Improvement in the Anal Function in Rats via Electrical Muscle Stimulation

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Abbreviations:

ISR - intersphincteric resection
EMS - electrical muscle stimulation
SNM - sacral neuromodulation

ABSTRACT

Background: Intersphincteric resection (ISR) has become a common procedure for lower rectal cancer. However, while the anus can be preserved, thereby avoiding colostomy, anal sphincter dysfunction remains a troublesome complication. This is a serious problem with no effective therapies available at present. We established a novel rat model with long-term anal sphincter dysfunction. The objective of this experiment was to assess anal functional recovery after electrical muscle stimulation (EMS) of the anal sphincter in anal dysfunction model rats.

Methods: Twenty female Sprague-Dawley rats underwent resection of the approximately half of the internal and external anal sphincter. Rats were divided into two groups: one treated by electrical stimulation to anal sphincter (EMS group, n=10), and another with no treatment after surgery (control group, n=10). The EMS group rats had an electrode pad placed at the anus and received electrical stimulation. The rats were then evaluated for the anal function by measuring their resting pressure before surgery and on postoperative days. Additionally, the rats were histologically examined for the assessment of smooth muscle.

Results: The pressure of the anus was not significantly different between the two groups. However, the rate of the improvement of the anal pressure was greater in the EMS group than in the control group (293.4% vs. 129.6%, $p < 0.005$) significantly. Pathologically, the internal sphincter muscle of the bundles of the EMS group became significantly thicker (1.22 times thicker than the external ones, $p < 0.05$).

Conclusion: EMS to the anal sphincter has the therapeutic possibility to resolve anal sphincter dysfunction following anorectal surgery, including ISR.

Key words: transcutaneous electrical nerve stimulation, anal dysfunction, electrical muscle stimulation, rat, intersphincteric resection

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INTRODUCTION

It was reported that the use of intersphincteric resection (ISR), anal-preserving surgery, could be adapted for patients with tumors localized to the lower

third of the rectum in 1994 (1). The physical perceptions and lifestyle could be preserved by ISR instead of permanent colostomy. Also, it has been reported that the oncological outcomes of ISR and abdominoperineal resection were comparable, and the functional assessment of ISR were reasonable (2,3). However, while fecal incontinence after ISR has improved gradually, some patients are unable to completely recover for the rest of their lives. Fecal incontinence influences the quality of daily life and thus remains a critical issue after ISR.

The therapeutic choices for fecal incontinence are usually medication, diet and surgery (4). Surgical treatment can be applied for repairing a impaired anal sphincter, especially the external anal sphincter; however, the function of the sphincter muscles become deteriorated over long-term follow up period, therefore effective therapeutic management for internal anal sphincter dysfunction are highly desired (5). Several novel therapeutic options such as bulking agents, artificial bowel sphincter etc., have been proposed, but they are not completely accepted yet. Lately, several articles have reported that mesenchymal stem cells (MSC) can improve the function of the anal sphincter after an injury in animal models, including one from our own laboratory (6,7).

One of our co-authors, M.O., previously reported the effect of transcutaneous electrical nerve stimulation (EMS) for induced muscle contracture and fibrosis in rats (8). In addition, we previously reported an experimental rat model of anal sphincter dysfunction with anal sphincter resection (9). In the present study, we attempted to use EMS to treat incontinence using our animal model of surgically induced incontinence.

MATERIALS AND METHODS

All procedures were performed in accordance with the protocols approved by the Animal Care and Use Committee of Nagasaki University.

The experiment was conducted by randomly assigning 10 animals to the electrical stimulation group (EMS group) and 10 to the control group. The animals were kept in a temperature - and humidity-well controlled environment with a 12-h light-dark cycle. They were allowed free access to food and water *ad libitum*.

The animals were anesthetized by inhalation of isoflurane. After anesthesia, via a posterior incision, all animals underwent sphincterotomy by the removal of a left semicircle of both the internal and external anal sphincters, as reported previously (9).

Electrical stimulation was applied by attaching electrical pads around the anus of the rat using an

electrical stimulator, which was provided by Alcare Co. Ltd. (Tokyo, Japan) for this experiment. The stimulation program was executed with a stimulator with a regimen of 4-mA amplitude at 50 Hz for 60 min, 3 times per week, for 56 days after the surgery, which was referred from our previous paper with some modification (10).

The anal pressure was measured and recorded immediately before resection as a baseline and on post-operative days (PODs) 1, 7, 14, 21, 28, 35, 42, 49 and 56 after the surgery (9). Under inhalation anesthesia, the resting anal pressure was measured using a catheter with a microminiature silicon strain gauge-type sensor installed at one end (CODMAN MICROSENSOR ICP Transducer; Codman, Raynham, MA, USA), connected with a low profile connector (TEC-10D cable; Millar Instruments, Houston, TX, USA), pressure control unit (TCB-500; Millar Instruments) and digital data recording system (PowerLab 4/30; ADInstruments, Nagoya, Japan) as described in our previous study (9). The mean pressures at each time point were measured and compared the control group with the EMS group.

On POD 56, the animals were killed with an overdose of inhaled anesthetic agent. The histological changes in the rectum and anal canal of the both groups were investigated. The anal portion of the rats were dissected in parallel along the dorsal and ventral sides, and fixed in 4% paraformaldehyde phosphate-buffered solution (Wako Chemical Industries, Miyazaki, Japan), embedded in paraffin, sectioned (5 μ m) and stained with hematoxylin and eosin (H&E).

GraphPad Prism 5.0 software (Graph Pad Software Inc., San Diego, CA, USA) was applied to analyze the current data set. Experiments were analyzed using the Mann-Whitney test. The probability of $p < 0.05$ was considered statistically significant. All values are shown as the mean $\pm$ standard deviation.

RESULTS

Preoperatively, the resting anal pressure was 23.02 mmHg in the control group and 22.18 mmHg in the EMS group. One day after the surgery, these values dropped to 3.32 mmHg in the control group and 2.25 mmHg in the EMS group. The anal pressure in the control group remained low until eight weeks after the surgery. However, the rate of improvement of the anal pressure was significantly greater in the EMS group than in the control group (293.4% vs. 129.6%, $p < 0.005$) (fig. 1).

Histologically, the width of whole thickest wall of the sphincter bundle did not differ significantly between the groups (fig. 1). However, the internal anal sphincter

Figure 1 - Improvement rate of pressure.
The rate of improvement was significantly improved in EMS group. Improvement rate: (pressure of dayX / day1) x 100

muscle bundles of the EMS group were significantly thicker at 2 months after the surgery (1.22 times thicker than the external ones, $p < 0.05$) (fig. 3a) while no such change was noted in the external anal sphincter muscle bundles (fig. 3b).

DISCUSSIONS

The main result of this study was that the non-pharmacological intervention TENS resulted in improvement in the intrarectal pressure at 1 month and after partial sphincter muscle resection. These results suggest that low-intensity muscle contraction exercise is beneficial for resolving incontinence after rectal surgery.

Electrical stimulation of the anal sphincter, such as sacral neuromodulation (SNM) has the possibility to improve anal sphincter dysfunction induced by ano-rectal surgical procedure, including ISR (11-13). However, SNM requires invasive surgery to install the stimulation apparatus, which is cumbersome and expensive. In contrast, EMS, which has an external stimulation system, requires only an electrical power source, making it very safe and cost-effective. In addition, little evidence exists concerning the mechanism by which SNM resolves fecal incontinence in small animal models, as most such experiments have been done for urinary incontinence rather than fecal incontinence. Therefore, we believe that EMS could be used more simply for patients with dysfunction in anal

Figure 2 - Histological evaluation
There was no significant difference in the width of whole anal sphincter muscles

Figure 3 - (a) Histological evaluation
There was a significant difference in the width of the width of internal anal sphincter bundles.

(b) There was no significant difference in the width of the width of external anal sphincter bundles

sphincter due to anorectal surgical procedure such as ISR.

The rate of improvement of the anal pressure was greater in the EMS group than in the control group in the present study and may improve the anal pressure significantly. The new and improved EMS protocol shown here may help make the anal sphincter thicker. However, only the internal anal sphincter muscle bundles of the EMS group became significantly thicker in the present study. An additional study is needed to reveal the reason why only the internal anal sphincter muscle bundles, and not the external ones, became thicker in this study.

Several limitations associated with the present study warrant mention. First, we did not collect any blood samples or perform muscle biopsies in the present study. Data on humoral factors, the local expression of growth factors, genetic signaling and detailed information about muscle hypertrophy obtained by these approaches may strengthen our findings. It is very important to perform massage in exercise therapy and prescription. Second, in the present study, we were unable to demonstrate that the increases in muscle mass and strength with our training method led to an improvement in the overall performance. Although it is difficult to measure muscle

strength in a rodent model, it could be ideal to show the improvement of muscle contraction force using mechanic approach. Third, the surgical procedure in this study was sphincterotomy by the removal of a left half of both the internal and external anal sphincters through a posterior incision. It is somewhat different from ISR for human patients with rectal cancer. Finally, combination therapy with EMS and regenerative therapy may synergistically increase the effects of each treatment (7).

CONCLUSIONS

EMS to the anal sphincter has the possibility to ameliorate dysfunction of the anal sphincter induced by anorectal surgery, including ISR.

Statement of Ethics

Ethics Committee approval whole experimental procedure and concepts.

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Disclosure Statement

All authors declare no conflict of interest regarding this article.

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