Intraurethral Injection of Autologous Minced Skeletal Muscle: A Simple Surgical Treatment for Stress Urinary Incontinence

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Abbreviations and Acronyms

cSUI = complicated SUI

ICIQ-SF = International Consultation on Incontinence Questionnaire-Short Form

PVR = post-void residual urine

SUI = stress urinary incontinence

uncSUI = uncomplicated SUI

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Purpose: Intraurethral injection of in vitro expanded autologous skeletal muscle derived cells is a new regenerative therapy for stress urinary incontinence. We examined the efficacy and safety of a simpler alternative strategy using freshly harvested, minced autologous skeletal muscle tissue with its inherent content of regenerative cells.

Materials and Methods: A total of 20 and 15 women with uncomplicated and complicated stress urinary incontinence, respectively, received intraurethral injections of minced autologous skeletal muscle tissue and were followed for 1 year. Efficacy was assessed by the number of leakages in a 3-day diary and by ICIQ-SF scores. We calculated the rates of cure, defined as zero leaks in 3 days plus an ICIQ-SF score of 5 or less, and improvement, defined as simultaneous decreases in each outcome measure.

Results: Significant reductions were observed in each group in the mean number of leakages ($p < 0.01$) and in ICIQ-SF scores ($p < 0.001$). In the uncomplicated group cure and improvement were observed in 25% and 63% of patients, and in the complicated group they were noted in 7% and 57%, respectively. No voiding dysfunction developed and only minor adverse events were noted.

Conclusions: Intraurethral injection of minced autologous muscle tissue is a simple surgical procedure that appears safe and moderately effective in women with uncomplicated stress urinary incontinence. It compares well to a more complicated regenerative strategy using in vitro expanded muscle derived cells.

Key Words: urethra; urinary incontinence, stress; regeneration; quadriceps muscle; stem cells

Intraurethral injection of in vitro expanded autologous skeletal muscle derived cells is a new and potentially useful regenerative therapy for female and male SUI.[1–14] However, the cell expansion procedure is categorized as an advanced therapy medicinal product by EMEA (European Medicines Agency) (European Union Regulation 1394/2007) and the need for expertise and regulatory approval is a limiting factor for the cost-effectiveness and clinical relevance of the treatment.[14] Convincing evidence also suggests that the expansion process has a negative influence on cell regenerative potential.[14–16] A technically simple, less advanced approach would be advantageous.[14]

Minced skeletal muscle grafts have remarkable capacity for muscle repair, as discovered and described in detail decades ago by Studitsky[17] and Carlson.[18] This technique was

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recently reintroduced as a potential tissue engineering therapy for volumetric loss of muscle tissue.[19] The muscle cells of the minced fibers die but some donor muscle stem cells (satellite cells) survive, are activated by the inflammatory process and divide into proliferating myoblasts, which ultimately fuse to form new, innervated muscle fibers. As suggested, this simple technique may have been overlooked in the general pursuit of more technically advanced regenerative therapies.[19] However, the potential usefulness of single muscle fibers,[15,16,20] muscle fragments[19,21,22] and muscle strips[23] to deliver satellite cells and other muscle derived regenerative cells in various animal models was confirmed by others.

Accordingly in a clinical phase I trial the effect of surgical implantation of a free muscle strip around the urethra was recently examined in 5 women and 5 men with intrinsic sphincter deficiency and SUI.[24] During the 12-month followup no serious side effects were observed. Subjective effects in men were minimal but in 4 of the 5 women continence improved significantly and 2 recovered normal continence.

The current phase I/II pilot clinical trial was designed to examine the efficacy and safety of a technically simple surgical procedure by which freshly harvested autologous muscle biopsies with their inherent content of satellite cells and other regenerative cells and components[19,20,22,23,25] were minced and injected immediately into the urethral wall in women with SUI. This procedure is categorized as a normal surgical procedure since it involves only minimal manipulation of autologous tissue, which is excised and autotransplanted during the same surgical procedure as specified by EMEA regulations (No. 1394/2007, Annex I) and Danish tissue law (No. 273, January 4, 2006).

METHODS

Patients and Inclusion

Initially a protocol in 20 women classified with uncSUI according to ICI-RS (International Consultation on Incontinence-Research Society) definitions was approved by the local ethics committee in 2009. A feasibility study was performed in the first 4 patients. In 2011 the study was expanded with 15 women classified with cSUI according to ICI-RS definitions and 5 women in the uncSUI group were offered reoperation. The combined protocols are registered on www.clinicaltrials.gov (NCT01323426). The study was performed from April 2010 to June 2013. Written and oral informed consent was obtained from all patients before enrollment. Before study inclusion all patients received conservative treatment, including at least 3 months of pelvic floor muscle training supervised by a physiotherapist. The Appendix lists study inclusion and exclusion criteria for each group.

Muscle Biopsy and Mincing

All procedures were performed in an outpatient clinic and patients were released home 2 to 4 hours postoperatively. The first 4 patients received general anesthesia and the remainder received a combination of local analgesia supplemented with intravenous infusion of fentanyl.

Open muscle biopsy was taken from the vastus lateralis muscle since it is large and easily accessible. In the first 4 procedures the muscle specimen was collected in a stainless steel cup with 20 ml sterile isotonic saline. It was immediately fragmented with a scissor until it could pass through a 14 gauge injection needle and through subsequently smaller needles ending with 22 gauge. The solution was centrifuged and the pellet was resuspended in 3 ml isotonic saline. Without further manipulation the muscle suspension was used for injection. The mincing procedure required 20 to 30 minutes.

In the remaining 36 procedures the muscle specimen was collected in 6 ml sterile isotonic saline, immediately fragmented with a scissor and needles ending with 18 gauge and then used for injection. The new procedure was quicker (10 minutes) and potentially preserved satellite cell activating substances released from the fragments.

Periurethral Injection

To visualize the urethra a vaginal ultrasound probe was placed in front of the external urethral orifice. Injection sites were at the 9, 12 and 3 o'clock positions at 0.5 to 1 cm from the orifice. At each site the needle was inserted along the urethral wall just outside the smooth muscle layer and introduced to approximately 1 cm from the bladder neck. During needle retraction the muscle suspension was released at the presumed location of the rhabdosphincter in an area corresponding to the mid urethra. At each injection site 1 ml muscle suspension was released in the first 4 patients and 2 ml were released in the remainder.

Primary and Secondary Outcomes

As the primary outcome, efficacy assessment was based on a 3-day leakage diary preoperatively and also performed at 6 and 12 months of followup. As secondary outcomes, efficacy was further assessed by the ICIQ-SF with a score range of 0 to 21. Cure was defined as a decrease to zero leaks in 3 days and a simultaneous decrease in ICIQ-SF score to 5 or less. Improvement was defined as any simultaneous decrease in the number of leaks and in ICIQ-SF score. Safety was assessed by the occurrence and severity of adverse events during surgery and followup. The maximum flow rate and PVR were measured to evaluate potential voiding dysfunction.

Urethral pressure measurements were made at rest and during squeezing using urethral pressure reflectometry as previously described[26,27] except in the first 4 patients, for whom the method was not available.

Statistical Analysis

Statistical analysis was done with InStat® 3.1. Baseline and followup data were compared using repeated measures ANOVA followed by the Dunnet test. Differences between groups were analyzed using parametric or nonparametric tests as appropriate. Correlations were tested with the Spearman test. Statistical significance was considered at $p < 0.05$.