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CLINICAL ARTICLE

Intrasphincteric autologous myoblast injections with electrical stimulation for stress urinary incontinence

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ABSTRACT

Objective: To assess the feasibility and safety of ultrasound-guided autologous myoblast injections into the external urethral sphincter followed by electrical stimulation (ES) as a possible 2-step treatment for stress urinary incontinence (SUI). *Methods:* Autologous myoblasts isolated from a biceps muscle sample were injected under transurethral ultrasound guidance into the external urethral sphincter of 38 female patients. The patients also underwent ES postoperatively to enhance cell integration. Treatment feasibility, as well as possible intraoperative and postoperative complications, was assessed 6 weeks after the injections. Additionally, the effects of the myoblast injections followed by an ES cycle were compared to those of a preoperative ES cycle undergone by the same patients. *Results:* No serious adverse events or complications were noted and the procedure was well tolerated. Compared with the objective and subjective measurements collected after the preoperative ES cycle, the corresponding measurements obtained 6 weeks postoperatively, after the completion of a second ES cycle, indicated considerable improvement. The results to the stress test were negative for 29 (78.4%) of the patients, 5 (13.5%) considered their SUI cured, and 29 (78.4%) reported improvement. *Conclusion:* Intrasphincteric autologous myoblast injections followed by ES is minimally invasive and feasible, and safely produced promising initial results.
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1. Introduction

Stress urinary incontinence (SUI) is a highly prevalent condition in women [1]. The integrity of the urethral sphincter complex is necessary to maintain continence, especially that of the external rhabdosphincter, an omega-shaped muscle which compresses the urethra voluntarily [2]. Vaginal delivery [3], surgical injury, and aging [4], however, affect the morphologic and functional integrity of these muscles. Methods to treat SUI have long existed, but their limitations have encouraged researchers to investigate new approaches, including within the field of regenerative medicine, to preserve or improve tissue function. Animal models [5–12] have paved the way to clinical trials of tissue engineering as a treatment option. In these trials autologous myoblasts were injected in the rhabdosphincter to determine whether these progenitor muscle cells would mature, multiply, and restore muscle function [13–15], perhaps lastingly [16].

Treating SUI by transurethral ultrasound-guided injections of autologous myoblasts into the rhabdosphincter is not the only minimally invasive investigational approach devised. Electrical stimulation (ES) has long been widely applied in clinical and research settings as a form of physical therapy. In 2006, an in vitro study reported that ES accelerated the maturation and organization of myoblasts into myotubes, that the newly produced muscle fibers were spontaneously contracting, and that these events probably accelerated the restoration of tissue function [17]. Exercise was also shown to increase long-term graft success by enhancing the recruitment and fusion of transplanted myoblasts [18]. Thus, the muscle regeneration that follows myoblast injections might be accelerated by ES as a form of physical therapy.

The primary objective was to assess the feasibility and safety of autologous myoblast injections followed by an ES cycle. Additionally, we hypothesized that this 2-step treatment would improve SUI symptoms more than ES alone. To verify this hypothesis, we first compared the baseline measurements (subjective as well as objective) associated with SUI evaluation with the corresponding measurements obtained after a first, preoperative ES cycle. Then, we compared these second measurements with those obtained after completion of the 2-step treatment.

2. Materials and methods

The study was approved by the National Medical Ethics Committee and the National Agency for Medicinal Products and Medical

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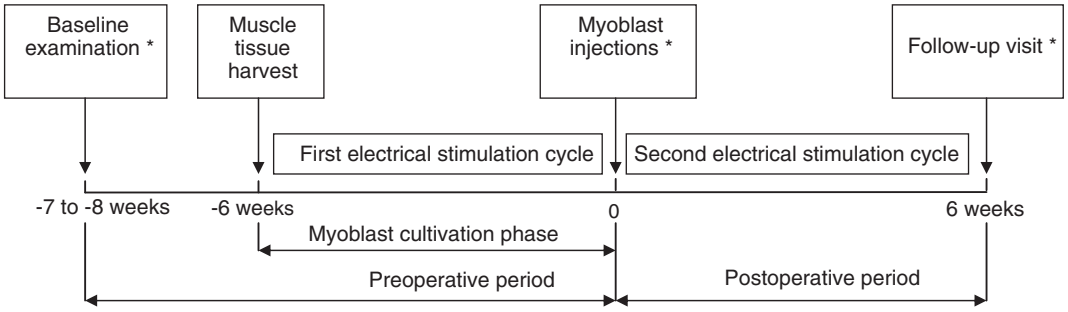

Fig. 1. Flow chart of the study. The asterisks indicate the times of data collection (the studied variables are those shown in Table 1).

Devices. All procedures were conducted in accordance with the National Act on Quality and Safety of Human Tissues and Cells for the purposes of medical treatment and good clinical practice. All participants gave written informed consent. Safety and feasibility were the main concerns of this single-center, academic, investigator-initiated, explorative, noncomparative clinical trial. The flowchart of the trial is presented in Fig. 1.

The eligibility criteria were the following: age between 18 and 75 years, primary symptoms of SUI, more than 10 incontinence episodes weekly and more than 1 daily, a positive stress test result, a positive 1-hour pad test result (defined as a pad weight increase >1 g), normal detrusor activity on a filling cystogram, bladder capacity greater than 300 mL, and failure of a noninvasive treatment acknowledged at least 3 months prior to enrolment. Major exclusion criteria were severe urethral hypermobility, defined as a 45° rotation of the proximal urethra and bladder neck during a Valsalva effort and detected by the movement of an inserted Q-Tip; a pelvic organ prolapse (POP) noted from the anterior, apical, or posterior aspect of the vagina (corresponding, respectively, to a cystocele, uterine prolapse, or rectocele) more severe than stage I in the POP quantification (POP-Q) system; and previous anti-incontinence surgical treatment. A total of 38 patients were treated at the Division of Gynecology, University Medical Centre Ljubljana, Ljubljana, Slovenia, from September 23, 2009, to August 26, 2010.

In each participant, approximately 0.5 cm^3 of muscle tissue was taken under local anesthesia from the upper arm corresponding to the nondominant hand. The tissue was then placed in a transport medium and sent to a Good Manufacturing Practice–certified laboratory (Innovacell Biotechnologie, Innsbruck, Austria) for myoblast isolation, culture, harvest, and storage.

Five to 14 weeks later, in an operating theater, 2 mL of liquid myoblast suspension (1×10^6–5×10^7 cells in 2 mL of a solution consisting of 90% of DMEM/F12 medium and 10% of autologous serum) was injected in the rhabdosphincter. A rotating, high-frequency (15–20 MHz), transurethral 8F ultrasound microprobe associated with an injection device (Sonoject; AMI, Feldkirch, Austria) enabled visualization of the rhabdosphincter and injection of the myoblast suspension with precision. In a relatively simple procedure the probe was gently inserted up the urethra into the bladder. The bladder neck and the omega-shaped muscle, located at mid-urethra, were ultrasonographically identified by slowly moving the probe. After the rhabdosphincter was identified, 26 injections of 50 to 100 μL were performed transurethrally under direct visualization at 2 different levels of the rhabdosphincter (Fig. 2). To minimize leakage of the myoblast suspension at the puncture sites, the needle was extracted only a few seconds after each injection was made. Upon completion of the injection procedure, hypoechogenic spots were clearly seen in the rhabdosphincter. The first 18 participants received intravenous anesthesia but the remaining 20 patients only received local anesthesia with a lidocaine gel.

Immediately following the myoblast injections, the participants self-administered ES intravaginally at home for 5 weeks to enhance cell integration. The device was the contic+ (tic Medizintechnik, Dorsten, Germany) and it was used according to the manufacturer's instructions. However, to ensure that any clinical improvement 6 weeks after the injections would not be due to ES alone, the participants had previously undergone a first 5-week ES cycle, just after muscle tissue was harvested. The objective and subjective measurements obtained upon completion of each ES cycle were then compared (Fig. 1).

To ensure safety, vital signs and common laboratory values for urine and blood were monitored. Moreover, particular attention was paid to possible immediate and short-term onset of complications of the muscle tissue harvest and myoblast injections. Possible complications were surgical injury, local inflammation, urinary tract infection, pelvic pain, urinary retention, voiding dysfunction, de novo urgency, hyperplasia, and tumor formation.

Physical examination notes, fixed bladder volume stress test result, entries in the 3-day voiding diaries for urinary incontinence episodes (UIE) and number of voids (NOV), degree of trouble living with SUI on a visual analog scale (VAS), score on the modified Patient Global Impression of Improvement scale (PGI-I), and score on the Incontinence Quality-of-Life questionnaire (I-QOL) were recorded at baseline; after completion of the first ES cycle that followed the muscle tissue harvest; and 6 weeks after the myoblast injections, after the second ES cycle was completed.

The values obtained after completion of each ES cycle for the different variables of interest were compared using the paired *t* test

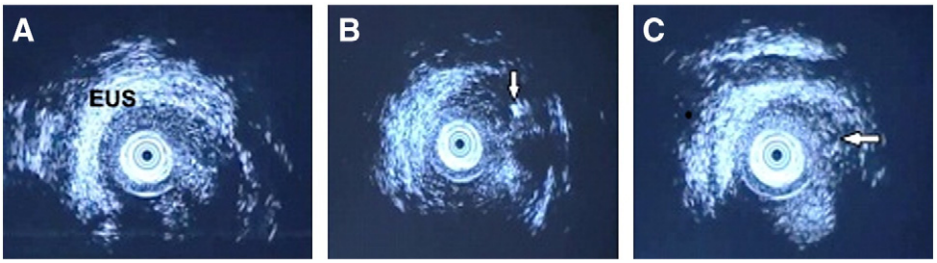

Fig. 2. Transurethral ultrasound-guided myoblast injection. A, The external urethral sphincter (EUS) was identified. B, The vertical arrow indicates the site of an injection. C, The horizontal arrow indicates small myoblast deposits within the sphincter.

and the Wilcoxon signed rank test in cases of parametric and nonparametric variables, respectively. The χ^2 test was used for categoric variables and the Fisher exact test when sample sizes in the contingency tables were small. Spearman correlation coefficients were calculated to analyze any association between number of myoblasts injected and the various variables. $P<0.05$ was considered statistically significant.

3. Results

Thirty-eight women participated in the trial (median age at baseline, 52 years; median parity, 2 [range, 1–4]; mean ± SD body mass index [calculated as weight in kilograms divided by the square of height in meters], 26.6 ± 4.4; and mean maximal urethral closure pressure, 57 ± 24 mm Hg). One participant did not present to the follow-up visit at 6 weeks because of other obligations but her available data were included in the analysis.

No serious adverse events were reported in the course of the study. There were no notable changes in vital signs or laboratory values. One participant was referred to physiotherapy immediately after the myoblast injections because tenderness had developed at the site where muscular tissue was taken and 2 participants had acute cystitis at the follow-up visit. All conditions resolved with treatment. There were no postoperative cases of urinary retention (defined as a postvoiding urine volume >50 mL), nor were there any cases of de novo urgency, hyperplasia, or tumor development.

A gynecologist performed all phases of the 2 surgical procedures but these procedures were supervised at first, either directly or by reviewing the patients' records. A plastic and reconstructive surgeon supervised muscle tissue harvest in the first patients whereas sphincter identification and myoblast injections were supervised by a radiologist. Both procedures were simple to perform. There were no problems shipping the muscle samples to a laboratory located abroad or receiving the myoblast solutions from the laboratory. Removing the needed amount of muscle tissue took approximately 10 minutes and the mean duration of the myoblast injection procedure was 15 minutes (range, 10–30 minutes), with shorter durations associated with later patients and longer durations associated with the 18 earlier patients who underwent general anesthesia. Six (33.3%) of these 18 participants required longer monitoring and were discharged on the day following the procedure whereas the 20 patients who underwent the myoblast injections under local anesthesia were discharged after the first voiding, 3 to 6 hours after the procedure. None of the 38 patients required postoperative analgesics. The procedure was well tolerated by all patients, although satisfaction was higher among those who received local anesthesia.

At the follow-up visit 29 participants (78.4%) had a negative result to the stress test, the median UIE count was significantly decreased, and the median VAS and I-QoL scores were significantly improved (Table 1). Whereas no significant changes from baseline were observed following the first ES cycle, there was a significant decrease in the median number of voidings ($P<0.001$). Moreover, 5 participants (13.5%) answered on the modified PGI-I scale that their SUI was "cured", 29 (78.4%) that it was "improved," and 3 (8.1%) that it was "unchanged". There were no significant correlations between any of the studied variables and the numbers of myoblasts injected.

4. Discussion

The present clinical study is among the first to use only autologous muscle-derived myoblasts for the treatment of women with stress urinary incontinence [13–15]. It is also the only one to combine this novel treatment with ES in an effort to enhance postoperative cell integration. No serious adverse events occurred in the 38 participants and the procedures were easily feasible. Six weeks after the myoblast injections, as had been hypothesized, the numbers of participants with positive results to the stress test, the numbers of UIEs, and the numbers of voidings were significant decreased whereas I-QoL scores, VAS scores, and modified PGI-I scores were significantly improved.

No significant correlations were noted between any of the studied variables and the size of the myoblast populations injected, a finding which concurs with a recent study [19]. The latter study reports no dose-dependent effect on SUI improvement in 2 groups of patients treated with myoblast populations similar to those used in the present study. However, it reports a somewhat better SUI improvement among 2 groups of patients treated with populations 2 and 4 times larger than the largest population used in the present study.

Our intraoperative and short-term postoperative safety outcomes concur with those published [13–15,20,21]. Unlike other surgical SUI treatments, myoblast injections were not associated with de novo urgency. Additionally, compared with ES results only, the subjective and objective measurements were significantly improved 6 weeks postoperatively, which implies an effect of the myoblast treatment. The myotubes and new muscle tissue derived from the myoblasts injected in the rhabdosphincter promote sphincter regeneration [22,23] and therefore may lead to the restoration of the sphincter's function [6,12]. On the other hand, new muscle tissue may also cause bulking [13,24]. Improvements were noted 3 to 8 months after the initial injection in a North American trial [13] and first complete responses to treatment were noted after 3 months in a French trial [15], whereas improvement and complete responses were noted 6 weeks postoperatively in the present one.

Table 1
Changes for 38 patients with SUI treated first with ES alone and then with autologous myoblasts injections followed by ES.[a]

Variable	Preoperatively		6 weeks after	*P* value [b]
	Baseline (n = 38)	After 1st ES cycle (n = 38)	myoblast injections followed by a 2nd ES cycle (n = 37)	
No. of UIEs [c]	13 (4–41)	12 (1-35)	5 (0-33)	<0.001
No. of patients with a negative stress test result	0	1	29	<0.001
No. of patients with SUI [d]				
Cured	0	0	5	<0.001
Improved	0	7	29	
Unchanged	38	31	3	
I-QoL score [e]	56.5 (28–92)	63 (29–99)	78 (41–105)	<0.001
VAS score [f]	8 (3–10)	7 (4–10)	3 (0–9)	<0.001

Abbreviations: I-QoL, Incontinence Quality of Life Questionnaire; SUI, stress urinary incontinence; UIE, urinary incontinence episode; VAS, visual analog scale.
[a] The values are given as median (range) unless otherwise indicated.
[b] For comparisons between values obtained after completion of the first ES cycle and those obtained after completion of the ES cycle that followed the myoblast injections.
[c] As noted in a 3-day voiding diary.
[d] The 3 categories are those provided in the modified Patient Global Impression of Improvement instrument.
[e] The possible scores ranged from 22 (worst) to 110 (best).
[f] The scale provided a measure for the trouble of living with SUI; the possible scores ranged from 0 (no trouble) to 10 (unbearable trouble).

The 38 patients self-administered a postoperative ES cycle aiming at improving myoblast integration and new muscle formation following the injections. Since ES alone is considered a successful conservative treatment for the amelioration of pelvic muscle function [25], a control group would have been useful. However, one of the study's inclusion criteria was the failure of a conservative treatment (such as pelvic floor muscle training or ES, whether at home or supervised) acknowledged at least 3 months before inclusion. In the preparation phase of the study the patients indicated that they were unwilling to undergo the same or a similar treatment merely to be part of a control group. To avoid the possibility that the outcome at 6 weeks be predominantly due to the effect of ES, all participants thus self-administered 2 identical ES cycles, one prior to the myoblast injections and another following the procedure, and the outcomes were compared. As expected from the self-reported lack of success of the conservative treatments that they received prior to recruitment, our participants received less benefit from their preoperative ES cycle than would be expected in a larger population of women with SUI. Hence, the vast postoperative improvement observed in the participants was probably an effect of the cellular treatment rather than a cumulative effect of ES.

The present study demonstrates both the safety and feasibility of the 2-step treatment and the rapidity with which it improved SUI. The myoblast injections into the rhabdosphincter were performed in a precise, standard pattern under ultrasound visualization. The method and devices permitted reproducibility as well as accuracy in all patients.

The study's safety and efficacy results were reached without randomization or blinding and after only 6 weeks. Besides, it is impossible to know at this time whether the efficacy results are dose-dependent, whether they will last, and whether they are due to proper tissue regeneration or bulking. A long-term follow-up would enable assessment of the true value of the myoblast injections and, perhaps, determination of the mechanisms of tissue regeneration.

In conclusion, intrasphincteric transurethral ultrasound-guided autologous myoblast injections followed by ES is a safe, precise, and minimally invasive treatment option for SUI. It is well tolerated and relatively easy to learn, but the long-term results of ongoing trials, especially from multicenter trials, are needed to confirm the favorable initial results.

Conflict of interest

The authors have no conflicts of interest.

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