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ARTICLE

Feasibility of minimally invasive, same-day injection of autologous adipose-derived stem cells in the treatment of erectile dysfunction

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ABSTRACT

Objective: To investigate feasibility and safety of a new minimally invasive same-day method of autologous adipose derived stem cell (ADSC) transplantation in men suffering from ED.

Materials and methods: Prospective case series of 10 men with an IIEF-EF domain score <17. The IIEF questionnaire was filled out at baseline and 1, 2 and 3 months after treatment. Side effects were assessed by investigations and interviews until 6 months after treatment. The myStem® X2 kit was used for preparation of ADSC: Adipose tissue was harvested from the patient himself under local anesthesia and immediately prepared and injected into the penis. Primary endpoints were feasibility and safety. Secondary outcomes included effects on ED and changes in the remaining IIEF domains.

Results: Ten men were included. Only one adverse event in the form of minor blue discoloration at the fat harvest site was registered. There were statistically significant improvements in IIEF-EF at one, two and three months after treatment compared to baseline with the median score increasing from 5.5 to 10.5, 10.5 and 10, respectively. Considering the individual patients, 3/10 men achieved an improvement equal to or greater than the minimal clinically important difference according to their baseline IIEF-EF score.

Conclusions: Our study confirms the feasibility and safety of this minimally invasive, same-day delivery of ADSC. Due to the design and size on the study, conclusions should not be drawn regarding efficacy, but the method seems worthy of further study.

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Introduction

Erectile dysfunction is a common condition affecting about 30% of men above the age of 40, potentially interfering with quality of life [1]. Medical treatments have evolved over the last several decades with the development of intracavernosal injection therapy, followed by intraurethral formulations before oral medications in the form of phosphodiesterase-5-inhibitors (PDE5-Is) were first marketed in the late 1990s [2]. Especially the latter revolutionized the management of ED and was unquestionably a breakthrough. However, an important drawback to all available medical treatments, is that they do not offer a cure for ED and require continues treatment. This has been identified as a major unmet need for patients [3]. Therefore, an increasing interest in regenerative medicine for erectile problems has emerged over recent years. Within this area, stem cell therapy is arguably one of the most interesting venues.

Stem cells are defined as cells with the ability to self-renew and to differentiate into different specialized cell lines depending on their environment. Stem cell therapy seeks to take advantage of this ability by injecting the cells into damaged tissues in the hope of subsequent differentiation and tissue regeneration [4]. In relation to ED, preclinical studies have suggested that different kinds of mesenchymal stem cells may differentiate into endothelial and smooth muscle cells when injected into the corpora cavernosae [5,6]. Further, mesenchymal stem cells have also been found to induce regeneration of endothelium, smooth muscle, blood vessels, and damaged nerves by means of paracrine action or delivery of growth factors [7,8,9]. Subsequently, the safety and a potential effect of the treatment has been indicated in clinical pilot studies. However, even though the first such study was published more than 10 years ago, high-quality trials are still lacking [10]. Important obstacles for the application of the treatment modality include the need for donors and the risk of tissue reactions with allogeneic cells and invasiveness of the approach with most autologous preparations requiring cell harvest under full and/or preparation of cell cultures over several weeks. The aim of this pilot study was to investigate feasibility and safety of a minimally invasive same-day method of autologous adipose derived stem cell (ADSC) transplantation in men suffering from organic ED using a commercial kit named myStem® X2 (MYSTEM LLC, Wilmington, Delaware, United States).

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Patients and methods

We conducted a prospective case series as a pilot study in 10 men referred to the Department of Urology, Zealand University Hospital, Roskilde with organic ED. We included sexually active men in stable heterosexual relationships who were between 30 and 70 years old and had suffered from moderate or severe ED clinically assessed to be of vasculogenic origin in all situations for at least 6 months as documented by an IIEF-EF domain score <17. Patients were excluded if they suffered from known neurological, psychiatric or endocrinological disease or if they had previous trauma, surgery or radiation to the pelvic area. Likewise, men with Peyronie's disease, uncontrolled cardiac comorbidity or previous episodes of priapism were excluded. Finally, the use of anti-androgens, prednisolone and/or excessive alcohol intake were not permitted. Those using erectogenic aids had to undergo a wash out period of at least four weeks and were not permitted to resume this treatment until 3 months following the injections.

The regional ethical committee approved the study (registration number SJ-820), and all participants provided oral and written consent. Patient age, duration of ED, relationship status, comorbidities, BMI, smoking status and use of medications were registered. Blood tests including lipids, fasting plasma glucose and total testosterone were performed. All participants filled out the complete IIEF questionnaire at baseline and again 1, 2 and 3 months after treatment. As patients were permitted to use other erectogenic treatments after this point, we did not collect further questionnaires. Since this was a pilot study designed to assess feasibility and safety of the stem cell treatment no invasive diagnostic measures for ED were considered.

Any treatment related side effects were assessed on the day of injections and by phone calls the following day, after two weeks and at 1, 2, 3 and 6 months following treatment. The sites of aspiration and injection were initially inspected, and patients were asked an open-ended question about side effects followed by specific questions regarding pain, bleeding and swelling.

The myStem® X2 kit was used for stem cell preparation. This is a Single-Use kit for autologous stromal tissue graft preparation. The autologous graft prepared contains a viable native tissue fraction that supports regeneration and healing processes. The kit contains all disposable materials for manual liposuction, fractionation, purification and concentration and the filtration technology separates the solid and liquid fraction in a few seconds. The lipose aspirate is initially fractionated through the myStem® bag, to a liquid and a solid fraction in a ratio of about 60/40. The bag contains up to 100 ml of lipoaspirate, but if more is needed, the bag can be refilled after extraction. The procedure is shown in Figure 1.

The primary endpoints were feasibility and safety as assessed by completed procedures and registered adverse events. Secondary outcomes included changes in erectile function as well as changes in the remaining IIEF domains. Analyses were performed by MedCalc® Statistical Software version 20.009 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Due to the small sample size, the data was not normally distributed; therefore, we used median and interquartile range (IQR) for demographic data description and paired Mann–Whitney test to compare the change in IIEF score of individual patients

According to Rosen and coauthors, the minimal clinically important difference (MCID) was defined for each ED category with 5 points in moderate ED (IIEF-EF 11-16) and 7 points in severe ED (IIEF-EF 0-10) [11]. As this was a pilot study pre-planned to include ten men, no power calculation was performed.

Results

Ten men were included between April 2020 and November 2020. The median age was 61 (IQR; 54-65). The median duration of ED was 36 months (IQR; 24-84). Baseline characteristics, including comorbidities, are shown in Table 1. At baseline, three patients suffered from moderate ED, while seven suffered from severe ED.

All participants completed the treatment and the six months follow-up including IIEF scores at 3 months. Fat harvest and penile injections were associated with minor discomfort, but no serious side effects were observed in any of the ten men. Only one adverse event in the form of minor blue discoloration at the fat harvest site was registered. This resolved spontaneously within a few days. No new side effects were reported beyond this point.

There were statistically significant improvements in IIEF-EF at one, two and three months after treatment compared to baseline with the median score increasing from 5.5 to 10.5, 10.5, and 10 respectively. No statistically significant changes in the remaining IIEF domains were seen, except for an increase in the overall satisfaction score at two months after treatment (Table 2). Considering the individual patients, 3/10 men achieved an improvement equal to or greater than the MICD according to their baseline IIEF-EF score.

Discussion

Our pilot study in 10 patients suffering from organic ED shows that outpatient treatment with autologous ADSC is feasible and safe. We consider it unlikely that further side effects would arise later than six months after a single injection. Regarding potential efficacy, we cannot draw any conclusions based on our study as it was neither designed nor powered for such an assessment. In the analyses we conducted we saw a statistically significant increase in the median IIEF-EF score and three of the patients experienced the MCID according to the previous findings of Rosen et al. [11], which is arguably one of the most strict criteria for clinical significance. Meanwhile, the overall median IIEF-EF score of 10 at three months is still a low score indicating that further treatment would be needed to obtain erections sufficient for intercourse. Further, a placebo effect cannot be assessed due to the lack of a comparator arm. This is especially important since ED often has a psychological component and since the main outcome is derived from a subjective patient reported questionnaire. Further, it was not

Figure 1. The myStem® X2 procedure.

1. The working field was sterilized and the donor site was infiltrated with Ringer solution 50 ml+ 10 ml lidocaine (10 mg/ml), in a volume equal to or greater than the desired volume harvested. We used 60 ml on each site and waited for 10 min.
2. 50–60 ml adipose tissue was harvested from each site and homogenized by shaking the syringe thoroughly for a minimum of 30 sec. Lipoaspirate was transferred to the MyStem bag and the fractionation was relieved by gently squeezing the aspirate into the filter.
3. The liquid and solid fractions were transferred to separate syringes. The liquid stromal vascular fraction (SVF) was concentrated by gently pulling the purified material through the loose filter. The SVF cells were trapped in the filter, so for every 20 ml passed through the filter, 2 ml of sterile isotonic saline solution was returned through the filter to collect the purified and concentrated product. Using the solid fraction, the adipocytes were lysed through mechanical stress (emulsification), thereby releasing a large portion of the bound SVF. Using the solid fraction, concentrated SVF and solid fraction were mixed in a 1/1 ratio for injection.
4. Finally, 4 ml of this fraction was injected into a single site in the corpus cavernosum without the use of a penile constriction band. The entire procedure took about 90–120 min. All patients were sent home after the procedure without the need for further observation.

Table 1. Baseline characteristics.

Age (median, IQR)	61 years (54–65)
Duration of symptoms (median, IQR)	36 months (24–84)
Systolic Blood pressure (median, IQR)	135.5 (128–151)
Diastolic blood pressure (median, IQR)	81 (77–86)
Puls (median, IQR)	81.5 (69–86)
Total serum testosterone (median, IQR)	11.25 (10.5–12.3) nmol/L
Lipids (median, IQR)	5.1 (3.950–5.775) mmol/L
Glucose (median, IQR)	6.4 (5.450–8.275) mmol/L
BMI (median, IQR)	27.358 (26.297–30.041)
Diabetes (no., %)	3/10 (30%)
Previous myocardial infaction (no., %)	4/10 (40%)
Neurological disease (no., %)	1/10 (10%)
Smoking	
Never (no., %)	3/10 (30%)
Previously (no., %)	4/10 (40%)
Current smoker (no., %)	3/10 (30%)

investigated if the potential effect was durable beyond three months. This means that our study cannot be taken as evidence of an effect but rather it indicates that autologous ADSC are worthy of further study.

In our study, we used the myStem kit, which has been previously described in a trial investigating healing in fingertip injuries [12]. In this study, flow cytometric analysis documented the presence of ADSC through expression of the surface antigens CD90+, CD105+, CD73+, CD45−, CD34−, and CD31− in the aspirate. Interestingly, reduced healing time compared to control subjects was documented. Importantly, reduced healing time compared to control subjects was seen with the injections. However, the authors did not document that the stem cells were in fact transformed into new cells within the healing tissue. Our study includes a similar drawback and in fact no clinical trial on ED has directly shown that stem cells are transformed into endothelial and smooth muscle in the penile tissue. However, this would require pre- and post-treatment biopsies, which would likely be difficult to obtain patient consent for.

Following several promising pre-clinical studies, stem cell injection into the corpora cavernosae as a treatment for ED has been investigated in a series of small pilot studies in humans [13]. Importantly the only adverse effects reported

Table 2. Comparison of IIEF score before and after stemcell treatment.

IIEF domains	n	Median IIEF score	Median paired differences	*p* Value
Erectile function				
Baseline	10	5.5	–	
One month vs. Baseline	10	10.5	3.5	0.0039*
Two months vs. baseline	10	10.5	4	0.0039*
Three months vs. baseline	10	10	3	0.0078*
Orgasmic function				
Baseline	10	6	–	
One month vs. baseline	10	4	−2	0.375
Two months vs. baseline	10	4	−2	0.8203
Three months vs. baseline	10	6	0	0.5625
Sexual desire				
Baseline	10	6.5	–	
One month vs. baseline	10	6	0	0.5781
Two months vs. baseline	10	6	0	0.5781
Three months vs. baseline	10	7.5	0	0.1875
Intercourse satisfaction				
Baseline	10	1.5	–	
One month vs. baseline	10	4.5	1	0.2188
Two months vs. baseline	10	4.5	1.5	0.1484
Three months vs. baseline	10	4	0.5	0.2969
Overall satisfaction				
baseline	10	4	–	
One month vs. baseline	10	5.5	0.5	0.0625
Two months vs. baseline	10	6	1	0.0313*
Three months vs. baseline	10	5.5	0.5	0.0625

have been minor pain at the injection site and occasional self-limiting hematomas. This is in accordance with our findings and confirms that the treatment is safe although long term reports are generally lacking. The previous studies have also indicated that stem cell treatment may have a clinical effect, which is worth exploring further. In a 2010 study, Bahk et al. used allogenic human umbilical cord stem cells in 7 diabetic men awaiting penile implants [10]. By the second month following the injections 6/7 reported return of morning erections, however, none of the participants achieved spontaneous erections sufficient for intercourse and at 11 months, only one patient reported some sustained effect with the ability to penetrate his partner using PDE5-inhibitors. No improvements in erectile function were reported in a blinded control group of 3 patients who received saline injections. Another study used placental matrix-derived mesenchymal stem cells in 8 men [14]. This treatment failed to increase overall IIEF score significantly at neither 6 weeks, 3 months or 6 months. The authors did report increases in peak systolic velocity on penile Doppler ultrasound examination. Further, it was noted that 3 participants had regained the ability to achieve spontaneous erections and that another 4 had gone from needing injection therapy to only requiring oral medications. Meanwhile, the quality and clinical significance of these erections were not described.

A third study used autologous bone marrow-derived stem cells at different doses in 12 men with severe ED and no effects of medications at 6 months to 3 years following radical prostatectomy [15]. All patients received one injection consisting of 2×10^7, 2×10^8, 1×10^9, or 2×10^9 stem cells. The authors saw an increase in mean IIEF-EF domain score from 7.3 at baseline to 17.4 at 6 months with sustained benefit at 12 months. Additionally, 9/12 patients were able to penetrate their partner with the use of medications and the best effects tended to be in men receiving the two highest doses of stem cells. Side effects related to bone marrow aspiration included post-operative pain after aspiration and a small drop in mean hemoglobin levels of -1.9 ± 0.7 g/dl. Aspiration of bone marrow was performed under general anesthesia. The use of two consecutive intracarnosal injections of autologous bone marrow-derived stem cells in 4 diabetic patients with ED was reported in 2018 by Al Demour et al. [16]. There were statistically significant improvements in the IIEF-15 score at 1 and 3 months including the erectile function sub-score without any side effects aside from pain on aspiration and injection. For this study, aspiration of bone marrow was performed in local anesthesia but after initial preparation, cells were cultured for 10–14 days before they were harvested for use.

A few studies have reported on the use of ADSCs as in our trial. Haahr et al. studied 17 men with ED 5–18 months following radical prostatectomy [17]. In contrast to our method, liposuction was performed under general anesthesia but aside from minor pain in two men and three small self-limiting hematomas, no side effects were noted. During the 6-month study, 8 men achieved erections sufficient for sexual intercourse. Meanwhile, the increase in IIEF score was not statistically significant except for in a post hoc sub analysis of continent men. Further, the participants were encouraged to use erectogenic medications for the duration of the study and spontaneous erectile function recovery is known to occur following this type of surgery. Both issues may have influenced the results. Recently, Protogerou et al., administrated autologous ADSC resuspended in platelet lysate plasma (PLP) derived from the patients' own blood [18]. This achieved mixing the stem cells with growth factors, potentially inducing a dual mechanism of regenerative action. Men with ED of mixed etiology received intracavernosal injections with either ADSC and PLP ($n = 5$) or PLP only ($n = 3$). No severe side effects were seen and statistically as well as clinically significant improvements in the IIEF-5 score was reported in both groups at 1- and 3-months following

injections with no difference between groups. Although a very small study, these results suggest that effects of ADSC are induced by paracrine effects rather than stem cell differentiation as also implied by preclinical data [9]. However, the findings are hampered by the use of erectogenic medications in all of the participants to various degrees throughout the study. It was not reported what kind of anesthesia was used during liposuction and cells were cultured for several weeks.

Our study adds to a growing body of literature on stem cells in the treatment of ED. Importantly, the approach used in our trial is relatively non-invasive and offers simple and fast preparation of autologous ADSC. This means that we were able to perform same day aspiration and treatment without the need for general anesthesia and without the need of a donor. This is a potentially important step forward in stem cell therapy for ED as it improves the chance to bring the treatment modality into clinical practice. Meanwhile, our study includes the same draw backs as previous pilot studies, namely a low number of participants and a lack of control group. Further, we have only evaluated one specific method of ADSC delivery and from our study it is not clear if potential effects were exerted by differentiation of stem cells or by paracrine effects. Thus, larger randomized trials with longer follow-up are needed to draw conclusions on potential efficacy.

Conclusions

We have demonstrated the feasibility and safety of a minimally invasive same-day method of ADSC transplantation in men with ED. However, based on our study and the available literature, stem cells are unlikely to be a one-stop universal cure for ED in all patients. Larger, randomized and placebo-controlled trials are needed to elucidate their potential role, including the optimal stem cell preparation and patient groups. When designing such studies there is a need to use validated questionnaires with predefined outcomes in the assessment of clinical effects. Meanwhile, the temptation to use purely subjective measures with unclear clinical significance, and to employ post hoc analyses resulting in statistical significance should be avoided. The task is to assess if stem cell therapy has an effect in the treatment of ED - not simply to confirm our hopes that it does.

Disclosure statement

Mikkel Fode is a speaker for Boston Scientific and Astellas Pharma. No potential conflict of interest was reported by the author(s).

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