Autologous myoblasts and fibroblasts for female stress incontinence: a 1-year follow-up in 123 patients

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Level of Evidence 4

OBJECTIVE

To assess the efficacy and safety of the application of autologous myoblasts and fibroblasts for treating female stress urinary incontinence (SUI) after a follow-up of ≥1 year.

PATIENTS AND METHODS

In all, 123 women with SUI (aged 36–84 years) were treated with transurethral ultrasonography-guided injections with autologous myoblasts and fibroblasts obtained from skeletal muscle biopsies. The fibroblasts were suspended in a small amount of collagen as carrier material and injected into the urethral submucosa, while the myoblasts were implanted into the rhabdosphincter. All patients were evaluated before and 12 months after the injection using the Incontinence and Quality of Life Instrument (I-QOL) scores, urodynamic variables, and morphology and function of the urethra and rhabdosphincter.

RESULTS

At 1 year after implanting the cells, 94 of the 119 women (79%) were completely continent, 16 (13%) had a substantial improvement and nine (8%) a slight improvement. Four patients were lost to follow-up.The incontinence and I-QOL scores, and the thickness, contractility and electromyographic activity of the rhabdosphincter were significantly improved after treatment.

CONCLUSIONS

These results show the efficacy and safety of transferring autologous myoblasts and fibroblasts in the treatment of female SUI, after a follow-up of 1 year.

KEYWORDS

autologous myoblasts, fibroblasts, endoscopic injection, stress urinary incontinence, rhabdosphincter

INTRODUCTION

Stress urinary incontinence (SUI) is a common condition in women, with a prevalence of 35% for Western countries; 78% of incontinent women have SUI or mixed UI [1,2]. Therefore, the urethra and the rhabdosphincter, the so-called urethral sphincter complex, must be the main targets of treatment in most cases.

Urethral smooth and striated muscle tone, and the supportive properties of the urethral mucosa and submucosa, in particular the vascular submucosal layer, are important in the closure of the urethra. The rhabdosphincter in the female urethra is composed of small type I fibres located predominantly around the middle third of the urethra. It surrounds the urethra at its ventral and lateral aspects [3]. Previous ultrasonographic studies confirmed experimental and clinical data indicating that the rhabdosphincter represents the major muscular portion of the urethral closure mechanism [4]. Resting tone and contractility of the rhabdosphincter are markedly reduced in incontinent patients, thus preventing the urethra from being closed completely [4]. Damage to this muscle might result from maternal injury during childbirth or be iatrogenic. Furthermore, spontaneous apoptosis contributes to an age-dependent loss of the striated muscle cells of the rhabdosphincter [5].

Recent animal experiments indicated a role for the combined use of autologous myoblasts and fibroblasts in reconstructing the urethral sphincter complex [6,7]. Myoblasts have been used in these preclinical studies to regenerate the rhabdosphincter, and fibroblasts were injected to treat atrophy of the mucosa. Therefore, the combined application of these cells might restore normal morphology and function of the rhabdosphincter and urethral submucosa in incontinent patients.

In the present study we present the results at 1 year after treatment using transurethral ultrasonography (TUUS)-guided injections with autologous myoblasts and fibroblasts in female SUI, with special emphasis on efficacy and safety.

PATIENTS AND METHODS

After approval by the Ministry of Health of the Federal Government of Austria, the first set of patients was investigated and subsequently treated in 2004 until the end of 2005, 123 women were treated (mean age 62.8 years, SD 10.5). In the present study the results at 1-year for 119 patients are presented. Informed consent was obtained from all patients.

All patients had urodynamically confirmed SUI associated with only mild hypermobility of the

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urethra and the bladder, or intrinsic sphincter insufficiency. All patients had performed pelvic-floor exercises with no improvement of their symptoms before the initial examination; 68 had had previous surgery, including tension-free vaginal tape, hysterectomy and colposuspension. Urodynamic and clinical tests, including cystoscopy, pressure–flow studies and the Q-tip test (mobility ≤45°) were used to investigate the lower urinary tract before therapy, to exclude patients with urge UI and marked hypermobility of the urethra. Before the injection treatment the variables used to define success were again assessed in all patients.

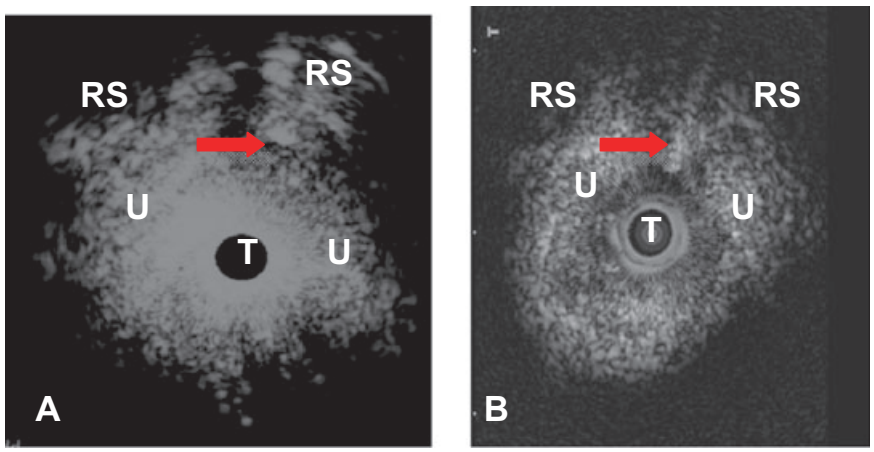
The patients had used pelvic-floor exercises before the treatment and were instructed to regularly train the rhabdosphincter and use transvaginal electrical stimulation for 4 weeks after treatment, to support the integration of the cells and their regeneration. At 1 year after treatment the variables used to define success were assessed in all patients.

To quantify the success rates, patients were evaluated before and after therapy using the following four criteria, derived from an extensive literature search. The Incontinence Score, validated in previous clinical studies [8], was the primary variable used to assess the success of treatment, comprising three different criteria, i.e. a 24-h voiding diary, 24-h pad test and patient questionnaire [8]. The total score range was 0 (continent) to 6 (completely incontinent).

TUUS (high-frequency transducers, 8 F, 15–20 MHz) was used to evaluate the lower urinary tract before and after therapy. Previous studies showed that this is the only currently available imaging technique allowing an investigation of the morphology and function of the urethra and rhabdosphincter [4]. The distance between the TUUS transducer and the inner aspect of the rhabdosphincter was measured at rest and during voluntary contraction of the muscle. The difference between these readings was the variables used for contractility of the rhabdosphincter (Fig. 1) [3,4]. The thickness of the urethra and of the rhabdosphincter, as well the contractility of the rhabdosphincter, were defined as primary outcome measures. The TUUS measurements were made by experienced radiologists.

The Incontinence Quality of Life (I-QOL) instrument, which assesses QOL before and after treatment for UI, was used as a secondary outcome measure [9]. The total score for all answered questions is between 22 (no QOL) and 110 (no restrictions on QOL).

FIG. 1. TUUS-guided injection of myoblasts (A) and fibroblasts (B). (A) A tiny depot of myoblasts (marked with an arrow) has been injected at the inner aspect of the rhabdosphincter, which is atrophic and has an irregular TUUS pattern. (B) A small depot of fibroblasts (marked with an arrow) has been injected into the urethral submucosa. The volume of both injections is only 75 μL. Altogether, ≈50 injections with myo- and fibroblasts were used in every patient. RS, rhabdosphincter; U, urethra; T, TRUS transducer.



Urodynamic tests, including pressure–flow studies and urethral pressure profiles, were defined as secondary outcome measures for determining whether there was obstruction of the lower urinary tract after therapy, and to show any therapeutic effect on urethral closure pressures [1]. In addition, kinesiological electromyography (EMG) measurements were made using periurethral surface electrodes, to assess the patterns of individual muscle activity before and after treatment, both at rest and during voluntary contraction [1].

Muscle biopsy and TUUS-guided injection with adult autologous myoblasts and fibroblasts in the urethra were administered to the lower urinary tract, as previously described [10] A muscle biopsy (from the biceps muscle) was taken (0.5–2 mL) and transported to a Good Manufacturing Practice facility with official authorisation for the production of myoblasts and fibroblasts (IGOR, Wels Austria; Innovacell Biotechnologie GmbH, Innsbruck, Austria) for the therapy of SUI.

Myoblasts and fibroblasts, two different types of muscle-derived autologous cells, were isolated from these muscle biopsies, grown separately for 6–8 weeks and then harvested. All the materials used were of high quality and certified for Good Manufacturing Practice use.

Thereafter the myoblasts were suspended in 1.4 mL of Dulbecco's modified Eagle medium (DMEM)/F12 with 20% autologous serum, and the fibroblasts in 1 mL DMEM/F12 with 20% autologous serum mixed with 2.5 mL of collagen (Contigen®, Bard, Covington, GA, USA) as carrier material to prevent them from migrating from the site of injection, as fibroblasts are mobile after application. Collagen has been shown to stabilize the cells so that they stay in place and produce their own extracellular matrix. The fibroblasts and myoblasts were then filled into separate sterile syringes and transported to the operating room.

First, the TUUS transducer was carefully inserted into the urethra to visualize the urethral wall and rhabdosphincter, whose main portion was situated in the mid-portion of the urethra. Using a specially designed injection device, 15–18 aliquots (50–100 μL per depot) of the myoblast suspension were injected directly into the omega-shaped rhabdosphincter at two different levels, to promote regeneration of the muscle. Then 25–30 depots (50–100 μL per depot) of the fibroblast/collagen suspension were injected into the submucosa circumferentially at three levels, slightly cranial to, slightly caudal to, and between the levels of the injected myoblasts, to treat atrophy of the urethral

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FIG. 2. Human myoblast culture (×20).

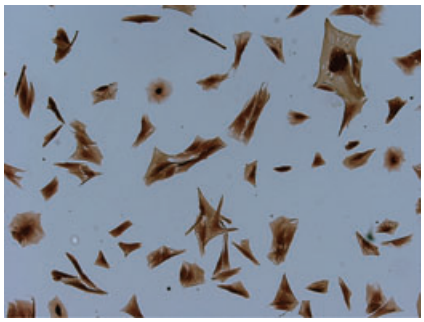

FIG. 3. EMG (measured in μV) and urinary flow (mL/s) in a patient before and after therapy, showing markedly increased EMG activity of the rhabdosphincter after treatment, indicating that the increase in thickness and contractility of the rhabdosphincter measured by TUUS was due to formation of new muscle tissue in the rhabdosphincter, as reflected by the higher electrophysiological activity of the muscle.

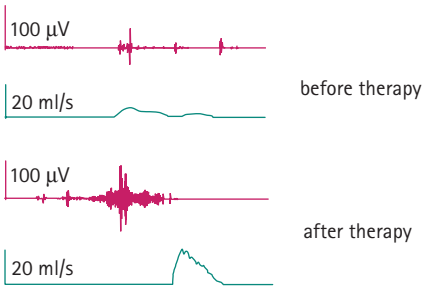

TABLE 1 The characteristics of the 119 patients treated with autologous cells at baseline and the 1-year follow-up. The statistical differences between before and after treatment were assessed using the Wilcoxon test. The incontinence score and IQOL score are shown as the median (range), and all numeric variables as the mean (SD)

Variable	Before	After (1 year)	P
Incontinence score	6 (5–6)	0 (0–4)	0.001
IQOL score	51 (27–70)	108 (29–110)	0.001
Thickness of urethra, mm	3.5 (0.8)	4.9 (0.9)	0.001
Thickness of rhabdosphincter, mm	2.1 (0.3)	3.4 (0.4)	0.001
Contractility of rhabdosphincter	0.65 (0.3)	1.39 (0.3)	0.001
Maximum:			
residual urine, mL	49.2 (131.7)	12.5 (67.8)	0.001
urinary flow rate, mL/s	21.6 (9.1)	25.2 (8.1)	0.001
detrusor pressure during flow, cmH_2O	36.6 (25.7)	31.2 (16.7)	0.001
bladder capacity (ml)	425.9 (129.6)	450.5 (118.0)	0.570
closure pressure at rest, cmH_2O	28.8 (12.3)	40.5 (15.8)	0.001
closure pressure during voluntary contraction of rhabdosphincter, cmH_2O	51.8 (35.8)	78.1 (56.2)	0.001
Periurethral EMG, μV			
at rest	34.0 (11.0)	45.1 (15.0)	0.001
during voluntary contraction of rhabdosphincter	43.1 (11.8)	55.4 (15.3)	0.001

submucosa and improve its sealing effect. Urethroscopy was performed at the end of the operation.

Results were expressed as the mean (SD) for numeric and as the median (range) for ordinal variables; the Wilcoxon test was used to compare values before and after treatment, with $P \leq 0.05$ considered to indicate statistical significance.

RESULTS

The TUUS-guided injection with adult autologous myoblasts and fibroblasts was administered in all 123 patients, with no complications during or after application (Fig. 1). On average, all patients received injections with 3.8×10^7 fibroblasts (5.4×10^6–6×10^7) and 2.8×10^7 myoblasts (range 5.1×10^6–3.6×10^7) (Fig. 2). In 10 patients a catheter had to be placed after the injection until the first day after treatment, and in two until the second day. During the clinical follow-up no severe side-effects, e.g. pelvic pain, obstruction, inflammation or de-novo urgency, were detected. No strictures, scars or tumours were detected on TUUS and cystoscopy.

At 1 year of follow-up 94 of the 119 patients (79%) were completely continent and did not need to wear pads during daily life. A further 16 patients (13%) had a substantial improvement, whereas nine had only a slight improvement. Four patients were lost to follow-up. In no patients was there any deterioration of incontinence.

The Incontinence Score was significantly improved at the 1-year follow-up (median values 6 vs 0). The changes were similar for the IQOL score (median 51 vs 108). In addition, the mean (SD) maximum urethral closure pressure during voluntary contraction of the rhabdosphincter improved from 51.7 (35.8) to 78.1 (56.2) cmH_2O, the thickness of the rhabdosphincter improved from 2.1 (0.3) to 3.4 (0.4) mm, and the rhabdosphincter contractility from at 0.65 (0.3) to 1.39 (0.3). The EMG activity at rest increased from 34.0 (11.0) vs 45.1 (15.0) μV and during voluntary contraction of the rhabdosphincter from 43.1 (11.8) to 55.4 (15.3) μV (Fig. 3).

These marked changes were clinically relevant, showing substantially improved sphincter function in the patients (Table 1).

The urodynamic variables showed a slight but statistically significant increase in maximum urinary flow rate and a statistically significant decrease in residual urine during voiding. As there was no significant change in detrusor pressure, there was no sign of obstruction of the lower urinary tract after treatment. There were statistically significant improvements in all variables used to assess success, except for maximum bladder capacity (Table 1).

DISCUSSION

Intrinsic sphincter deficiency and hypermobility of the urethra are the two main components postulated to contribute to the pathophysiology of SUI. Scientific data suggest that SUI can be considered as a continuous spectrum rather than existing as a dichotomy [11]. The pathophysiology of SUI also has to be correlated with the overactive bladder, as there is an association between detrusor overactivity-related filling symptoms and SUI [12].

Conventional surgical procedures to treat SUI, including retropubic colposuspension and the

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suburethral sling procedures, can be expected to generate a short-term mean cure rate of up to 80% at 1 year after surgery, but generally there is a decrease in continence rate with time [1].

To date, injectable substances have included collagen, silicone, and carbon-coated beads; these 'bulking agents' are injected into the periurethral tissue at the bladder neck, often under local anaesthetic and in an outpatient setting [13]. Short-term cure rates are ≈50% and success rates (cure plus improved) are 76%, representing a favourable alternative to standard surgery in old or frail women, especially with the associated low complication rates and morbidity. However, injectable substances do not have durability and there is often a need for multiple re-injections over time [12,14].

These injected or implanted materials and the surgical procedures currently used to treat SUI can cause severe side-effects. Some injected materials have been shown to migrate into the brain and other organs [15], and the injection of 'bulking agents' and implantation of synthetic slings can lead to chronic inflammation, foreign-body giant cell responses, periurethral abscesses, erosions, obstruction of the lower urinary tract, urinary retention, and severe voiding dysfunction. The surgical techniques and materials used to date have also been reported to cause passive obstruction of the urethra, and do not lead to regeneration of the rhabdosphincter and urethra [15,16]. Therefore, the ideal surgical treatment for SUI has yet to be defined, and especially in view of the limited efficacy and side-effects of current therapies, there is obviously a need for new treatments.

The present study showed a significant improvement in UI in patients at 1 year after injection with autologous myoblasts and fibroblasts. The injection of myoblasts led to an increase in thickness and improved contractility of the rhabdosphincter. In addition, periurethral EMG measurements showed increased activity of the rhabdosphincter, resulting in increased resting tone and voluntary contractile force. From these data we conclude that additional muscle tissue was formed in the rhabdosphincter and that this new muscle tissue was functionally active.

In the present study only patients with SUI and with no marked hypermobility of the urethra and bladder were treated. This new therapy has not been used in women with pronounced hypermobility, as injection with cells cannot reconstruct the supporting structures of the lower urinary tract.

The results presented, and the EMG and TUUS data, suggest that an injection with myoblasts and fibroblasts, unlike that of 'bulking agents' such as collagen, leads to regeneration of the rhabdosphincter and the urethral submucosa. The present data show that the effects of the autologous cell injections cannot be due to a simple 'bulking effect', as the cells were injected in several small depots and the overall amount of injected material was low. Therefore, the use of 2.5 mL of collagen as a carrier material for the fibroblasts could not be the reason for the good clinical results. In addition, it was shown that collagen is normally absorbed within 3–6 months after injection, and that the 1-year cure rates are poor. Herschhorn *et al.* [17] showed that in the few patients who were cured after collagen injection, the amount of collagen was very variable and that >10 mL was injected in some patients.

To date, injectable agents have been delivered under endoscopic control. Recently, non-endoscopic injection devices have been developed, allowing outpatient treatment and avoiding the need for surgical facilities [18]. Nevertheless, based on animal experiments and on long-term experience in implanting cells, TUUS-guided application is crucial in this new treatment approach, as it allows accurate and precise injection of cells into the lower urinary tract. Endoscopic guidance does not provide the precision and control that is needed for injected regeneration of urethral submucosa and rhabdosphincter.

As to the safety of injections with autologous myoblasts and fibroblasts, previous studies showed that these cells do not proliferate excessively, due to contact inhibition and the absence of growth medium after injection [19]. In addition, mononucleated myoblasts can only proliferate if they have not fused and formed myofibres [19]. In previous studies to date, no serious side-effects, e.g. the development of hyperplasia, scars, tumours or inflammation, were reported to date after implantation of autologous myoblasts and fibroblasts [19,20]. Unlike surgical procedures such as colposuspension and suburethral slings to treat hypermobility of the lower urinary tract, myoblast injection is not associated with the onset of *de novo* urge symptoms.

The present study suggests that the combined approach using myoblasts for regeneration of the rhabdosphincter and fibroblasts for regeneration of the urethra leads to improved closure of the urethral lumen, with no side-effects. The present data support earlier data showing that this new and minimally invasive therapeutic approach is effective. If long-term and multicentre data are as good as those in the present study, this new technique has the potential to become a standard therapeutic method in urology and urogynaecology in the future.

In conclusion, the TUUS-guided application of autologous myoblasts and fibroblasts represents an effective and safe new therapeutic method to cure female SUI. Ongoing trials, including multicentre studies and long-term data will be need to confirm the results of this promising treatment approach.

CONFLICT OF INTEREST

Dr M. Fusssenegger is co-owner of IGOR, and Dr H. Strasser and Dr R. Marksteiner are founders and co-owners of Innovacell Biotechnologie GmbH. Both companies run certified GMP facilities where the autologous cells were grown. Dr E. Margreiter, an employee of Innovacell, was responsible for the cell cultures. Innovacell Biotechnologie GmbH and IGOR provided the cells. IGOR and Innovacell Biotechnologie had no role in study design, in the collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

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Abbreviations: **(S)UI**, (stress) urinary incontinence; **I-QOL**, Incontinence Quality of Life (questionnaire); **EMG**, electromyography; **DMEM**, Dulbecco's modified Eagle medium; **TUUS**, transurethral ultrasonography.

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