published in 2016 summarized these studies. As stated by those authors, these preclinical trials have provided ample data on the utilization of both bone marrow stem cells and ADSCs for ED. Almost all of the studies reported improved erectile function in various animal models of CN injury, vascular insufficiency, diabetes mellitus, hyperlipidemia, and aging.

It is important to note that preclinical studies have also raised concerns about the effects of stem cells on the growth of malignancies. One paper described the effects of transplantation of ADSCs on grafted prostate tumors in athymic mice. The average size of the tumors in the treated mice was larger than in the control group, and ADSCs were identified inside of the tumors of ADSC-treated mice. Capillary density was twice as high in the tumors of the ADSC-treated mice as in the control mice. The authors concluded that prostate cancer cells recruited ADSC via the CXCL12/CXCR4 axis and that ADSCs helped tumor growth by increasing tumor vascularity, which was mediated by fibroblast growth factor 2 [55]. Concerns about the possible promotion of malignant growth remain paramount as studies exploring the utilization of stem cells for benign human diseases progress.

CLINICAL TRIALS

Human data on stem-cell therapy for ED are finally emerging approximately 10 years after the first reports on animal models. To date, 4 published human clinical trials have employed stem cells in patients with ED, and these trials are summarized below.

In 2010, Bahk et al [53] in Korea reported a single-blind study on the effect of intracavernosal injections of umbilical cord stem cells in 7 men (mean age, 69.5 years; range, 57～87 years) with type 2 diabetes-related ED. The men had been diabetic for a mean of 29.4 years (range, 12～52 years) prior to the study and were already scheduled for penile prostheses. The authors injected 1.5×10^7 human umbilical cord blood stem cells into the bilateral corpora cavernosa after placing a clamp at the base of the penis; the clamp was removed after 30 minutes. The 3 men in the control group were injected with normal saline in a similar

fashion. The authors assessed outcomes utilizing the international index of erectile function-5 (IIEF-5), the Sexual Encounter Profile, Global Assessment Question, and an erection diary at 9 months, and the patients were followed for a total of 11 months. The controls reported no changes in erectile function after injection. In the experimental group, by 2 months after treatment, 6 of 7 patients reported the return of morning erections, which was maintained for at least 3 months. Six patients reported increased penile hardness. With the addition of 100 mg of sildenafil, 2 patients were able to achieve erection adequate for coitus, and this effect was retained at the fifth month. By the ninth month, one of these patients reported the inability to penetrate even with the addition of the oral agent. Three of the 7 patients agreed that stem-cell therapy had some effect on ED, although it was insufficient. Five of the 7 patients regarded stem-cell therapy as effective for ED when combined with a PDE5i. The authors also reported improvements in blood glucose levels after treatment with stem cells, suggesting that the stem cells had a systemic effect in addition to a local effect. No serious adverse effects were reported.

In 2016, Yiou et al [51] from France reported the results of a 1-year, nonrandomized dose-escalation, phase I-II pilot trial of the intracavernous injection of bone marrow mononuclear cells in post-prostatectomy patients with vasculogenic ED. This study enrolled 12 men (aged 45～70 years old; mean age, 63.6 years) with penile arterial insufficiency and/or venous occlusive dysfunction at 6 months to 3 years after prostatectomy for localized prostate cancer, and administered 1 of 4 escalating doses of treatment (2×10^7, 2×10^8, 1×9^9, or 2×10^9 stem cells). The effects of treatment on erectile function and penile vascular parameters were assessed using the IIEF-15 and Erection Hardness Scale (EHS) questionnaires and by color duplex Doppler ultrasound. Endothelial function was assessed using the penile NO release test by measuring the percentage of post-occlusive changes in the cavernosal artery diameter. At 6 months, EHS and Doppler ultrasound parameters had significantly improved in patients receiving higher doses and were more pronounced in combination with pharmacotherapy; patients also reported significant improvement in the intercourse satisfaction and erectile function domains of the IIEF-15. Significantly greater improvements in spontaneous erections were reported with higher doses. Overall, 9 of 12 patients reported successful intercourse with vaginal penetration when using medication. Clinical benefits were associated with improvements in peak systolic velocity (at 6 months, this parameter improved to normal in 7 of the 11 patients) and percent penile NO release test (at 6 months, 8 of the 11 patients were in the normal range, versus 2 of 11 at baseline), and these benefits were sustained after 1 year. No serious adverse side effects were reported.

In 2016, Haahr et al [52] from Denmark reported on the safety and potential effect of a single intracavernous injection of autologous ADSCs in 17 men (aged 46～69 years; median age, 63) with severe, refractory, post-prostatectomy ED resulting from surgery 5～18 months prior to enrollment. This was a 6-month, prospective, open-label, phase I single-arm study. Erectile function was assessed by IIEF-5 scores. The men received between 8.4×10^6 and 37.2×10^6 ADSCs immediately after cell isolation from liposuction. Eight of the 17 men recovered erectile function with the ability to accomplish sexual intercourse. Their IIEF-5 scores continued to improve over the course of the 6-month study in continent participants. The EHS results had significantly improved at 6 months, but no change was noted at 1 month and at 3 months. Of note, positive effects of treatment were absent in incontinent men, who reported IIEF-5 scores and EHS scores at 1, 3, and 6 months that did not significantly differ from those at the time of inclusion into the study. No serious adverse events were reported.

In 2016, Levy et al [67] reported on the feasibility and efficacy of managing ED with placental matrix-derived stem cells. The authors injected 8 patients with placental matrix-derived stem cells and followed them for 6 months with Doppler parameters and the IIEF questionnaire. At 6 months, the peak systolic velocity was found to have improved to a statistically significant extent, from 25.5～56.5 cm/s at 3 months to 50.7～73.9 cm/s. Changes in end-diastolic velocity and IIEF scores were not statistically significant. At a 2-month follow-up, 2 patients were able to have erections using PDE5is. At a 3-month follow-up, 3 patients could attain erections with pharmacologic assistance from PDE5is, whereas previously they could not (Table 1).

Table 1. Summary of the 4 published clinical trials on stem-cell therapy for ED

First author (year)	Number of men	Cause of ED	Treatment	Assessment	Results
Bahk (2010) [53]	7	Diabetes	Umbilical blood SC	IIEF-5, SEP, GAQ	Improved rigidity in 2/7, able to penetrate with PDE5i
Levy (2016) [67]	8	Organic	Placental-derived SC	PSV, IIEF	3/8 improved erection; IIEF change not significant
Haahr (2016) [52]	17	5～18 months after radical prostatectomy	Adipose-derived SC	IIEF-5	8/11 continent men and 0/6 incontinent men recovered erection
Yiou (2016) [51]	12	22 months after radical prostatectomy	Bone marrow mononuclear cells	IIEF-15, EHS, color Doppler ultrasound	1/12 hard erection; 9/12 needed ICI, PDE5i, or VCD. Improved EHS and IIEF

ED: erectile dysfunction, SC: stem cell, IIEF: international index of erectile function, SEP: Sexual Encounter Profile, GAQ: Global Assessment Question, PSV: peak systolic velocity, EHS: erectile hardness score, PDE5i: phosphodiesterase type 5 inhibitor, ICI: intracavernous injection, VCD: vacuum constriction device.

A review by Casiraghi et al [21] included data from trials across a wide range of specialties, including hematology, oncology, cardiology, neurology, and orthopedics, among others. The safety data from clinical trials incorporating more than 700 patients receiving autologous or third-party bone marrow or adipose-tissue derived mesenchymal stem/stromal cells does not suggest that serious adverse events are a clinically important issue [21].

IMMUNOCOMPATIBILITY

Autologous stem cell transplantation is the least immunogenic type of stem cell transplantation. However, a surgical procedure is required for the harvest of ADSCs (liposuction) and bone marrow stem cells (bone marrow biopsy), and the procedure itself may impact the outcome of stem-cell treatment. More recent preclinical studies have increasingly utilized allogeneic and xenogeneic stem cells transplants as a substitute for autologous stem cells. Allogeneic and xenogeneic stem cells do not incite an immune response, as they do not possess T-cell co-stimulatory molecules [68].

FUTURE DIRECTIONS

Stem-cell therapy is rapidly developing into a viable treatment option for ED patients. With ample preclinical data, 4 published clinical trials, and multiple ongoing clinical trials, it seems that both clinicians and patients are eager to embrace this cutting-edge technology. Despite this overwhelming enthusiasm, several critical questions remain to be answered before the widespread application of these complex techniques. First and foremost, the mode of action still needs to be determined and the safety of the treatment established. Next, the most efficacious delivery method has yet to be ascertained, although intracorporal injection seems to be the route of choice based on the clinical trials that have so far been published [69,70]. Additionally, the ideal timing, type, and dosage of stem cell for treatment still needs to be established, incorporating such considerations as type (adipose-derived, bone marrow, adipose-derived stromal vascular fraction, etc.), source (allogeneic versus autologous), ease of isolation and culture, risk, efficacy, and cost. Finally, long-term surveillance studies are needed to determine the possible adverse effects of stem cell treatment on cell growth and to determine the consequences of the secretome on coexistent subclinical conditions.

ON THE HORIZON

As scientists and clinicians, we must always continue to contemplate the next step in treatment for our patients. On the horizon for the treatment of ED is low-intensity pulsed ultrasound (LIPUS), a non-invasive method of upregulating endogenous mesenchymal stem cells [71]. Studies

have shown that LIPUS stimulates cell proliferation through activation of integrin receptors and the Rho/ROCK/Src/ERK signaling pathway [72], and by promoting the multilineage differentiation of mesenchymal stem/progenitor cell lines through the ROCK-Cot/Tpl2-MEK-ERK signaling pathway [73]. Additionally, low-energy shock wave therapy (LESWT)-induced endogenous progenitor cell recruitment and Schwann cell activation was associated with angiogenesis, tissue regeneration, and nerve generation in a rat model of pelvic neurovascular injury [74].

In human cardiovascular patients, LIPUS has demonstrated an angiogenesis-promoting effect believed to involve upregulation of vascular endothelial growth factor. Additionally, LESWT has been shown to markedly improve erectile function in patients with organic ED [75] through downregulating receptors for advanced glycation end products [76] and the recruitment of endogenous mesenchymal stem cells [77]. As an indication of the widespread enthusiasm it has received from urologists, LESWT has already been discussed in the first-line therapy section (Section 3.5.3; page 24) of the European Association of Urology Sexual Dysfunction Guidelines [78], but has not yet been recommended, as further studies to define the mechanism of action and the best treatment protocol have yet to be carried out.

By utilizing these non-invasive methods, if we are successful, we will be able to potentially mitigate or eliminate the safety and methodological concerns about stem cell acquisition, preparation, and delivery that were discussed above. As with stem-cell therapy, the underlying mechanisms of therapeutic ultrasound and the biological effects it has on the human body remain to be investigated, and well-designed rigorous studies are needed to further define the mechanism of action, safety profile, and efficacy of treatment.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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