Functional analysis of urethral continence reflexes using simultaneous bladder and urethral pressure recordings in a rat model of SUI induced by multiple simulated birth traumas.

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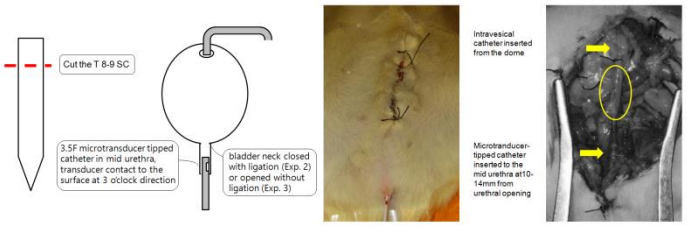
Aims of study

One of the major aetiology of stress urinary incontinence (SUI) in women is vaginal childbirth. Rats with simulated birth trauma induced by vaginal distention (VD) has been used as a model of child birth-related SUI; however, the SUI condition in this model is usually short lasting (7~10days) [1]. Also, multiparity with 3 or more live births reportedly increases the risk of SUI [2]. In addition, the measurement of leak point pressure (LPP), which is the bladder pressure at urine leakage, has often been used to access the urethral function; however, the actual changes in urethral pressures during LPP evaluation are not well characterized. Therefore, we used a novel method capable of measuring LPP and urethral pressure simultaneously under the bladder neck open condition, and investigated the functional changes in the urethral continence function in a rat model of multiple simulated birth traumas.

Ref) *1. J Urol. 2014 Feb;191(2):529-38.*
2. Climacteric. 2013 Dec;16(6):653-62.

Study design

Forty-nine female SD rats were used. All rats underwent spinal cord transection at the T8-9 level to block the spino-bulbo-spinal voiding reflex under isoflurane anesthesia.
1. First, after a PE60 catheter was inserted into the bladder through the dome, LPP without a urethral catheter was measured under urethane anesthesia. Thereafter, a microtransducer-tipped catheter was inserted into the mid urethra at 10-14 mm from the urethral meatus.



2. Then, LPPc (leak point pressure with a urethral catheter), UBP (urethral baseline pressure), dUP (differential values of urethral pressure during intravesical pressure elevation), Puc (vesical pressure when urethral contraction begins), Pno (vesical pressure when bladder neck begins to be opened, and MUP (maximal urethral pressure) were measured under urethane anesthesia (Fig. 1.). LPP and LPPc were defined as the intravesical pressure at which the fluid leakage occurred by increasing intravesical pressure gradually using a water reservoir connected to the bladder. UBP was measured as the urethral pressure at 0 cmH_2O intravesical pressure for more than 2 minutes. dUP was defined as the differences between MUP and UBP. Puc was measured as the bladder pressure when reflex urethral contractions began to increase. Bladder neck opening was confirmed by the beginning of amplitude increases of urethral contraction or the steep elevation of urethral pressure. Pno was measured as the vesical pressure when bladder neck begins to be opened. MUP was calculated as the urethral pressure at which bladder neck is opened. **The timing of bladder neck opening was determined by the time of appearance of urethral pressure fluctuations (point F in Fig. 1), which corresponded to the timing when urethral pressure reached a plateau despite the increase of intravesical pressure in rats with ligated bladder neck (Fig. 2).**

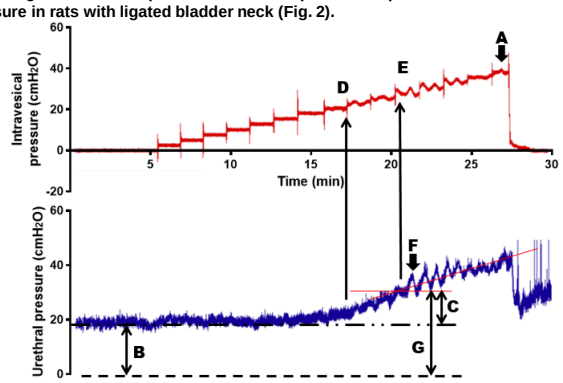


Fig. 1. Representative recordings of LPPc (A; leak point pressure with urethral catheter), UBP (B; urethral baseline pressure), dUP (C; differential values of urethral pressure during intravesical pressure elevation), Puc (D; vesical pressure when urethral contraction begins), Pno (E; vesical pressure when bladder neck is opened), the point at which bladder neck is opened (F), and MUP (G; maximal urethral pressure)

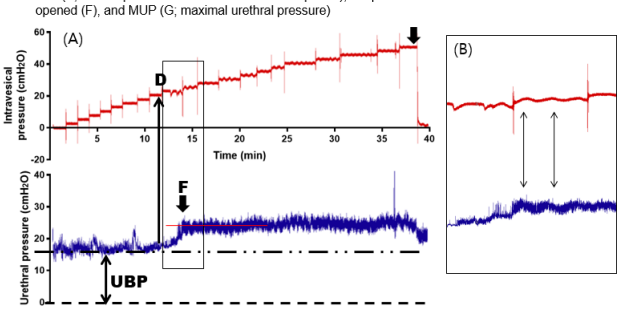
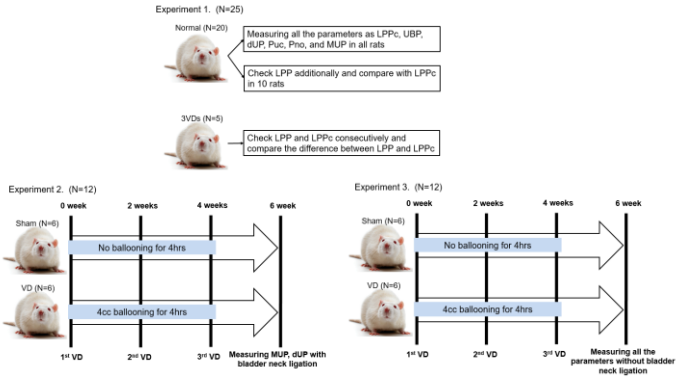


Fig. 2. Representative recordings of simultaneous bladder and urethral pressure in the rat with bladder neck ligation (A). Urethral pressure begins to increase when intravesical pressure exceeds the UBP (poin t D). At this same time, small detrusor and urethral contractions occurs synchronously (B). Thereafter, urethral pressure gradually increases as the bladder is distended, and then the urethral pressure is plateaued from the point F and , afterwards, does not increase any more even though intravesical pressure continued to be increased. Because the urethral pressure continues to elevate beyond the point F in the bladder neck opened condition (Fig. 1), it is considered that the bladder neck opens at the point F to induce the continued urethral pressure elevation with urethral pressure fluctuations due to fluid leakage into the proximal urethra as seen in Fig.1, but not in the bladder neck closed condition as in this figure.

Experiment 1: 20 untreated rats and 5 rats with 3-times VDs were used to validate the methodology to confirm that simultaneous recordings of bladder and urethral pressures with urethral catheter insertion does not affect LPP values, which were obtained without urethral catheter insertion.
Experiment 2: 12 rats were divided into 2 groups; (1) sham-operated rats without VD (n=6) and (2) SUI rats with 3-times VDs (n=6), which were conducted every 2 weeks. VD was induced with a balloon catheter inflated in the vagina for 4 hours under isoflurane anesthesia. After bladder neck ligation using a thread, urethral pressure profiles such as MUP and dUP were measured at 2 weeks after the last VD. These parameters were compared to those in rats with opened bladder neck (Exp. 3).
Experiment 3: 12 rats were divided into 2 groups; (1) sham-operated rats without VD (n=6) and (2) SUI rats with 3-times VDs (n=6), which were conducted every 2 weeks. VD was induced with a balloon catheter inflated in the vagina for 4 hours under isoflurane anesthesia. Simultaneous recordings of bladder and urethral pressures were performed at 2 weeks after the last VD to compare LPPc and other parameters between 2 groups under the opened bladder neck condition.



Results

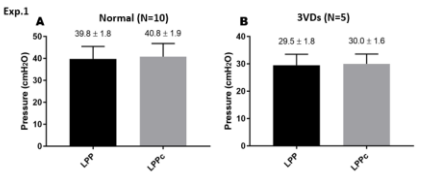


Fig. 3. Comparison between LPP and LPPc in the same rats. p=0.351 in normal group (A) and p=0.705 in 3VDs group (B) by Wilcoxon's rank test

Urethral catheter insertion does not affect the LPP values in normal rats.

Urethral catheter insertion does not affect the LPP values in VD rats, either.

Table. 1. Mean values of all parameters in normal rats

	N	Mean ± SEM
LPPc (cmH_2O)	20	41.1 ± 1.2
UBP (cmH_2O)	20	21.4 ± 1.1
dUP (cmH_2O)	20	8.6 ± 0.4
Puc (cmH_2O)	19	20.7 ± 0.9
Pno (cmH_2O)	19	24.6 ± 1.1
MUP (cmH_2O)	20	30.0 ± 1.2
LPPc-MUP (cmH_2O)	20	11.2 ± 1.0
Pno-UBP (cmH_2O)	19	3.9 ± 0.9
LPPc-Pno (cmH_2O)	19	16.4 ± 1.0

1 missing value for Puc, and Pno, respectively.

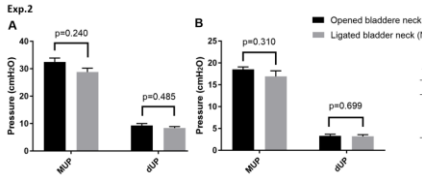


Fig. 4. Comparison of urethral parameters between two experimental groups with opened and ligated bladder neck. Sham group (A), VD group (B). Statistic analysis by Mann-Whitney's U test.

The parameters related to urethral pressure represent no difference between the experiments with opened bladder neck and ligated bladder neck.

Table. 2. Spearman's correlation coefficient between Puc, Pno, and UBP

	Puc	Pno	UBP
Puc	1.000	0.466**	0.501**
Pno	0.466**	1.000	0.645**
UBP	0.501**	0.645**	1.000

Missing values are excluded (N=31). * P < 0.05, ** p < 0.01

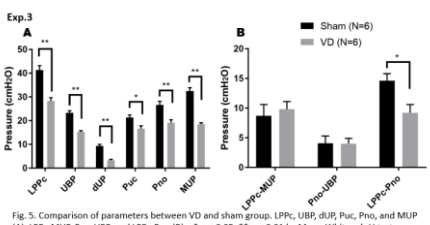


Fig. 5. Comparison of parameters between VD and sham group. LPPc, UBP, dUP, Puc, Pno, and MUP (A). LPPc-MUP, Pno-UBP, and LPPc-Pno (B). * p < 0.05, ** p < 0.01 by Mann-Whitney's U test.

Table. 3. Comparison of parameters between VD and sham group.

Parameters	Sham (N=6)	VD (N=6)	p
LPPc (cmH_2O)	41.3 ± 1.9	28.3 ± 1.4	0.002
UBP (cmH_2O)	23.3 ± 0.9	15.3 ± 0.5	0.002
dUP (cmH_2O)	9.3 ± 0.7	3.3 ± 0.4	0.002
Puc (cmH_2O)	21.3 ± 1.1	16.7 ± 1.1	0.015
Pno (cmH_2O)	26.7 ± 1.5	19.2 ± 1.2	0.009
MUP (cmH_2O)	32.5 ± 1.4	18.5 ± 0.6	0.002
LPPc-MUP (cmH_2O)	8.7 ± 1.9	9.8 ± 1.3	0.699
Pno-UBP (cmH_2O)	4.1 ± 1.2	4.0 ± 0.9	0.818
LPPc-Pno (cmH_2O)	14.6 ± 1.2	9.2 ± 1.4	0.015

All values are presented as mean ± SEM. Statistic analysis by Mann-Whitney's U test

Interpretation of results

Experiment 1: These results indicate that; (1) simultaneous recordings of bladder and urethral pressure using an intravesical catheter and a urethral microtransducer-tipped catheter are useful for detailed characterization of the urethral continence function in a SUI animal model and (2) the urethral catheter insertion does not affect the measurement of LPP during passive intravesical pressure elevation.
Experiment 2: We observed that urethral pressure elevation due to urethral sphincter muscle contraction started when the intravesical pressure exceeded UBP (point D of Fig. 1), and lasted until fluid leakage occurred (point A of Fig. 1) in the bladder neck opened condition. By the comparison of the data between bladder neck opened or closed conditions, it seems that there are two stages of urethral continence mechanisms; (1) the first stage of bladder neck opening when urethral pressure elevation reaches a plateau in the bladder neck closed condition (point F in Fig. 2) and, afterwards, urethral pressure fluctuations appear, and continuous urethral pressure elevation due to fluid leakage into the proximal urethra is continuously seen in the bladder neck open condition and (2) the second stage when the external sphincter opens and actual leakage form the urethral orifice occurs (point A in Figs. 1 & 2).
Experiment 3: In the multiple birth trauma model with 3-times VDs, urethral contractions started earlier (at lower Puc), and increases of urethral pressure (dUP) were smaller in association with significant reductions in other parameters (LPPc, UBP, and MUP) compared to sham rats (Fig. 5), indicating the impaired urethral continence reflex activity in multiple VD rats. It seems that a higher pressure (9-11 cmH_2O) in the bladder than in the urethra (LPPc-MUP) is needed to induce fluid leakage in both sham and SUI rats. In addition, intravesical pressure should exceeds UBP (Pno-UBP) by 4 cmH_2O for opening the bladder neck in both sham and SUI rats. However, a smaller amount of intravesical pressure increase from the Pno (LPPc-Pno) is enough for inducing fluid leakage in SUI rats compared to sham. **These results suggest that multiple VDs impair the external sphincter function evident as the decrease of LPPc-Pno, but not the internal sphincter function shown by no change in Pno-UBP during bladder distention while the reduction in UBP in VD rats may reflect the additional damage of internal sphincter function at baseline.**

Conclusions

- Rats with multiple VDs, which showed the relatively long-lasting SUI condition (> 2weeks after VDs), would be a reliable model to study the pathophysiology of multiple child birth related injuries inducing SUI.
- Our new methods of simultaneous recordings of bladder and urethral pressures would be useful to fully evaluate the functional changes in urethral continence function in SUI models.

Conflict of interest: None

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