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IMPROVEMENT OF URETHRAL SPHINCTERIC FUNCTION BY PERIURETHRAL INJECTION OF LOW SERUM CULTURED ADIPOSE-DERIVED STEM CELLS: EXPERIMENTAL INVESTIGATION IN RATS

Hypothesis / aims of study
Cell therapy for the regeneration of injured tissues has recently been extensively investigated at an experimental level, and its clinical application in a variety of fields has also been in progress. Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can proliferate in culture and are able to differentiate into a variety of mesenchymal cell phenotypes. Thus far, MSCs have been mainly harvested from bone marrow, a tissue source that has many limitations. Adipose tissue contains multipotent cells that are similar to MSCs, and the abundance of stem cells in the adipose tissue is 100-fold higher than that in the bone marrow. This finding has generated major interest because, unlike bone marrow cells, adipose tissue can be easily and safely harvested in large quantities with minimal morbidity, making it an appealing source for cell therapy.
The purpose of the present study was to assess the effects of periurethral injection of cultured adipose-derived stem cells (ADSCs) and to develop a new strategy for autologous cell therapy for stress urinary incontinence.

Study design, materials and methods
The present study was performed to test the effects of injected ADSCs on rat urethral sphincteric function. ADSCs ($3x10^6$), GAX collagen, or vehicle (control) was injected to the periurethral portion of rats (each n=7). At 2 and 4 weeks after injection, a spinal cord transection was performed on anesthetized rats. Leak point pressure (LPP) was measured before and after the resection of the pelvic nerves (with and without urethral closure reflexes) and compared among the 3 groups. The urethral tissues were taken for histological observation.
A cell trace experiment was also performed by injecting the ADSCs obtained from green fluorescent protein (GFP) transgenic rats to nude rats (n=6). ADSCs were collected from GFP-transgenic rat and were cultured and injected into the periurethral zone of the nude rats.

Results
At 2 weeks, both the ADSCs and collagen groups showed significantly higher LPP than the control group. At 4 weeks, the increase in LPP in the ADSC group remained, while LPP in the collagen group decreased to baseline levels. In the absence of urethral closure reflex after transection of the pelvic nerves, LPP in the ADSC group was significantly higher than that in the other two groups. Histologically, periurethral injection showed compression of the urethral lumen in the ADSC and collagen groups, although collagen was reduced in volume at 4 weeks. At 4 weeks, injected ADSCs were observed at the injected site, and most of the cells were positive for myogenic antigens including alpha smooth muscle actin (alpha-SMA), desmin, and calponin I. In the cell trace experiment, most of the injected ADSCs (64%) were merged with alpha-SMA positive cells at 4 weeks.

Interpretation of results
The ADSCs injected in the periurethral area remained at the injected site over a long period and changed into smooth muscles. It was supposed that the periurethrally injected ADSCs would increase the urethral resistance not only by providing bulking effects but also by acquiring contractile function.

Concluding message
Periurethral injection of autologous ADSCs capable of myogenic differentiation causes a long-lasting increase in urethral resistance in rats. ADSCs cultured in low serum medium are a potential stem cell source for the treatment of stress urinary incontinence.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**the Animal Experimentation Guidelines of Nagoya University Graduate School of Medicine**