W19: Final Frontier in LUTS; Targeting the Urothelium to Treat OAB, IC/BPS, Hypersensitive Bladder and Ketamine Cystitis

Workshop Chair: Hann-Chorng Kuo, Taiwan

21 October 2014 09:00 - 12:00

Start	End	Topic	Speakers
09:00	09:05	Introduction	• Hann-Chorng Kuo
09:05	09:35	The Role of Urothelium in Bladder Function and Lower Urinary Tract Dysfunction	• Lori Birder
09:35	10:00	Urological Diseases Associated with Urothelial Dysfunction –Bladder Hypersensitivity, Overactive Bladder, Interstitial Cystitis/Bladder Pain Syndrome and Ketamine Cystitis	• Yao-Chi Chuang
10:00	10:30	Intravesical Botulinum Toxin A Injection in The Treatment of Overactive Bladder, IC/BPS and Hypersensitive Bladder	• Hann-Chorng Kuo
10:30	11:00	Break	None
11:00	11:20	Concept of Drug Delivery Across the Urothelium –	• Michael Chancellor
11:20	11:35	Clinical Experience of Liposome in Treatment of IC/BPS – Animal Model and Human	• Yao-Chi Chuang
11:35	11:55	Clinical Experience and evidence of Liposome Encapsulated Botulinum Toxin A in Treatment of Overactive Bladder	• Chun-Hou Liao
11:55	12:00	Discussion	All

Aims of course/workshop

Urothelial dysfunction might play a role in the abnormality of expression of sensory receptors or release of transmitters in the suburothelial nerves or interstitial cells. In this regard, intravesical treatment to inhibit receptor expression or transmitter release might provide good therapeutic effects in the treatment of sensory urgency, interstitial cystitis/bladder pain syndrome, and overactive bladder (OAB). Intravesical pharmacotherapy has been used for the treatment of refractory OAB and IC/BPS, however, an important obstacle in the success of intravesical drug delivery arises from the low permeability of bladder epithelium. This workshop helps participants select suitable intravesical treatment for urothelial associated LUTD.

Urothelial Signaling

Lori Birder, Karl-Erik Andersson

Departments of Medicine and Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; and the Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston Salem, North Carolina

The urothelium, which lines the inner surface of the renal pelvis, the ureters, and the urinary bladder, not only forms a high-resistance barrier to ion, solute and water flux, and pathogens, but also functions as an integral part of a sensory web which receives, amplifies, and transmits information about its external milieu. Urothelial cells have the ability to sense changes in their extracellular environment, and respond to chemical, mechanical and thermal stimuli by releasing various factors such as ATP, nitric oxide, and acetylcholine. They express a variety of receptors and ion channels, including P2X3 purinergic receptors, nicotinic and muscarinic receptors, and TRP channels, which all have been implicated in urothelial-neuronal interactions, and involved in signals that via components in the underlying lamina propria, such as interstitial cells, can be amplified and conveyed to nerves, detrusor muscle cells, and ultimately the central nervous system. The specialized anatomy of the urothelium and underlying structures, and the possible communication mechanisms from urothelial cells to various cell types within the bladder wall are described. Changes in the urothelium/lamina propria ("mucosa") produced by different bladder disorders are discussed, as well as the mucosa as a target for therapeutic interventions.

Urothelial Signaling. *Physiol Rev* 93: 653–680, 2013; doi:10.1152/physrev.00030.2012.

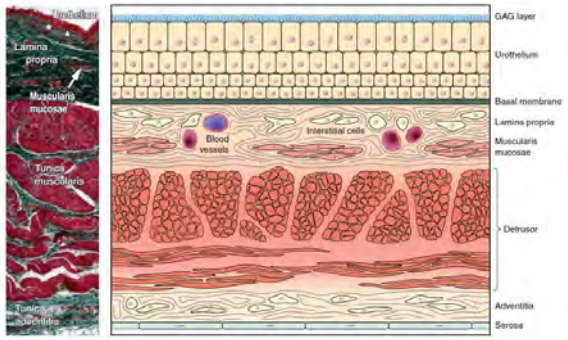
Urological Diseases Associated with Urothelial Dysfunction –Bladder Hypersensitivity, Overactive Bladder, Interstitial Cystitis/Bladder Pain Syndrome and Ketamine Cystitis

Yao-Chi Chuang
Professor
Kaohsiung Chang Gung Memorial Hospital, Taiwan

Agenda

- Structure and function of urothelium
- Bladder Hypersensitivity (BH)
- Overactive Bladder (OAB)
- Interstitial Cystitis (IC)/Bladder Pain Syndrome (BPS)/Painful bladder syndrome (PBS)
- The role of urothelium NGF, ATP, and TRPV1 receptor on OAB and IC/BPS/PBS
- Ketamine Cystitis

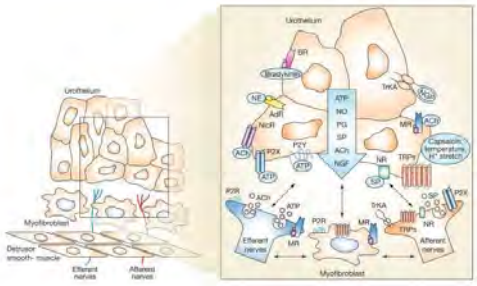
Components of bladder wall



(Birder and Andersson., Physiol Rev , 2013)

Mechanisms of disease: involvement of the urothelium in bladder dysfunction

(Birder and deGroat, Nat Clin Pract Urol 2007)



Sensing Bladder Fullness: Cross-talk Between the Urothelium and the Nervous System

(Apodaca, Traffic, 2004)

- Epithelium receive and transmit signals to submucosal neurons
- Afferent nerves are found within the urothelium and in a nerve plexus just below the basal cell layer
- Urotheliuum may communicate bladder fullness to the underlying nervous system through a paracrine signaling pathway involving ATP release

Disruption of urothelial function induced lower urinary tract dysfunction

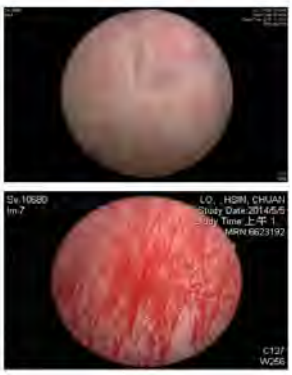
- Modification of the urothelium and/or loss of epithelial integrity in a number of pathologic conditions can result in the passage of toxic and irritating urinary constituents through the urothelium, or release of neuroactive substances from the urothelium.
- Changes in the properties of sensory nerves and sensory symptoms such as urinary frequency and urgency.
- Chemical communication between the nervous system and urothelial cells might have an important role in the generation of urinary bladder dysfunction.

Definiton of IC/PBS/BPS

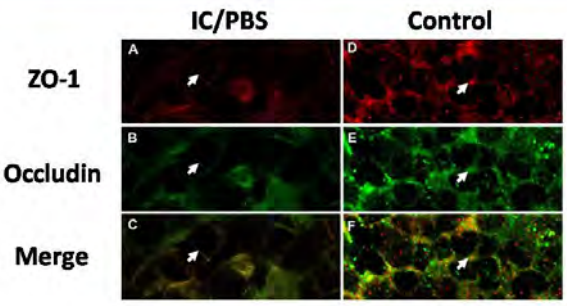
- PBS is defined by the ICS as "suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology
- AUA definition- an unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of more than 6 weeks' duration, in the absence of infection or other identifiable causes
- EAU- "chronic pelvic pain, pressure, or discomfort perceived to be related to the urinary bladder accompanied by at least one other urinary symptom such as persistent urge to void or urinary frequency. Confusable diseases as the cause of symptoms must be excluded

IC/BPS/PBS

- IC/PBS primarily affects women, with a female-to-male ratio of 5:1.
- Potential pathophysiologic causes proposed include inflammatory, neurogenic, autoimmune, vascular, or lymphatic disorders; self-destruction by loss of the glycosaminoglycan layer from superficial cells; and the presence of toxic substances in the urine.
- IC may have multiple etiologies, all of which result in a similar clinical manifestation.



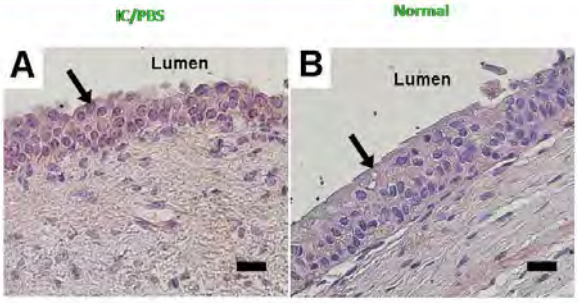
Double Stain



Magnification: 1600x

Lee and Lee. J Formos Med Assoc 2013 (in press).

Thickness of urothelium



Scale bar = 20 mm

Basic and Translational Science

Increased Expression of Hypoxia-inducible Factor-1α and Vascular Endothelial Growth Factor Associated With Glomerulation Formation in Patients With Interstitial Cystitis

Jane-Dar Lee and Ming-Huei Lee

Urology 2011;78:971.e11-5.

IC/PBS (compared with control)

- ✓ HIF-1a expression: cell ischemia ↑
- ✓ VEGF expression: immature vessels ↑
- ✓ Location: Overexpression in Apical cells

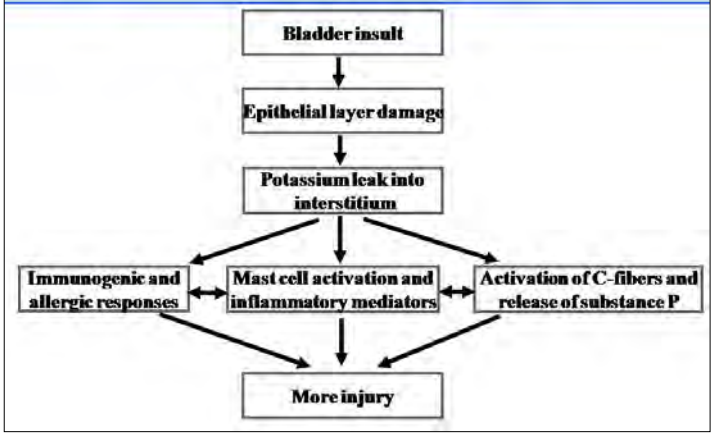
⇒ Glomerulation formation

Hypothesis

- Bladder urothelial dysfunction of IC/PBS patients:
- 1. Tissue ischemia.
- 2. Increased cell apoptosis.
- 3. Decreased tight junction proteins.
- 4. Increased permeability. (leaky urothelium)

Pharmacological treatment of IC

mucosal protection, anthistamine, tricyclic antidepressant, intravesical therapy



Symptom Definitions: 2002 International Continence Society Terminology

- Overactive bladder—urgency, with or without urge incontinence, usually with frequency and nocturia
  - urgency is the complaint of a sudden compelling desire to pass urine, which is difficult to defer

Abrams P et al. *Neurourol Urodynn.* 2002;21:167-178.

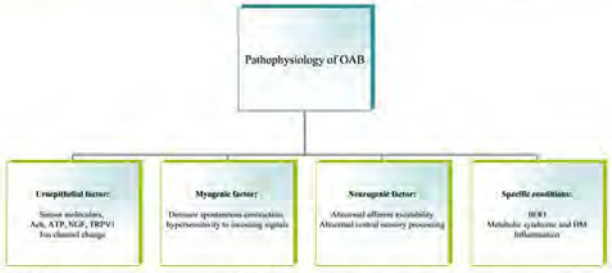
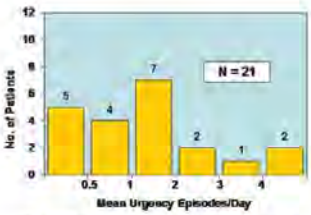


Fig. 1. Possible pathophysiology of overactive bladder. Ach, acetylcholine; ATP, adenosine triphosphate; BOO, bladder outlet obstruction; DM, diabetes mellitus; NGF, nerve growth factor; TRPV1, transient receptor potential vanilloid 1.

Meng and Chuang et al., LUTS, 2012

Defining Overactive Bladder as Hypersensitivity (Yamaguchi et al., Neurourol Urodyn, 2007)

- Difficult for patients to differentiate urgency from normal urge
- 43% of patients seeking medical care, urgency episodes occurred less than once/day, and some patients had days without urgency
- At volumes exceeding 40% of the maximum bladder volume (MBV), urgency episodes occurred frequently and independently of the bladder volume, indicating that 40% of the MBV may be a threshold of bladder volume to induce urgency.



Urge sensation increased markedly at any given bladder volume among patients with OAB compared normal subjects (Yamaguchi et al., Neurourol Urodyn, 2007)

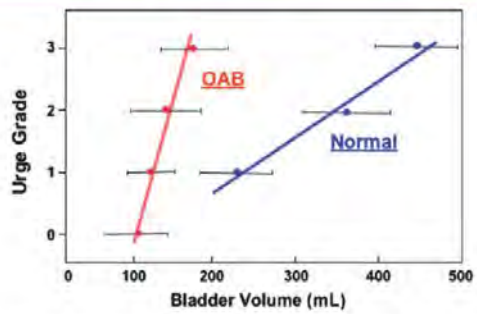


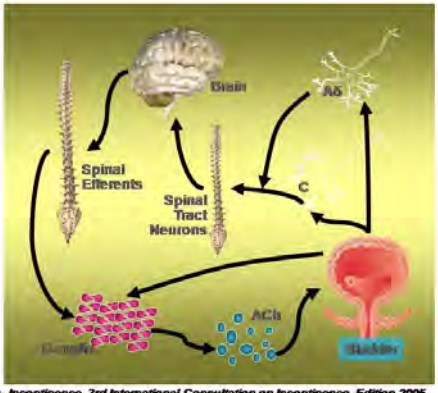
Fig. 5. Relationship between urge intensity and bladder volume in patients with OAB and in normal healthy subjects. The graph shows the results from 21 patients with OAB (OAB) and 10 healthy women without OAB (Normal). Bladder volumes (means ± SE) are shown at each urge grade.

Urgency

- Urgency in OAB is characterized by sudden onset and/or fear of leakage. Urgency in IC is of a persistent nature, and is associated with the fear of pain. (Diggs et al., Urology, 2007)

Potential Urgency Pathways

- Abnormal urothelial-afferent interactions
- Intrinsic detrusor muscle activity
- Abnormal afferent nerve excitability
- Neurally evoked detrusor activity
- Abnormal central sensory processing



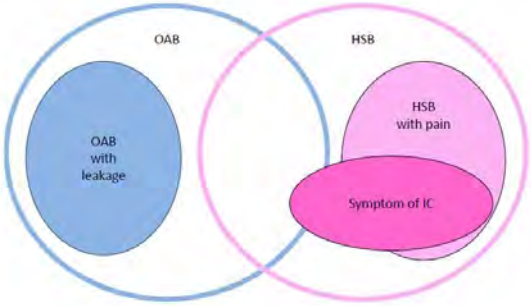
Abrams P, et al, eds. *Incontinence, 3rd International Consultation on Incontinence, Edition 2005.* Plymouth; Health Publications Ltd; 2005

Hypersensitive Bladder (HSB)

(Homma, In J Urol, 2013)

- HSB symptoms are defined as "increased bladder sensation, usually associated with urinary frequency and nocturia, with or without bladder pain".
- HSB suggests pathophysiological hyperactivity of sensory nerves.

HSB related terms

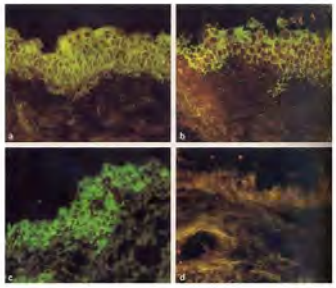


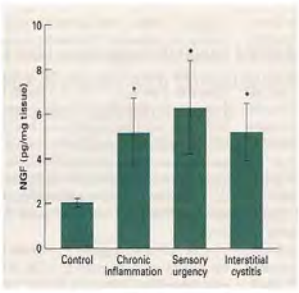
(Homma., Int J Urol, 2014)

Nerve Growth Factor (NGF) and bladder disorders

- NGF involved in regulation of neural function, inflammation and pain
- NGF produced by bladder urothelium and smooth muscle
- NGF levels elevated in the bladder of BPH, IC, and idiopathic OAB

Increase NGF levels on the urinary bladder



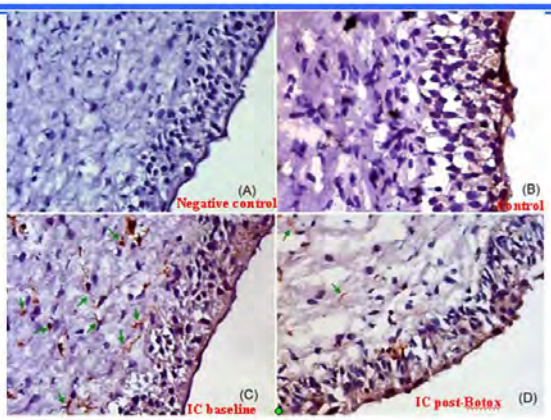


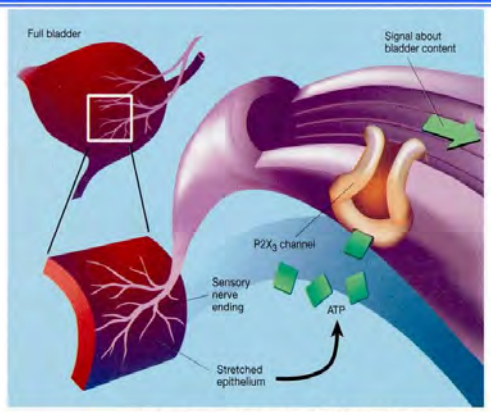
a: idiopathic sensory urgency
b: chronic inflammation
c: interstitial cystitis
d: control

Lowe et al. Br J Urol 1997, 79

Increased Bladder NGF expression in IC

(Liu and Kuo, Urology, 2007)





Nature, vol. 407, Cook and McCleskey

Involvement of ATP in bladder dysfunction

- Augmented ATP release from the urothelium can cause painful sensations by excitation of purinergic (P2X) receptors on sensory fibers.
- There is speculation that this type of noncholinergic mechanism could have a role in a number of bladder pathologies (e.g. idiopathic detrusor instability, interstitial cystitis and bladder outflow obstruction), as well as in the aging bladder

The molecular basis of urgency: regional difference of vanilloid receptor expression in the human urinary bladder (Liu et al., NeuroUrodyn, 2007)

- The symptoms of sensory urgency (SU) were associated with the increased expression of TRPV1 mRNA in the trigonal mucosa.
- No upregulation or regional differences of TRPV1 mRNA were seen in IDO patients. TRPV1 may play a role in SU and premature first bladder sensation on filling.

Transient receptor potential vanilloid receptor subtype 1 in painful bladder syndrome and its correlation with pain. (Mukerji G et al., J Urol, 2006)

- There was a marked increase in suburothelial nerve fibers expressing transient receptor potential vanilloid receptor subtype 1 (TRPV1) in painful bladder syndrome in comparison with that in controls ($p < 0.0001$).
- The ratio of (TRPV1) fibers to neurofilaments was also significantly increased in painful bladder syndrome, suggesting over expression of (TRPV1) ($p < 0.0001$).
- The pain score correlated significantly with the relative nerve fiber density of (TRPV1) in the suburothelium ($r = 0.6862$, $p = 0.0002$) as well as the ratio of (TRPV1) fibers to neurofilaments ($r = 0.5554$, $p = 0.004$).
- Urothelial (TRPV1) showed a tendency toward an increase in the painful bladder syndrome group but it did not achieve statistical significance. No correlation was found between (TRPV1) immunoreactivity of urothelium or neurofilament fibers and the pain score.

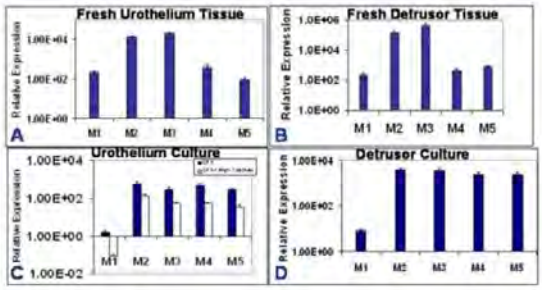
Intravesical resiniferatoxin for the treatment of storage lower urinary tract symptoms in patients with either interstitial cystitis or detrusor overactivity: a meta-analysis (Guo et al., PLoS one, 2013)

- Bladder pain was significantly reduced after RTX therapy in patients with either IC or DO. The average decrease of the visual an alogue pain scale was 0.42 after RTX treatment ($p = 0.02$).
- The maximum cystometric capacity (MCC) was significantly increased in patients with DO (MCC increase, 53.36 ml, $p = 0.006$) but not in those with IC (MCC increase, -19.1 ml, $p = 0.35$).
- No significant improvement in urinary frequency, nocturia, incontinence or the first involuntary detrusor contraction (FDC) was noted after RTX therapy ($p = 0.06$, $p = 0.52$, $p = 0.19$ and $p = 0.41$, respectively).

The role of acetylcholine and muscarinic receptors in the overactive bladder

- Muscarinic-receptor antagonists prevent the stimulation of postjunctional muscarinic receptors by acetylcholine released from bladder efferent nerves and result in increased bladder capacity
- The urothelium expresses the full complement of muscarinic receptors (M1–M5).
- Since antimuscarinic agents effectively enhance the storage phase of micturition, when parasympathetic nerves are silent, it is postulated that the release of acetylcholine from the urothelium might contribute to detrusor overactivity.

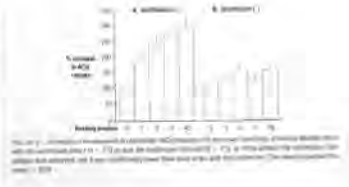
Qualitative and Quantitative Expression profile of muscarinic receptors in Human Urothelium and Detrusor



Tyagi and Chancellor, J Urol, 2006

Management of detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging (Yoshida et al., Urology, 2004)

- Purinergic transmission increases with age, whereas cholinergic transmission decreases.
- These effects are most likely because of decreased release of ACh and increased release of adenosine triphosphate (ATP) from postganglionic parasympathetic axons innervating the bladder.
- The release of nonneuronal ACh increases with age and detrusor stretch.



Treatment

OAB

Behavioral modifications
- Lifestyle changes
- Bladder training
- Pelvic floor exercises
- Biofeedback

and/or

Oral pharmacotherapy
- Tolterodine
- Oxybutynin
- Solifenacin
- Darifenacin
- Trospium chloride

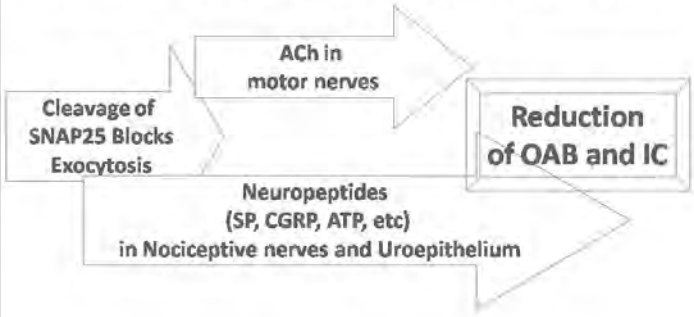
Failure

Specialist management
- Cystoscopy
- Urodynamics
- Botulinum toxin A*, resiniferatoxin*
- Sacral nerve neuromodulation
- Bladder augmentation / auto-augmentation / urinary diversion

*Investigational

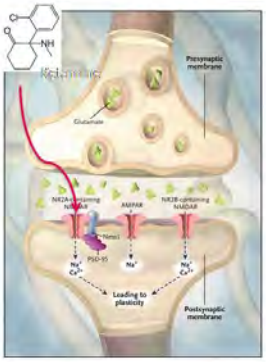
Adapted from Hashim H, et al. *Drugs*. 2004;64:1643-1656.

Botulinum toxin- reduction of Neurotransmission and reduction of OAB and IC



Aberrant Cross Talk Between Uroepithelium and Sensory Nerves- the Consequence of HS, OAB, IC/BPS/PBS

KETAMINE

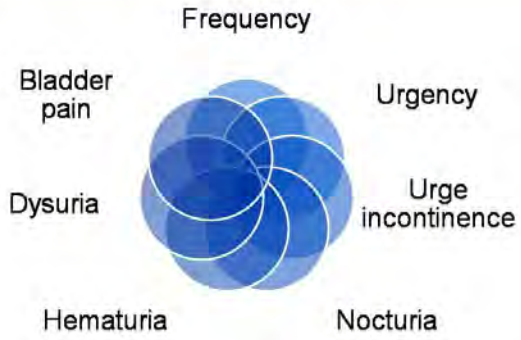


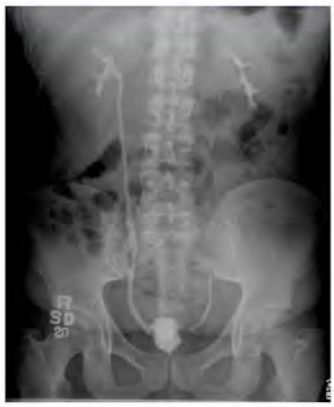
- N-methyl-D-aspartate (NMDA) receptor antagonist
- Mainly affects central nervous system
- For general anesthesia induction and minor surgery process that muscle relaxation is not required

Ketamine abuse

- The 5th popular recreational drug in Taiwan
- The common dosage is 50-100mg/kg each use (3-6 gm/day)
- In powdered form, it can be insufflated, injected, or placed in beverages
- Peak age: 16-35 years
- Approximately 20–30% of ketamine abusers suffer from LUTS

Ketamine related urinary tract symptoms



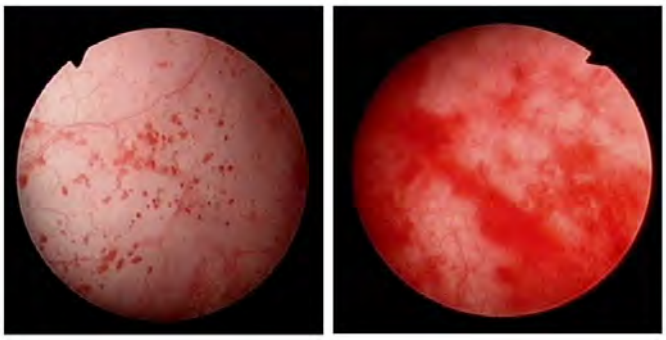


Urodynamic characteristics

- Detrusor overactivity
- Decreased bladder compliance
- Small bladder capacity
- High maximal urethral closure pressure

Chu, P. S., et al. (2008). *BJU Int* **102, 1616-22**
Tsai, T. H., et al. (2009). *Int J Urol* **16, 826-9**

Multiple petechial hemorrhages and neovascularization (Courtesy from Professor En Meng)



Postulated mechanism related to ketamine or its metabolites

- Direct toxic damage to the urinary tract
- Microvascular damage
- Autoimmune effect
- Unrecognized bacteriuria
- Increased apoptosis and suburothelial inflammation

Treatment

- Ketamine withdrawal
- NSAIDS? Anticholinergic therapy? Steroids?
- Intravesical instillation of Hyaluronic acid?
- Intravesical botulinum toxin injection?
- Augmentation of bladder

Intravesical Botulinum Toxin A Injection in Treatment of Overactive Bladder, Interstitial Cystitis and Hypersensitive Bladder

Hann-ChorngKuo, M.D.

Department of Urology, Buddhist Tzu Chi General Hospital and Tzu Chi University, Hualien, Taiwan

Correspondence: Dr. Hann-Chorng Kuo, Department of Urology, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan
Tel: 886-3-8561825, ext. 2117; **Fax:** 886-3-8560794
E-mail: hck@tzuchi.com.tw

Botulinumtoxin A (BoNT-A) are well known for their ability to potently and selectively disrupt and modulate neurotransmission and treat muscular hypercontractility. In addition, recent studies also suggest that BoNT-A has effects on modulation of sensory function, inflammation, and glandular function, like prostate. BoNT-A has approved by FDA for urological use in overactive bladder (OAB) and neurogenic detrusor overactivity (NDO), urologists have become interested in the application of BoNT-A in patients with detrusor and sphincter overactivity, bladder hypersensitivity, lower urinary tract symptoms suggestive of benign prostatic hyperplasia and other urological disorders in the recent decade.

Pathophysiology of OAB and Mechanism of Action of BoNT-A in OAB and Bladder Oversensitivity

The urothelium of the bladder exhibits both sensor and transducer functions. The suburothelial afferent nerves and urothelial cells have neuron-like properties, which express capsaicin sensitive receptor transient receptor potential vanilloid receptor subtype 1 (TRPV1) and adenosine triphosphate (ATP)-gated ion channel purinergic receptors $P2X_3$. In addition, the interstitial cells may receive transmitters from bladder nerves or chemical from urothelial cells and increase sensory nerve excitability causing sensory abnormalities of the urinary bladder. The urothelium in human bladder can transmit mechanosensation through release of neurotransmitters such as acetylcholine (ACh), ATP, substance P, and the expression of TRPV1 and $P2X_3$. The suburothelial myofibroblasts or interstitial cells may respond to ATP to activate ATP-gated P2Y receptors. The urothelial release of ACh and ATP on bladder filling increases with ageing and in patients with spinal cord injury (SCI) and NDO. Treatment targeting the abnormal release of these neurotransmitters may provide beneficial effects on DO. In patients with IDO immunoreactivity of $P2X_3$ expression

in suburothelial fibers was found to decrease after intra-detrusor onabotulinumtoxionA injections. The decrease of $P2X_3$ expression correlated with the improvement of urgency sensation.

Clinical Application of OnabotulinumtoxinA in Overactive Bladder Syndrome/Detrusor Overactivity

Overactive bladder/detrusor overactivtiy (OAB/DO) is a highly prevalent disease. Although antimuscarinics are used as first line therapy, many people cannot tolerate the side effects. Intravesical onabotulinum toxin-A (BoNT-A) injection, a minimally invasive procedure, is an alternative treatment used worldwide. However, there is no standard protocol to treat patients with OAB/DO, and no optimal dose is used. Injections of 200 U of BoNT-A provide good therapeutic results for a long duration, but the rate of side effects is high. There were similar success rates between intravesical BoNT-A 100 U and 200 U injections. Although a short therapeutic duration was noted in patients who received 100 U BoNT-A, the complication rate was obviously lower than with 150 U and 200 U injections. For patients at risk of urine retention after treatment, bladder base/trigone injection relieved the urgency sensation but did not increase the risk of urine retention. Common adverse effects, such as difficult urination and a large post-voided residual, did not affect the success rate at 3 months after administration and in long-term follow-up.

Intravesical botulinum toxin A (BoNT-A) injection is effective and has been approved in the treatment of OAB in patients who are refractory or intolerable to antimuscarinic therapy. Intravesical BoNT-A injection increases bladder capacity, decrease detrusor pressure and reduce the urgency sensation in OAB patients. Although clinical experiences have demonstrated a dose-dependent therapeutic effect of BoNT-A, the adverse events such as acute urinary retention, voiding difficulty, large post-void residual and subsequent urinary tract infection remain problematic and increase with higher doses and in the frail elderly patients. Currently, 100U of onabotulinumtoxinA has been approved by many countries for treatment of patients with non-neurogenic OAB. The duration of therapeutic effect is around 6 to 9 months, and usually remains the same after repeat treatments. The injection sites can involve the bladder wall with or without sparing the trigone. Gathered experience has also shown BoNT-A injection is also effective in treatment of OAB symptoms in children, and in patients with stroke or Parkinson’s disease. Before BoNT-A injection, physicians should learn the injection technique and inform the potential adverse events to patients who desire this treatment.

Pathological mechanism of the therapeutic effect of BoNT-A on interstitial cystitis/ bladder pain syndrome (IC/BPS)

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic bladder condition characterized by bladder pain, frequency and nocturia. There is no definite treatment providing a long-term cure for IC/BPS. Recent studies have demonstrated that intravesical BoNT-A has promising effects on IC/BPS. Repeated BoNT-A injections might provide long-term symptom relief and decrease glomerulations after cystoscopic hydrodistention. Our previous studies demonstrated bladder tissue nerve growth factor (NGF) is elevated in IC/BPS bladders and decreased in responders to BoNT-A injection associated with decreased visual analog pain scores. Another study revealed that increased urothelial cell apoptosis, decreased cell proliferation, increased mast cell activation and impaired expression of junction protein E-cadherin were significant in IC/BPS bladders. Further study of apoptotic markers and inflammatory protein expression also revealed that apoptotic signaling molecules, including Bad, Bax, and caspase 3, were increased in the bladder tissues of patients with IC/BPS. Theapoptosis and growth arrest of bladder tissues of IC/BPS patients could be due to upregulation of inflammatory signals, including p38 mitogen-activated protein kinase and tumor necrosis factor alpha.

BoNT-A is an inhibitor of acetylcholine release at the presynaptic neuromuscular junction. Inhibition of acetylcholine release results in regional decreased muscle contractility at the injection sites. This chemical denervation is a reversible process, and axons resprout in about 3-6 months. Vanilloid receptors VR1 are co-localized with P2X3, CGRP, or substance P in the urothelium and suburothelial sensory fibers. A significant decrease was noted in P2X3-immunoreactivity of suburothelial fibers at 4 weeks with a further decrease at 16 weeks after BoNT-A injection in the responders of DO. The study speculated that onabotulinumtoxinA might reduce production/uptake of neurotrophic factors, and regulate expression of VR1 and/or P2X3. In an animal model, Chuang et al found that intravesical onabotulinumtoxinA blocked acetic acid induced bladder pain responses and inhibited CGRP release from afferent nerve terminals. Intravesical onabotulinumtoxinA injections might not only reduce bladder sensitivity in IC/BPS patients but also induce desensitization in the central nervous system through affecting the over-expression of activated proteins in the dorsal horn ganglia.

Clinical Experience of Intravesical Botulinum Toxin A on IC/BPS

Smith et al first treated 13 IC/BPS patients with 100 U to 200 U of Dysport or onabotulinumtoxinA submucosally in the trigone and bladder base and found that 69% of patients had subjective improvement after onabotulinumtoxinA injections. The symptom index improved by 71%, problem index by 69%, and bladder pain by 79%. The effect of

onabotulinumtoxinA on IC/BPS patients was further confirmed by recent studies. Giannantoni et al treated 14 patients with injections of 200 U of onabotulinumtoxinA in 20 mL saline at 20 sites in the trigone and bladder base. Twelve patients (85.7%) reported subjective improvement at 1 and 3 months, scores on the visual analog scale (VAS) decreased, frequency decreased and bladder capacity increased significantly. Two patients reported dysuria and intermittent clean catheterization was needed. The same authors evaluated the one-year efficacy and tolerability of intravesical onabotulinumtoxinA injection. Among 13 patients 86.6% reported subjective improvement at the 1 and 3-month follow-ups. At the 5-month follow up the beneficial effects persisted in 266%, and at 12 months after treatment pain recurred in all patients. Dysuria persisted in 4 patients at 3 months and in 2 at 5-month follow up. Nevertheless, the authors found that intravesical onabotulinumtoxinA treatment reduced bladder pain, improved psychosocial functioning, and well-being.

Kuo et al. have compared the clinical effectiveness of intravesical onabotulinumtoxinA injections followed by cystoscopic hydrodistention and hydrodistention alone in 67 patients with IC/BPS. OnabotulinumtoxinA 200 U and 100 U were given in 15 and 29 patients and hydrodistention alone in 23 patients. The IC symptom score significantly decreased in all three groups, but VAS reduction, increases of functional bladder capacity and cystometric bladder capacity were significant only in the onabotulinumtoxinA groups at 3 months. Of the 44 patients in the onabotulinumtoxinA groups 31 (71%) had a successful result at 6 months, 24 (55%) at 12 months and 13 (30%) at 24 months. Another recent study using 100 U onabotulinumtoxinA to treat women with IC/BPS by 10 trigonal injection sites, Pinto, et al. found all patients had subjective improvement at 1- and 3-month follow-up. The treatment remained effective in more than 50% of the patients for 9 months. The authors concluded that trigonal injection of onabotulinumtoxinA is a safe and effective treatment for refractory IC/BPS.

The largest cohort of onabotulinumtoxinA treatment for patients with IC/BPS was recently reported by the authors. Intravesical injection of 100 U of onabotulinumtoxinA immediately followed by cystoscopic hydrodistention under intravenous general anesthesia was performed in 67 patients with IC/BPS. Significant improvement was shown after intravesical injection of 100 U of onabotulinumtoxinA. Baseline and 6 months after injection scores were: ICSI and ICPI (23.6 ± 5.9 versus 15.2 ± 8.5, P = 0.000), VAS (5.3 ± 2.2 versus 3.3 ± 2.4, P = 0.000), functional bladder capacity (136 ± 77.6 versus 180 ± 78.2, P = 0.000) and GRA (0.3 ± 0.8 versus 1.4 ± 1.0, P = 0.000). Intravesical onabotulinumtoxinA injection appears to be a safe and effective therapeutic option for analgesia and increased bladder capacity for patients with IC/PBS.

Repeated onabotulinumtoxinA injections plus hydrodistention might provide a better outcome in treating IC/PBS. If repeated onabotulinumtoxinA injections can relieve bladder

pain and increase bladder capacity in responders, the result might provide evidence of urothelial repair and reduction of suburothelial inflammation in IC/BPS responders. Chronic suburothelial inflammation might alter urothelial function and cell differentiation, and onabotulinumtoxinA injection might reduce the inflammation and restore a healthy urothelium, thereby improving the clinical symptoms of IC/BPS.

Intravesical Botulinum Toxin Injection for OAB and IC/BPS – What We Can Learn from Previous Clinical Trials

Intravesical BoNT-A injection has been demonstrated effective in treating OAB and IC/BPS refractory to conventional treatment. In the past 5 years, there have been several clinical trials using BoNT-A targeting OAB and IC/BPS, and the therapeutic results are promising. Recent investigations have revealed that urothelial dysfunction and abnormality of sensory receptor expression or transmitter release in the suburothelial nerves might contribute to OAB refractory to antimuscarinics. On the other hand, chronic inflammation causing urothelial dysfunction and overexpression of the sensory receptors on the urothelium were noted in the IC/BPS bladders. Intravesical BoNT-A treatment to inhibit abnormal receptor expression or transmitter release in the sensory nerve terminals in the suburothelial space provides good therapeutic effects in the treatment of OAB. Intravesical BoNT-A injection can also decrease inflammation and restore urothelail homeostasis and normal function on IC/BPS bladders. Intradetrusor or suburothelial BoNT-A injections with small or large doses of BoNT-A in the bladder body or bladder base can achieve satisfactory results. However, BoNT-A impairs detrusor contractility and causes a large postvoid residual (PVR) after injection in some patients. This adverse effect induces acute urinary retention and difficult bladder emptying in the early postoperative period. Urinary tract infections can occur in patients with a large PVR. Although adverse effects may not influence the therapeutic outcome, they might prohibit wide application of BoNT-A in the treatment of refractory OAB. Patients with a high risk of a large PVR or urinary retention should be taught clean intermittent catheterization. Analysis of patient characteristics and urodynamic variables reveals that patients who are ageing, have low detrusor contractility at baseline, and have chronic medical diseases are at risk of adverse effects. Therefore, careful adjustment of the dose and injection site and patient selection is mandatory to achieve satisfactory results with intravesical BoNT-A therapy.

Concept of Drug Delivery Across the Urothelium – New Therapeutic Approach in Treatment of Urothelial Dysfunction Associated LUTD

Michael B Chancellor, MD

Department of Urology, Oakland University, William Beaumont School of Medicine, Royal Oak, Michigan, USA

Intravesical therapy is the routine first line of treatment for effectively delaying or preventing the recurrence of bladder cancer. This route of drug administration has also shown tremendous promise in treatment of interstitial cystitis/painful bladder syndrome (IC/PBS) and potentially overactive bladder to justify investments for further improvements. Ongoing efforts to advance the field of intravesical drug delivery include development of sustained-release drug implants and efforts to improve delivery of biotechnological products including large protein acting as neurotoxins and small interfering RNAs.

Bladder Cancer

Intravesical therapy is the routine first line of effective treatment for delaying or preventing recurrence of bladder cancer. The standard of care, intravesical chemo and immunotherapy reduces tumor progression through either direct cytoablation or immunostimulation, which halts implantation of tumor cells after transurethral resection of bladder tumor and eradicates residual disease. Bacillus Calmette-Guerin (BCG) is the most commonly used first-line agent immunotherapeutic agent for prophylaxis and treatment of carcinoma in situ and high-grade bladder cancer. Other immunotherapeutic options include the interferons, interleukins 2 and 12, and tumor necrosis factor, all of which have activity in BCG refractory patients, although with low durable remission rates.

IC/PBS

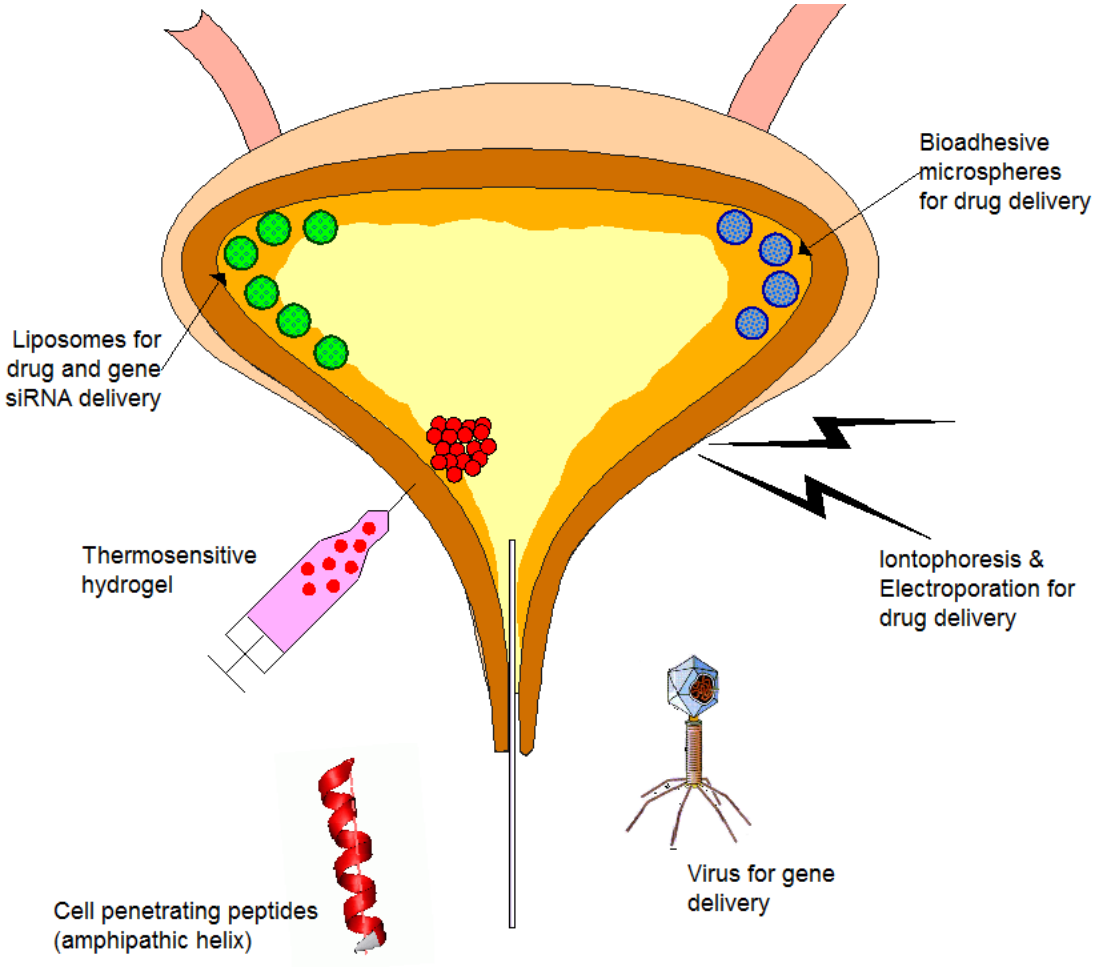
A large body of evidence support the notion that symptoms of this painful pelvic disease emanate from underlying inflammation in bladder. Studies on animal models of IC/PBS have reported infiltration of neutrophils, enhanced activation of several inflammatory cytokines in the bladder and increase in inflammatory gene expression. It is believed that activation of mast cells and disruptions in bladder permeability barrier are the other key events in the bladder inflammation associated with IC/PBS.

Intravesical route offers new and promising adjunctive therapies for immediate symptom relief during symptom flare up of IC/PBS. Given the multi-factorial nature of the

disease, therapy is often tailored to improve therapeutic outcomes with multimodal treatment through pharmacological and non-pharmacological approaches such as hydrodistention acting via different mechanism of action.

Overactive bladder (OAB)

Oral anti-cholinergic medications are the current standard of care for OAB patients with limited benefits. The new therapeutic options are aimed at reducing to the maximum symptomatology, as well as the induced side effects. Intravesical delivery of anti-cholinergics is becoming a promising alternative for patients who fail oral therapies.



Schematic Diagram to Illustrate Advanced Delivery Options for Intravesical Drug or gene Delivery. Anatomic location of bladder allows development of various drug delivery platforms such as virus, liposomes, microspheres, polymeric hydrogel and cell penetrating peptides.

Conclusions

Advances in the development of bladder coating with liposomes and drug delivery are expected to further improve the efficacy and safety of pharmacotherapy for bladder diseases in the future. Liposomes can not only provide a biocompatible interface with affinity for bladder surface but it can also facilitate absorption of high molecular weight drug and biologic agent by vesicular traffic. Latest developments in the field of nanotechnology can bring this mode of therapy as new hope to the forefront of disease management for the lower urinary tract.

Clinical Experience of Liposome in Treatment of IC/BPS – Animal Model and Human

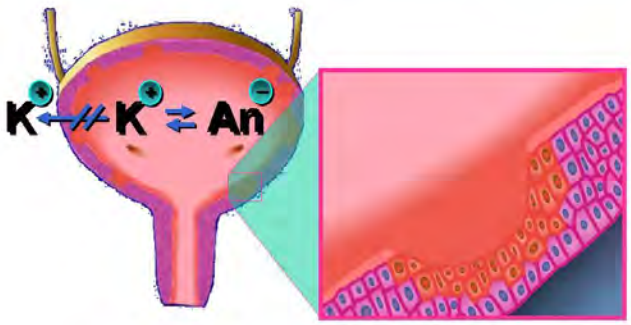
Yao-Chi Chuang

Professor

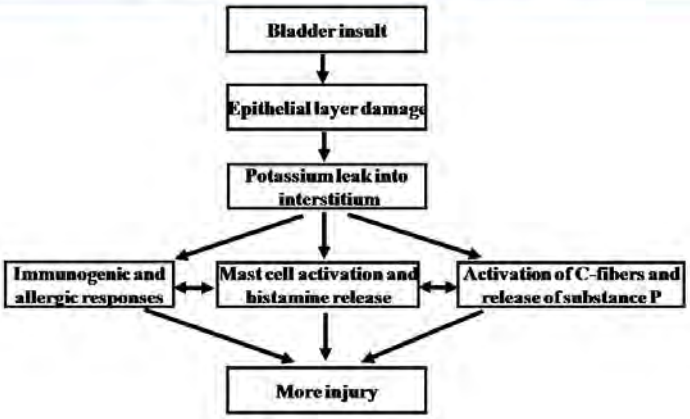
Department of Urology ,

Kaohsiung Chang Gung Memorial Hospital, Taiwan

Is IC Defective Urothelial Barrier?



Proposed Pathogenesis of IC



Mucosal Surface Protection

- Pentosanpolysulfate (Elmiron) (Oral, IVES)
- Heparin (IVES, SQ)
- Hyaluronic acid (Cystistat) (IVES)
- Chondroitin sulfate (oral)
  - Elmiron could also suppress mast cell activation (Theoharides and Sant)

Discovered by accident when exploring an intravesical NGF -delivery technology at UPMC 2000

- Liposomes are typically used to carry drugs or active agents
- Liposomes are stable self assembled phospholipid BUBBLES filled with water which adhere to a surface
- Surprised that empty liposome vehicle also had efficacy in animal model of IC/BPS (Fraser, Chuang, Chancelor et al., Urology, 2003)

Why Liposomes ?

- Empty liposomes have been used to improve wound healing & barrier function of broken skin (Jeschke et al 2005 & Wutzler et al 2003)
- Most consistent finding in IC is a compromised bladder barrier function
- Liposomes have long history of safe clinical use

INTRAVESICAL LIPOSOME ADMINISTRATION—A NOVEL TREATMENT FOR HYPERACTIVE BLADDER IN THE RAT

MATTHEW D. FRASER, YAO-CHI CHUANG, PRADEEP TYAGI, TERUHIKO YOKOYAMA, NAOKI YOSHIMURA, LEAF HUANG, WILLIAM C. DE GROAT, and MICHAEL B. CHANCELLOR

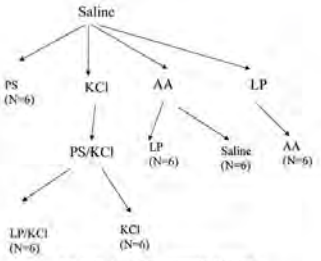


FIGURE 1. *Experimental design.*

TABLE IV. *Effects of saline, acetic acid, and liposomes on CMG parameters*

	ICI (min)	Compliance (mL/cm H_2O)	PT (cm H_2O)	Amplitude (cm H_2O)
Saline	15.3 ± 2.2	0.296 ± 0.040	6.8 ± 0.9	28.7 ± 1.7
AA	2.4 ± 0.5*	0.071 ± 0.016*	8.5 ± 0.9	56.8 ± 6.5
LP	8.7 ± 1.5*	0.114 ± 0.020*	10.2 ± 1.8	36.9 ± 4.9

Figure 1

CMG tracing during control, protamine sulfate in potassium chloride (PS/KCl), and liposomes in KCl (LP/KCl) or KCl treatment. PS/KCl elicited bladder hyperactivity. LP/KCl partially reversed the irritative effect of PS/KCl, which was maintained after switching to KCl.

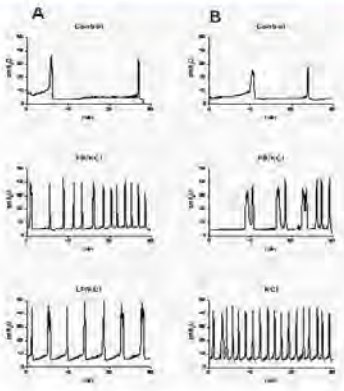
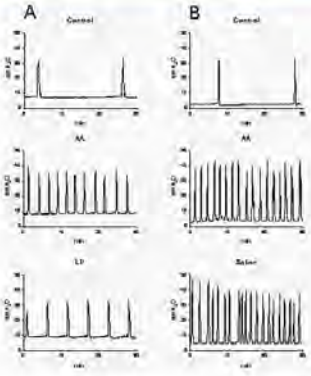
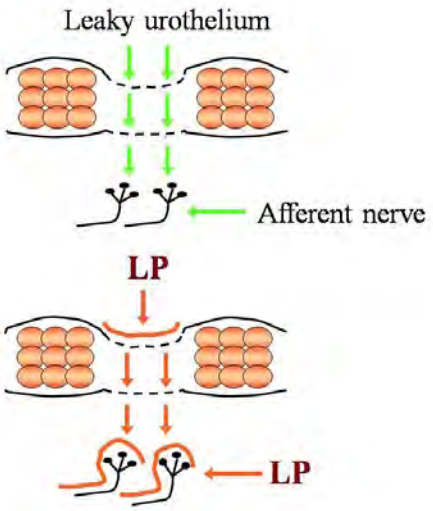


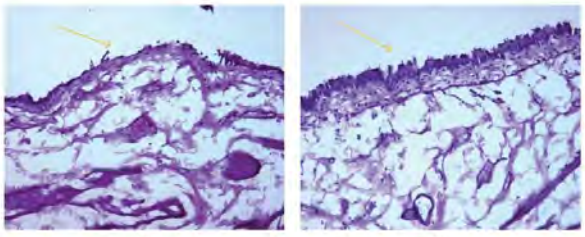
Figure 2

CMG tracing during control, acetic acid (AA), and liposomes (LP) or saline treatment. AA elicited bladder hyperactivity. LP partially reversed the irritative effect of AA, which was maintained after switching to saline.



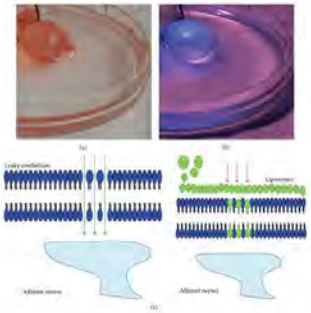


Protective effect of liposomes evaluated by histology in rat bladder injured by protamine sulfate. (Tyagi et al., ISRN Pharmacology, 2014)



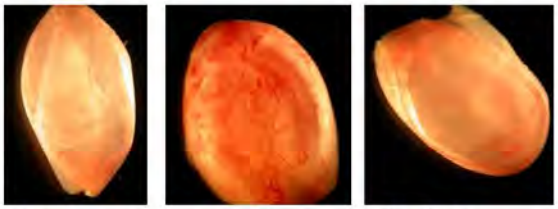
(a) (b)

Liposomes coating the bladder surface are invisible in visible light photograph (a) but is indicated by blue colored coating on the bladder luminal surface in NIR light (b).



(Tyagi et al., ISRN Pharmacology, 2014)

Animal Model

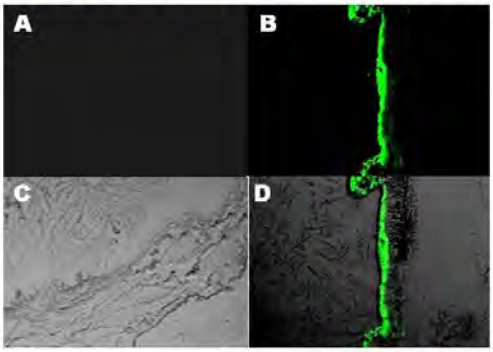


Normal Bladder | AA Bladder | AA Bladder with Liposome

Tyagi, et al., JU 171:483, 2004

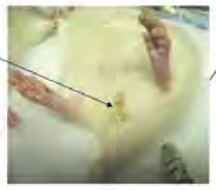
Bladder surface of rat instilled with saline

Liposomes having fluorescent lipid Adhering to bladder surface



Experimental Method

Empty Liposome With ^{14}C lipid



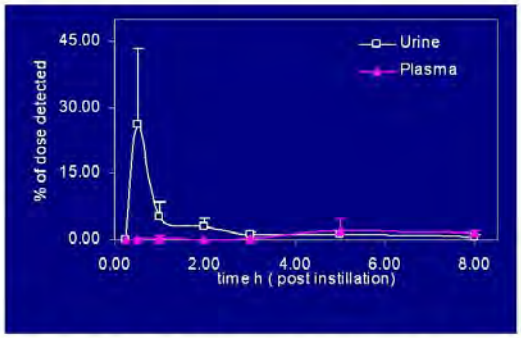
Instilled Under Anasthesia

Urine Collected and Radioactivity Measured

Method

- 24 female Sprague-dawley rats (240-260gm) were instilled under isoflurane anesthesia
- Radiolabelled liposomes prepared with 1.64%w/w ^{14}C radioactive lipid (Perkin Elmer) of the total lipid (1mg in 0.5ml) in liposomes
- Radioactive dose for each rat was 0.97µCi or ~81000 disintegrations per minute (dpm)

Bladder Residence Time

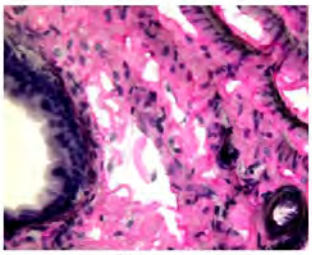


35.51±7.12 % of dose excreted into urine within 3h and only **0.74± 0.20 %** absorbed into plasma

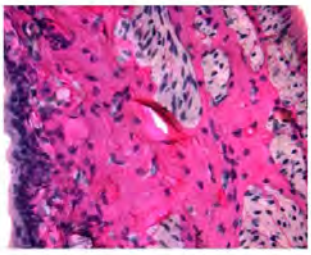
Radioactivity Localized In Urothelium

- Serial sectioning of harvested Bladder
- Radioactivity determined in tissue sections
- Urothelium has Two fold higher dose fraction than detrusor

Histology



Sham



Liposome Treated

Conclusions

- Intravesical delivery of liposomes provides a significant bladder urothelium targeting advantage with long bladder residence time
- The pharmacokinetic studies taken together with efficacy and toxicity studies support the use of empty liposomes as a local therapy for PBS/IC and refractory overactive bladder

A Randomized Comparative Study of the Safety and Efficacy of Liposome Topical Solution and Oral Pentosan Polysulfate in Patients with Interstitial Cystitis

Yao-Chi Chuang, Wei-Chiang Lee, Wei-Chia Lee, Po-Hui Chiang

Division of Urology, Chang Gung Memorial Hospital Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan

J Urol, 2009

Purpose

- A previous preclinical study showed that intravesical instillation of Liposomes (LPs), a mucosal protective agent, could reduce the bladder hypersensitivity.
- We evaluated the safety and efficacy of intravesical LPs in comparison to oral pentosan polysulfate sodium (PPS) in treating interstitial cystitis (IC).

Empty Liposomes As IC Treatment

- Easy to prepare with lipids from egg yolk
- Developed a proprietary method based on Freeze- drying
- Shelf life of 2 years at -20°C
- Reconstituted at site of instillation

Materials and Methods

- A total of 24 patients diagnosed with IC were randomized in a 1:1 ratio to receive intravesical liposomes (LPs, Lipella Pharmaceutical Inc., 80 mg/40 cc) once a week or 100 mg Pentosanpolysulfate (PPS) 3 times daily for a period of 4 weeks.
- The primary outcome was the change in the O'Leary-Sant Interstitial Cystitis Symptoms/Problems Index from baseline to week 4, and 8.
- Other outcomes included: the changes in visual analogue scale for pain, urgency scale, voiding log, and patient global assessment.

Table 1.
Baseline patient characteristics by treatment group

	Mean ± SD	
	Liposome	Pentosan Polysulfate Sodium
Patient age (years)	47.8 ± 11.1	51.9±14.3
Frequency in 24 hrs	17.5 ± 6.0	16.5±5.7
Nocturia episodes	3.1±1.1	2.4±1.2
Mean voided volume (ml)	98.4 ± 37.1	135.2±60.1
O'Leary-Sant symptom score (range 0-36)	29.3 ± 4.0	28.9±5.3
IC symptom index (0-20)	15.3 ± 2.5	15.3±3.1
IC problem index (0-16)	14.0 ± 1.9	13.7±2.5
Pain score (0-5)	3.6±1.5	3.1±0.8
Urgency (0-5)	4.3±0.9	3.7±1.2
Qmax (ml/sec)	11.4±5.3	10.8±5.0
RU (ml)	27.1±27.7	28.1±30.3

There were no statistically significant differences in baseline parameters between the 2 groups.

Results

- Both Intravesical LPs and oral PPS improved clinical outcome parameters and the effects were maintained at week 8.
- The change in the total score of O'Leary-Sant Interstitial Cystitis Symptoms/Problems Index from baseline to week 4 and 8 among the LPs treated group (-6.1±6.6 and -7.0±9.1, respectively) was similar to that of the PPS treated group (-6.1±9.9 and -5.8±11.1, respectively).
- The clinical response rate at week 4 and 8 for LPs and PPS was 50.0% and 58.3%% vs 58.3% and 50.0%, respectively.
- There were no major adverse events between both groups.

Table 2. Changes in IC symptoms from baseline

Outcomes	Liposome (n=12)		Pentosan Polysulfate Sodium (n=12)	
	4 week	8 week	4	8
O'Leary-Sant total score (0-36)	-6.1±6.6*	-7.0±9.1*	-5.4±9.7	-5.0±10.9
O'Leary-Sant ICSI (0-20)	-3.5±3.7*	-3.9±4.6*	-3.5±5.0*	-3.2±5.8
O'Leary-Sant ICPI (0-16)	-2.6±3.6	-3.1±5.0	-1.9±5.0	-1.8±5.3
Pain scale (0-5)	-1.8±1.5*	-1.5±1.5*	-0.9±1.2*	-0.6±1.3
Urgency scale (0-5)	-1.7±1.7*	-1.6±1.8*	-1.1±1.9	-0.9±1.9
Voiding frequency	-2.5±4.3	-2.6±3.4*	-3.6±2.9*	-3.9±3.9*
Voided volume (ml)	7.1±39.9	27.1±50.1	7.2±32.9	14.7±39.6
Nocturia	-1.0±1.3*	-1.0±1.6	-0.5±0.9	-0.5±0.7*
Qmax (ml/sec)	2.3±2.4*	2.0±4.2	0.8±1.4	0.5±2.4
RU (ml)	-6.1±11.3	-5.2±14.2	0.02±10.1	4.7±15.2

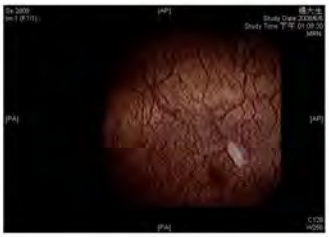
Results were expressed as mean+/-SD. *The paired Student's t-test (changes from baseline to each end point within each group) $p<0.05$. There was no statistical difference at the changes from baseline to each end point between treatment group (Mann-Whitney U test)

Table 3.
Level of improvement among responders at the end of the trial

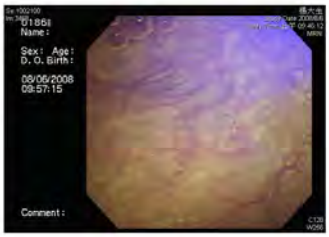
Pt Global Assessment	%(No./total No.) LP		%(No./total No.) PPS	
	4W	8W	4W	8W
Level of improvement :				
Mild improved	25 (3/12)	41.67 (5/12)	25(3/12)	25(3/12)
Moderately improved	16.67(2/12)	8.33 (1/12)	8.33 (1/12)	8.33(1/12)
Greatly improved	8.33(1/12)	8.33(1/12)	25(3/12)	16.67(2/12)

LPs Suppress Bladder Inflammation in IC Patient

Pretreatment



Posttreatment



Safety and dose flexibility clinical evaluation of intravesical liposome in patients with interstitial cystitis or painful bladder syndrome

Wei-Ching Lee, Yao-Chi Chuang*, Wei-Chia Lee, Po-Hui Chiang

Kaohsiung Journal of Medical Sciences (2011) 27, 437e440

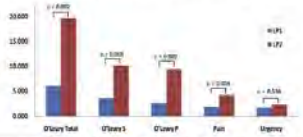


Figure 1. Treatment parameters at Week 4. The O'Leary-Sant Questionnaire Problems score, O'Leary-Sant total Score, and pain score showed significantly greater improvement in the biweekly group (LP2) than the weekly group (LP1) at the Week 4. LP = liposome; SD = standard deviation.

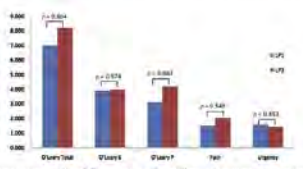
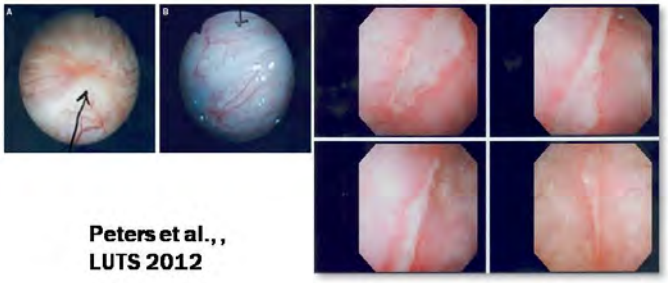


Figure 2. Treatment parameters at Week 8. There were no significant differences in treatment parameters between the biweekly group (LP2) and the weekly group (LP1) at Week 8. LP = liposome; SD = standard deviation.



Peters et al., ,
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Clinical Experience and evidence of Liposome Encapsulated Botulinum Toxin A in Treatment of Overactive Bladder

Chun-Hou Liao, MD

Department of Urology, Cardinal Tien Hospital and School of Medicine, Fu-Jen Catholic University, Taipei, Taiwan

Introduction

Intravesical onabotulinumtoxinA (BoNT-A) injection has been proven effective in decrease of urgency and urgency urinary incontinence (UUI) in patients with overactive bladder syndrome (OAB). This treatment has been widely used for the OAB patients refractory to antimuscarionic therapy and has gained license from countries in the United States and Europe. However, increased postvoid residual (PVR) urine volume and urinary tract infection (UTI) remain risks yet to be resolved. Because intravesical BoNT-A injection requires sedation or anesthesia and the high rate of AEs with injection usually limit the OAB patients willing to accept the treatment, research interests have moved from injection to intravesical instillation. If clinicians can deliver BoNT-A to the urothelium without injection, the acceptance of treatment by patients will increase. We speculated that the penetration of BoNT-A delivered by liposomes might be lower than with injection; thus the therapeutic effects might be limited to the urothelial sensory nerves without compromise to detrusor contractility. This treatment might prevent undesired detrusor underactivity after BoNT-A injection, especially in elderly patients who have impaired detrusor contractility.

Treatment with liposome Encapsulated Botulinum Toxin A (Lipotoxin)

We had reported a pilot proof-of-concept study, which designed as a randomized double-blind parallel controlled trial to evaluate whether liquid liposomal delivery of BoNT-A (liposome BoNT-A [Lipotoxin]) could penetrate the bladder urothelium without an injection in patients with refractory OAB [1]. Patients with confirmed OAB were randomly assigned to receive intravesical instillation of either Lipotoxin (treatment group) or normal saline (N/S; control group). Sphingomyelin liposomes are available for preparation at a concentration of 2 mg/ml (2.84 mM) in N/S containing 500 mM KCl (LP-08, Lipella Pharmaceuticals Inc., Pittsburgh, PA, USA). Lipotoxin was prepared before application by hydrating 80 mg freeze-dried LP-08 in 40 ml N/S and 200 U BoNT-A (Botox, Allergan, Irvine, CA, USA) in 10 ml N/S to make a total volume of 50 ml at room temperature. A 50 ml N/S solution served as the control arm. Lipotoxin or N/S solution blindly obtained from the pharmacy was instilled into the bladder through a 6F Nelaton tube. The study drug (Lipotoxin or N/S) remained in the bladder for 60 min.

Clinical Efficacy and Safety

A total of 24 patients were eligible for the treatment including 10 men and 14 women with a mean age of 67 year (range: 38–82) [1]. A statistically significant reduction versus baseline in urinary frequency and urgency episodes with intravesical installation of Lipotoxin was found but not with normal saline: respectively, frequency decreased from 34 to 24.5 episodes per 3 day versus from 29 to 27.0 episodes per 3 day, whereas urgency decreased from 32 to 22 episodes per 3 day versus from 27.5 to 24.5 episodes per 3 day. However, the UUI episodes did not change after Lipotoxin treatment. In contrast, only small changes in residual urine were observed, and they were neither statistically significant nor clinically relevant (from 25.5 ml to 33 ml vs from 21.0 ml to 24.5 ml, respectively). An emerging need for catheterization or occurrence of a UTI was not observed in any patient.

Another two-center, double-blind, randomized, placebo controlled study enrolled patients with OAB who were inadequately managed by antimuscarinics. Patients were randomly assigned to intravesical instillation of Lipotoxin (N=31) or normal saline (N=31). At week 4 after treatment, lipotoxin significantly decreased total frequency per 3-day (-4.64 for Lipotoxin versus -0.19 for placebo; p= 0.0366). Total urgency (-7.43) and overactive bladder symptom score (-1.86) significantly decreased in Lipotoxin group. Urgency severity score (USS) improved by 39.29% and 14.29% for Lipotoxin and placebo, respectively, though the difference was not statistical significance between groups. There was inconclusive effect on UUI due to a relatively low baseline incidence. There were no adverse events in either group.

Immunohistochemistrical Evidences

The biological effects of BoNT-A seem to have both efferent and afferent effects by modulation ATP release and inhibiting acetylcholine release. The therapeutic effect can be demonstrated by the immunohistochemistrical staining of the cleaved synaptosomal-associated protein-25 (cSNAP-25) and decreased expression of the purinergic receptors P2X3. We retrospectively evaluated the clinical results of 20 OAB patients treated with 100U BoNT-A injection and 23 OAB patients who received intravesical instillation of liposome encapsulated BoNT-A (Lipotoxin, 14 patents) or normal saline (9 patients). Bladder tissues were harvested at baseline and after treatment.

Our results demonstrated that BoNT-A injection can decrease both P2X3 expression in the urothelium and cleave SNAP-25, the therapeutic effects involve both sensory and motor mechanisms. On the other hand, Lipotoxin instillation can only deliver BoNT-A to the superficial urothelium, therefore, only P2X3 expression was decreased but the cSNAP-25 cannot be detected in immunohistochemistry staining. These evidence correlate with the clinical therapeutic results that BoNT-A injection increases bladder capacity, decreases

detrusor contractility and also decrease frequency urgency episodes. However, patients treated with Lipotoxin instillation can only have decrease of frequency urgency episodes but not the urgency urinary incontinence episodes.

Conclusions

Our pilot study demonstrated that intravesical Lipotoxin instillation can effectively reduce frequency and urgency episodes 1 month after treatment in OAB patients. The PVR did not increase, and all patients were free of UTI after the treatment. We further demonstrated that BoNT-A injection can effectively cleave SNAP-25 and decrease P2X3 receptors in the urothelium, whereas liposome encapsulated onabotulinumtoxinA can decrease P2X3 expression in the urothelium but the SNAP-25 was not significantly cleaved. These results support that BoNT-A delivered by liposomes might be lower than with injection; thus the therapeutic effects might be limited to the urothelial sensory nerves without compromise to detrusor contractility.

References

1. Kuo HC, Liu HT, Chuang YC, Birder LA, Chancellor MB. Pilot Study of Liposome-encapsulated OnabotulinumtoxinA for Patients with Overactive Bladder: A Single-center Study. Eur Urol. 2014; 65(6):1117-24.