Vaginal Distention Rodent Model for Fecal Incontinence: A Pilot Study on the Effect on Defecation Behavior

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Abstract

Objectives Vaginal balloon inflation simulates the compressive forces on the pelvic floor during the second phase of natural delivery. The foremost use of this animal model of vaginal distention (VD) is to study the mechanisms underlying urinary incontinence. As damage to the pelvic floor during natural birth is a common cause of fecal incontinence, the present paper aimed to investigate the effect of VD on defecation behavior in adult rats.

Methods Vaginal distention was performed in 8 rats for 2 hours, and in 3 rats for 4 hours, and sham inflation was performed in 4 rats. With the use of a latrine box in the rat home-cage and 24/7 video tracking, the defecation behavior was examined. The time spent in and outside the latrine was monitored for two weeks preoperatively and three weeks postoperatively, and a defecation behavior index (DBI; range: 0 [continent] to 1 [incontinent]) was defined. Pelvic floor tissue was collected postmortem and stained with hematoxylin and eosin.

Results Vaginal balloon inflation for 2 hours resulted in fecal incontinence in 29% of the animals (responders) whereas the DBI scores of non-responders (71%) and control animals did not **change** in the postoperative phase compared with the baseline score. A 4-hour balloon inflation resulted in fecal incontinence in 1 animal and caused a humane

endpoint in 2 animals with markedly more tissue damage in the 4-hour responder compared with the 2-hour responders.

Conclusions   Vaginal balloon inflation, with an optimum duration between 2 and 4 hours, can be used as a model to study changes in defecation behavior in rats induced by pelvic floor damage.

Introduction

Fecal and stress urinary incontinence are a possible consequence of natural birth due to excessive compressive forces on the pelvic floor that cause damage to it.[1–3] Presently, the preclinical research[4,5] into the treatment and mechanisms of action (MoAs) of fecal incontinence is limited, and only two preclinical models related to defecation behavior have been published in literature to date. These studies utilized either retro-uterine balloon inflation[4] or transvaginal retro-uterine intrapelvic balloon inflation.[5] While both balloon-inflation models showed signs of fecal incontinence, the responder rate was limited; for instance, with the retro-uterine balloon inflation model, it did not exceed 32% of animals.[4] The retro-uterine intrapelvic balloon inflation model resulted in a small effect on the behavioral outcome, and this very small window and this did not enable the analysis of future treatment effects.[5]

Currently, the vaginal distention (VD) model is commonly used for stress urinary incontinence.[6,7] This model is characterized by intravaginal balloon inflation, which mimics the compressive forces on the pelvic floor during the second phase of natural delivery. The advantage of this model over the aforementioned models is that it is induced using a physiological approach as it does not require an open procedure, and, at the same time, it very closely resembles natural delivery in humans. Morphological studies[8,9] into urinary incontinence have shown significant muscle disruption, inflammatory damage, and acute edema as a result of VD. Remarkably, the VD model has been exclusively focused on urinary incontinence, and/but not on fecal incontinence and defecation behavior.

Therefore, the present pilot study aimed to investigate the effect of the relatively non-invasive vaginal balloon inflation on defecation behavior in adult rats to establish a reproducible animal model for fecal incontinence.

Methods

Animals
All experiments were performed in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). The Central Dutch Authority for Scientific Procedures on Animals (Centrale Commissie Dierproeven, CCD, in Dutch) granted ethical approval to all experiments (Project License 2018–005–018). We used 19 nulliparous female Sprague Dawley rats (Harlan Sprague Dawley, Inc. Indianapolis, IN, United States) weighing 160 g to 200 g and aged 8 weeks at the start of the experiment. The animals were housed individually in custom-made cages designed for this experiment in a 12-hour reversed day/night cycle with constant temperature (20°C) and humidity (55%). During the entire experiment, cages were randomly placed at the wall rack to avoid environmental influences. Food and water were available ad libitum. The present study consisted of two experiments. Initially, before the beginning of the experiments, we detected a loss of volume during balloon inflation in a pilot with three animals; therefore, a clamp was used right behind the balloon to prevent loss of volume due to high compliance in the tubing. In **experiment A**, 12 Sprague Dawley rats (weight: 230–270 g) were included, 8 of which underwent balloon inflation (Rüsch gold foley balloon catheter, CH8, Teleflex Medical, Wayne, PA, United States) with 4 ml of saline (at room temperature) for 2 hours (experimental group), and 4 animals underwent sham inflation (control group). In **experiment B**, 4 Sprague Dawley rats (weight: 200–250 g) were included, 3 of which underwent balloon inflation with 4 ml of saline for 4 hours, and 1 rat underwent sham inflation. All animals were randomly assigned to one of the groups using a randomization software at the time of balloon inflation. The researchers were blinded for the condition of the animal during the entire experiment, including the analysis.

Vaginal Distention and Balloon Inflation
Thirty minutes prior to surgery, buprenorphine (0.025 mg/kg, subcutaneous [s.c.]) was administered. Rats were anesthetized with isoflurane (4%), which was maintained at concentrations of 1.5% to 2.5%. The depth of the anesthesia was constantly monitored during surgery to insure proper anesthesia in all rats. Body temperature was maintained at $37.5 \pm 0.5°C$ using an automated heating pad and heat lamp. The rats were placed on their back and the balloon was placed intravaginally. The balloon was then inflated using a constant inflation rate of 400 µL/min, and counter pressure was applied to prevent the balloon from popping out easily. After 10 minutes, the balloon was inflated with 4 ml of saline and clamped to avoid loss of volume. A plunger of a 5 ml syringe was placed against the balloon to keep it in place. After two or four hours, the balloons were deflated, carprofen (at dose of 4–5 mg/kg) was administered, and the rats were placed in the home cages to recover. When discomfort was observed after surgery, an extra dose of carprofen (4–5 mg/kg) was administered. The rats in the control group underwent the same procedure with sham inflation (0 ml).

Fig. 1 Home-cage of the rats.

Defecation Behavior Task

The defecation behavior task as used in the present study was first published by Devane et al.[4] In short: a latrine box was placed into the rat's home-cage (which measured 40×60 cm) in the edge furthest away from the food and water. The latrine box was filled with bedding material, and the rest of the cage was filled with paper bedding, and contained nesting and playing material, as shown in ►**Fig. 1**. A one-week continence training period was started, in which rats were trained in pairs to defecate in the latrine box by placing all pellets in the latrine twice a day (see the timeline in ►**Fig. 2**). Following one week of training, the rats were housed individually and the pellets in the latrine and non-latrine areas were counted daily. With the use of infrared video-tracking, the location of the rat was then tracked for 24 hours a day, and the time spent in and outside the latrine was monitored. A defecatory behavior index (DBI) was used to examine fecal incontinence. The DBI (range: 0–1) was defined as the amount of pellets per hour outside the latrine divided by the amount of pellets per hour in total.[4] A DBI of 0 implies a completely continent rat with all pellets in the latrine, whereas a DBI of 1 refers to a completely incontinent rat with all pellets deposed randomly throughout the cage. The baseline defecation behavior was measured for two weeks, and the postoperative defecation behavior was studied for three weeks. The animals were considered incontinent if the post-operative DBI had doubled in comparison to the baseline index and if DBI was higher than 0.3.

Postmortem Analysis

The animals were euthanized using CO_2, and fresh pelvic floor tissue was collected. The tissue was further submitted to immersion fixation in paraformaldehyde (4%) for 14 days, decalcified in a solution containing formic acid (8%), and embedded in paraffin. Sections of 5 µm were cut using a microtome and mounted on glass slides coated with Polysine (Polysciences, Inc., Warrington, PA, United States). Sections were incubated overnight at a temperature of 37°C and stained with hematoxylin and eosin (H&E).

Statistical Analysis

The statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA, United States) software, version 5.00 for Windows, and the data are presented as mean $\pm$ standard error of the mean (SEM). The baseline data of all animals appeared to be normally distributed through visual inspection of the histogram. Repeated-measures analysis of variance (ANOVA) was performed to determine the significance of the DBI over time. Comparisons between the groups were performed over the third postoperative week using an unpaired t-test.

Results

Experiment A: 2-hour Balloon Inflation

Out of 12 animals, 2 did not successfully complete the continence training (DBI < 0.3) and were excluded from the study. From the remaining 10 animals, 7 were allocated to the experimental group and 3, to the control group (sham surgery). The results are shown in ►**Fig. 3A**. The DBI of the control group did not change in the postoperative phase as compared with the baseline (0.19 ± 0.03 versus 0.13 ± 0.04; $p = 0.4438$). Likewise, in the experimental group, the 2-hour balloon inflation did not significantly change the DBI (baseline: 0.16 ± 0.03; postoperative: 0.24 ± 0.11; $p = 0.4073$). As already described by Devane et al.,[4] within the experimental group subgroups of responders and non-responders could be discerned. The DBI in the subgroup of
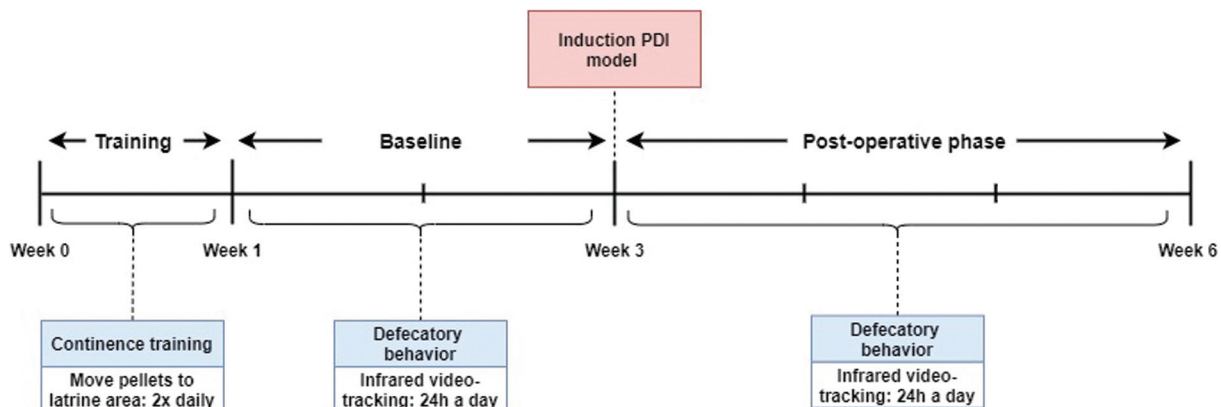
Fig. 2 Timeline of the experiments.

A. 2h balloon inflation

B. 4h balloon inflation
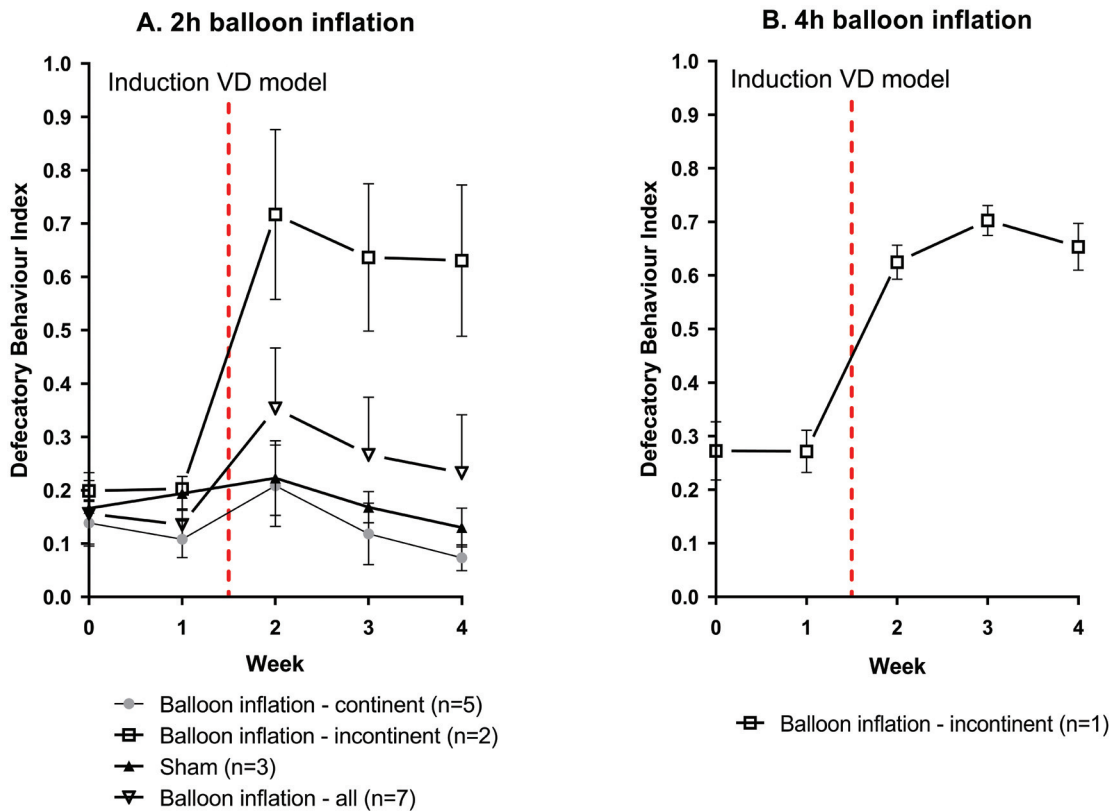
Fig. 3 Effect of balloon inflation on defecation behavior. (**A**) Defecatory Behavior Index of experiment A overtime, and (**B**) Defecatory Behavior Index of experiment B overtime. Data are presented as the mean ± standard error of the mean.

non-responders ($n = 5$; 71%) did not significantly change in the postoperative phase as compared with the baseline (0.11 ± 0.03 versus 0.07 ± 0.02; $p = 0.0671$). The DBI in the subgroup of responders ($n = 2$, 29%) clearly increased in the postoperative phase compared with the baseline (0.20 ± 0.02 versus 0.65 ± 0.12; $p = 0.0295$) and was significantly higher than the DBI of the control group (0.65 ± 0.12 versus 0.13 ± 0.04; $p = 0.0150$). The side effects observed in this responder subgroup were absence of defecation for two days after surgery and small motor deficits of the hind paws.
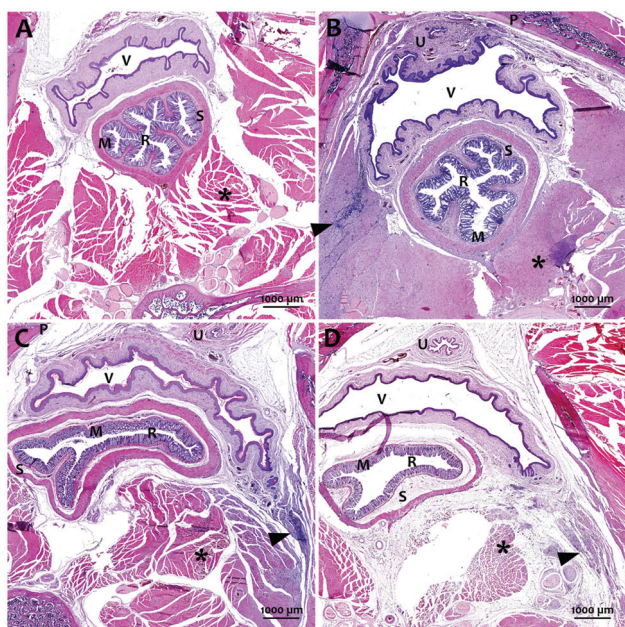
Experiment B: 4-hour Balloon Inflation

Out of 4 animals, 1 did not successfully complete the continence training (DBI < 0.3). The remaining 3 animals were allocated to the experimental group. To enable blinding of the researcher during the experiment, the animal that was not continent underwent the whole procedure but was excluded from the analysis. The results of the 4-hour inflation are shown in ►**Fig. 3B**. Two animals developed absence of defecation after surgery and reached a humane endpoint after two days. Absence of defecation, albeit to a smaller degree, was also observed in the responders of Experiment A. The DBI of one animal increased after surgery compared with the baseline (0.27 ± 0.05 versus 0.65 ± 0.04).

Postmortem Analysis

Sections of the pelvic floor stained with H&E are shown in ►**Fig. 4**. Continent animals (VD non-responders; ►**Fig. 4B**) showed minor signs of inflammation, but no signs of fibrosis, edema or dilation as compared with the control animals (►**Fig. 4A**). In contrast, inflammation with fibrosis was observed in the levator ani muscle surrounding the colon, with markedly more damage after the 4-hour balloon inflation (►**Fig. 4D**) as compared with the 2-hour inflation (►**Fig. 4C**). Furthermore, signs of edema in the submucosa and rectum dilatation were noted after the 4-hour balloon inflation (►**Fig. 4D**) and to a lesser extent after the 2-hour inflation (►**Fig. 4C**). In addition, a slightly thinner muscularis externa was observed after the 4-hour balloon inflation (►**Fig. 4D**). The mucosa seemed unaffected in all animals of all groups. So regardless of the condition. In my opinion this sentence is clear and I don't know how to write it otherwise.

Discussion

The present methodological paper showed the effect of vaginal balloon inflation on defecation behavior in adult Sprague Dawley rats. The experimental group did not significantly differ from the control group after the 2-hour balloon inflation. Within the experimental group, 29% of the animals developed fecal incontinence, and 71% did not develop fecal incontinence.

Fig. 4 Sections of the pelvic floor with hematoxylin and eosin Staining. (**A**) Control group; (**B**) 2-hour balloon inflation – continent group; (**C**) 2-hour balloon inflation – incontinent group; (**D**) 4-hour balloon inflation – incontinent group. Ø = levator ani muscle; ► = inflammation. Abbreviations: M, mucosa; P, pubic bone; R, rectum; S, submucosa; U, urethra; V, vagina.

All animals responded to the 4-hour vaginal balloon inflation; 1 animal developed fecal incontinence, and 2 animals showed severe discomfort and therefore reached a humane endpoint. In the postmortem analysis, dilatation of the colon and damage to the levator ani muscle were observed after the 4-hour inflation, and to a lesser extend after the 2-hour inflation.

The VD model has the advantage of being more closely related to natural birth as compared with the retro-uterine balloon inflation model[4] or the transvaginal retro-uterine intrapelvic balloon inflation model.[5] The responder rate following the 2-hour vaginal balloon inflation (29%) is comparable to the responder rate of the retro-uterine balloon inflation model (32%) as described by Devane et al.[4] Unfortunately, the responder rate is relatively low, which makes the animal experiments, from an ethical point of view, rather complicated. Interestingly, the level of incontinence in responders following the 2-hour vaginal balloon inflation (DBI = 0.65 ± 0.12) seems higher as compared with the retro-uterine balloon inflation model. This profound increase in DBI with the VD model provides an adequate window of opportunity, which may be useful to investigate the effect of the effect of possible interventions. To further increase the responder rate and, with it, the usability of the VD model, Experiment B with an inflation of 4 hours was performed. Although the responder rate increased, regrettably, severe complications occurred, and some animals reached a humane endpoint. However, the sample size for the 4-hour group in the present study was low, and future studies should therefore aim to replicate and extend our findings.

It is complicated to compare the defecation behavior of the VD model to that of the transvaginal retro-uterine intra-

pelvic balloon model, since[5] the behavior was examined with two different approaches. Nevertheless, the treatment window for the transvaginal retro-uterine intrapelvic balloon model appeared to be relatively small, whereas the one for the VD model appears to be substantially wider.

In contrast to these other models in which the balloons are inflated for a period of one hour, the inflation in the present VD model lasted for two or four hours. The responder rate following the 2-hour inflation was moderate (29%), whereas the rate following the 4-hour inflation appeared to be substantially higher, but too severe in terms of discomfort. After the 4-hour inflation, all animals showed signs of bowel dysfunction, 1 of which developed fecal incontinence, and 2 animals developed severe postoperative absence of defecation with signs of ileus, which did not allow us to complete the analysis in them.

Damage of the levator ani muscle was noted in the postmortem analysis of the VD responders, and this confirms the importance of this muscle for bowel continence. Indeed, Fernández-Fraga et al.[10] showed that the severity of fecal incontinence is correlated to an impaired function of the levator ani muscle and less strongly related to EAS (External Anal Sphincter) function. This was supported by the clinical improvement after treatment, which was observed without significant improvement of the EAS.[10]

With the development of this VD model for fecal incontinence, various technical aspects should be noted. As described, the balloon should be clamped to avoid loss of volume. In addition, it is important to apply counter pressure to the balloon from the perineal side to keep it in place, as without this counter pressure the balloon will take the path of least resistance and pop out of the vaginal canal. A limitation of the present study might be the use of opioids, and the possible effect of these drugs on the gastrointestinal tract. In the present study, buprenorphine (0,025 mg/kg, s.c.) was applied to the animals preoperatively. Studies[11] have shown that buprenorphine is associated with impaired gastrointestinal motility and postoperative ileus, especially after long abdominal surgical procedures. Several animals in experiments A and B developed absence of defecation, which, in 2 out of 3 animals from the 4-hour group even led to exclusion from the experiment. It is therefore of utmost importance to minimize the use of opioids for preoperative pain in gastrointestinal surgery. Hence, the absence of defecation might be related to the administration of opioids such as buprenorphine and its effect on the gastrointestinal tract. However, it can also be caused by pain-induced reflex-mediated pelvic floor hypertonicity. In the present study, it is unlikely that pain-induced hypertonicity occurred, as we administered perioperative and postoperative analgesia.

From these experiments, it can be concluded that the vaginal distention model with 2-hour balloon inflation was not enough to cause fecal incontinence with a substantial responder rate. Moreover, the 4-hour inflation exceeded the discomfort needed to cause fecal incontinence. The optimum duration of inflation mimicking the physiological trauma caused by natural delivery will probably be between 2 and 4 hours. Importantly, the responders showed a significant

and adequate treatment window and responder rates were relatively low with an inflation duration of 2 hours. From this we presume that the VD model can be used in future studies on the possible effect of interventions such as sacral neuromodulation. This will allow the optimization of these therapies for clinical application and the investigation of the underlying mechanisms of action.

Conflict of Interests
The authors have no conflict of interests to declare.

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Authorship Statement
PD, GAvK, EAJ, SOB and JM designed and conceptualized the study. PD and GF performed the experiments. PD analyzed the data and wrote the manuscript. JD performed the vaginal distention and balloon inflation. All authors have approved the final version of the manuscript.

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