Combination of bladder ultrasonography and novel cystometry method in mice reveals rapid decrease in bladder capacity and compliance in LPS-induced cystitis

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VARIOUS DISEASES CAUSE BLADDER dysfunction with lower urinary tract symptoms (11). Some diseases, such as overactive bladder, interstitial cystitis, and neurogenic bladder, lead to storage failure, and other diseases, such as underactive bladder and bladder neck constriction, lead to voiding failure. Objective evaluation of bladder function is indispensable for accurate diagnosis and development of appropriate treatment strategy for these diseases. Bladder ultrasonography, which can evaluate postvoiding residual volume and bladder wall thickness, is noninvasive and the most common examination for bladder function (5). Cystometry, which can identify detrusor overactivity, low bladder compliance (BCP), and sensory urgency, is an invasive but gold standard examination for bladder function (8). Thus these examinations have played an important role in the clinical practice of bladder dysfunction. Although the prevalence of these diseases is considerably high (14), the pathophysiology of these diseases is not completely elucidated, and effective therapies have not been established in several diseases. Therefore, further research is needed to elucidate the pathophysiology of bladder dysfunction in detail and resolve these clinical problems (2).

To investigate the molecular bases of bladder dysfunction, some animal models have been used and have provided important information on bladder physiology and pharmacology (1). Because of its feasibility for genetic modification and abundant databases, the mouse has become a highly useful model for biological research. Actually, previous studies using genetically modified mice have revealed important contributions of several genes to bladder function, such as TRPV channels (3, 9) and P2X receptors (6). Despite several advantages over other animals, mice remain less popular animal models in research into bladder function, the major reason being the difficulties in functional analysis of the mouse bladder. In vivo cystometry is a most common method of analyzing bladder function, but it is rather difficult to perform reliably in mice (1). Actually, the cystometric voided volume (VV) of the normal mouse varies widely between 29 and 168 µl according to previous reports (9, 17, 23, 24). However, these cystometric VVs are remarkably small compared with mouse spontaneous VVs estimated from voided spots on paper (ranging from 200 to 520 µl) (12, 16, 22). It has been suggested that the low VV as measured by mouse cystometry can be attributed to the inhibition of bladder function caused by anesthesia (25), laparotomy, and bladder catheter placement. Because bladder catheter placement will affect bladder wall movement in cystometry, the catheter position in the mouse bladder is one of the most important factors. In almost all previous studies, the bladder catheter was placed into the bladder apex during mouse cystometry (1); therefore, we wonder whether bladder filling and emptying are physically inhibited by the catheter. For the accurate examination of bladder function, the physical inhibition of bladder wall movement caused by the bladder catheter should be minimized, and the most reliable cystometric method with appropriate catheter placement should be established. Furthermore, for the precise...
and detailed analysis of bladder function, the maximum bladder capacity and postvoiding residual volume should also be examined. Although these parameters are quite important for the examination of human bladder function in clinical practice, there are few reports analyzing these factors in rodents (12). In the present study, we examined the efficacy of transabdominal bladder ultrasonography for analysis of mouse bladder function. Then, we investigated the influence of the bladder catheter on bladder function by a combination of ultrasonography and cystometry and established the most reliable cystometry method with novel placement of the bladder catheter.

Lipopolysaccharide (LPS) is a component of the gram-negative bacteria cell wall and a common virulence factor. LPS initiates the innate immune response via Toll-like receptor 4 and induces inflammation (13). LPS has been used in animal models of inflammation including urinary tract infections, and LPS-induced cystitis has been an experimental animal model of acute bacterial cystitis (4, 10, 15, 19). Previous studies revealed that intravesical LPS instillation causes histological inflammation in the bladder wall. Therefore, bladder function also could be altered by LPS, but there have been few studies on functional changes in the mouse bladder induced by LPS instillation. Determining the influence of LPS on bladder function will contribute to the elucidation of mechanisms of voiding dysfunction and pelvic pain due to acute bacterial cystitis. In this study, we analyzed functional changes in the bladder caused by LPS-induced cystitis by a combination of ultrasonography and a newly established cystometry method.

MATERIALS AND METHODS

Animals

Male mice (C57BL/6, 12–16 wk old), weighing 25.0–31.0 g, were used in this study. Animals were housed under a 12:12-h light-dark cycle with controlled humidity and temperature and free access to food pellets and tap water. The experimental protocol was approved by the Animal Ethics Committee of Osaka University, Osaka, Japan.

Bladder Ultrasonography

Transabdominal bladder ultrasonography was performed in mice with a Vevo 770 Imaging System equipped with a 25-MHz transducer (Visual Sonics, Toronto, ON). Each mouse was placed in the supine position, and the ultrasonography probe was delicately placed on the lower abdomen of the mouse with sonographic jelly, and the maximum sagittal cross section of the bladder was visualized.

For the examination of spontaneous voiding, mice were anesthetized with 1.2 g/kg urethane subcutaneously (sc; Sigma, St. Louis, MO), and upon the absence of pedal reflex, the lower abdomen was shaved to obtain a clear image, and a small incision in the lower abdomen was made as a sham operation (Sham group; n = 5). The incision was soon sutured. The ultrasonography study was performed 6 h after the sham operation. Before analysis, mice were fed with 1 ml distilled water to increase the output of urine. The prevailing largest and postvoiding smallest cross-sectional areas (CSA) of the bladder were recorded.

Bladder Catheter Placement

Mice were delicately placed in an induction chamber, and anesthesia was induced with 3% isoflurane (Abbott Laboratories, Tokyo, Japan) to prevent the bladder from emptying before implantation of the bladder catheter. After induction, mice were anesthetized with urethane (1.2 g/kg sc), and upon the absence of a pedal reflex, the operation was started.

The bladder catheter was implanted in two different ways: one was in the bladder apex (Apex group; n = 5), and the other was in the bladder anterior wall (Anterior group; n = 5). In the Apex group, the urinary bladder was exposed by way of an abdominal midline incision. A catheter (PE-50; BD Japan, Tokyo, Japan) with a collar was inserted through the bladder apex and secured with a 7-0 nylon suture (NESCOSUTURE; Alfresa Pharma, Osaka, Japan). The catheter exited near the xiphoid process (Fig. 1A). In the Anterior group, the bladder anterior wall was exposed by way of a 1-cm lower abdominal midline incision. A catheter (PE-50; BD Japan, Tokyo, Japan) with a collar was inserted through the bladder apex and secured with a 7-0 nylon suture (NESCOSUTURE; Alfresa Pharma, Osaka, Japan). The catheter exited near the pubic bone (Fig. 1B). Only the fascia layer of the surgical wound was closed; the skin layer was left open to obtain a clear image for bladder ultrasonography.

Fig. 1. Surgical procedure for bladder catheter placement. A: in the Apex group, the catheter was placed in the bladder apex and exited near the xiphoid process. B: in the Anterior group, the catheter was placed in the center of the bladder anterior wall and exited the lower abdomen 1 cm above the pubic bone.
Combination of Cystometry and Bladder Ultrasonography

Cystometry was started 6 h after placement of the bladder catheter and was combined with bladder ultrasonography. Mice were placed in the supine position, and the ultrasound probe was carefully placed on the lower abdomen so as not to change the position of the catheter. The catheter was connected with a T stopcock to an infusion pump (TE-351; Terumo, Tokyo, Japan) and a pressure transducer (DX-360; Nihon Kohden, Tokyo, Japan). Bladder activity was monitored by way of the catheter connected to the pressure transducer. Variations in intravesical pressure were recorded in response to continuous infusion (1.5 ml/h) of room-temperature saline. The signal from the pressure transducer was amplified by an MEG-6108 multichannel amplifier (Nihon Kohden) and acquired on the computer using PowerLab and Chart 5 (ADInstruments, Bella Vista NSW, Australia). The cystometric parameters measured in this study were pressure threshold (PT; cmH₂O), which was the intravesical pressure to induce a voiding contraction; maximum voiding pressure (MVP; cmH₂O), which was the peak intravesical pressure during voiding; resting pressure (RP; cmH₂O), which was the lowest pressure immediately after a voiding; and VV (µl), which was calculated by multiplying infusion rate by micturition interval; and BCP (µl/cmH₂O), which was calculated as the increase in bladder volume divided by the increase in bladder pressure during the storage phase. The ultrasonographic parameters measured in this study were prevoiding largest CSA (mm²) and postvoiding smallest CSA (mm²) of the bladder. In all animals, cystometry and bladder ultrasonography were recorded for more than 1 h.

LPS-Induced Cystitis Model

LPS prepared by phenol extraction from *Escherichia coli* serotype 0111:B4 was purchased from Sigma. LPS (1 mg/ml) in normal saline was continuously infused into the bladder (1.5 ml/h) for 1 h. After LPS infusion, normal saline alone was again infused into the bladder at the same 1.5-ml/h rate. Effects of LPS on bladder function were...
evaluated 1 h after stopping the LPS infusion. At the end of each experiment, mice were euthanized with pentobarbital sodium (200 mg/kg ip).

Statistical Analysis

Results are given as mean values and SE. The ultrasonographic differences among the Sham group, Apex group, and Anterior group were analyzed by ANOVA followed by a post hoc Tukey’s HSD test. The ultrasonographic and cystometric differences between the Apex group and Anterior group were analyzed by Student’s t-test. The cystometric and ultrasonographic changes by LPS instillation were analyzed by a paired t-test, and the interactions were tested by mixed effects models. Statistical significance was considered at P < 0.05. Statistical analyses were performed using JMP version 9.0.3 software (SAS Institute, Cary, NC) and Stata version 12.1 (Stata, College Station, TX).

RESULTS

Ultrasonographic Findings of Voiding With or Without Bladder Catheter

We performed transabdominal ultrasonography in the mice, and sagittal cross sections of the bladder were recorded during the voiding process (Fig. 2; Supplemental Videos 1–3; all supplemental material for this article is available on the journal website). In spontaneous voiding without the bladder catheter (Sham group), the bladder apex made a dynamic movement (Fig. 2A). However, the position of the bladder anterior wall (Fig. 2A (Sham group), the bladder apex made a dynamic movement (Fig. 2A). In addition, VV and BCP were significantly lower in the Apex than Anterior group (Apex vs. Anterior: 7.4 ± 0.7 vs. 5.1 ± 2.0 cmH2O; P = 0.0046) (Fig. 3C). In addition, VV and BCP were significantly lower in the Apex than in the Anterior group (Apex vs. Anterior: 7.4 ± 0.7 vs. 5.1 ± 2.0 cmH2O; P = 0.0046) (Fig. 3C). Meanwhile, there were no significant differences in voiding smallest CSA between the three groups (Sham vs. Apex vs. Anterior: 7.4 ± 0.7 vs. 5.1 ± 2.0 vs. 8.4 ± 1.3 mm2, P = 0.3000) (Fig. 2D). These data revealed that the movement of the bladder wall was affected by catheter placement, but the ultrasonographic findings of the Anterior group were more comparable to spontaneous voiding than those of the Apex group.

Bladder Functional Analyses in Cystometry With Different Catheter Placement

We performed cystometry to compare the bladder functional parameters between the Apex and Anterior groups (Fig. 3, A and B). PT was significantly higher in the Apex than Anterior group (Apex vs. Anterior: 7.2 ± 0.5 vs. 4.4 ± 0.5 cmH2O, P = 0.0046) (Fig. 3C). In addition, VV and BCP were significantly lower in the Apex than in the Anterior group (Apex vs. Anterior: VV, 149.2 ± 9.1 vs. 262.5 ± 22.7 μl, P = 0.0017; BCP, 27.0 ± 3.7 vs. 82.0 ± 9.8 μl/cmH2O, P = 0.0008) (Fig. 3C). Meanwhile, there were no significant differences in MVP and RP values between the two groups (Apex vs. Anterior: MVP, 28.8 ± 1.8 vs. 29.7 ± 0.8 cmH2O, P = 0.7961; RP, 1.3 ± 0.5 vs. 1.0 ± 0.3 cmH2O, P = 0.6898) (Fig. 3C).

Fig. 3. Bladder functional analyses in cystometry with different catheter placements. Cystometrogram from the Apex group (A) and Anterior group (B) are shown. C: cystometric parameters were compared between the Apex (n = 5) and Anterior (n = 5) groups. PT, pressure threshold; MVP, maximum voiding pressure; RP, resting pressure; VV, voided volume; BCP, bladder compliance. Values are means ± SE. Asterisks mark significant differences (*P < 0.01, Student’s t-test).
Bladder Functional Analyses in LPS-Induced Cystitis Model by Combination of Ultrasonography and Cystometry

The LPS-induced cystitis model is an experimental animal model of acute bacterial cystitis. We performed bladder functional analyses in this model by a combination of bladder ultrasonography and cystometry. Cystometry was performed by a conventional method (Apex group) and a new method (Anterior group). Cystometrograms and ultrasonographic images showed that intravesical LPS instillation decreased the intervals between consecutive voiding contractions (intercon-

Fig. 4. Bladder functional analyses in LPS-induced cystitis by combination of ultrasonography and cystometry. Cystometrograms and ultrasonographic images after intravesical LPS instillation in the Apex (A) and Anterior groups (B) are shown. C: changes in cystometric and ultrasonographic parameters caused by LPS instillation were compared between the Apex (n = 5; ○) and Anterior groups (n = 5; ●). The extent of the decreases in VV and BCP was greater in the Anterior than Apex group. Values are means ± SE. Asterisks and number sign mark significant differences (*P < 0.05, **P < 0.01, paired t-test. #P < 0.05, mixed effects models).
traction interval), largest CSA, and postvoiding smallest CSA in both groups (Fig. 4, A and B). The effects of LPS instillation on cystometric and ultrasonographic functional parameters were examined and compared between the two groups. In the Apex group, LPS instillation significantly decreased MVP (from 24.7 ± 2.3 to 18.0 ± 0.9 cmH_2O, P = 0.002), VV (from 152.2 ± 10.1 to 81.5 ± 11.7 μl, P = 0.0026), largest CSA (from 32.9 ± 0.5 to 23.9 ± 0.8 mm², P = 0.0014) and smallest CSA (from 4.5 ± 1.0 to 1.3 ± 0.1 mm², P = 0.0418). There were no significant changes in PT (from 6.1 ± 0.5 to 4.5 ± 0.3 cmH_2O, P = 0.0728), RP (from 1.3 ± 0.3 to 1.5 ± 0.2 cmH_2O, P = 0.5428) or BCP (from 32.6 ± 2.4 to 27.9 ± 4.1 μl/cmH_2O, P = 0.3905). In the Anterior group, LPS instillation significantly decreased VV (from 272.0 ± 14.2 to 147.0 ± 12.7 μl, P = 0.0011), BCP (from 76.7 ± 4.1 to 56.5 ± 4.6 μl/cmH_2O, P = 0.0062), largest CSA (from 44.3 ± 1.3 to 312 ± 1.1 mm², P = 0.0031) and smallest CSA (from 7.2 ± 0.8 to 2.0 ± 0.3 mm², P = 0.007). There were no significant changes in PT (from 4.7 ± 0.3 to 3.8 ± 0.1 cmH_2O, P = 0.0556), MVP (from 30.0 ± 1.5 to 25.8 ± 2.6 cmH_2O, P = 0.066) or RP (from 1.1 ± 0.2 to 1.2 ± 0.1 cmH_2O, P = 0.5012). It was noteworthy that the extent of decrease in VV and BCP were greater in the Anterior than the Apex group (P = 0.017 and 0.0374, respectively).

DISCUSSION

In this study, we demonstrated the usefulness of transabdominal bladder ultrasonography for analysis of mouse bladder function and the validity of a new cystometry method with the bladder catheter placed in the bladder anterior wall. In addition, we revealed rapid bladder functional changes induced by intravesical LPS instillation by a combination of ultrasonographic and cystometric analyses.

Usefulness of Transabdominal Bladder Ultrasonography

Although the VV can be estimated by cystometry or measuring voided spots on paper (22), maximum bladder capacity and postvoiding residual volume cannot be examined at all by these methods. Postvoiding residual volume measured by ultrasonography is a very important parameter with which to evaluate bladder function in humans and is frequently examined in clinical practice (18). However, these parameters have rarely been investigated in rodents (12). This is the first report, to our knowledge, on the transabdominal ultrasonographic investigation of bladder dynamics, maximum bladder capacity, and postvoiding residual volume in mice. The correlation between CSA and intravesical volume was analyzed, and CSA was confirmed to be a useful parameter correlating with intravesical volume in both the Apex and Anterior groups (Fig. 5). Bladder ultrasonography revealed the inhibition of bladder distention by the bladder catheter and the decrease in maximum bladder capacity and postvoiding residual volume caused by LPS instillation. Therefore, ultrasonographic analyses in animal models will enable us to examine detailed bladder function more accurately.

Validity of the New Cystometry Method With the Catheter Placed in the Bladder Anterior Wall

In this study, we have established a novel cystometry method with the catheter placed in the bladder anterior wall. Bladder ultrasonography revealed that the catheter conventionally placed in the bladder apex (Apex group) inhibited bladder apex movement and bladder distention. In contrast, catheter placement in the bladder anterior wall (Anterior group) did not inhibit bladder apex movement, and the bladder was well distended in the Anterior group. Therefore, we considered our new cystometry method to reflect more spontaneous urination than the conventional method. Cystometric analysis of bladder function combined with bladder ultrasonography showed that PT was lower in the Anterior than Apex group. In addition, VV, BCP, and largest CSA were higher in the Anterior than Apex group. These findings indicated that bladder wall movement in the Anterior group was free from catheter compression. Because mouse bladder function is susceptible to catheter placement due to the small size of the bladder, the effect of the catheter should be minimized. Our new cystometry method appears to minimize the effect of the catheter and may provide a more accurate and reliable analysis of mouse bladder function. In fact, the cystometric VV measured by this new method was more comparable to the spontaneous VV than that reported in previous studies (9, 17, 23, 24). Moreover, this new method enabled us to determine the decrease in BCP that occurs in LPS-induced cystitis, which was not detectable by the conventional cystometry method. Thus our new cystometry method should contribute to more accurate and detailed analysis of bladder function in mouse disease models. We have demonstrated the usefulness of our new cystometry method in a model of bladder hyperactivity. However, it has not been studied in a model of increased bladder capacity.
Further study is needed to demonstrate its usefulness in such models.

**Bladder Functional Changes in LPS-Induced Cystitis**

Acute bacterial cystitis is one of the most common urinary tract infections, and typical symptoms are a burning sensation during urination, frequent urge to urinate, and lower abdominal pain. Previous studies have shown that intravesical instillation of LPS causes histological inflammatory changes in the bladder wall, such as infiltration of inflammatory cells, edema, and hemorrhage (4, 10, 15, 19). However, there have been few studies on functional changes to the bladder induced by LPS. By combining bladder ultrasonography with our new cystometry method, we revealed that LPS instillation caused decreases in VV, BCP, maximum bladder capacity, and postvoiding residual volume. Interestingly, these functional changes were observed 1 h after LPS instillation, when histological inflammatory changes were not seen yet, according to previous reports (10). This rapidity of functional change is reasonable in terms of biological defensive responses to bacterial infection; however, the mechanisms of these rapid changes are not clear. LPS has been reported to lead to rapid secretion of inflammatory cytokines such as interleukin-6 from urothelial cells (17, 21), and inflammatory cytokines have been suggested to be involved in bladder activity (5) and hyperalgesia (20). These findings suggest that the chemical mediators secreted from urothelial cells may cause the rapid changes in bladder function. However, further study is necessary to elucidate the precise mechanisms by which chemical mediators cause rapid decreases in bladder capacity and compliance in LPS-induced cystitis.

**Conclusion**

We demonstrated the usefulness of bladder ultrasonography for the analysis of mouse bladder function and established a new cystometry method with the catheter placed in the bladder anterior wall. The combination of bladder ultrasonography and this new cystometry method provided more accurate and detailed analysis of mouse bladder function than the conventional method. By combination analyses, we revealed decreases in bladder capacity and BCP caused by intravesical LPS instillation. Our new method may become a powerful tool for analysis of mouse bladder function and could contribute to the development of new treatments for bladder dysfunction caused by various diseases.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**

