Original article

Cell therapy for urinary incontinence. Does it really work?

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Abstract: Stress urinary incontinence (SUI) is the most common form of urinary incontinence, a condition that affects approximately two hundred million people worldwide, significantly reduces the quality of life and exacerbates co-morbidities. The causes of stress urinary incontinence are urethral hypermobility, intrinsic sphincter deficiency, or both. There are different ways of treatment of SUI, inter alia: a gold standard procedure - retropubic repair or urethral sling placement. In selected cases the injection of bulking agents or installation of an artificial sphincter are performed. These methods have advantages as well as disadvantages. That is why the new ways of treatment are sought. Newly emerging technologies in tissue engineering may provide novel methods for the treatment of SUI. The deficiencies of urethral muscle and connective tissue can be regenerate by stem-cell therapy, which is currently at the forefront of incontinence research. The stem-cell therapy is very important in a way of replacing, regenerating, or enhancing the biological function of damaged tissue or organs. The choice of stem cell source is determined by ease of harvest, population density and differentiation potential. Results of in vivo experiments as well as clinical application of injecting stem cells are promising but also have some limitations.

Key words: Stress Urinary Incontinence; Mesenchymal Stem Cells; Stem Cell Therapy.

INTRODUCTION

Stress urinary incontinence is the most common form of urinary incontinence, a condition that affects approximately two hundred million people worldwide.[1,2] SUI is characterized by an involuntary passing of urine, synchronous with exertion, sneezing or coughing. SUI also significantly reduces the quality of life and exacerbates co-morbidities.[3]

Generally, SUI affects approximately 20% of young women but increases to 50% in elderly women.[4,5] Moreover, the number of patients presenting with this urologic health problem will rise as the baby-boomer generation continues to age.[6]

The aetiology of SUI involves many factors, such as the functional impairment of pelvic muscles, connective tissue and their associated innervating nerves. These factors occur secondary to pelvic floor damage following vaginal childbirth, advancing age, and hormonal status.[7-10] The causes of stress urinary incontinence are urethral hypermobility, intrinsic sphincter deficiency (ISD), or both.11 ISD is characterized by a malfunction of the urethral muscle closure mechanism, whereas urethral hypermobility occurs following the loss of bladder neck support and a lack of intra-abdominal pressure transmitted to the proximal urethra. It seems reasonable that SUI varies between the extremes of intrinsic sphincter deficiency and urethral hypermobility, thus the majority of patients presenting with stress incontinence often present with elements of ISD and hypermobility.[11-13]

MATERIALS AND METHODS

What is a current treatment for SUI?

SUI can be treated with physicobehavioral therapy alone or combined with pharmacotherapy, whereas surgical intervention is considered based on SUI intensity and etiological criteria. Therefore SUI can be divided into moderate, mild and severe.[14] The treatment of mild SUI is based on pelvic and floor exercises, electric stimulation of the pelvic floor and pharmacotherapy whereas moderate and severe SUI needs urethral sling or retropubic repair. In selected cases the injection of bulking agents or installation of an artificial sphincter are performed. The therapeutic effect of injections and pharmacology are usually disappointing.[14]

The current treatment of SUI is suburethral sling placement, which is very popular and good working method. This procedure involves applying autologic, allogenic, xenogenic or various artificial materials to suspend the bladder neck or urethra. The graft options for sling therapy include: autografts (eg, rectus fascia, fascia lata, vaginal wall), allografts (cadaveric tissues, including dura mater, dermis, fascia lata), xenografts (porcine small intestinal submucosa, porcine dermis), and synthetic artificial materials (polytetrafluoroethylene, polypropylene, silicone elastomers, polyglactic acid, polyester). Selection of each graft material depends on its own inherent advantages and disadvantages because the ideal sling material should be readily available, durable and does not trigger immune response while performing its intended function.[6] There is also the possibility of using artificial urinary sphincters that offer reliable continence and are highly efficacious. However, the cost of the artificial urinary sphincter remains and after such implant operations, functional disorders and local tissue erosion have been observed.[15]

Bulking agents and SUI treatment

Injectable bulking agents have become popular in the treatment of stress urinary incontinence due to intrinsic sphincter deficiency (ISD). Urethral bulking agents offer a less-invasive support for the urethra than sling procedures or artificial sphincters. By adding bulk to the bladder neck and the proximal segment of the urethra, the increased coaptation of the urethral mucosa protects against the increases of intravesical pressure by improving the resistance to the outflow of urine.[16] Injectable bulking agents that are frequently used include Teflon, bovine collagen, silicone particles, carbon beads, and autologous ear chondrocytes. Although chondrocytes injection is a cell therapy, the target of this method is not a muscle tonus improvement. It works as a 'closing mechanism', which does not lead to muscle regeneration.

Bulking agents have been used successfully, however they are known to induce chronic inflammatory reactions leading to periurethral abscesses, erosion of the urinary bladder or the urethra, obstruction of the lower urinary tract with resultant urinary retention, severe voiding dysfunction, migration to inner organs, and pulmonary embolism.[17]

The above mentioned techniques are considered unsatisfactory because the "bulking" of the urogenital tract can cause obstruction of the urethra and a passive sealing of the urethral lumen, without restoring the sphincter function. Also the closure apparatus may become inflexible and rigid.[18]

Modern techniques of sphincter regeneration have come from "bulking agent therapies". Cells can be used as a "natural bulking agent" when transplanted into the space between bladder neck and urethra. Bulking agents need large volume of cell suspension, in which cell viability is low. Bulking agents only narrow the light of the urethra while stem cells are designed to regenerate the sphincter, a method derived from the original concept of injecting bulking agents.[19-20]

Different sources of stem cells in treatment of SUI

Newly emerging technologies in tissue engineering may provide novel methods for the treatment of SUI. The deficiencies of urethral muscle and connective tissue that results in ISD can be regenerate by stem-cell therapy, which is currently at the forefront of incontinence research.[21] The stem-cell therapy is very important in a way of replacing, regenerating, or enhancing the biological function of damaged tissue or organs. Stem cells injected into muscle area over the middle urethra can restore the contractility of rhabdosphincter. The type of stem cells, which can be potentially used in the treatment of stress urinary incontinence are adult stem cells.[22] Over the past few years, mesenchymal stem cells (MSC) have been derived from various types of tissues including bone marrow, umbilical cord blood, adipose tissue, skin, periosteum, and dental pulp.[23-28] Cell-based therapies are most often associated with the use of autologous multipotent stem cells. The choice of stem cell source is determined by ease of harvest, population density and differentiation potential.

The new autologous sources are: muscle-derived stem cells (MDSCs) and adipose-derived stem cells (ADSCs). Both are advantageous because cells can be easily obtained in large quantities under local anesthesia. MDSCs injection therapy, often referred to as myoblast transfer therapy, will not cause an immunogenic reaction, because it is an autologous cell transplantation.[29-31] Muscle-derived stem cells are also physiologically capable of improving urethral function, as it has been proposed that the newly formed myofibers and myotubes may receive excitable stimulus as part of the syncitium.[19, 20, 32]

Zuk and associates demonstrated that ADSCs can differentiate *in vitro* into adipogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors.[25] In addition, Rodriguez and colleagues reported that smooth muscle cells derived from ADSCs exhibit the functional ability to contract and relax in response to pharmacologic agents.[33] Thus providing the experimental basis that supports the injection ADSCs to improve the function of impaired urethra sphincter muscle. Human adipose-derived stem cells may also represent an alternative stem cell source for the treatment of stress urinary incontinence.[34]

Above mentioned cell therapies using MDSCs and ADSCs offer a promising technology for the treat of stress urinary incontinence.

Bone marrow stem cells and muscle differentiation.

The bone marrow stroma is commonly described source of multipotent stem cells. Bone marrow contains several cell populations one of which is MSC compartment.[35] Autologous mesenchymal stem cells are capable of differentiating into adipogenic, chondrogenic, osteogenic, and myogenic cell lines.[35-38] Although there are some limitations of using MSCs, such as the painful process of harvesting autologous bone marrow (often requiring the use of general or spinal anesthesia) and the low numbers of growing cells (the necessity of differentiation and the inability to predict or track differentiation), the MSCs are very popular, evolutionarily youngest cells and commonly used in cell therapy.[39] Muscle cells have been generated in vitro from human bone marrow using 5-azacytidine, an analogue of cytidine which induces DNA hypomethylation.[40] 5-azacytidine was also shown to induce mouse 10T1/2 fibroblasts to differentiate into skeletal myoblasts by reactivation of the transcription of silenced genes including MyoD family.[41] Similar results have been achieved by co-culturing MSCs with muscle cells and exposure of the mesenchymal stem cells to low bovine or horse serum concentration.[42] However, most of the data regarding the differentiation of muscle cells from bone marrow come from *in vivo* experiments. Different studies demonstrated that damaged muscles may be repaired either after whole bone marrow transplantation or by direct injection of bone marrow cells into damaged muscles.[40,43,44] The myogenic repair can be promoted by the fusion to existing and/or damaged myocytes that paracrine release cytokines and factors.[42] Both *in vitro* and *in vivo* studies have indicated that several factors such as, microenvironment, cell-to-cell contact and extracellular matrix play a key role in determining the function and differentiation of mesenchymal stem cells.[40,42,43,44] Engler and colleagues showed that during ex vivo culture of MSCs, lineage differentiation could be directed by the elasticity of the matrix on which the cells are grown.[45] Moreover, authors demonstrated that mesenchymal stem cells differentiate into myogenic precursors when cultured on gels of varying elasticity.

Regardless of the success achieved with mesenchymal stem cells, the level of differentiation *in vitro* has raised a number of questions that remain unanswered. It is not known whether pre-differentiation of MSCs will be essential in clinical applications and whether undifferentiated stem cells differentiate into the host muscle cells *in vivo*.[43]

Results of *in vivo* experiments

Cannon and colleagues performed injection of muscle derived progenitor cells into denervated female rat urethras.[46] The injection of muscle derived progenitor cells into the denervated sphincter significantly improved fast-twitch muscle contraction amplitude. Two weeks following injection, immunohistochemistry revealed a large amount of new skeletal muscle fiber formation at the injection site of the urethra with minimal inflammation. The subsequent experiments showed that allogenic MDSCs significantly improved the LPP in nerve transected animals after one and four weeks. Authors observed coincidence between doses and improvement. [21,47] Likewise two, four and six weeks after the cauterization of periurethral tissue the mean LPP in rats that received MDSCs was markedly increased compared to the sham procedure group. MDSCs have been also seeded onto a urethral sling with positive effects. That means urethral slings could be an effective delivery mechanism for these cells.[48] MDSCs are able to multipotent differentiation in the host tissue or have the capacity to elict a paracrine effect resulting in a more complete regenerative muscle-nerve healing response, what was observed in a rat model of SUI.[46] Chermansky and colleagues showed that MDSCs had integrated four weeks after injection within the striated muscle layer of the cauterized middle urethra.[49] Additionally, the striated muscle layer of the MDSCs-injected urethra was contiguous and better innervated than the cauterized urethra injected with only saline solution. Furthermore, in the groups of rats injected with MDSCs the increase in leak point pressure (LPP) was noticed as significant when compared with the cauterized rats injected with saline solution. Importantly, the difference in LPP observed four and six weeks after the MDSCs injection was not significant when compared with the uncauterized control rats. Kwon and colleagues compared MDSCs and fibroblasts using LPP as a marker of improvement.[47] The comparison was made with

regard to their potential for restoration of urethral function after injection. The short-term experiment with equal cell dosage exhibited no significant difference between MDSCs and fibroblasts or their combination. When the dosage was varied by two 10-fold increases, only a high dose of injected fibroblasts led to urinary retention, while high doses of MDSCs did not result in such adverse events. That's mean the fibroblasts may produce a bulking effect and make the tissue less compliant.[47]

The potential use of ADSCs with biodegradable microbeads in a rat model of SUI has been suggested. Improvement in LPP and urethral function was reported.[50]

Results of clinical application of injecting stem cells

Carr and coworkers have conducted clinical studies with MDSCs biopsy from lateral thigh muscle.[51] Eight patients were included in the first trial using either a periurethral or transurethral MDSCs injections into the middle urethra and external urethral sphincter (EUS). The measurable improvement was observed in two patients who underwent periurethral injection and two patients who received transurethral injections with using a 10-mm needle. Two patients with initial injections using an 8-mm needle had no benefit. Five of eight patients followed up for more than one year reported significant improvement. That's why these results are the potential for pure cellular therapy in treating stress urinary incontinence and emphasize the importance of proper cell placement in resulting effectiveness.

The I phase of next clinical trial, in which new therapeutic strategy for urethral sphincter insufficiency is developed, has finished in October 2008.[52] Women and men aged 40-75 years suffering from urinary incontinence since at least six months and candidate for a surgical treatment (artificial urinary sphincter, synthetic compressive tapes or adjustable balloons) were enrolled in this trial. This study developed a new therapeutic strategy for stress urinary incontinence based on the implantation whole myofibers with satellite cells. The scientific background of this therapy relies on the activation *in vivo* of the satellite cells. Satellite cell activation is concomitant with myofiber death that occurs after their implantation. Activated satellite cells proliferate, fuse and form myotubes replacing the parental myofibers. It leads to the regeneration of the muscle volume. Preliminary studies on the pig model showed the regenerated muscle tissue in the urethra was innervated by urethral nerves and developed tonic contractions which acts like a new sphincter. This procedure does not include a phase of satellite cells amplification *ex vivo*, as standard methods of satellite cell transfer. Thus, the procedure of cell transfer into the urethra is considerably simplified and can be performed in one step in the operating room. This therapeutic strategy could represent an alternative method to the artificial urinary sphincter implantation.[52] Additionally two trials are open to test the efficacy and safety of autologous muscle derived cells transplantation. Eighteen years and older women, whose stress urinary incontinence symptoms had not improved within six months of conventional therapy are enroll. This study is currently recruiting participants.[53] The another attempt of SUI treatment was made by Mitterberger and coworkers, who investigated the injections of autologous myoblasts and fibroblasts. Twenty female patients, whom injected fibroblasts and myoblasts into the urethral submucosa and into the rhabdosphincter respectively, were included in this study. The authors reported that two years after SUI therapy, sixteen of eighteen patients were cured, two patients were improved, and two patients were lost to follow-up. Moreover, observations after therapy revealed that thickness of urethra and rhabdosphincter were significantly increased.[54] Despite many objections against quoted work, it has to be treated with a grain of salt, but this work was written and published and it's hard to deny it's accuracy.

Why cell therapy can fail?

Tissue engineering techniques hold promise for the future in SUI treatment. Unfortunately, aforementioned results have a number of weaknesses that should be clarified. A number of challenges however still need to be overcome. *In vitro* studies on autologous stem cell differentiation have some limitations. Biochemical characteristic of stem cells do not necessarily translate to *in vivo* usefulness and the *in vitro* findings may not mimic the signal transduction pathway that occurs in host.[42] The presence of some antigens may change *in vitro*, due to specific culture condition and the duration prior to individual passages. Some antigens may be found on freshly isolated mesenchymal stem cells, but their expression disappears in culture[55,56,57,58]. Furthermore such *in vitro* conditions can activate the DNA damage and may lead to a senescence phenotype.[59] A further questions arises, whether the grafted stem cells can maintain their undifferentiated state and build new niches, thus supporting the therapeutic effect on a long term basis.[55] It has also been observed that mesenchymal stem cells may differentiate into unwanted phenotypes *in vivo* such as osteocytes and adipocytes which are undesirable for therapeutic application in the muscle repair.[60] Some observations indicate on fusion of mesenchymal stem cells and endogenous differentiated cells *in vivo*, although it's extremely rare event.[55,61] For clinical applications, the choice of stem cells must have a high regenerative potential, however, it is not known whether single or multiple injections would be sufficient to achieve a stable functional improvement over a given time period. Moreover, homing mechanisms are not well defined, thus it is unclear whether systemic or direct graft administration of stem cells to the target organ would be most effective.[61] Another issue that needs to be addressed before the administration of functional cell populations *in vivo* is the type and number of viable cells injected delivered at the graft site and the prevention of neoplasm formation due to contamination of the graft with remaining undifferentiated cells or potentially embryonic like cells.[62,63] It has been shown that *in vitro* expansion affects the stem cells differentiation capabilities and regenerative potential.[61,64,65,66]

CONCLUSIONS

The current gold standard for the treatment of SUI is to surgically lift and reposition the urethra with a sling, supporting the muscles and ligaments of the middle urethra. Experimental stem cell injection therapy has the potential to restore the contractile response of the rhabdosphincter and has been at the forefront of incontinence research. However, the available data is derived from studies with small treatment groups. Furthermore, we still do not know whether the grafted stem cells can maintain their undifferentiated state or differentiate into unwanted phenotypes. The way of stem cells administration is not specified as well. All aforementioned issues related with stem cell therapy indicate that it is very promising. Nevertheless, additionally studies with significantly larger groups are required for proof of this concept.

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