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Establishment of a new anal sphincter injury model in rats based on cardiotoxin☆,☆☆

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ABSTRACT

Background/purpose: Mainstream models for anal sphincter injury use large animals. We developed a simple and stable anal sphincter injury model in a small animal (i.e., rats) to obtain manometry measurements by using a miniaturized probe and applying cardiotoxin.
Methods: The histological structure of the anal canal was evaluated by using manometry in normal rats ($n = 40$). We damaged the internal and external anal sphincters by locally administering snake poison (cardiotoxin; 20 uM, 100 μL 8 points). We evaluated the anal canal function through manometry measurements ($n = 5$) and examined the histology using hematoxylin–eosin staining (at each time point, $n = 3$; total $n = 15$).
Results: The manometry parameters and structure of the anal canal of normal rats were similar to those of humans, because rats have resting pressure, rectoanal reflex in the manometry, and an external and internal anal sphincter. After inducing injury, the following findings were observed: rhythmic wave loss and a remarkable reduction in the anal sphincter resting pressure; and local bleeding and advanced infiltration of the inflammatory cells (day 1) and the loss of muscle fibers (day 3).
Conclusion: This new rat model will contribute to increasing the knowledge on the anal canal.

Two types of manometry are used to measure anal canal function. The recently introduced solid-state manometry is thought to be more advantageous than the conventional water-perfused manometry, because it offers a high-frequency response while being equally reliable [1,2].

Most of the studies that have measured the anal canal function by using manometry were performed in large animals such as rabbits [3], dogs [4,5], and pigs [6–9]; few manometry measurements have been reported in smaller animals such as rats [10–13]. This is most likely because of the difficulty in obtaining accurate manometry measurements with the use of a large probe, considering the size of the inner cavity of the anal canal in rats. Usually, electromyography (EMG), in which an electrode is implanted into the anal sphincter, is used to evaluate the anal canal function in rats [14,15].

The anal canal is surrounded by an internal anal sphincter (IAS) consisting of smooth muscle and an external anal sphincter (EAS) consisting of skeletal muscle. The IAS is located at the distal end of the swollen myenteron, proximal to the anal canal opening. Outside of the IAS is the longitudinal muscle, which is thought to play an important role in bowel function [16]. The EAS has straight muscle layers and lies further outside, and it is thought to be divided into three sections. The IAS is involved in maintaining the constraint (endurance) of bowel movement by creating a high-pressure zone, and it relaxes to assist the bowel movement process. The latter function, intervening locally, is called the rectoanal reflex (RAR).

Most injury models mimic obstetric injuries [14] and rely on methods used to induce an anal sphincter injury, such as sphincterotomy, pudendal nerve transaction, and balloon dilatation. Using these methods, an injury is generated in the anal sphincter, and the result largely depends on the proficiency of the surgical technique.

However, a skeletal muscle injury can be induced by using a myotoxic agent [17] such as cardiotoxin (CTX), which is a cobra toxin that causes muscle fibers to melt by inducing a rapid depolarization of the cellular membrane when administered locally [18]. Additional methods and reasons for damaging the skeletal muscle include the local administration of other myotoxic agents such as bupivacaine [19], CTX [20], and netoxin [21]; direct damage from frostbite [22] or crushing injuries [23]; and surgical mechanical trauma due to skeletal muscle exchange transplantation [24] or neurovascular blocking [25].

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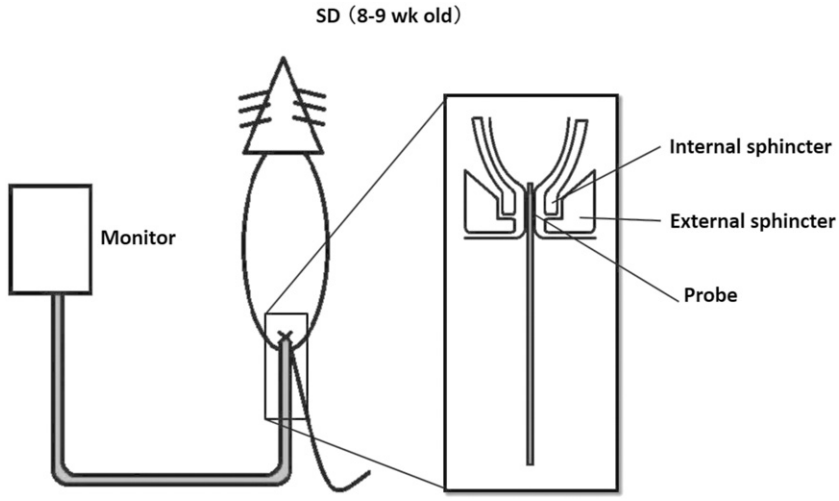

Fig. 1. The manometric method in rats using solid-state manometry. Preparation is performed by using 10 mL of saline; for awakening after anesthesia, the remaining saline in the anorectum is released. The measurements are repeated using inhalation anesthesia. After inserting a probe into the anus of the Sprague–Dawley (SD) rats in the dorsal position, manometry measurements are continuously performed for 5–10 minutes at the highest point of the anal sphincter resting pressure.

Skeletal muscle injury with CTX starts with massive tissue destruction, and the tissue is regenerated within 3 weeks [26,27]; therefore, this is an appropriate and widely used method for inducing acute injury.

In this study, we describe a simple and stable anal sphincter injury model in small animals (i.e., rats), in which we induced anal sphincter injuries with CTX and evaluated the anal canal function using solid-state manometry.

1. Materials and methods

1.1. Animals

Six 8-week-old female Sprague–Dawley rats were purchased from CLEA Japan (Tokyo, Japan).

This study was approved by the local ethics review board, and the animal experiments were conducted in compliance with the Animal

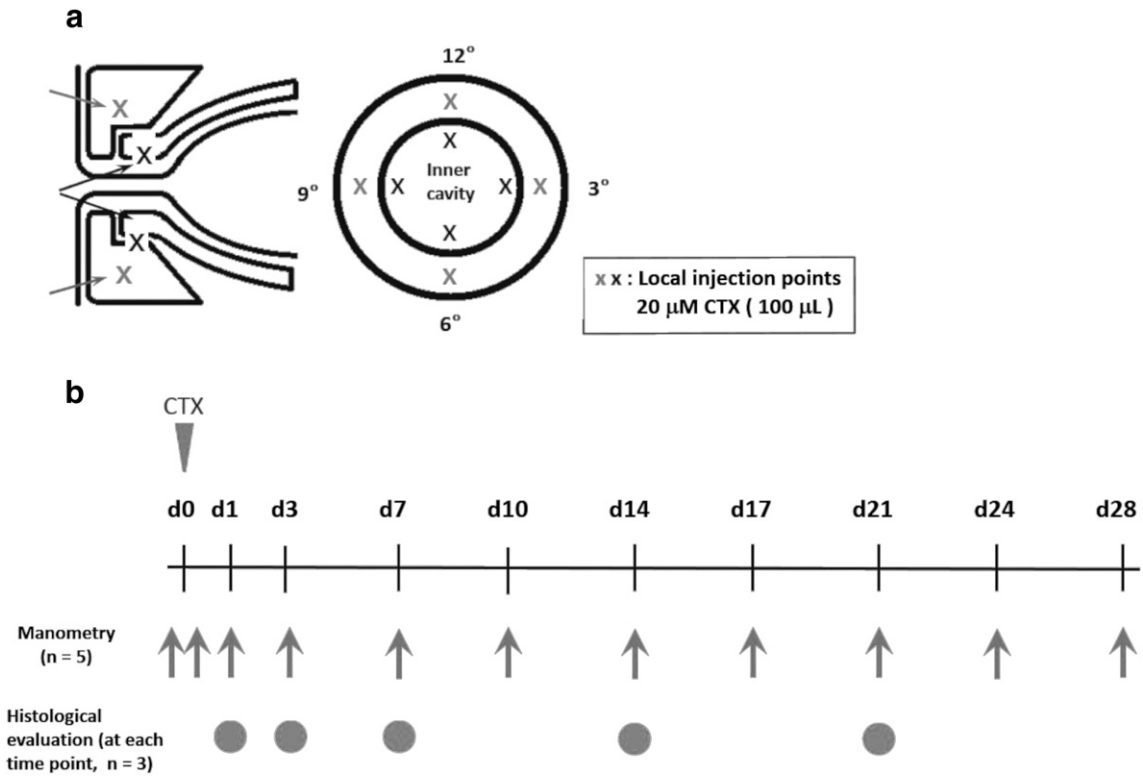

Fig. 2. Methods for the preparation and evaluation of the rat anal sphincter injury model based on cardiotoxin (CTX). A model using 8–9-week-old Sprague–Dawley (SD) rats is prepared by administrating 20 μM of CTX (100 μL each) locally at the 3-, 6-, 9-, and 12-o'clock positions in the anal inner cavity side and the perianal region (a). The functional evaluation of the anal sphincter with solid-state manometry consists of measuring the manometry parameters over time, before and immediately after CTX administration as well as on days 1, 3, 7, 10, 14, 17, 21, 24, and 28 after CTX administration ($n = 5$). Furthermore, the histological evaluation is based on hematoxylin–eosin staining of the dissected anal canals on days 1, 3, 7, 14, and 21 after CTX administration (at each time point, $n = 3$).