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Original Article

Exploring the dynamics of progenitor cells in the urethra after simulated birth trauma in mice

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ABSTRACT

Objective: To examine the alteration in the cellular dynamics of the urethral tissue after a simulated birth trauma in a mouse model.
Materials and Methods: A total of 36 B6 mice received vaginal distention treatment, and four untreated mice were used as controls. Specimens were collected every 24 hours after the injury for 9 consecutive days and examined using immunofluorescent staining for cell markers including c-kit, smooth muscle actin (SMA), and vimentin. Confocal microscopy was used to localize the stained cells and determine the cell number.
Results: The number of c-kit positive cells increased after the 1st day and peaked on the 3rd day. The amount of SMA positive cells rapidly reduced to its lowest count on the 1st day and maintained a statistically significant low cell number than that at the basal level for 4 days after vaginal distension. The cell number finally returned to basal level on the 9th day. The amount of vimentin positive cells increased dramatically after the 1st day and plateaued from the 3rd day to the 9th day. The number of vimentin positive cells in the plateau phase was significantly higher than that of the control group.
Conclusion: Our study confirmed that the dynamic change in different cell types after the urethral injury was dependent on the nature and physiology of the wound repairing cells during the tissue healing process. It might be a simple animal model to study birth trauma repair; however, the varied progenitor cell activity in different species should also be considered.

Introduction

The mechanisms of birth trauma caused by vaginal delivery (VD) are complicated [1]. Traumatic damage to fascial and muscular support structures in the vagina and urethra during childbirth may be an important contributor to incontinence and organ prolapse [2,3]. Determining the wound healing process at the lesion site might be a suitable way to explore the pathogenesis of diseases associated with birth trauma such as pelvic organ prolapse and stress urinary incontinence. As is known, the wound healing process imposes an ordered sequence of events at the lesion sites, including hemostasis, inflammation, proliferation, and remodeling by fibrosis [4–7]. Fibrosis results from tissue replacement by an excessive accumulation of distorted, nonfunctional, and excessive accumulation of scar tissue and results in many clinical problems [8].

In our previous study, we found that there might be two repair systems involving progenitor cells and fibroblasts located in the smooth muscle layer of the injured urethral tissue [9]. The results of this study indicated a need for an animal model to study tissue re-engineering in birth trauma. However, we were unable to confirm whether the recovery in the number of smooth muscle cells was statistically significant because experimental animal numbers were not adequate. It was also interesting to determine whether the simple animal model could really elicit a wound healing process by

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fibroblasts and progenitor cells. In this study, we used statistically adequate animal numbers to quantify the dynamic pattern of urethral wound healing at the cellular level. For more statistical reliability and evidence, we extended the number of mice following the same procedure in our previous animal study.

Injured urethral tissue is an important factor in the study of urinary incontinence associated with birth trauma, and in our previous experiments, the c-kit positive cells only appeared in the urethral circle. Based on this result, we chose this area as a hot spot to analyze the pattern of cell number change in this present study. The findings may be of merit in the study of VD pathophysiology, potential therapeutic targets for treating postpartum urinary incontinence, and the regeneration of injured urethral tissue resulting from birth trauma in a simple animal model.

Materials and methods

A total of 40 virgin female C57BL/6J mice from the National Laboratory Animal Center, at roughly 9 weeks of age, were used in this study. A lubricated, spherical head of 7.5 mm in diameter was inserted to enlarge vaginal capacity for 30 minutes under isoflurane anesthesia. The vaginal distention procedure was modified from a previously described protocol to induce urethral tissue trauma [10]. For the injury groups of 36 mice, single distention was given; the remaining four mice received no treatment but just received the same anesthesia procedure, and served as the control group.

Tissue specimens were collected from four of the mice at each time point on Days 1 through to Day 9 after injury. After euthanization under isoflurane anesthesia, all tissues including the whole urethra, vagina, lower segment of the rectum, and surrounding connective tissues were removed by transverse cuts. Mice were sacrificed by decapitation after tissue removal. The specimens were embedded in Optimal Cutting Temperature (OCT) compound. Specimens were then taken out to conduct immunofluorescent staining. A confocal microscope was used for imaging. The animal study protocol was granted approval by the Institutional Animal Care and Use Committee at Chang Gung Memorial Hospital, Keelung, Taiwan. In addition, all of our care and work involving animals was in line with the guidelines of the European Commission Directive 86/609/EEC.

The specimens were subjected to histological examination and immunofluorescent staining. Various cell markers were used to identify the cell type at the urethral–vagina intersection and the urethra circle. The c-kit antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used to identify the progenitor cells. Smooth muscle actin antibody (alpha-SMA; Sigma–Aldrich, St. Louis, MO, USA) was used to identify smooth muscle cells. Vimentin antibody (Santa Cruz Biotechnology, Inc.) was used to examine fibroblasts.

Confocal microscopic examination, at a magnification of ×630 (10 × 63), was carried out to examine the sections from whole urogenital tissues. Pictures were taken from completely continuous viewpoints around the circumference of the urethra, including some parts of the anterior vaginal wall, as shown in Figure 1. This allowed us to count all target cells numbers completely but sample randomly, while limiting the volume of statistical analysis. The cell number was determined based on Hoechst staining for total cells, c-kit positive for progenitors, SMA positive for smooth muscle cells, and vimentin positive for fibroblasts.

For statistical analysis, each data point obtained from experiments of tetraplicate assays is presented as mean ± standard deviation. The statistical analysis was performed using Student *t* test.

Results

In the present study, all c-kit positive cells were still only located within the smooth muscle in the urethral–vagina intersection and the urethral circle. There were no c-kit positive cells in the layers of the vaginal fascia and the vaginal muscle. Conversely, the positions of vimentin positive cells were found predominately in the urethral and vaginal surroundings. They were located in the vaginal fascia, the vaginal smooth muscle, and the urethral smooth muscle layers. Briefly, we reconfirmed in the present study that our methods could reproducibly induce a real trauma reaction of progenitor cells and that the hot spots were located in the circumference of the urethra, especially within the smooth muscle in the urethral–vagina intersection.

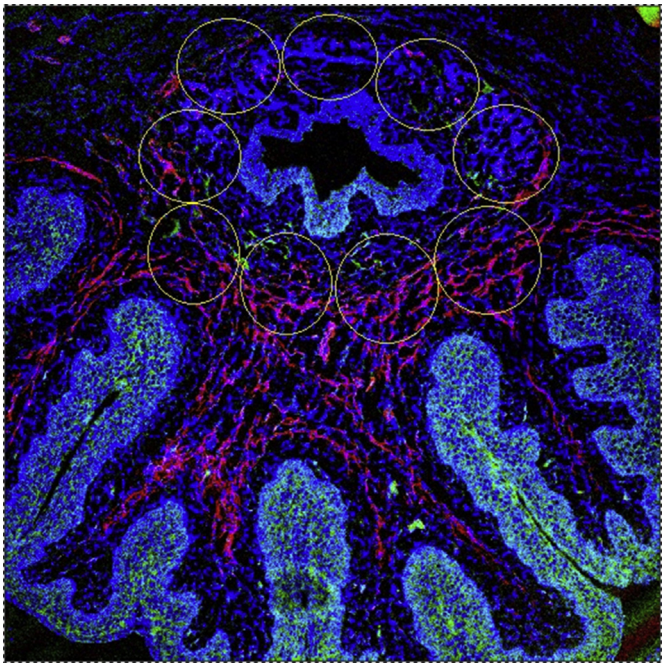

Figure 1. Pictures were taken from completely continuous viewpoints around the circumference of the urethra. Pictures of positive-stained cells were taken by confocal microscopy at a magnification of ×100. Cell number was counted in each observation area at a magnification of ×630. Circle: pictures were taken from continuous observation fields around the urethra and some parts of the anterior vaginal wall. Green stain = c-kit stain; red stain = smooth muscle; U = urethra; V = vagina.

Table 1 demonstrates the dynamic change of the three cell types at the urethral–vaginal intersection during the observation period. A graphical representation of this can be seen in Figure 2. The amount of SMA positive cells rapidly reduced to its lowest point on the 1st day after injury and maintained a low cell number for 4 days after trauma. The decreasing number of SMA positive cells showed a statistically significant difference to the basal level within 4 days after vaginal distension. The cell number of SMA positive cells increased beginning on the 5th day and returned to basal level on the 9th day after trauma. The ratio of SMA to Hoechst cells showed no statistically significant difference to the control group from the 5th day after trauma. This indicates that the number of smooth muscle cells in the local urethral area increased gradually until it was close to that of the control group.

Interestingly, the number of c-kit positive cells increased after the 1st day after injury and peaked on the 3rd day. It then dropped swiftly. The consecutive changes were below the baseline (i.e., the control group) until the 9th day. The number of c-kit positive cells only increased significantly on the 2nd day and 3rd day after vaginal dilatation. This is a little different from the results of our prior study, where the amount of c-kit positive cells increased from the 1st day after trauma before almost completely returning to a level similar to that in the control group on the 6th day after trauma.