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Adipose-derived stromal cell transplantation for treatment of stress urinary incontinence

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ABSTRACT

We aimed to investigate the application of adipose-derived stromal cells in the treatment of stress urinary incontinence (SUI). Animal models of stress urinary incontinence were established with Sprague–Dawley female rats by complete cutting of the pudendal nerve. Rat adipose-derived stromal cells were isolated, cultured and successfully transplanted into animal models. Effects of stem cell transplantation were evaluated through urodynamic testing and morphologic changes of the urethra and surrounding tissues before and after transplantation. Main urodynamic outcome measures were measured. Intra-bladder pressure and leak point pressure were measured during filling phase. Morphologic examinations were performed. Transplantation of adipose-derived stem cells significantly strengthened local urethral muscle layers and significantly improved the morphology and function of sphincters. Urodynamic testing showed significant improvements in maximum bladder capacity, abdominal leak point pressure, maximum urethral closure pressure, and functional urethral length. Morphologic changes and significant improvement in urination control were consistent over time. It was concluded that periurethral injection of adipose-derived stromal cells improves function of the striated urethral sphincter, resulting in therapeutic effects on SUI. Reconstruction of the pelvic floor through transplantation of adipose-derived cells is a minimally invasive and effective treatment for SUI.

1. Introduction

Urinary incontinence affects over 200 million people worldwide and its presence in the increased aging population is raising associated healthcare costs. Stress urinary incontinence (SUI) is diagnosed most often in women, influencing patients' daily work, home life, social activities, quality of life and family relationships. Neuroanatomical degeneration near the external urethral sphincter of the complex urethral sphincter system (urethral sphincter complex) has been implicated in SUI pathophysiology (Damaser et al., 2003). High mobility of the urethra and intrinsic urethral sphincter dysfunction are fundamental factors contributing to SUI development (Furuta et al., 2007a). Treatment focuses on the middle urethra and urethral sphincter because urethral closure, and thereby continence, is influenced by pudendal nerves innervating striated muscles and the striated muscle sphincter (rhabdosphincter) of the middle urethra (Furuta et al., 2007b,c). Commonly applied therapeutic measures include open surgery such as retropubic colposuspension or bladder neck suspension, or pelvic sling procedures using synthetic materials or tension-free transvaginal tape to correct urethral support defects (Harding and Thorpe, 2008). Although these procedures have a reported 5-year cure rate >80%, they may also result in complications or symptom recurrence (Roche et al., 2010), and transvaginal tape reinforces weak pelvic floor muscles without correcting urethral sphincter deficiency (Furuta et al., 2007c). Reconstructing a defective urethral sphincter through periurethral injection of filling agents (e.g., bovine collagen, silicone particles, autologous chondrocytes, and teflon) is an effective, minimally invasive treatment for SUI with combined intrinsic sphincter defect (Smaldone and Chancellor, 2008). However, even with ideal short-term effects, generalization in clinical practice is precluded by complications, including chronic inflammation, rejection, periurethral abscess, filling material displacement, and lower urinary tract obstruction (Smaldone and Chancellor, 2008).

Recent rapid development of molecular biology, especially stem cell biology, has enabled great progress in basic research of cell therapy, strengthening the promise of stem cell injection therapy for SUI (Furuta et al., 2007b,c; Bajada et al., 2008; Levac et al., 2005). Tissue engineering techniques targeting regeneration morphology and tissue and organ function especially benefit urology practice (Mizuno, 2009; El Tamer and Reis, 2009; Stock and Vacanti, 2001). Studies of cell-based therapies for SUI and other diseases have focused on the potential of autologous stem cells derived

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from bone marrow (Furuta et al., 2007b; Bajada et al., 2008; Levac et al., 2005), muscle tissue (Stangel-Wójcikiewicz et al., 2010; Carr et al., 2008; Strasser et al., 2007; Mitterberger et al., 2008) and adipose tissue (Taha and Hedayati, 2010; Strem et al., 2005; Schaffler and Büchler, 2007). Obtaining bone marrow stem cells (BMSCs) involves a painful procedure requiring general anesthesia and has a low harvest rate after processing, discouraging clinical application (Furuta et al., 2007b). Muscle-derived stem cells (MDSCs) are easily obtained from striated muscle biopsy, demonstrate self-renewal and multipotent differentiation, have better yield than BMSCs, and deliver neurotrophic factors for urethral sphincter regeneration (Smaldone and Chancellor, 2008). However, after many studies of BMSCs and MDSCs, abundant, fibroblast-like adipose-derived stromal cells (ADSCs) have become a new research focus.

Zuk et al. (2002) found that adipose tissue contains multipotentiated mesenchymal stem cells having the same embryonic layer origination and similar biological properties as BMSCs. Directly inducted ADSCs differentiate into nervous system cells, smooth muscle cells, cartilage cells, and hematopoietic cells (Smaldone and Chancellor, 2008; Bajada et al., 2008; Levac et al., 2005; Zuk, 2010). ADSCs are easily obtained by lipoaspiration, are abundant in adipose tissue, have vigorous proliferating capability in vitro and multi-directional differentiation potential similar to MDSCs. Further, their convenient isolation and cultivation techniques indicate good application prospects (Taha and Hedayati, 2010; Strem et al., 2005; Schaffler and Büchler, 2007; Guilak et al., 2006; Fu et al., 2010).

We evaluated the efficacy of transplanting ADSCs in established animal models of SUI, evaluating definitive effects of stem cell transplantation through urodynamic testing and observing morphological changes of the urethra and surrounding tissues in rats before and after transplantation.

2. Materials and methods

2.1. Animals

Four-week-old SPF-grade female Sprague–Dawley (SD) rats with average weight of 110 g were provided by Shanghai Slack Experimental Animals Ltd. (License number: SCXK (Shanghai) 2007-0005). The protocol for this animal study was approved by the Institutional Animal Care and Use Committee of Fujian Medical University.

2.2. Construction of SUI rat model

Rat models of SUI were constructed following the methods of Damaser et al. (Damaser et al., 2003; Hijaz et al., 2008), however the present study was modified by mutilating the pudendal nerve, not just injuring it. Briefly, female rats were anesthetized and fixed and dorsal midline skin incision and bilateral muscle incisions on the back were adopted. The pudendal nerve was identified at the ischiorectal fossa under microscope and was dissociated. Using a minimally invasive surgical clamp, a length of 0.5 cm bilateral pudendal nerve branches near the obturator were mutilated and the incision was closed. Successfully established rat models were confirmed by urodynamic tests. Early in model construction among 40 rats, 4 rats died under anesthesia, 3 died after model construction. Thirty-three rats (20 in transplantation group, 13 in control group) were retained during final model construction. The model construction started with 4-week-old rats and lasted 3 weeks. Therefore the rats were 7–8 weeks old when measurements were taken, accounting for differences compared to 2-week-old rats.

2.3. Adipose-derived stromal cells isolation, identification and prelabeling

Primary isolation, culture purification and passage of rat ADSCs were performed following methods of Wu et al. (2010). Briefly, an inguinal fat pad was collected from each rat aseptically. Fat tissue samples were cut into small pieces and digested with 0.75% type I collagenase (Sigma Corporation, USA). Cells were then filtered through 200 μm stainless steel mesh. After centrifugation, cells were re-suspended in complete DMEM high-glucose medium (HyClone Inc., USA) containing 10% fetal bovine serum (Sigma Corporation, USA) and incubated with 5% CO_2 at 37 °C overnight. Non-adhering cells were removed the next day. Cells were passaged when they reached 80% confluence.

To study cellular differentiation potential, second-generation cells in logarithmic phase were collected. Adipogenic and osteogenic differentiation function of ADSCs were identified according to adipogenic and osteogenic induction scheme. ADSCs were also identified by the detection of cell surface specific markers in flow cytometry analysis.

The cells for transplantation were labeled. One hour before transplantation, sterile Hoechst 33258 (30 mg/l) (HyClone Inc., USA) was added into third- or fourth-generation ADSCs to label the cells as described by Lee et al. (2003). Samples were incubated at 37 °C for 10 min and rinsed with PBS. Unbound Hoechst 33258 was removed and then resuspended in high-glucose DMEM and refrigerated at 4 °C for up to but not exceeding 1 h prior to transplantation while rats were being anesthetized. Normally the cells were used in transplantation directly after suspension preparation.

2.4. ADSC transplantation

In this study, the interval between pudendal nerve mutilation and ADSC transplantation was three weeks. Successfully established rat models received intraperitoneal anesthesia with injection of 10% chloral hydrate (0.3 ml/100 g rat body weight) and a lower abdominal midline incision was made. Urethral tissue was exposed and 300 μl labeled ADSC suspension (in serum-free medium) was injected at 3, 9, and 12 o'clock around middle and lower urethra using a micro-syringe under direct view (total cells approximately 5×10^6). Control group rats received injections of 300 μl serum-free medium. Rats were sacrificed 3, 7, and 14 days post-transplantation, and urethral tissue was removed and frozen sections of the urethra were made (7–8 μm). Migration, distribution, and proliferation of labeled cells in vivo were observed using the LSM510 laser confocal microscope imaging system (Zeiss, Germany). Ultrastructure changes of urethra tissue (striated urethral sphincter) were observed under electron microscope (type Hu-12A transmission electron microscope, Hitachi, Japan). Pathological changes were observed microscopically (inverted phase contrast microscope, Olympus, Japan) with H&E stain protocols and Masson's and Mallory's staining.

2.5. Urodynamic testing

Laborie UDS-600 urodynamic testing instrument (Laborie Company, Canada) was used to perform urodynamic tests. Briefly, rats were anesthetized with intraperitoneal injections of 10% chloral hydrate (0.3 ml/100 g rat body weight) and fixed in supine position. Two sterile epidural catheters were inserted into the urethra successively and were used for bladder pressure measurement and bladder perfusion. Rat bladder was emptied, and pressure measurement urethral catheter (1 mm diameter) was connected to a pressure sensor. The perfusion catheter was connected to the