Temporal Morphological and Functional Impact of Complete Urinary Diversion on the Bladder: A Model of Bladder Disuse in Rats

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Purpose: Urinary diversion has been used as a surgical option for some bladder diseases. We developed a urinary diversion model in the rat and examined the effects of urinary diversion on the bladder.

Materials and Methods: We distributed female Sprague-Dawley® rats into age matched control, sham urinary diversion and urinary diversion groups. Each group was subsequently evaluated 1 or 8 weeks after urinary diversion or sham operation. Diversion was done by surgical disconnection of the ureters from the bladder and implantation to the uterine cervix. Conscious cystometry was examined. Bladders were harvested for histological examination and quantification of smooth muscle, urothelium and collagen. Vaginal histology was assessed. Bladder muscarinic and purinergic receptor expression was examined.

Results: All rats survived the urinary diversion procedure. Bladder weight decreased in the diversion group. Cystometry showed decreased intercontractile interval and voided volume in the urinary diversion group compared to those in the control and sham operated groups. Compliance was decreased in diverted rats. Smooth muscle and urothelium were decreased as a percent of total bladder cross-sectional area. Collagen increased in 1 and 8-week diverted rats vs controls. Histological examination of the vaginal wall revealed mild swelling in 2 rats. Urinary diversion caused decreased muscarinic 3 and ligand gated purinergic 1 receptor expression but no change in muscarinic 2 or ligand gated purinergic 2 receptors.

Conclusions: Creating a urinary diversion model by ureterovaginostomy in the rat is feasible. Urinary diversion causes distinct functional and morphometric bladder alterations.

Key Words: urinary bladder; urinary diversion; atrophy; vagina; models, animal

Abbreviations and Acronyms

CMG = cystometry

M = muscarinic

P2X = ligand gated purinergic

UD = urinary diversion

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URINARY diversion has long been used as a surgical option in patients with various conditions, including bladder cancer, neurogenic bladder, congenital disorders or hemorrhagic cystitis, or by default before renal transplantation when patients with end stage renal failure cease to have urinary output.[1–4] The term disuse atrophy has been used for bladders subject to UD. However, little is known about the impact of bladder disuse on bladder morphology and functional status and, thus, about implications of such changes on future potential bladder use.

Previous studies show that UD results in rapid atrophy of bladder smooth muscle and in decreased contractile function in fetal sheep, young

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rabbits, dogs and humans.[1,5–8] However, there are few studies of the natural history of the disused bladder in an adult small rodent such as the rat.[9] We introduce what is to our knowledge a new UD model created by ureterovaginostomy in the rat and further examined bladder temporal functional, histological and molecular changes in this model.

MATERIALS AND METHODS

Experimental Animals and Design

We used 10-week-old female Sprague-Dawley rats matched by birth date. Rats were randomly allocated to 3 groups, including 20 age matched controls, 24 with sham UD and 24 with UD. UD was done by surgical ureterovaginostomy. Sham UD included laparotomy and ureteral identification. No surgery was done in controls.

Each group was subsequently evaluated 1 or 8 weeks after UD. At designated time points half of the animals were tested by conscious CMG and then sacrificed. The bladder was removed to examine muscarinic and purinergic receptors by immunoblotting. The remaining half of the rats was sacrificed directly. The bladder was removed at the level of the bladder neck, weighed and sectioned at the equatorial midline. The bottom half of the bladder was fixed in 10% formalin for histological staining. The urethra and vagina were fixed in 10% formalin. Rats were sacrificed by injection of pentobarbital (200 mg/kg intraperitoneally). The experimental protocol was approved by the Case Western Reserve University institutional animal care and use committee.

Urinary Diversion

Rats were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight). A lower ventral midline incision was made. The ureters were identified and ligated distally. Two small orifices were made using a Model 770 drill (Dremel, Racine, Wisconsin) in the uterine cervix. Ureter (1 to 2 mm) was brought through the orifice and secured to the cervix with 1 suture close to the medial side at the 3 o'clock position (fig. 1). The abdomen was closed. Antibiotics were administered for 72 hours. The vaginal cavity was assessed daily the first 2 weeks and once weekly thereafter to ensure that no crystals had formed.

Suprapubic Bladder Catheter Implantation and Conscious CMG

Catheter implantation was done using anesthesia.[10] The bladder was exposed and a circular purse-string suture of 7-zero silk was placed on the bladder wall. A small incision was made in the bladder wall. The polyethylene-50 tubing catheter with a flared tip was implanted and a purse-string suture was tightened around the catheter. The catheter was tunneled subcutaneously and externalized at the back of the neck out of reach of the animal. The distal end of the tubing was sealed. The skin and abdominal incisions were closed separately.

Two days later CMG was done as described previously.[10] Briefly, the bladder was filled via the catheter with 0.9% saline at 5 ml per hour while bladder pressure was recorded. Data on at least 5 representative micturition cycles were collected. The mean was calculated to analyze cystometric parameters, including bladder capacity, peak detrusor leak pressure and mean intercontraction interval. We also calculated bladder compliance.

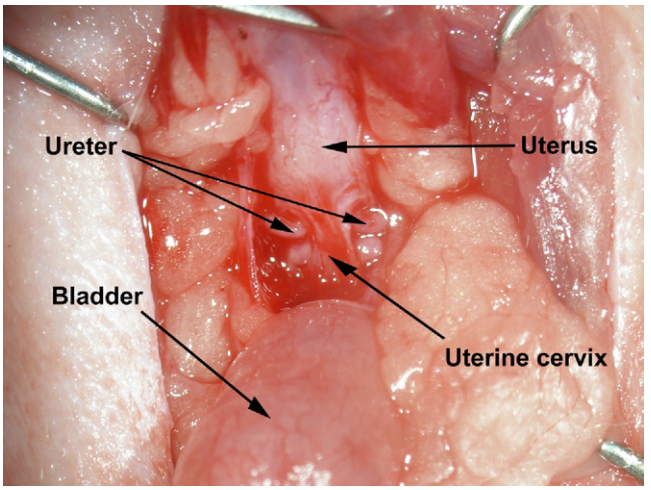

Figure 1. UD in rat was done by surgically disconnecting ureters from bladder and implantation to uterine cervix. Ureters were identified and ligated distal. Two small orifices were made using drill in uterine cervix, and 1 to 2 mm of ureter were brought through orifice and secured to cervix with 1 suture close to medial side at 3 o'clock position. Abdomen was closed.

Histology

The bladder, mid vagina and urethra were processed, embedded, sectioned transversely at 5 μm and stained with Masson's trichrome. Specimen cross sections were examined by light microscopy and photographed.

Image Analysis

We analyzed Mason's trichrome stained sections at the equatorial midline with Image-Pro® Plus 5.1 image analysis software, which can distinguish regions stained with different colors and accurately measure such areas.[11] We used this color segmentation method to determine the whole cross-sectional area, and the tissue area that stained pink (urothelium), blue (collagen) and red (smooth muscle). These 3 components are expressed as percent of total tissue area. In all cases image processing was done by 1 investigator blinded to treatment group assignments.

Immunoblotting

Frozen bladder tissues were homogenized. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Equal amounts of protein extract (40 μg) from the 3 groups at the same time points were distributed to the same gel to decrease nontreatment effects. Proteins were then transferred to nitrocellulose membranes, probed with primary antibody and incubated with secondary antibodies. Bands were visualized using enhanced chemiluminescence and HyBlot CL™ autoradiography film. The primary antibodies used were mouse anti-M2 (Affinity BioReagents, Golden, Colorado) (1:5,000), rabbit-anti-M3 (Sigma-Aldrich®) (1:200), rabbit anti-