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Protocol Article

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Treatment with stromal vascular fraction of Peyronie's disease – a study protocol

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ABSTRACT

INTRODUCTION. Peyronie´s disease is the result of an acquired fibrotic plaque in the tunica albuginea. It remains unknown why the condition appears, but it seems to affect up to 9% of all men. It can result in a debilitating curvature of the erect penis, which has extensive impact on the quality of life for many men. Thus, the disease may result in low self-esteem, depression and impaired sexual performance.

METHODS. This is a prospective pilot study investigating the feasibility and safety of stromal vascular fraction injection into the plaque of 22 men with Peyronie´s disease in the chronic phase. The stromal vascular fraction is obtained from a small liposuction of 250 ml fat in general anaesthesia and injected the same day.

CONCLUSION. We hope to show that injection of stromal vascular fraction is safe and effective in reducing curvature in men with Peyronie´s disease.

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In Peyronie's disease (PD), a fibrotic plaque located in the penile tunica albuginea (TA) results in fibrosis, pain, penile curvature and often subsequent erectile dysfunction (ED). The prevalence of PD varies between studies falling in the 5-9% range [1, 2].

The pathophysiology of PD is not fully understood but the underlying mechanism is generally thought to be multiple micro-trauma to the TA resulting in inflammation and subsequently fibrosis [3]. If the injury is not resolved or the healing process becomes de-regulated, the initial reversible phase can evolve into a permanent fibrotic reaction [4], causing malfunction of the organ. The condition is divided into an acute and a chronic phase. The acute phase is characterised by pain and initiation of penile deviation. When the deviation is stable for 4-6 months, a palpable plaque is usually present and the curvature is permanent and irreversible. With a

deviation of more than 30&; and in cases affecting the ability to have intercourse, treatment is recommended.

PD has a substantial impact on quality of life for men and their partners. Apart from physical molestation, patients are also burdened with bother, distress and impaired sexual performance [5].

Non-invasive and invasive therapies exist for PD.

There is evidence that extracorporal shock wave therapy (ESWT) may ease PD-associated pain [6]. In a single-arm prospective study from 2019, 325 patients with a mean PD duration of 16 months received ESWT. Apart from pain relief, they experienced reduced penile curvature and improved sexual function [7].

For the chronic phase, collagenase (Xiapex) injected into the plaque is currently the only approved drug. Collagenase dissolves the collagen, softening the plaque. Studies showed a reduction in curvature of 17&; [8]. The marketed product has been deregistered in Europe and the Middle East since the end of December 2019 for commercial reasons.

A third option is surgery to achieve symptomatic correction of the curvature when the disease is stable. This treatment may result in penile shortening, narrowing, pain or ED, why many men opt out of surgery with consequences for their quality of life.

The shortage of efficient treatments with few side effects generates a strong need for alternative treatment modalities by which the anatomy of the penis is recreated causing as few side effects as possible.

Lately, treatment of PD has focused on intervention in the earlier stages of the disease, thereby preventing the fibrosis from escalating.

Inflammation of the tissue and aggregation of extracellular matrix (ECM) is generally a reversible phase of the normal wound healing process. However, if the injury is not resolved, this phase may evolve into fibrosis. This pathological accumulation of ECM may lead to progressive tissue loss and/or organ function [4].

It is agreed that myofibroblasts play an important role in forming fibrosis as inhibition of myofibroblast transformation is effective in preventing fibrosis [9]. The mechanism producing this effect, however, remains unknown.

In a trial by Ilg et al. [10] comprising both an in vitro and an in vivo rat study, the authors aimed to identify compounds capable of inhibiting the formation of a PD plaque. The results suggest that phosphodiesterase 5 inhibitors and selective oestrogen receptor modulators inhibit myofibroblast transformation and ECM and could potentially be used in treatment of the PD patient in the acute phase.

Adipose-derived stem cells (ADSC) promote growth and repair, thereby regenerating tissue locally, primarily in a paracrine manner. They are thought to be released from a perivascular location where they are activated to regenerate the micro environment and regulate the local immune response, though some studies have also shown their effect by direct differentiation [11].

It is thought that injured tissue expresses receptors or ligands that facilitate transportation of ADSC to the site of the injury [11]. It is likely that ADSC only works in tissues with an on-going inflammation or in a regenerative state.

We have chosen to define the stromal cells as recommended by the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy. Adipose tissue-derived stromal vascular fraction (SVF) cells are obtained from liposuction after the SVF cells have been separated from the mature adipocytes by collagenase and centrifugation. When SVF is seeded into culture, elongated cells eventually adhere to tissue culture plastic ware. These cells, when purified and depleted of hematopoietic cell

population, represent adipose tissue-derived stromal cells (ASC) [12].

SVF has an advantage over ASC as it is easily acquired and re-injected within a short time span. This makes SVF feasible as a same-day procedure to harvest the cells with liposuction and inject them in the desired site only few hours later [13].

Animal studies with ASC and SVF have shown promising results. A study even showed a better effect with SVF than with ASC. In a comparative study of erectile function with SVF and ASC in a rat model of cavernous nerve injury, SVF treatment showed a statistically significantly higher smooth muscle/collagen ratio as an expression of reduced fibrosis than ASC treatment alone, though both were effective in restoring erectile function [14].

The first study to test ASC on a validated animal model of PD was published in 2013 [15]. ASC injected into the TA showed prevention of fibrosis and elastosis in the TA and corpus cavernosum of the rat in the acute phase of the disease. The same research group showed matching tendencies in another study from 2018 in a group of rats with PD in the chronic phase [16].

Lastly, in 2019, they showed that SVF was equally effective and clearly reduced fibrosis in penile TA in a rat model [13].

Only two human studies with stem cells on patients with PD have been conducted.

In 2015, Levy et al. injected placental matrix-derived mesenchymal stem cells into plaques on five patients. The study showed that seven out of ten plaques disappeared completely at three-month follow-up. The study does not mention whether the men were in the acute or the chronic phase of PD [3].

The other human study, from 2016 published by Lander et al., combined SVF injection in the plaque and ESWT. Eleven men with chronic stable PD were injected with autologous SVF. The authors reported overall improvement based on results from Peyronie´s Disease Questionnaire and an erection hardness score. Patients reported subjective straightening of the penis. The study did not rapport any objective measures in its results [17].

METHODS

The purpose of this study was to examine and understand men's bother, distress and degree of ED in relation to PD and to conduct a safety and feasibility study with SVF injection into PD plaques in 22 Danish men.

Prospective pilot study investigating feasibility and safety

P: Men with PD in the chronic phase

I: Injection of SVF into the plaque, completion of questionnaires

C: Historical group from Investigation for Maximal Peyronie's Reduction Efficacy and Safety Studies (IMPRESS) I and II [8].

Primary outcome measures

Penile curvature angle (degrees) (baseline, one, three, six, 12 months).

Secondary outcome measures

Safety of SVF injection into the penile plaque

Penile plaque size (mm^3) (at baseline and 12 months)

International Index of Erectile Function (IIEF-5) (at baseline, one, three, six and 12 months)

Stretched penile length from symphysis to meatus of the glans (cm) (at baseline and 12 months).

Plan for statistical analysis

The mean curvature (degrees ± standard deviation) in the IMPRESS I and II studies prior to intervention was 50° ± 14°.

A curvature of > 30° is considered clinically important, and the observed effect of treatment in the IMPRESS I and II [8] studies was 33% or 17°.

With 15 patients in this study, we will be able to detect a curvature correction of 17° (from 50° to 33°) with a power (beta) of 90% and a significance level (alpha) of 5%.

With an expected 33% drop out, a total of 22 men aged > 18 years with chronic state PD will be recruited for this study and compared with 22 from a historical group (the placebo group of IMPRESS I and II) [8]. The study has fulfilled the goal of statistical significance with 15 patients all following their controls.

The attendees

The patients are offered autologous tissue injection with SVF cells into the plaque. The DOSES tool will be used [18]. Patients will be recruited at the Hospital of South West Jutland and Odense University Hospital, Region of Southern Denmark.

Before inclusion, the patients are evaluated according to suggested PD criteria in evidence-based 2010 guidelines [19].

Additionally, the penis is evaluated with Doppler ultrasonography to measure plaque volume, which is calculated by multiplying plaque length, width and height.

Based on a patient-produced photograph in two planes of the erect penis before the treatment and at the one-, three-, six- and 12-month follow-up, the curvature is measured three times on the photograph with a goniometer. The angle is measured at the concave side. At baseline and 12 months, a medical erection is induced with alprostadil. This is to make the erection as standardised as possible.

The translated and culturally adapted Danish questionnaire for PD [20] and the IIEF 5 are completed before receiving treatment and at every control visit.

A total of 200-250 ml subcutaneous adipose tissue is harvested from the abdomen by liposuction in general anaesthesia and processed using an automated processing Celution 800/CRS system (Cytori Therapeutics, San Diego, California, USA), thereby producing SVF. For the digestion process, a product named Celase, containing both collagenase and neutral protease, is added.

Two and a half ml SVF is injected into and around the plaque. One ml SVF is processed in NucleocounterNC-100 for cell viability and cell size. After isolation and counting of cells, the cells are fixated in 1% neutral buffered formalin for ten minutes before being washed and kept refrigerated until further processing. Flow cytometry for surface markers (CD31, CD34, CD73 and CD90) is made on a BD FACS lyric flow cytometer. We strive to have 200,000 cells for each marker.

The study database is stored in the Odense Patient Data Explorative Network (OPEN) and a data-management plan will be described.

See **Table 1** for inclusion and exclusion criteria.

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria
Acquired penile curvature of 30°-90° associated with a palpable penile plaque on physical examination
In the chronic phase of the PD
1 or 2 penile plaques at ultrasound screening
Willingness to attend follow-up at 1, 3, 6 and 12 months
With sufficient abdominal fat to undergo liposuction of 200-250 ml
Understand and speak Danish
Exclusion criteria
Taking the medication coumadin, warfarin or a new oral anticoagulant
Unable to achieve adequate erection with penile injection to assess degree of curvature
Undergoing definitive treatment for prostate cancer, bladder cancer or other pelvic malignancies including surgery, external beam radiation therapy, brachytherapy and cryotherapy
With a prior history of prostate cancer, haematologic disorders, chronic liver disease including cirrhosis and hepatitis C, disorders affecting the immune system, including infection with HIV, or psychiatric disorders including, but not limited to, major depression, schizophrenia and bipolar disease
With a history of cerebrovascular incidents, a history of deep venous thrombosis within the past 5 years or a history of untreated or severe sleep apnoea
With clinically significant abnormal lab results which would place the subject at increased risk or compromise the integrity of the study data in the opinion of the investigator
Involved with any other projects with an investigational drug within 30 days
In treatment for any alcohol or drug abuse within 1 year
With congenital deviation of penis
Within 6 months treated with collagenase, ESWT and/or other therapeutic injection in the plaque treatment for PD

ESWT = extracorporal shock wave therapy; PD = Peyronie's disease.

Feasibility

The study is feasible because PD is a common phenomenon among men (5-9%). They are seen in all urological outpatient clinics.

Trial registration: The project was reported to ClinicalTrials.gov NCT04771442, EudraCT 2020-004297-22, the Danish Medicines Agency 2020090057, the National Ethics Committee 74705 and the Danish Data Protection Agency (record no. 1/21757).

DISCUSSION

Hopefully, the personal benefit for the subject participating is a straighter penis. For men to experience an altered appearance of his genitalia leading to dysfunction may have a considerable impact on their quality of life, affecting mood and even leading to depression and low self-esteem. Currently, the standard treatment is an operation in general or local anaesthesia resulting in a shortened penis and, in some cases, chronic pain. This treatment solves the problem so to speak but it does not remove it. Stem cell treatment has been used in various anatomical places to remove scar tissue. ADSC therapy contains great potential owing to the easy access to fat tissue and the positive results with scar tissue repair in animal studies. Our study will show if the same occurs in

men, so that the change of the scar tissue that caused the deviation in the penis produces in a more straight and more functional penis. A treatment with less side effects using the patient's own cells to repair the deformity will produce a therapeutic gain for the study subjects and future patients by giving these men an opportunity to restore their quality of life.

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REFERENCES

1. Hussein AA, Alwaal A, Lue TF. All about Peyronie's disease. Asian J Urol. 2015;2(2):70-78. doi: 10.1016/j.ajur.2015.04.019.
2. Russo GI, Milenkovic U, Hellstrom W et al. Clinical efficacy of injection and mechanical therapy for Peyronie's disease: a systematic review of the literature. Eur Urol. 2018;74(6):767-81.
3. Levy JA, Marchand M, Iorio L et al. Effects of stem cell treatment in human patients with peyronie disease. J Am Osteopath Assoc. 2015;115(10):e8-e13.
4. Milenkovic U, Albersen M, Castiglione F. The mechanisms and potential of stem cell therapy for penile fibrosis. Nat Rev Urol. 2019;16(2):79-97. doi: 10.1038/s41585-018-0109-7.
5. Hellstrom WJG, Feldman R, Rosen RC et al. Bother and distress associated with Peyronie's disease: validation of the peyronie's disease questionnaire. J Urol. 2013;190(2):627-34.
6. Palmieri A, Imbimbo C, Longo N et al. A first prospective, randomized, double-blind, placebo-controlled clinical trial evaluating extracorporeal shock wave therapy for the treatment of Peyronie's disease. Eur Urol. 2009;56(2):363-9.
7. Di Mauro M, Russo GI, Camera PAD et al. Extracorporeal shock wave therapy in Peyronie's disease: clinical efficacy and safety from a single-arm observational study. World J Mens Heal. 2019;37(3):339-46.
8. Gelbard M, Goldstein I, Hellstrom WJG et al. Clinical efficacy, safety and tolerability of collagenase Clostridium histolyticum for the treatment of Peyronie disease in 2 large double-blind, randomized, placebo controlled phase 3 studies. J Urol. 2013;190(1):199-207.
9. Jiang H, Gao Q, Che X et al. Inhibition of penile tunica albuginea myofibroblasts activity by adipose-derived stem cells. Exp Ther Med. 2017;14(5):5149-56.
10. Ilg MM, Mateus M, Stebbeds WJ et al. Antifibrotic synergy between phosphodiesterase type 5 inhibitors and selective oestrogen receptor modulators in Peyronie's disease models. Eur Urol. 2019;75(2):329-40. doi: 10.1016/j.eururo.2018.10.014.
11. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739-49.
12. Bourin P, Bunnell BA, Casteilla L et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics (IFATS) and Science and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15(6):641-8. doi: 10.1016/j.jcyt.2013.02.006.
13. Castiglione F, Hedlund P, Weyne E et al. Intratunical injection of stromal vascular fraction prevents fibrosis in a rat model of Peyronie's disease. BJU Int. 2019;342-8.
14. You D, Jang MJ, Kim BH et al. Comparative study of autologous stromal vascular fraction and adipose-derived stem cells for erectile function recovery in a rat model of cavernous nerve injury. Stem Cells Transl Med. 2015;4(4):351-8.
15. Castiglione F, Hedlund P, Van der Aa F et al. Intratunical injection of human adipose tissue-derived stem cells prevents

fibrosis and is associated with improved erectile function in a rat model of Peyronie's disease. Eur Urol. 2013;63(3):551-60.

16. Castiglione F, Hedlund P, Weyne E et al. Intratunical injection of human adipose tissue-derived stem cells restores collagen III/I ratio in a rat model of chronic Peyronie's disease. Sex Med. 2019;7(1):94-103. doi: 10.1016/j.esxm.2018.09.003.
17. Lander EB, Berman MH, See JR. Stromal vascular fraction combined with shock wave for the treatment of Peyronie's disease. Plast Reconstr Surg Glob Open. 2016;4(3):e631.
18. Murray IR, Chahla J, Safran MR et al. International expert consensus on a cell therapy communication tool: DOSES. J Bone Joint Surg Am. 2019;101(10):904-11.
19. Ralph D, Gonzalez-Cadavid N, Mirone V et al. The management of Peyronie's disease: evidence-based 2010 guidelines. J Sex Med. 2010;7(7):2359-74.
20. Wiborg MH, Laursen BS, Kallestrup EB et al. Peyronie's disease questionnaire: translation into Danish and cultural adaptation. Sex Med. 2023; 22;11(2):qfac022. doi: 10.1093/sexmed/qfac022.