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THE DEVELOPMENT OF NON-INVASIVE VOIDING FUNCTIONAL TEST IN CONSCIOUS MICE TO MEASURE POST-VOID RESIDUAL URINE BY TRANSRECTAL APPROACH WITH MINIATURE ULTRASOUND PROBE

Hypothesis / aims of study

In order to evaluate voiding dysfunction, ultrasound demonstration of post-void residual urine (PVR) is essential, and its non-invasive nature allows us repeatable examinations to monitor the voiding function in comparison under certain treatments in clinical setting. Not only humans but also any animals, the bladders can be visualized with ultrasonography at any time of the urination cycle, and PVR can thus be estimated from the bladder image. To our knowledge, however, there have been no reports on ultrasonographic estimation of PVR in small rodents such as mice. Recently, miniature ultrasound probes were develop for the initial purpose of the endoscopic examinations of the gastrointestinal tract, and were also found available for examination in human urinary tract. Here, we report to apply such newly-developed miniature ultrasound probe (2 mm in diameters) to visualize the mice bladder with transrectal approach. This technique has enabled us to estimate the bladder volume in conscious mice any time of the urination cycle, and to be the repeatable PVR test for monitoring the voiding function of conscious mice.

Study design, materials and methods

Using TRUS probe of ALOKA-560 with 20MHz (6Fr MP-PN20-06M) (Aloka, Tokyo, Japan), we first verified the precision of measurement of PVR in autopsied mouse; then, applied in conscious mouse. (i) Study in autopsied mouse: The bladder of autopsied mouse was exposed through an abdominal midline incision. Urine was drained with 24G small injection needle implanted into bladder. After emptying the bladder, seven kinds of volumes of saline pre-measured accurately (0.01, 0.02, 0.03, 0.05, 0.10, 0.20, 0.30ml) were injected into bladder. The probe was inserted gently with a little sonographic jelly into the anus of the mouse until the bladder was visualized. The bladder volume was estimated by maximum section planimetry, using the semiplanimetric ellipsoid formula ; PVR (ml) = (ventro-dorsal diameter (mm)) x **(**transverse diameter (mm))^2 x 0.5x 0.001. The examinations with saline leakage from external urethral orifice or pinhole were excluded from the analysis. The precision of the TRUS measurement was evaluated with parameter as follows: bladder volume measured by TRUS / saline volume injected into bladder. Measurement error was also evaluated with parameter as {(Volume measured by TRUS)-(Volume injected into bladder)} / (Volume injected into bladder) ×100. (ii) PVR measure in conscious mouse: The mouse was placed in a translucent cage and was observed carefully. Immediately after voluntary urination, the urine was wiped off with a pre-weighted small piece of paper towel, to determine the micturition volume by the weight gain of the paper towel on the assumption that the specific gravity of the urine is 1.0. The mouse was then restrained gently with the examiner's hand without anaesthesia, and TRUS test was performed as mentioned above, in comparison of the measure of PVR at the first urination after subcutaneous administration of atropine sulphate (0.01mg/g body weight).

Results

Pilot TRUS experiments using autopsied mice indicated that the bladder could be visualized clearly when the body weight of the animal was more than 10 g. In living mice, holding with an examiner's hand sometimes caused leakage of the urine, but this could be avoided by habituation of the mice and training of the examiner. With careful techniques, the probe insertion (1 – 2 cm from the anus) caused no apparent complications such as breeding or perforation. The precision of the urine volume measurement by TRUS were assessable for in vivo measurement of PVR. The parameter of precision (urine volume measured by TRUS / saline volume injected into bladder) were 0.74±0.15 (0.01ml injection, n=12), 0.99±0.16 (0.02ml, n=12), 1.02±0.16 (0.03ml, n=13), 0.95±0.09 (0.05ml injection, n=17), 0.98±0.06 (0.10ml injection, n=12), 0.99±0.05 (0.20ml injection, n=9) and 0.86±0.09 (0.30ml injection, n=5). (Table 1). Thus, our procedure is reliable for a wide range between 0.01 ml and 0.30 ml. In conscious mice, the TRUS measurement of PVR was repeatable without apparent complications such as breeding or perforation, to reveal the significantly increased PVR from 0.013ml before administration of atropine (n=20) to 0.124 ml after it ($p<0.001$). (Figure 2)

Interpretation of results

With careful gentle techniques, the probe insertion (1 – 2 cm from the anus) caused no apparent complications such as breeding or perforation in conscious mice. TRUS measure of the bladder in conscious mice is a reliable and useful method to monitor the voiding function.

Concluding message

Real-time transrectal ultrasonic imaging analysis of the bladder with miniature US probe is a useful modality to monitor the urinary function in conscious mice.

Injected volume	**0..01ml** (n=12)	**0.02ml** (n=12)	**0.03ml** (n=13)	**0.05ml** (n=17)	**0.10ml** (n=12)	**0.20ml** (n=9)	**0.30ml** (n=5)
Volume measured by TRUS (ml) Mean±SE	0.007±0.001	0.020±0.003	0.031±0.005	0.047±0.004	0.098±0.006	0.198±0.010	0.260±0.026
Precision of the measurement Volume measured by TRUS/Volume injected into the bladder	0.74±0.15	0.99±0.16	1.02±0.16	0.95±0.09	0.98±0.06	0.99±0.05	0.87±0.09
Measurement error (%) {(Volume measured by TRUS)-(Volume	-25.9±14.9	-0.7±16.1	-9.7±10.6	-3.4±11.9	-2.1±5.9	-0.8±5.0	-13.3±8.8

injected into the bladder)} /(Volume injected into the bladder) × 100							

Table 1. Precision of measurement: the bladder volumes measured by transrectal ultrasonography were compared with the saline volumes injected into bladder.

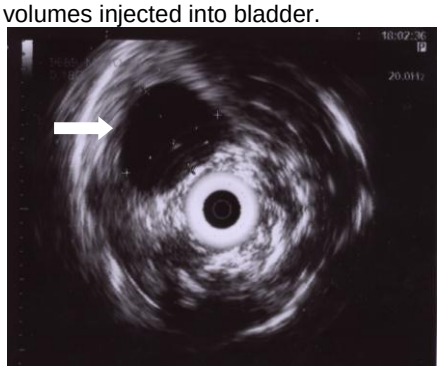

Figure 1. Postvoid residual urine (PVR) measured by miniature TRUS at 10 weeks old mouse. Arrows indicate the bladder with PVR.

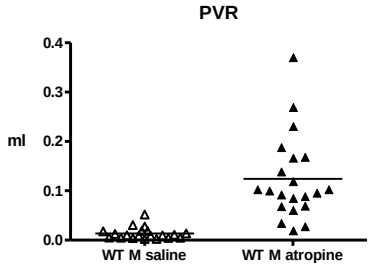

Figure 2 Postvoid residural urine measurements in conscious WT mice using transrectal ultrasonography after administration of saline and atropine.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee policies of Tokyo University**