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ORIGINAL ARTICLE

Usefulness of An Anal Sphincter Injury Mouse Model by Means of a Balloon Catheter and a New Method of Evaluating Anal Sphincter Function

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Abstract

Background: The incidence of fecal incontinence is ~2%, and the associated symptoms significantly impact daily life. New treatment methods including electrical stimulation and regenerative therapy using stem cells for fecal incontinence have been reported. We explored the usefulness of an anal sphincter injury mouse model by means of a balloon catheter and focused on the defecation status of mice as a novel method for evaluating anal function. We examined the utility of the mouse model of anal sphincter injury and the efficacy of electrical stimulation as a treatment modality using this model.

Methods: A 10-mm balloon catheter was used to create an anal sphincter injury model in mice. Sphincter function was evaluated in the noninjured (n = 4), injured (n = 4), noninjured electrical stimulation (n = 4), and injured electrical stimulation (n = 4) groups. Defecation status (defecation frequency in 24 h and fecal weight per stool) and pathological evaluation were used for comparison.

Results: The defecation frequency increased and the fecal weight per stool decreased significantly in the anal sphincter injury model. Pathological evaluation revealed that anal sphincter tears occurred the day after the injury. Meanwhile, the defecation frequency improved on d 7, and the fecal weight per stool gradually normalized to that of the control group and exhibited significant sphincter muscle hypertrophy in the electrical stimulation group.

Conclusion: Anal sphincter injury using a balloon catheter in mice allowed us to create a uniform model. The evaluation of defecation status in mice is a useful method for comparatively evaluating anal function.

KEYWORDS

anal sphincter, catheter, electrical stimulation, fecal incontinence

1 | INTRODUCTION

Fecal incontinence is defined as an "involuntary loss of liquid or solid stool that is a social or hygienic problem."[1,2] Its prevalence is reported to be ~2% and increases with age by ~11% and 26% in men and women >50 y of age, respectively.[3] Fecal incontinence has a significant impact on daily life.[4] Current treatment options in clinical practice include dietary medical management and pelvic floor rehabilitation as nonsurgical management, whereas sphincter repair and sacral neuromodulation are surgical management options.[5]

New treatment methods, including electrical stimulation, have recently been developed as novel, less invasive, and more effective treatment options. The usefulness of sphincter regeneration using stem cells has been reported in the field of regenerative medicine.[6-8] However, there is a lack of consensus regarding the clinical application of these therapies. For example, in electrical stimulation the appropriate power, frequency, and duration of stimulation are unclear, and in stem cell-based therapies, no established protocols have been proposed regarding which tissue-derived cells should be used (eg, autologous myoblasts, autologous adipose-derived mesenchymal stem cells, or others). Many considerations have not yet been addressed, and further studies are required.

To evaluate the efficacy of treatments, most previous reports have used rats in anal sphincter injury models; however, there is no uniformity regarding the method of injury, with some models using incisional injury and others using freezing injury; hence, a more uniform method is required.[9,10] Meanwhile, no studies have used mice, possibly because their smaller size makes it difficult to create a model and achieve uniform injury using conventional methods of anal sphincter injury.

However, mice are more widely used than rats as models for human diseases, and we believe that mouse models are effective for validating diverse models.[11] Therefore, we sought to explore the feasibility of an experimental mouse model using a balloon catheter to simulate anal sphincter injury. In this study we used a balloon catheter to create a model of anal sphincter injury in mice and examined the therapeutic effects of electrical stimulation. Anal function has generally been assessed based on anal pressure and pathological findings.[9,10] However, it has been reported that there is a disconnect between anal pressure and symptoms; in clinical practice, the patient's actual symptoms are considered the most important. Therefore, we focused on the defecation status of mice as a novel method to evaluate the effects of treatment and compared the findings with those of previous reports.

2 | METHODS

All procedures were approved by the Institutional Review Board and Animal Research Committee and were in accordance with the protocols approved by the Animal Care and Use Committee of Osaka University.

2.1 | Animals

Twenty7-week-old female BALB/c mice weighing 18–19 g were used (Nippon Clare Co.). The animals were bred in temperature- and humidity-controlled rooms. They were kept in an environment with free access to food and water.

Two of the 20 mice were used for pathological evaluation of the sphincter before balloon catheter injury, and two mice were used the day after balloon catheter injury.

The remaining 16 mice were randomly assigned to three groups: the noninjured group (negative control group; n = 4), injured group (positive control group; n = 4), noninjured electrical stimulation group (treatment control group; n = 4), and the injured electrical stimulation group (active treatment group; n = 4) in which the anal sphincter was injured and then treated with electrical stimulation.

2.2 | Damage procedure to the anal sphincter

To induce anal sphincter injury, a balloon catheter (outer diameter: 10 mm) was used. The balloon dilation time was set at 2 min and was performed twice. The damage procedure was performed under anesthesia.

2.3 | Electrical stimulation procedure

Electrical stimulation was performed by attaching an electric stimulation pad (1 cm × 3 cm) around the anus of the mouse. Electrical stimulation was applied at 4 mA amplitude and 50 Hz frequency for 10 min, three times per week, using a Uromaster device (SD-U2100, Star Medical Force). Electrical stimulation was performed under anesthesia. Mice were anesthetized using a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol (5 mg/kg).

2.4 | Complications of anal sphincter injury

Anal sphincter injury using a balloon catheter was performed in 10 mice. No complications were observed in the mice during this procedure.

2.5 | Evaluation of defecation status

To evaluate the defecation status, defecation frequency in 24 h and fecal weight per stool were measured before anal sphincter injury (d 0) and on d 1, 7, 14, 21, and 28 after anal sphincter injury.

The experiment overview is in Figure 1.

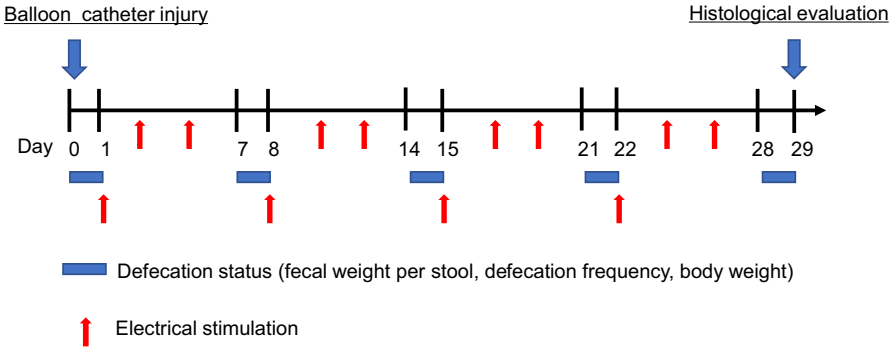

FIGURE 1 Experiment overview

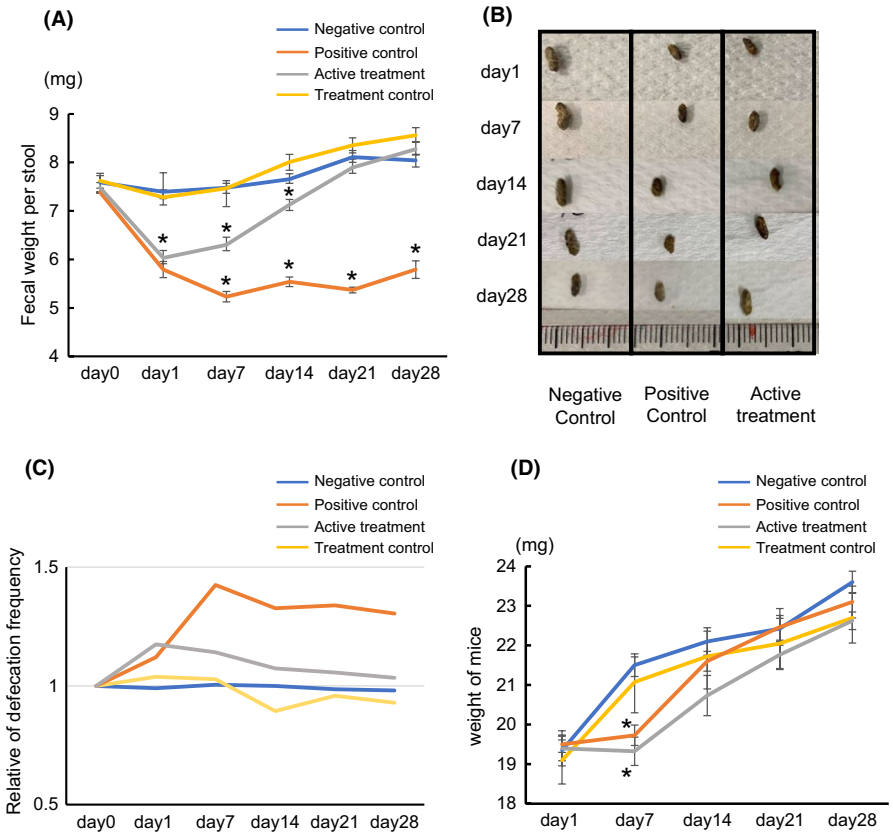

FIGURE 2 Comparison of defecation status (fecal weight per stool, defecation frequency, body weight) among four groups for 28 d. A: Fecal weight per stool in the positive control and the active treatment group relative to the negative control group and the treatment control group. Each bar represents the mean ± SEM of quadruple measurements (*$P < .05$). B: Representative photographs of stool. C: Defecation frequency. D: Body weight of the positive control and active treatment groups relative to the negative control group. Each bar represents the mean ± SEM of quadruple measurements (*$P < .05$)

2.6 | Evaluation of pathological changes in the anal sphincter

The perianal tissues of four mice were sampled before injury (n = 2) and after injury (n = 2).

The perianal tissues of the four groups were sampled on d 28 for pathological evaluation. Mice were euthanized in a chamber with 40% CO_2. The sampled specimens were fixed in 10% formalin solution, and paraffin blocks were prepared. The fixed paraffin blocks were cut into 5-μm slices and stained with hematoxylin and eosin for evaluation. The anal sphincter area was measured using ImageJ (NIH, Bethesda, MD) and compared among the groups.[12]

3 | RESULTS

3.1 | Defecation status

3.1.1 | Fecal weight per stool

The fecal weight per stool was measured every 24 h once a week in each group. From the day after anal sphincter injury, the fecal weight per stool significantly decreased in the positive control and active treatment groups compared with the negative control group and treatment control group. The fecal weight per stool in the active treatment group improved until there was no significant difference from that of the negative control group and treatment control

group on d 21 following the injury. However, the fecal weight per stool in the positive control group remained significantly decreased (Figure 2A,B).

3.1.2 | Comparison of defecation frequency

In each group the defecation frequency in 24 h was compared based on d 0. In the positive control group, defecation frequency increased 1.4 times compared to that before anal sphincter injury. However, in the active treatment group the defecation frequency increased 1.2 times on the day after the anal sphincter injury and then decreased with electrical treatment (Figure 2C).

3.2 | Comparison of weights in mice

The growth of mice in each group was evaluated based on weight changes. In the positive control and active treatment groups, weight gain significantly decreased 1 week after the anal sphincter injury. Subsequently, no significant differences in weight were observed among the groups (Figure 2D).

3.3 | Pathological evaluation

Evaluation of anal sphincter injury and changes in the anal sphincter muscle due to electrical stimulation were pathologically assessed.

A sectioned specimen of the anal sphincter in the negative control group is shown in Figure 3A. This indicates that the sphincter was formed circularly against the rectal mucosa. A section of the sphincter on the day after the injury (Figure 3B) showed that the sphincter was torn by the balloon catheter.

Sampled specimens of the anal sphincter of the positive control and active treatment group on d 28 after the injury are shown in Figure 4. To evaluate changes in the anal sphincter, the cross-sectional areas were compared among the four groups (Figure 4A–D). The findings revealed that the anal sphincter area in the injured electrical stimulation group (active treatment group) was significantly larger by the electrical stimulation (Figure 4E).

4 | DISCUSSION

Recently, techniques that allow the preservation of the anal sphincter and avoid the use of a permanent artificial anus have been widely applied for rectal cancer.[13] The number of patients with fecal incontinence, with or without anal sphincter injury, is expected to increase in the future, and more effective treatment methods are required. Recently, the field of regenerative medicine has seen rapid technological innovation, and many reports of muscle regeneration using stem cells have been published. To validate the therapeutic effects of various treatment methods on anal sphincter injury, a more uniform mouse model needs to be established, which can be widely used in fields such as immunology and organ transplantation.[11]

The difficulty in creating a mouse model of anal sphincter injury is the small size of the animal and an appropriate method to achieve anal sphincter injury is one of the main limitations of using mice models. The weight of rat is about 10 times as much as that of the mouse, and it may be relatively easy to create an injury model by surgical procedure.[9] In this study, considering the size of the mouse anus, we used a 10-mm balloon catheter. This method is simple and can be consistently applied. Pathological evaluation showed that the balloon catheter produced a tear in the sphincter muscle on the day following the injury. In the rat model of a previous study, the sphincter was surgically incised, but this study showed that a balloon catheter could produce a sphincter tear.[9]

Anorectal manometry is widely used to evaluate the anal function in animal models of anal sphincter injury. However, studies have reported that there are variations in the measured values of anorectal manometry, depending on the machine used. Normal values may differ depending on age and sex, and there is a discrepancy between anorectal manometry and clinical defecation assessment.[14-16] In clinical practice, the fecal incontinence severity index (FISI), the fecal incontinence quality of life (FIQL) scale, and the low anterior resection syndrome (LARS) score, which

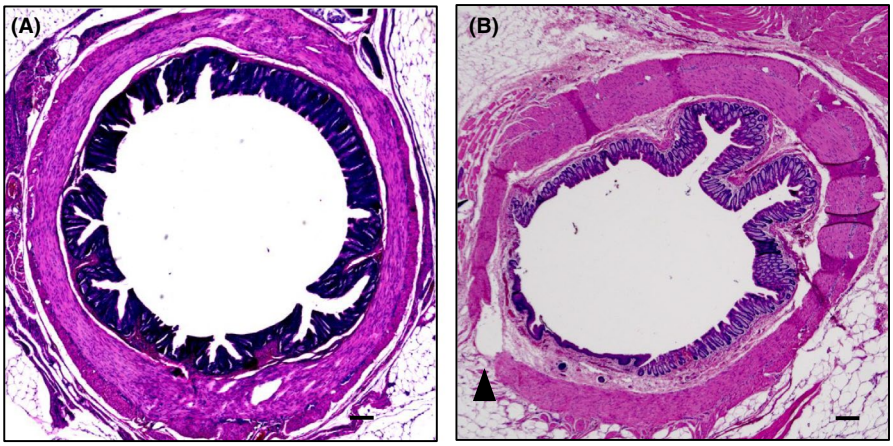

FIGURE 3 Photomicrographs of histological sections of anal sphincter with hematoxylin and eosin staining. A: Before balloon catheter injury. Scale bar: 200 μm. B: After balloon catheter injury anal sphincter tear (arrow). Scale bar: 200 μm

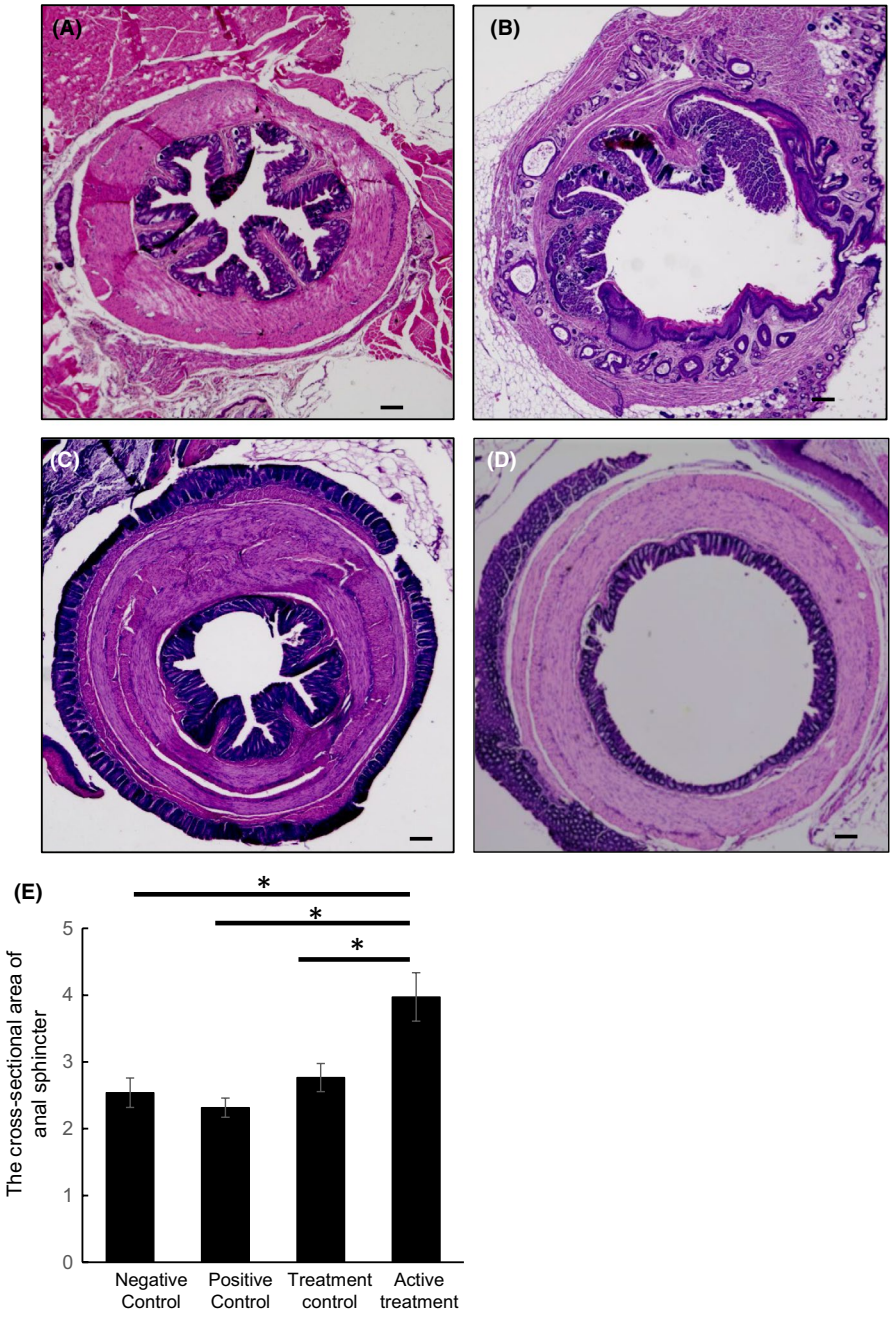

FIGURE 4 Photomicrographs of histological sections of anal sphincter 28 d after anal sphincter injury with hematoxylin and eosin staining. A: Noninjured group (negative control group). Scale bar: 200 μm. B: Injured group (positive control group). Scale bar: 200 μm. C: Active treatment group. Scale bar: 200 μm. D: Noninjured electrical stimulation group (treatment control group; n = 4). Scale bar: 200 μm. E: Bar plot of cross-sectional area of the anal sphincter at 28 d after anal sphincter injury. Negative control, positive control groups and treatment control group relative to active treatment group. Each bar represents the mean ± SEM of quadruple measurements (*$P < .05$)

mainly assesses the symptoms, are essential diagnostic tools in fecal incontinence.[17-19] However, to our knowledge, there are currently no established methods to assess similar clinical symptoms in animal models. In this study we attempted to use a new assessment method by measuring defecation rhythm to evaluate fecal incontinence in our mouse model. Although evaluating defecation rhythm as an index in animal models has its limitations, measuring defecation status (defecation volume in 24 h and fecal weight per stool) in mice can evaluate fecal incontinence in animal models as effectively as FISI, FIQL, and LARS.

Anal sphincter injuries caused by a balloon catheter resulted in a tear in the anal sphincter. This impaired the function of the sphincter and altered the defecation status; anal sphincter injury resulted in a significant decrease in fecal weight per stool and an increase in defecation frequency for 24 h in the positive control group compared with the negative control group. This trend was confirmed on d 28. This means that impaired function of the sphincter persisted for 28 d in the positive group, although there were no significant pathological differences detected in the area of the anal sphincter between the two groups. This indicated that the sphincter dysfunction due to anal sphincter injury caused by the balloon catheter was considered to have persisted for 28 d. Electrical stimulation of the anal sphincter was performed on the mice with injured sphincters. This induced sphincter muscle hypertrophy and improved the anal function, contributing to normalization of the defecation status.

The current findings on sphincter muscle hypertrophy are similar to those of previous reports of sphincter regeneration induced by electrical stimulation in rats.[20,21]

This study confirms that a mouse model of anal sphincter injury can be effectively created using a balloon catheter. We believe that the mouse model in this study will be useful when investigating the effectiveness of a new treatment for sphincter muscle damage, which is one of the causes of fecal incontinence.

Moreover, electrical stimulation was proven to be effective in this model.

However, this study had several limitations. First, the study population was small. Further, since we did not measure anal pressure, comparisons with previous reports are based only on pathological evaluations. In the future, we believe that new treatment and evaluation methods can be investigated using this new mouse model.

5 | CONCLUSION

We successfully established a mouse model of anal sphincter injury using a balloon catheter and validated the efficacy of electrical stimulation. The mouse model established in this study may be useful in the rapidly advancing field of regenerative medicine for anal sphincter regeneration and its clinical applications.

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DISCLOSURE

Conflict of Interest: The authors declare no conflicts of interest.

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