available at http://ajws.elsevier.com/tcmj

Tzu Chi Medical Journal

Original Article

Cystometric Changes in Pressure-guided Acute Distension Rat Model of the Underactive Bladder™

*Dae K. Kim[1], Jonathan Kaufman[2], Zhonghong Guan[3], Pradeep Tyagi[4], Naoki Yoshimura[5], Kim A. Killinger[4], Kenneth M. Peters[4], Michael B. Chancellor[4]**

[1]*Department of Urology, Eulji University, Daejeon, Korea*
[2]*Lipella Pharmaceuticals, Inc., Pittsburgh, PA, USA*
[3]*Department of Urology, State University of New York Downstate Medical School, New York, NY, USA*
[4]*Department of Urology, William Beaumont Hospital, Royal Oak, MI, USA*
[5]*Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA*

Article info

Article history:
Received: September 19, 2008
Revised: December 22, 2008
Accepted: December 26, 2008

Keywords:
Bladder
Muscle
Retention
Underactive bladder

Abstract

Objective: Acute bladder distension results in pressure ischemia, subsequent reperfusion injury, and ultimately damage to the detrusor. We hypothesize that changes in pressure may be a key factor to damage resulting from over-distension and developed a pressure-guided distension model to evaluate cystometric changes.

Materials and Methods: Three groups of adult female Sprague Dawley rats (250 g) were used: a sham distended control group, a 3-day (3D) and 7-day (7D) follow-up group after pressure-guided distension. Under pentobarbital anesthesia, the urethra was clamped and saline was infused (0.04 mL/min) under continuous intravesical pressure monitoring. After reaching 120 cmH_2O pressure, infusion was stopped and clamping was maintained for 30 minutes. For sham distension, all procedures except the saline infusion were done.

Results: There were no bladder ruptures during distension. Distension volumes needed to achieve the fixed pressure were variable (1.68–2.90 mL), but mean distension volumes were similar between the 3D and 7D groups (2.1 ± 0.1 mL *vs.* 2.2 ± 0.3 mL). After distension, maximal cystometric capacity and residual urine volume were increased at both time points. Voiding efficiencies were decreased significantly in both the 3D and 7D groups ($p < 0.05$) compared to controls. Maximal vesical pressure and bladder compliance showed no change before and after distension.

Conclusion: Our pressure-guided distension model exhibits cystometric characteristics of bladder decompensation. This model for the underactive bladder™ (UAB) may prove useful to further the development of targeted UAB™ treatments. (*Tzu Chi Med J* 2009;21(2):136–139)

*Corresponding author. Department of Urology, William Beaumont Hospital, 438, Medical Office Building, 3535 W. 13 Mile Road, Royal Oak, MI, USA.
E-mail address: chancellormb@gmail.com

1. Introduction

In the field of urology, overactive bladder syndrome (OAB) is a common medical problem and a number of antimuscarinic drugs are globally available. Yet, the inverse condition, underactive bladder™ (UAB), remains below the radar of academic experts and the pharmaceutical industry [1] even though bothersome symptoms and potential sequelae are significant. Although physicians treat patients with UAB™, the lack of a consensus definition is problematic in terms of syndrome recognition and developing efficacious treatments. The presence of urinary retention, high residual urine volumes, and incomplete bladder emptying have all been used as criteria for defining the disorder. Some of the established causes of UAB include neurogenic, myogenic, aging and medication side effects [2]. Detrusor underactivity is the antithesis to the OAB; however, symptoms may overlap. Common OAB symptoms include urgency, frequency, nocturia, and incontinence that may be overflow, urge and/or stress. Some symptoms more commonly associated with the UAB include hesitancy, sensation of incomplete emptying, straining to void and recurrent infections [3,4].

An interesting hypothesis is that the natural history of disease progression is from OAB toward UAB (Zhonghong Guan, MD, State University of New York Downstate Medical School; personal communication). How can the OAB progress to UAB? Recent research has demonstrated that in OAB, the bladder wall thickens in mass [5,6] and there is a rise in urine nerve growth factor levels [7–9]. Therefore, OAB may involve structural changes that may eventually lead to alteration of muscle and connective tissue structure and function that can result in impaired contractility. To understand UAB and its potential relationship to OAB and other urological conditions, defined and reproducible modeling of the UAB first needs to be established. In this study, we explored modeling UAB in a rat model that would allow us to study new therapies for the UAB.

2. Materials and methods

All experiments were performed on mature female Sprague Dawley (SD) rats (250–300 g) in accordance with the requirements and recommendations in the *Guide for the Care and the Use of Laboratory Animals*. Three groups of adult female SD rats (250 g) were used (n=4 in each group): a sham distended control group, a 3-day (3D) follow-up group and a 7-day (7D) follow-up group after pressure-guided distension.

Under pentobarbital anesthesia (30 mg/kg, i.p.), a PE-50 catheter was inserted via the urethra and connected with a Y connector to an infusion pump and Grass polygraph. A non-traumatic clamp was applied to the distal urethra and saline was infused (0.04 mL/min) under continuous intravesical pressure monitoring. After reaching 120 cmH_2O pressure, the infusion was stopped and clamping was maintained for 30 minutes. Higher pressure resulted in bladder rupture during model development. For sham distension, all procedures were done in the same manner without the saline infusion.

At 3 or 7 days following bladder sham or hydrodistention, transurethral cystometrograms were performed under urethane anesthesia (1.2 g/kg, s.c.). Cystometric parameters included pressure threshold, maximal vesical pressure, maximal cystometric capacity, residual urine volume, voiding efficiency and bladder compliance. The rats were sacrificed and bladder tissues from base of bladder were snap frozen using 2-methylbutane pre-cooled in liquid nitrogen. Serial 10-μm cryostat sections were obtained. Microscopic analysis of the sections was conducted after hematoxylin and eosin staining.

Data are expressed as arithmetic mean of "n" number of experiments and the standard error of the mean was computed. Voiding efficiency was calculated by dividing voided volume by bladder capacity and is expressed as a percentage. The statistical significance was analyzed by one-way analysis of variance followed by Newman Keuls test where appropriate. A level of $p<0.05$ was considered significant. For statistical calculations, the Prism3 statistical and graphic program (Graphpad, San Diego, CA, USA) was used.

3. Results

There were no bladder ruptures during bladder distension. None of the rats died or developed a urinary tract infection. Distension volumes to achieve the fixed pressure were variable (1.68–2.90 mL), but mean distension volumes were similar between the 3D and 7D follow-up groups (2.1±0.1 mL *vs.* 2.2±0.3 mL) (Table 1).

After distension, maximal cystometric capacity and residual urine volume were increased at both 3 and 7 day time points ($p<0.05$). Voiding efficiencies were decreased significantly in both 3D ($p<0.05$) and 7D groups ($p<0.05$) compared to controls. Maximal vesical pressure and bladder compliance showed no change before and after distension (Table 1). Examination of bladder tissues revealed no significant inflammation between control and hydrodistended groups on histology at 3 and 7 days after hydrodistension.

4. Discussion

Treatment options for the UAB include double voiding, straining to void, and indwelling or intermittent

Table 1 — Cystometric variables of each experimental group*

Group after hydrodistension (n=4/group)	Pressure threshold (cmH_2O)	Maximal vesical pressure (cmH_2O)	Maximal cystometric capacity (mL)	Residual urine volume (mL)	Voiding efficiency† (%)	Bladder compliance (cmH_2O/mL)
Control	5.9±0.3	37.1±2.7	0.84±0.08	0.25±0.05	71.1±4.8	5.9±0.3
3 days	8.5±0.9	34.6±1.6	1.91±0.25‡	1.61±0.31‡	18.1±0.7‡	4.2±0.2
7 days	9.4±1.1§	34.5±0.4	1.94±0.18‡	1.43±0.14‡	26.3±4.6‡	4.8±0.9

*Data are presented as mean±standard error of the mean; †voiding efficiency is expressed as a percentage of voided bladder volume; ‡$p<0.01$ *vs.* control; §$p<0.05$ *vs.* control.

catheterization. Standard pharmacotherapy includes the use of α-adrenergic blocker to reduce urethral outlet resistance and muscarinic agonists such as bethechanol. Misoprostol, cholinesterase inhibitor and cholinergic agents are potential treatment candidates, but safety and lack of benefit is a concern [4,10]. Treating UAB with novel muscarinic receptor manipulation such as presynaptic M2 receptor antagonists or postsynaptic allosteric receptor enhancement may yield promising results. Prokinetics used in gastroenterology and smooth muscle ionotropic agents used in cardiology may also warrant careful consideration for UAB treatment. Neuromodulation and intravesical electrical stimulation have also been reported to be potentially beneficial in selective patients. Experimentally, we have been interested in the use of trophic factors such as insulin growth factor and nerve growth factor to improve muscle and nerve function in the lower urinary tract. Use of stem cells and regenerative medicine may allow the weak detrusor to improve contractility [11,12], and finally gene therapy to increase weak individual myosite's contractility with SERCA gene therapy [13].

We have previously explored selective area UAB modeling using cryoinjury [11,12]. An acute invasive bladder injury model was elaborated in our lab by short intense cooling of the bladder muscle. Under our experimental conditions, the cryoinjured bladder smooth muscle exhibited reproducibly impaired contractile parameters such that it may be applied as a model of insufficiently functioning smooth muscle. For example, cryoinjury may be applied for testing of therapies to improve detrusor contractility such as tissue engineering. Similar cryoinjury models have been used to model myocardial necrosis and to test myoblast transplantation in injured heart muscles. Striated muscles have also been subjected to cryoinjury to evoke degraded muscle function [11,12]. One weakness of our model is that urodynamic measurements were done under urethane anesthesia. Although we have not seen decreased voiding efficiency with anesthesia, micturition under anesthesia is not the same as awake and future studies should consider avoidance of anesthesia. Another advancement for future studies would be use of longer duration of distension at lower elevated pressure which may more mimic chronic urinary distension in the clinical setting.

Currently, detailed epidemiologic and market data are lacking for UAB. However, it is likely that a percentage of men and women currently using α-blockers are being treated for UAB [14]. However, the breakdown of these numbers is unknown. Muscarinic agonists are not highly effective and have common side effects. It is possible that the market for UAB treatments, if proven safe and efficacious, can potentially exceed industry expectations as antimuscarinics did for OAB syndrome in the late 1990s. The pharmaceutical and biotechnology industries that have a pipeline in urology and women's health should consider the UAB as a potential target condition for developing new treatments. Our study demonstrated urodynamic and physiological changes after distension. Saito and Miyagawa [15] previously noted that after acute distension of the rat bladder via urethral clamping, dysfunction related to hypoperfusion and partially caused by free radicals may have an important role in bladder dysfunction during acute urinary retention.

The results of our study indicate that our pressure-guided distension model exhibits cystometric characteristics of bladder decompensation in short-term follow-up. Despite variability in maximal distension volume, when the bladder is clamped to sustain high pressure, voiding efficiency significantly decreased. Since the life span of a research rat is typically less than 2 years, a research duration of 3–7 days may suggest a clinical relevant duration of several months, with the limitation of extrapolating animal research to clinical conditions. More research is needed to further establish sound animal models of UAB in order to allow for accurate testing of potential therapeutic agents for the treatment of UAB. A refinement of our model may also be used to test the interesting hypothesis that the OAB may progress to UAB.

References

1. Jonas U, Fowler CJ, Chancellor MB, et al. Efficacy of sacral nerve stimulation for urinary retention: results 18 months after implantation. *J Urol* 2001;165:15–9.

2. Blaivas JG, Chancellor MB. Chapter 25: Urinary retention and dysfunctional voiding. In: Chancellor MB, Blaivas JG, eds. *Practical Neuro-Urology: Genitourinary Complications in Neurologic Disease.* Stoneham, MA: Butterworth Heinemann, 1995:239–46.
3. Yoshimura N, Chancellor MB. Chapter 56: Physiology and pharmacology of the bladder and urethra. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, eds. *Campbell's Urology*, 9th edition. Philadelphia: Saunders, 2007:1922–72.
4. Chancellor MB, Kaufman J. Case for pharmacotherapy development for underactive bladder. *Urology* 2008;72: 966–7.
5. Ukimura O, Kojima M, Iwata T, Inaba M, Miki T. Ultrasonic measurement of bladder weight as a novel urodynamic modality. *Adv Exp Med Biol* 2003;539:311–5.
6. Khullar V, Salvatore S, Cardozo L, Bourne TH, Abbott D, Kelleher C. A novel technique for measuring bladder wall thickness in women using transvaginal ultrasound. *Ultrasound Obstet Gynecol* 1994;4:220–3.
7. Kim JC, Park EY. Nerve growth factor and prostaglandin in the urine of female patients with overactive bladder. *J Urol* 2006;175:1773–6.
8. Liu HT, Chancellor MB, Kuo HC. Decrease of urinary nerve growth factor levels after antimuscarinic therapy in patients with overactive bladder. *BJU Int* 2009 Feb 11. (Epub ahead of print)
9. Liu HT, Chancellor MB, Kuo HC. Urinary nerve growth factor level could be a biomarker in the differential diagnosis of mixed urinary incontinence in women. *BJU Int* 2008; 102:1440–4.
10. Sasaki K, Nishiguchi J, Chancellor MB. Chapter 12: Pathophysiology of the areflexic bladder. In: Corcos J, Schick E, eds. *Textbook of the Neurogenic Bladder.* London: Martin Dunitz, 2004:143–56.
11. Somogyi GT, Szell EA, Yokoyama T, et al. Effect of cryoinjury on the contractile parameters of bladder strips: a model of impaired detrusor contractility. *Brain Res Bull* 2002;59:23–8.
12. Huard J, Pruchnic R, Yokoyama T, et al. Muscle based gene therapy and tissue for urological dysfunction. *Gene Therapy* 2002;9:1617–26.
13. Kim DK, Chancellor MB. *A Pilot Study of AAV1/SERCA2a Gene Transfer in the Rat Stress Urinary Incontinence Model.* American Urological Association Podium Presentation, 2008.
14. Rigby D. Underactive bladder syndrome. *Nursing Standard* 2005;19:57–64.
15. Saito M, Miyagawa I. Bladder dysfunction after acute urinary retention in rats. *J Urol* 2001;165:1745–7.