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A bladder diverticulum model in rabbits

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Summary

Purpose
Shuttling of some of the bladder volume into the bladder diverticulum (BD) can cause urinary retention, lower urinary tract dysfunction, infection, and stone formation. This experimental study is the first to create a rabbit BD to study micturition physiology (urodynamics and pathology) that mimics clinical findings.

Materials and methods
The study included 16 New Zealand adult male rabbits in the BD group and 16 sham-operated controls. BD creation consisted of a lower midline laparotomy and bladder entry via the spacing between the detrusor muscle fibers and the mucosa, posterolaterally from the bladder wall. The detrusor was excised to provide a mucosal prolapsus, creating a narrow BD neck (Figure). The sham group underwent bladder exposure with a midline incision. All rabbits underwent urodynamic study preoperatively and postoperatively, consisting of postmicturition residue (PMR), maximum bladder capacity (MBC), voiding detrusor pressure (VPdet), filling detrusor pressure (FPdet), compliance, and urine flow (Qflow). The animals were then sacrificed and their bladders assessed for pathology and stone formation.

Results
Preoperative MBC, Pdet, and Qmax were within reference ranges. No animals had PMR or urinary tract infections (UTIs). The BD group showed urodynamic and pathologic bladder changes, including decreased (28%) cystometric bladder capacity and compliance (Sham: 26.8 $\pm$ 0.4; BD: 4.46 $\pm$ 1.08, $p = 0.0001$) and increased post-void residual PMR (8.3 $\pm$ 2.4 mL). Pathology revealed increased bladder detrusor thickness correlated with urodynamic findings of increased filling detrusor pressures (Sham: 1.58 $\pm$ 0.2; BD: 4.89 $\pm$ 0.93, $p = 0.0001$). Urodynamics revealed intermittent BD bladder contraction during the filling phases. Eight BD group rabbits had UTIs; five had stone formation (4—9 mm).

Discussion
In the literature, it has not been determined whether lower urinary tract disorders (LUTD) could cause diverticula, or if a congenital diverticula could be reason for LUTD. Anatomical or neurological reasons for a low-compliance bladder can cause diverticulosis. As demonstrated in our study with rabbits, the congenital diverticulum could further reduce the compliance of the bladder. Further, a decrease in compliance logically correlates with the progressive decompensation of the bladder.

Conclusions
The nature of diverticula remains undetermined. All the information presented for this model is relevant to our clinical observations. We conclude that the rabbit bladder can be used for research into experimental diverticulum-induced changes in the activity of the bladder and for experimental detrusor research.

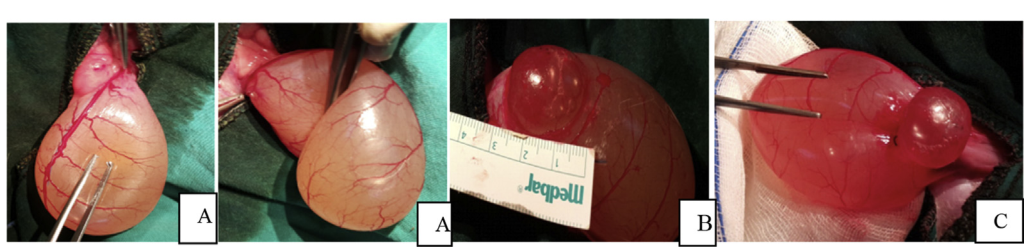

Figure Experimentally created diverticulum model. (A) The bladder was explored through a lower midline laparotomy. (B) A space was created and the detrusor excised to provide a mucosal prolapsus. (C) Creation of a small neck. To narrow the entrance hole of the diverticulum, a 5/0 prolen suture was applied around the hole of the diverticulum from the surrounding detrusor layer.

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Introduction

A bladder diverticulum (BD) is believed to arise secondary to bladder outlet obstruction. Primary or congenital diverticula, however, which can appear in smooth-walled bladders, can occur in the absence of bladder outlet obstruction [1]. The BD arises because of high pressure voiding over time, which results in a herniation of the bladder mucosa through the smooth muscle layer. This could happen prenatally, or later in patients in their 70s because of benign prostatic hyperplasia [2].

BDs are often thin-walled and lack the ability to contract. A significant amount of the bladder volume can be shuttled up into a BD [3], causing stasis of urine, micturition difficulties, enuresis, or urinary obstruction, as well as serving as a nidus for stone formation and infection [4].

To date, BDs have garnered only a small amount of interest in the urological literature. Prospective, randomized clinical trials and experimental models have not been emphasized; rather, all available studies are retrospective studies [5,6] or case reports [7,8], with little or no long-term follow-up. Consequently, the possibility of bias must be considered and the importance of this urinary dysfunction [9], in terms of pathophysiological alterations developing in the bladder because of the response to diverticula, has not totally been elucidated. The lack of knowledge of the effect of BDs on the nature of the bladder—in terms of micturition function, detrusor coordination, and contraction, or on involuntary activity of the bladder—has led to considerable debate regarding how symptomatic diverticula should be managed when encountered. Therefore, an experimental technique for generating data is important for the modern practicing clinician, who may not see this condition frequently.

The rabbit bladder provides a good opportunity to look at the physiological, histological, and biochemical properties of the functioning bladder [10]. This experimental study is the first to create a bladder diverticulum for the study of micturition function with urodynamic and pathologic findings. The aim was to determine how well this rabbit model mimics clinical diverticula in human patients.

Method

Animals

The Animal Welfare Committee approved the experimental protocol. Between January 2015 and July 2015, 24 New Zealand adult male rabbits, weighing 3 $\pm$ 0.2 kg, were used in this study. Rabbits were divided into two groups: 16 rabbits were a sham operation group and 16 rabbits were the BD group.

Technique and study groups

Prophylactic antibiotics were used preoperatively. Oral feeding was stopped 3 h before the operation. General anesthesia consisted of introduction of ketamine 25 mg/kg i.m. + xylazine 0.4 mg/kg i.m., analgesic butorphanol (0.3 mg/kg i.m.) in a single injection at the start of the surgery and anesthetic propofol (15 mg/kg i.v.) continuously throughout the operation. First, the bladder was explored through a lower midline laparotomy with the rabbits in a supine position. After entering between the detrusor muscle and bladder mucosa, a space was created and the detrusor fibers were excised to provide a mucosal prolapsus. Care was taken to maintain mucosal integrity. As even a large diverticulum will have a small neck, the entrance hole of the diverticulum was reduced by applying a 5/0 prolen suture from the detrusor layer surrounding the diverticulum (not from the mucosal layer). The neck diameter was reduced to a size to pass an 8F urethral catheter.

The sham group underwent only bladder exploration with a midline incision. Postoperatively, all rabbits received free access to water and food in the first day. All rabbits underwent urodynamic study preoperatively and 1 month postoperatively.

Urodynamic study

Urodynamic study was performed comfortably after a little relief on the table, with no anesthesia administered before and during urodynamic study.

After completely emptying the bladder, a 6F transurethral urodynamic catheter was introduced to the bladder and a rectal catheter was introduced 2 cm beyond the anal margin. The bladder was filled with saline at an infusion rate of 2 mL/min [10]. Pressure-flow measurement and recording was performed with a videourodynamics system (Aymed Locum, Aymed Medical Technology, Istanbul, Turkey). CMGs were repeated three times and the averaged urodynamic findings were recorded. Cystometric bladder capacity (MBC) was defined as the infused saline volume before urination, and bladder compliance was calculated as the change in volume divided by the change in pressure [11]. Filling detrusor pressure (FPdet), maximum voiding pressure (VPdet), and post-void residual urine volume (PMR) were also measured. Uninhibited contractions were considered as those detrusor involuntary contractions with low vesical volume, regardless of whether they yielded simultaneous urinary leakage [10].

A UTI was diagnosed when one or more organisms were present at quantitative counts of at least 100 colony-forming units (CFU) per milliliter. At the end of the study, the animals were sacrificed and the bladders were exposed and assessed. Bladders were retrieved, trimmed of all fat, and cut into small pieces. Formalin-fixed specimens were embedded in paraffin and sectioned at 6 μm. Standard H&E and Masson-Trichrome stains were used for histological evaluation and morphometric analysis. Each slide was evaluated in random order by standard light microscopy by two pathologists blinded to the rabbit groupings. Detrusor thickness, nerve innervations, and semiquantitative increases in connective tissue between the muscle fibers in the muscularis propria were evaluated and given a score ranging from 0 to 3, where a score of 3 indicated obvious connective tissue growth, 2 indicated intermediate growth, and 1 indicated a slight increase. Nerve innervation was