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Review

Mesenchymal stem cell-based gene therapy for erectile dysfunction

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Despite the overwhelming success of PDE5 inhibitor (PDE5I), the demand for novel pharmacotherapeutic and surgical options for ED continues to rise owing to the increased proportion of elderly individuals in the population, in addition to the growing percentage of ED patients who do not respond to PDE5I. Surgical treatment of ED is associated with many complications, thus warranting the need for nonsurgical therapies. Moreover, none of the above-mentioned treatments essentially corrects, cures or prevents ED. Although gene therapy is a promising option, many challenges and obstacles such as local inflammatory response and random transgene expression, in addition to other safety issues, limit its use at the clinical level. The use of stem cell therapy alone also has many shortcomings. To overcome these inadequacies, many scientists and clinicians are investigating new gene and stem cell therapies.

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INTRODUCTION

Penile erection is a complex response requiring functional integrity of nitrergic nerves, the endothelium and smooth muscles in the penis.[1] The treatment of ED has received much attention in recent years. The pharmacological approach using PDE5 inhibitors (PDE5I) is regarded as the first-line treatment for patients with ED. This treatment is highly effective; however, some patients require second-line therapy primarily consisting of intracavernous injections.[2] In addition, in diabetes mellitus (DM)-induced ED and cavernous nerve injury-induced ED, it is known that limitations in treatment outcomes exist.[3]

Regarding the success rate of PDE5I in men with concomitant medical conditions, the lowest value (43%) was found in patients who underwent radical prostatectomy and the second lowest value (44%) was found in patients with uncontrolled DM.[4–6] Moreover, PDE5I provides only symptomatic relief from ED and does not offer a cure for the disease. Therefore, it is important to evaluate other potential treatments including herbs, gene therapy and stem cell transplantation. Among these, using stem cell transplantation along with gene therapy is a promising new approach for the treatment of patients showing limited response to PDE5I (Table 1).

These strategies include cell-based therapies involving intracavernous injections of mesenchymal stem cells (MSCs) and therapeutic genes such as the endothelial nitric oxide synthase (eNOS) gene or the vascular endothelial growth factor (VEGF) gene, often by using an adenoviral vector.[7] MSCs derived from the bone marrow are capable of transforming into various cell types, thereby enabling tissue repair and regeneration. Furthermore, they do not induce local immune reactions and are stable.[3]

The penis is a potential target tissue for gene therapy because of its accessibility and the ubiquity of endothelial lined spaces. Gene therapy is, therefore, a promising therapeutic strategy for the treatment of ED. Both MSC injection therapy and gene therapy with eNOS or VEGF have some limitations when used individually. To overcome these limitations, combinational treatments with MSCs and gene therapy have been introduced. A novel approach for the treatment of ED that could prevent random distribution of the transgene and reduce the possibility of an inflammatory response involves the use of MSCs, also known as marrow stromal cells, alone or with *ex vivo* genetic modification using eNOS.[7–10]

The aim of this study is to evaluate the status of a combinational MSC-based gene therapy in ED.

PROPERTIES OF MSCS

It has been shown that MSC injection into the corpus cavernosum improves erectile functions in diabetic[11] and hyperlipidemic[12] rat models, as well as in neurogenic ED models.[13]

Human MSCs have been isolated from a large number of adult tissues including bone marrow, adipose tissue and skeletal muscles.[3] MSCs have been of particular interest in the treatment of ED because relatively easy methods are available for their acquisition.[14]

MSCs are capable of self-renewal and differentiation into various phenotypes.[15] However, they also produce characteristic immunomodulatory, proangiogenic, anti-apoptotic, anti-fibrotic and anti-inflammatory effects, mainly through the secretion of bioactive trophic factors.[16,17]

In addition, MSCs differentiate into multi-lineage cells that can survive for long periods after autologous transplantation without inducing an immune response. MSCs express low levels of MHC class 1 molecules and do not express MHC class 2 molecules, indicating that they are minimally immunogenic. This has led to the use of both allogeneic and autologous sources of MSCs in various preclinical and clinical studies with promising efficacy and safety data.[18] Transplantation of patients' own autologous

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Table 1. Preclinical trials of stem cell transplantation with gene therapy for the treatment of ED

Authors	*Animal model*	*Stem cell*	*Gene therapy*	*Transplantation*	*Structural changes*	*Functional outcomes*
Gou *et al.*[73]	Rat, DM model	EPC	$VEGF_{165}$- EPC	ICI	In $VEGF_{165}$- EPC-treated group, the corpus cavernosum showed numerous sites of neovascularization. Transplanted EPCs showed cell differentiation into endothelial cells.	Significant effects on improving ICP in response to CN stimulation.
Qiu *et al.*[72]	Rat, DM model	MSC	$VEGF_{164}$-MSC	ICI	Higher contents of smooth muscle and endothelium in the corpus cavernosum in $VEGF_{164}$-transfected MSC-treated group.	Significant effects on improving ICP and peak ICP/MAP ratio in response to CN stimulation.
Liu *et al.*[99]	Rat, DM model	ADSC	$VEGF_{165}$-ADSC	ICI	In $VEGF_{165}$- ADSC-treated group, the percentage of smooth muscle markers and the number of cells expressing pericyte markers significantly increased.	Significant effects on improving ICP and peak ICP/MAP ratio in response to CN stimulation.
Bivalacqua *et al.*[37]	Rat, aged	MSC	eNOS-MSC	ICI	eNOS-MSC-treated group showed improved endothelium signaling and differentiation into penile cells expressing endothelial and smooth muscle markers.	Significant effects on improving ICP, total ICP and the peak ICP/MAP ratio in response to CN stimulation.
Ouyang *et al.*[53]	Rat, DM model	Human USC	FGF_2-USC	ICI	The number of cells expressing smooth muscle markers within the corporal tissue and the cell/collagen ratio were significantly increased in the FGF_2-USC-treated group.	Significant effects on improving ICP and peak ICP/MAP ratio in response to CN stimulation.
Kim *et al.*[66]	Rat, nerve injury model	MSC	rAd/hBDNF-MSC	Injection into MPG	A greater extent of preservation of smooth muscle was observed in rats treated with MSCs infected with rAd/hBDNF than that observed in mice treated with MSCs alone.	Significant effects on improving peak ICP/MAP ratio in response to CN stimulation.
Bochinski *et al.*[65]	Rat, nerve injury model	ESC	EGFP-BDNF	Injection into MPG	Neurofilament staining was significantly better in the experimental groups injected with ESCs.	Significant effects on improving peak ICP in response to CN stimulation
He *et al.*[85]	Rat, DM model	MSC	KCNMA1-MSC	ICI	Not checked.	Significant effects on improving the peak ICP/MAP ratio in response to CN stimulation.
Gokce *et al.*[86]	Rat, tunica albugineal fibrosis model	ADSC	ADSCs-IFN	Injection into intraunical space	Various degree of collagen bundle disorganization and clumping with loss of the typical wavy appearance and presence of focal areas of nodule-like clumps of collagen bundles and tendon-like fibrous connective tissue reduced Peyronie's-like manifestations. Decrease in the expression of tissue inhibitors of metalloproteinases.	Significant effects on improving ICP. Changes in ICP and peak ICP/MAP ratio in response to CN stimulation.
Kendrici *et al.*[77]	Rat, nerve injury model	Multipotent stromal cell	p75dMSC	Injection into MPG	Surviving engrafted MSCs and p75dMSCs had a mesodermal (fibroblastic) morphology rather than a neuronal morphology.	Significant effects on improving ICP and mean/MAP ratio in response to CN stimulation.

Abbreviations: ADSC, adipose-derived stem cells; ADSC-IFN, ADSC-expressing human interferon α-2b; DM, diabetes mellitus; EGFP-BDNF, enhanced green fluorescence protein-brain-derived neurotrophic factor; EPC, endothelial progenitor cells; ESC, embryonic stem cell; FGF, fibroblast growth factor; ICI, intracavernous injection; ICP, intracavernous pressure; MAP, mean arterial pressure; MPG, major pelvis ganglion; MSC, mesenchymal stem cell; rAd/hBDNF, recombinant adenovirus expressing human brain-derived neurotrophic factor; USC, urine-derived stem cell; VEGF, vascular endothelial growth factor.

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adipose-derived stem cells (ADSCs) can be the best candidate for clinical application.

MSCs can express smooth muscle and endothelium-specific markers like α-SMA, calponin, von Willebrand factor and CD31 after transplantation into the corpus cavernosum.[19] MSCs can also secrete a variety of soluble factors with various advantageous effects including immunomodulation,[20] inhibition of fibrosis[21] and apoptosis,[22] and enhancement of vascular repair.[23,24]

MECHANISM

MSC-based cell therapies with or without gene therapy have similar mechanisms in restoring and recovering erectile function. The main mechanism underlying recovery from ED lies in the improvement of functional and histological components. In terms of identification of stem cell differentiation, no direct evidence has been described yet[25,26] to suggest the importance of paracrine action as a principal therapeutic mechanism in stem cell treatment of ED. Zhang *et al.*[27,28] found that the cytokine CXCL5 was abundantly secreted by cultured stem cells and it exhibited potent angiogenic and neurotrophic activities *in vitro*. Besides this paracrine action of stem cells, the other potent mechanism lies in the roles of NO and VEGF.

Penile erection is initiated by neuronal nitric oxide synthases (nNOS) and maintained by eNOS.[29] Relaxation of corporal smooth muscle is essential for normal erectile activity, and accumulated evidence supports NO as a major mediator of corporal smooth muscle relaxation and penile erection.[30,31] Release of NO from the endothelium and nitrergic nerves innervating the penile vasculature serves to activate NO-sensitive guanylyl cyclase and increase penile tissue cyclic guanosine monophosphate (cGMP) levels. cGMP activates a cGMP-dependent protein kinase (PKG) and phosphorylation of downstream proteins results in decreased intracellular calcium concentration and vasodilation.[32] The interaction of the superoxide anion with NO is responsible for the decreased NO bioavailability.[33–36]

Administration of eNOS-transduced MSCs improves the erectile response to cavernous nerve stimulation by enhancing the release of endothelium-derived NO.[37] Endothelial- and neuronal-derived NO has a pivotal role in the regulation of erectile physiology in penile vasculature.[32,38,39] Several putative explanations of the mechanism underlying the upregulation of eNOS expression and NO release in the corpus cavernosum have been proposed. The first explanation is that the maintenance of NO-dependent erectile response in mice lacking the gene for nNOS was a compensatory upregulation of eNOS to fulfill insufficient nNOS expression. A more recent explanation for the intact NO-dependent erectile response in these mice is the existence of nNOS gene variants resulting from alternative mRNA splicing of the nNOS-beta and nNOS-gamma alternative translation in exon 1.

However, a paracrine action, possibly the secretion of growth factors by MSCs to promote NO signaling, may occur after the transplantation of MSCs.[23,24,36,40]

Recently, the role of VEGF has been an important issue for DM-induced ED models. The importance of VEGF in the pathogenesis of ED is related to the condition in which VEGF receptors are downregulated.[41] Impaired VEGF signaling pathway in the corpus cavernosum is another key contributing factor to diabetic endothelial dysfunction.[42,43]

VEGF leads to hypertrophic and hyperplastic remodeling of the penile vascular structures. Furthermore, VEGF may also exert anti-apoptotic effects, protect the endothelium in response to acetylcholine receptors, restore the levels of sex hormones and increase the expression of eNOS and stimulate its phosphorylation.[3]

MSCS AND VEGF GENE THERAPY FOR ED

VEGF is one of several polypeptides with significant angiogenic activity *in vitro* and *in vivo*. A number of VEGF mRNA isoforms are expressed in both rat and human penises, and the most abundant form is a variant encoding a 164-amino acid protein.[44]

VEGF is a cytokine with strong angiogenic properties. It can stimulate proliferation, delay senescence, suppress apoptosis and promote survival of various cell types.[45] VEGF is known to improve the survival of transplanted MSCs in a myocardial infarction model.[46]

Rogers *et al.*[47] showed that VEGF treatment reversed cavernosal leakage in venogenic ED, suggesting that intracavernous injection of the VEGF gene may contribute to preservation of erectile function in patients. VEGF has been proven to alleviate neurogenic and vasculogenic ED associated with hypercholesterolemia in preclinical studies.[48]

VEGF may provide a protective effect to the endothelium and smooth muscle in the corpus cavernosum. Yamanaka *et al.*[49] demonstrated that intracavernous injection of VEGF restored erectile function through inhibition of apoptosis in the corpus cavernosum of diabetic rats. VEGF has also been shown to increase the NO-producing activity of endothelial cells, which has an important role in regulating cavernous smooth muscle relaxation.[50]

Low transfection, risk of chromatin integration, the potential malignant transformation and not tightly regulated gene expression cause adverse effects. Angiomyolipoma or venous leakage from the premature vascularization by VEGF may be considered for clinical trial.[51]

MSCS AND ENOS GENE THERAPY FOR ED

Many gene therapy strategies have focused on the NO/cGMP pathway. All three NOS isoforms, endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS have been used in gene therapy to improve erectile function. Different viral and non-viral vectors have been used to transfer the genetic material to the target cell or tissues, with varying results.[52] Deng *et al.*[7] conducted a series of experiments that showed the feasibility of successfully transferring the eNOS gene. The gene was inserted in an adenovirus and MSCs were transduced *ex vivo* to induce subsequent protein production without interfering with the totipotency of the MSCs. The calcitonin gene-related peptide gene was also expressed in a similar manner.[7] These MSCs infected with adenoviral vectors expressing specific NOS genes were transplanted into the corpus cavernosum of old rats. Seven days after the transplantation of transduced MSCs, there was an improvement in ED and a reduction in the inflammatory reaction. Finally, intracavernous injection of both wild-type MSCs and gene-modified MSCs after 21 days increased eNOS expression and improved ED. Inflammation response and random expression for the transgene may limit the clinical effects after transplantation.

Bivalacqua *et al.*[37] also confirmed that intracavernous transplantation of unmodified wild-type MSCs improved erectile function 21 days after injection. The putative mechanisms involved improved endothelium-derived NO/cGMP signaling as well as the differentiation of MSCs into penile cells expressing endothelial and smooth muscle markers.[37]

MSCS AND FIBROBLAST GROWTH FACTOR GENE THERAPY FOR ED

Fibroblast growth factors (FGFs) are multifunctional proteins with a wide variety of functions. They are most commonly mitogens but also have regulatory, morphological and endocrine effects.[53]

FGF is also known as a 'pluripotent' growth factor because of its varied interactions with multiple cell types.[54,55] One important

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function of FGF1 and FGF2 is the promotion of endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures *in vitro*.[56] They induce angiogenesis and enhance the growth of new blood vessels from pre-existing vasculature.[57] Both FGF1 and FGF2 are more potent angiogenic factors than is VEGF or platelet-derived growth factor.[58] FGF1 expression is mainly localized to the central nervous system, while FGF2 is expressed in all adult tissues.[59,60] In addition, FGF2 is reported to be more essential than VEGF, epidermal growth factor and insulin-like growth factor for endothelial differentiation of MSCs[61] because in the absence of VEGF, insulin-like growth factor or epidermal growth factor, MSCs may also display endothelial properties when grown in an FGF2-supplemented medium.

Ouyang *et al.*[53] reported that urine-derived stem cells (USCs) or USCs genetically modified with FGF2 enhance the expression of endothelial cell markers, smooth muscle contents and improve neurogenic-mediated erectile responses in type 2 diabetic ED rats. The improvement in diabetic ED in a rodent model after administration of USCs or USCs-FGF2 is similar to that observed with cell therapy using other types of MSCs. Paracrine action of USCs may have an important role in recruiting resident endothelial and smooth muscle cells to participate in tissue repair within the cavernous tissue.

MSCS AND BRAIN-DERIVED GROWTH FACTOR GENE THERAPY FOR ED

Among the various neurotrophins, brain-derived neurotrophic factor (BDNF) has an important role in the recovery of ED in a cavernous nerve injury model.[62]

Exogenous BDNF could produce a significant outgrowth of neurons via the Janus Kinase (JAK)/signal transducer and activator of transcription (STAT) molecular pathway.[63,64] In response to cavernous nerve transection, mRNA and protein expression of BDNF is significantly elevated in the major pelvis ganglion in a time-dependent manner by activation of the JAK/STAT pathway.[63,64]

Bochinski *et al.*[65] reported that neuronal embryonic stem cells transduced with enhanced green fluorescence protein-BDNF showed improved erectile function in a rat model of neurogenic impotence. Recently, Kim *et al.*[66] reported that erectile function was preserved to a greater extent after injection with MSCs infected with recombinant adenovirus expressing human BDNF in rats with ED caused by cavernous nerve injury.

MSC-BASED GENE THERAPY IN EACH DISEASE MODEL

As men age, a significant weakness in erectile function occurs.[3] With increasing age, endothelial cell function is altered; age-related impairments in erectile function include increased penile vascular tone, endothelial dysfunction and reduced NO bioavailability.[32,67,68]

Bivalacqua *et al.*[37] reported that the administration of MSCs alone or eNOS-transduced MSCs was associated with increased eNOS protein expression, calcium-dependent NOS activity and cGMP levels in aged corporal tissue. These molecular changes in the penis, mediated by MSC therapy, evoked the relevant physiological changes in neurogenic-mediated erectile function.

Endothelial dysfunction is a result of diminished phosphorylation of eNOS. Reduction of eNOS activity and endothelial NO bioavailability in the aging penile vascular bed have been reported as causes of age-associated ED.[3] It has been reported that eNOS gene therapy can improve neurogenic or endothelial-dependent erectile responses in aging rat models.[8,32,69,70] In addition, VEGF gene therapy has been shown to be effective in aging models. It has been demonstrated that VEGF gene transfer improved endothelial and smooth muscle areas in the corpus cavernosum of hypercholesterolemic rats.[71]

DM is frequently associated with ED, and PDE5Is are commonly used for treatment of ED in such cases. However, its efficacy is limited. To overcome these limitations, various therapies including stem cell therapy and gene therapy have been actively evaluated in DM-induced ED models. Qiu *et al.*[72] investigated the effects of bone marrow-derived MSC transplantation on erectile function in an experimental model. Intracavernous transplantation of MSCs confirmed its beneficial effects on erectile function through an increase in the content of the endothelium and smooth muscle in the corpus cavernosum.

Gou *et al.*[73] examined the effects of transplantation of EPCs that were transfected with the $VEGF_{165}$ viral gene in the corpus cavernosum of diabetic rats with ED.

Transplantation of EPCs transfected with $VEGF_{165}$ in the corpus cavernosum of diabetic rats with ED could restore erectile function. The same group of authors evaluated the effects of MSC transplantation transfected with the $VEGF_{164}$ gene through an adenovirus (Ad-$VEGF_{164}$) in diabetic mice with ED.[72,74]

The concentrations of VEGF, nerve growth factor and BDNF were measured in the bone marrow-MSC-conditioned medium. MSCs produced detectable levels of VEGF, nerve growth factor and BDNF, while the intracavernosal transplantation of MSCs resulted in an improvement of erectile function in diabetic rats. However, after the injection, a time-dependent reduction in MSCs occurred. This treatment strategy has also proven effective in improving nerve regeneration in diabetic rats, most likely through a mechanism that involves paracrine factors produced by the MSCs.[3,75]

Mangir *et al.*[76] recently presented findings similar to those of previous studies reporting improvement in erectile function after stem cell injection therapies in animal models of neurogenic ED.[13,77,78]

They reported that the use of either autologous or allogeneic cell sources did not result in an improvement in erectile function. Although a direct comparison of autologous and allogeneic cells in this experimental set-up has not been performed yet, both autologous[79] and allogeneic MSCs[80,81] were shown to be similarly effective in animal models of cavernosal nerve injury.

Hyperlipidemia and atherosclerosis are important metabolic factors,[12,82] which cause ED through neuronal and endothelial dysfunction, leading to a reduction in cavernosal NO levels.[12,82] In this field, stem cell transplantation combined with gene therapy has not been introduced because many studies have demonstrated successful outcomes with endothelial progenitor cells or by combining angiopoietin therapy with VEGF gene therapy.[4,25,48,71]

OTHER GENE THERAPIES

The gene KCNMA1(ref. 83) encodes pore-forming potassium large-conductance calcium-activated channel proteins in the cell membrane. Its expression can cause functional ion channel-mediated intracellular K^+ outflow, membrane hyperpolarization and a decrease in cell excitability.[84] Research about the functions of KCNMA1 has been mainly focused on maintaining intracellular and extracellular K^+/Ca^{2+} concentration balance, regulating vascular smooth muscle cell contraction, and maintaining membrane potential. He *et al.*[85] reported that KCNMA1 was able to enhance the positive effect of MSCs in the treatment of diabetes-associated ED. Recently, Gokce *et al.*[86] reported the efficacy of intratunical injection of ADSCs expressing human interferon α-2b (ADSCs-IFN) to decrease fibrosis and restore erectile function in a rat model of tunica albugineal fibrosis. In their report, there was more favorable outcome in ADSCc-IFN group compared with ADSCs-alone group. Recently, Kendrici *et al.*[77] reported that intracavernous injection of p75-derived multipotent stromal cells after bilateral cavernous nerve crush

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injury resulted in a significantly higher recovery of erectile function.

Another potential approach is represented by hMaxi-K gene transfer in men with ED. hMaxi-K is a 'naked' DNA plasmid carrying human cDNA encoding hSlo (for human slow-poke), the gene for the alpha, or pore-forming, subunit of the human smooth muscle Maxi-K channel.[3]

Induced pluripotent stem cells or induced neural progenitor cells could be promising options for treatment of ED. However, no studies have introduced pilot outcomes in preclinical studies. Recently, direct reprogramming or conversion into neural progenitor cells using chemical cocktails, induced hypoxia or diverse transcription factors ((Ascl1, Pou3f2 and Myt1l) have been introduced.[87–89] Direct conversion has the advantage of avoiding the use of transfecting virus and reprogramming oncogene. However, no study has been introduced for clinical application.

DISCUSSION

MSCs have the advantage of exhibiting all the characteristics of stem cells including self-renewal capacity, totipotency and *in vivo* tissue regeneration capacity.[3] In addition, they can be easily obtained in large numbers by a single bone marrow aspiration.[3]

The other advantages of MSC-based cell therapy in ED include enhanced endothelial nitric oxide (NO) synthase expression, display of endothelial and smooth muscle cell markers, increased content of smooth muscle and endothelium in the corpus cavernosum, enhanced neovascularization in the corpus cavernosum, increased content of nNOS-positive nerve fibers in penile dorsal nerves, inhibition of apoptosis in the corpus cavernosum, and inhibition of fibrosis and apoptosis.[3]

The main disadvantages include potential adverse effects, low transfection efficiency, risk of chromosomal integration and the potential for malignant transformation.[3] MSCs have a limited survival period; Song *et al.*[90] attempted to immortalize these cells using a viral vector encoding the myc gene and assessed whether they maintain the ability to differentiate or mutate into endothelial or smooth muscle cells.[90]

The least immunogenic stem cell transplantation could be achieved by using autologous stem cells. However, even with the easiest method of stem cell extraction such as in the case of ADSCs, a surgical procedure is still involved and that, by itself, may adversely affect the outcome of stem cell transplantation.[4] Therefore, more studies need to be conducted using allogeneic and xenogeneic stem cell transplantations as alternatives.

Although adeno or adeno-associated virus-mediated gene transfer of eNOS, nNOS, VEGF, BDNF or superoxide dismutase, or a dominant-negative RhoA mutant can augment erectile responses in aged or diabetic rat models, the possible occurrence of an inflammatory response and random expression of the transgene may limit the clinical utility of these interventions.[8,32,34,35,37,48,69,91–95]

Most of the animal models used in the DM study were streptozotocin-induced rat models of type 1 diabetes, which is different from type 2 diabetes in many characteristics, including insulin resistance and body mass index. Moreover, most cases of diabetes are type 2 diabetes.[96,97]

Although the adenovirus carrying the VEGF gene can induce therapeutic angiogenesis, VEGF expression is not under tightly regulated and might therefore cause unwanted side effects, such as angioma formation.[98] Possible side effects of VEGF need to be assessed before consideration of these combined stem cell and gene therapy by clinical trials.

As with other disease treatment settings, the most important issue is that there are limited long-term longitudinal data of ED treatment models using MSCs alone or in combination with gene therapy.

CONCLUSIONS

Experimental studies have revealed that both MSC transplantation and gene therapy have limitations with respect to their levels of effectiveness in the treatment of ED when used individually. To overcome this issue, combination treatment with MSC and gene therapy using specific transduction has been introduced, and it has shown favorable outcomes in preclinical studies. This combined strategy of MSC transplantation and gene therapy could be a promising option for the treatment of DM-induced and age-associated ED. MSCs together with gene therapy involving genes such as eNOS, VEGF and BDNF currently represent a promising treatment option in the field of vascular regenerative therapy for ED. However, before considering its potential applications in clinical settings, its disadvantages and limitations need to be addressed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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