14

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IMPLANTATION OF AUTOLOGOUS BONE MARROW-DERIVED CELLS RECOVERS URETHRAL SPHINCTER IN RABBITS

Hypothesis / aims of study

Stress urinary incontinence (SUI) can be grouped into two major categories, urethral hypermobility and intrinsic sphincter deficiency. SUI resulting from intrinsic sphincter deficiency, which is characterized by malfunction of urethral closure mechanism, has few effective treatments. In the urinary continence system, urethral closure pressure for prohibiting the leakage of urine is produced by the urethral sphincter, composed of striated and smooth muscle. Thus, replacement, enhancement, or recovery of the striated and smooth muscle in the urethral sphincters has great potential of treatment for the intrinsic sphincter deficiency-related SUI. The aim of this study was to determine if autologous bone marrow-derived cells implanted into the injured urethral sphincters could differentiate into striated and smooth muscle cells and reconstruct urethral sphincters.

Study design, materials and methods

Sixteen female New Zealand White (NZW) rabbits at postnatal week 10 were used, and anesthetized by inhalation of sevoflurane in each experiment during this study. Bone marrow cells were flushed out from a femur, which were inserted with two pediatric bone marrow needles. The harvested bone marrow cells were cultured on type I collagen-coated culture flasks for 10 days. At 8 days of the culture, the cells were transfected with green fluorescence protein (GFP) gene. At 10 days of the culture, GFP-labeled adherent, proliferating bone marrow-derived cells were used. Seven days prior to implantation, urethral sphincters located at the bladder neck were cryo-injured by spraying the liquid nitrogen for 15 sec. At 10 days of the culture, and 7 days after the injury operation, the cultured autologous bone marrow-derived cells ($2x10^6$ cells) were implanted into the injured regions (n=8). For the cell-free injection control group, the cell-free solution was similarly injected (n=8). At 7 and 14 days after implantation, leak point pressure (LPP) values of the rabbits (n=4) were measured by cystometric investigations with anesthesia. After the cystometric investigation, the bladder necks were fixed and embedded for immunohistochemistry investigations. The urethral sphincters were estimated by using myoglobin, alpha-smooth muscle actin (SMA), Pax7, and GFP antibody was used as a marker of striated muscle, smooth muscle, myoblast, and implanted bone marrow-derived cells, respectively.

Results

Prior to the cell-implantation experiments, we examined 7-day old cryo-injured urethral sphincters. The cryo-injured internal urethral orifices appeared to be relaxed, creating a larger orifice. The injured urethral sphincters lost muscle layers that were composed of the myoglobin-positive striated and SMA-positive smooth muscle cells. The LPP of the animals having the injured urethral sphincter, 7.33±0.27 cmH_2O, was significantly lower ($P<0.01$) than in the sham-injured animals, 12.58±1.26 cmH_2O.

The both 7- and 14-day cell-free control injected regions had a few myoglobin- and SMA-positive cells. In contrast, the both 7- and 14-day cell-implanted regions had abundant myoglobin- and SMA-positive cells compared to control regions. The proportion of myoglobin- and SMA-expressing areas in the cell-implanted regions was significantly higher than these in the control ones. At 7 and 14 days after implantation, some of the implanted cells identified by the presence of GFP antibody were simultaneously positive for myoglobin antibody. These double positive cells showed that the implanted autologous cells differentiated into striated muscle-like cells (figure). Also, some GFP-positive implanted cells were simultaneously positive for SMA or Pax7 antibody. These double positive cells showed that the implanted cells differentiated into smooth muscle- or myoblast-like cells. The GFP-positive cells that were positive for myoglobin or SMA appeared to form contacts among themselves, creating striated or smooth muscle layer-like structures. The GFP-positive cells that were positive for Pax7 were widely distributed within the reconstructed muscle layers. The LPP of 7-day old cell-implanted rabbits, 13.15±2.82 cmH_2O, tended to be higher than that of the 7-day old cell-free control group, 8.13±2.43 cmH_2O, but the difference was not statistically significant. At 14 days, the LPP of the cell-implantation group, 17.82±1.31 cmH_2O, was significantly higher than that of the control group, 11.78±3.23 cmH_2O ($P<0.05$).

Interpretation of results

Just prior to the implantation, the cultured cells were positive for GFP antibody, however, they were negative for myoglobin or SMA antibody. At 7 days, the majority of both GFP and myoglobin or SMA double-positive cells were mononuclear. While we cannot definitively exclude the possibility of cellular fusion, the findings suggest that the number of these double-positive cells formed by cellular fusion was small. Therefore, the GFP-labeled implanted cells differentiated into myoglobin-positive striated muscle-like cells and SMA-positive smooth muscle-like cells within the injured regions.

The majority of the patients having intrinsic sphincter deficiency-related SUI are elderly and have other diseases. Aging and/or disease processes may affect the potential of bone marrow cells for differentiation, and this needs to be investigated the appropriate animal models. However in the study reported here, we used young and healthy rabbits to investigate the potential for urethral reconstruction as first step investigation.

At 7 and 14 days after cell implantation, some GFP-labeled implanted cells were simultaneously positive for Pax7, suggesting that they had myoblast-like properties. The myoblasts properly differentiate into striated or smooth muscle cells according to surrounding environment. In the event that the newly differentiated striated and/or smooth muscle-like tissues and structures spontaneously regress or are lost for other reasons, the presence of the myoblast-like cells could ensure the replacement of the lost cells. Thus, the effects of treatments might be maintained for long time.

While our animal model of intrinsic sphincter deficiency SUI may have some limitations, our experimental rabbits lost urethral sphincter tissues composed of typical striated and smooth muscle cells, and exhibited lower LPP compared to sham-injured rabbits. At 7 days, the LPP of cell-implantation group increased slightly compared to the cell-free injected controls, and by 14

days the improvement was statistically significant. The close correlation of these changes with the accompanying structural changes in the sphincter suggested that the improvement was related to the recovery of muscle layers.

Concluding message

The autologous bone marrow-derived cells implanted into injured rabbit urethral sphincters differentiated into striated or smooth muscle-like cells. The differentiated cells became organized into layered muscle structures. Recovery of the urethral sphincters was accompanied by improved urethral closure pressure for prohibiting the leakage of urine. Therefore, the implantation of autologous bone marrow-derived cells has great potential to be an effective treatment for the intrinsic sphincter deficiency-related SUI.

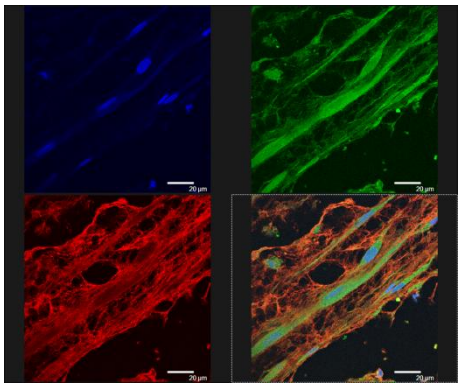

Figure. Some of the GFP-positive cells (green, right, top) were simultaneously positive for myoglobin antibody (red, left, bottom). These double positive cells (right, bottom) showed that the implanted cells differentiated into striated-like cells. Blue, left, top: nuclei. Bar: 20 μm.

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Is this a clinical trial?	**No**
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	**National Institutes of Health Animal Care Guidelines and the Animal Ethics Committee of Shinshu University School of Medicine**