Intravesical Drug Delivery for Dysfunctional Bladder

Yao-Chi Chuang
Professor and Chief, Division of Urology, Kaohsiung Chang Gung Memorial Hospital, Taiwan
成長於台南

建興國中
台南一中
Yao-Chi Chuang

In connection with the presentation, I disclose COI with the following companies/organizations.

A position of a advisor: Lipella, Allergan Pharmaceuticals, Inc.

Honoraria for lectures: Allergan Pharmaceutical Co, Ltd.
Outline

- Liposomes for IC/PBS- basic science and clinical results
- Liposomal botulinum toxin delivery- basic science and clinical results from OAB study
- Liposomal tacrolimus- for inflammatory cystitis and hemorrhage cystitis, view from rats study
Is IC Defective Urothelial Barrier?

K ← K ≡ An
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
Intravesical liposome administration--a novel treatment for hyperactive bladder in the rat (Fraser, Chuang, Chancellor* et al., Urology, 2003)
Leaky urothelium

Afferent nerve

LP

LP
Urodynamic and immunohistochemical evaluation of intravesical capsaicin delivery using thermosensitive hydrogel and liposomes

(Tyagi et al., J Urol, 2004)
Fig. 3  Fluorescent labeling shows liposomes coating bladder wall.

Hsu, Chuang*, Chancellor, Int J Urol, 2013
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)
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
Empty Liposomes (LP08, Lipella) 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
# Table 1. Baseline patient characteristics by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD LP</th>
<th>Mean ± SD PPS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt age</td>
<td>47.8 ± 11.1</td>
<td>51.9 ± 14.3</td>
</tr>
<tr>
<td>Frequency (No. hrs)</td>
<td>17.5 ± 6.0</td>
<td>16.5 ± 5.7</td>
</tr>
<tr>
<td>No. nocturia episodes</td>
<td>3.1 ± 1.1</td>
<td>2.4 ± 1.2</td>
</tr>
<tr>
<td>Mean voided vol (ml)</td>
<td>98.4 ± 37.1</td>
<td>135.2 ± 60.1</td>
</tr>
<tr>
<td>Qmax (ml/sec)</td>
<td>11.4 ± 5.3</td>
<td>10.8 ± 5.0</td>
</tr>
<tr>
<td>RU (ml)</td>
<td>27.1 ± 27.7</td>
<td>28.1 ± 30.3</td>
</tr>
</tbody>
</table>

* No statistically significant differences vs LP.
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)
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, 437-440

Figure 1. Treatment parameters at Week 4. The O'Leary-Sant Symptom/Problem 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 difference in treatment parameters between the biweekly group (LP2) and the weekly group (LP1) at Week 8. LP = liposome; SD = standard deviation.
Liposomal bladder instillations for IC/BPS: an open-label clinical evaluation.
(Peters and Chancellor et al., Int J Urol and Nephro, 2014)

- Fourteen symptomatic IC/BPS subjects were treated with intravesical liposomes once a week for 4 weeks.
- No treatment-related AEs.
- Urgency VAS scores significantly decreased at 4 weeks (p = 0.0029) and 8 weeks (p = 0.0112) post-treatment.
- Pain VAS scores significantly decreased at 4 weeks post-treatment (p = 0.0073). Combined ICSI and ICPI scores improved significantly at 4 and 8 weeks (p = 0.002 for both time points) post-treatment.
- Responses to GRA showed improvement at 4 weeks post-instillation. No significant decrease in urinary frequency was found.
From Botulinum Toxin to Lipotoxin for IC and OAB Treatment
Intravesical Botulinum Toxin A Administration Produces Analgesia Against Acetic Acid Induced Bladder Pain Responses In Rats (Chuang et al., J Urol, 2004)

Fig. 1

Fig. 3
Intravesical Botulinum Toxin A Administration Inhibits COX-2 and EP4 Expression and Suppresses Bladder Hyperactivity in Cyclophosphamide-Induced Cystitis in Rats

Yao-Chi Chuang\textsuperscript{a,*}, Naoki Yoshimura\textsuperscript{b}, Chao-Cheng Huang\textsuperscript{c}, Moya Wu\textsuperscript{a}, Po-Hui Chiang\textsuperscript{a}, Michael B. Chancellor\textsuperscript{d}

(Eur Urol., 2009)
Bladder BoNT A Injection Can Benefit Patients with Radiation and Chemical Cystitis

(Chuang et al., BJU Int, 2008)
Intravesical Botulinum Toxin A Injection Therapy for IC and OAB- Effective, but with Limitation

- Limitation: drug leakage, hematuria, pain on injection sites, uneven distribution, UTI, and urinary retention

Chuang, Kuo, Chancellor*, BJU Int, 2010
Liposome for Drug Delivery

- Drug crystallized in aqueous fluid
- Lipid-soluble drug in bilayer
- Lipid bilayer
- DNA
- Protective layer against immune destruction
Why Liposomes for Botulinum Toxin Delivery

- **BoNT-A**: 150 kilo Daltons, hard to access the submucosal nerve plexus using saline as a vehicle without direct injection to pass the urothelium barrier.

- **Liposome**: carrier potential, characteristics of adsorption and fusion with cells, may be used as delivering vehicle for BoNT-A without the need for injection.
Acetic acid Infusion on Day 8 Decreased the ICI by 57.2% and 56.0% in the LPs and BoNT-A pretreated rats, respectively. Mean ± SE; n=6

Lipotoxin (LPs + BoNT) pretreatment blunted the AA induced decrease in ICI to only 21.1%
SNAP-25 positive neuronal fibers were detected in the bladder samples of LPs and BoNT-A pretreated animals. However, SNAP-25 positive neuronal fibers were rarely seen in the Lipotoxin pretreated animals. Western blotting demonstrated that mean SNAP-25 protein level was 66.4% decrease and 58.1% decrease compared to the LPs and BoNT-A pretreated group, respectively.
Instilled BoNT liposomes

BoNT Entrapped In liposomes

Cleavage of SNAP-25 By endocytosed BoNT
- The bladder urothelium expresses the intracellular targets and the binding protein for cellular uptake of BoNT/A; and that the toxin is able to suppress the levels of these targets as well as hypotonic-evoked ATP release.

- Intravesical treatment with BoNT/A suppresses bladder reflex and sensory mechanisms by affecting a number of urothelial functions including release of transmitters.
Table 1 – Median changes of voiding diary and uroflow parameters in the Lipotoxin and control groups at baseline and primary end point (1 mo) after intravesical treatment with Lipotoxin or normal saline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lipotoxin (n = 12)</th>
<th>N/S (n = 12)</th>
<th>p value (BL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency, 3 d</td>
<td>34 (28.3–42.8)</td>
<td>29 (26.5–32.5)</td>
<td>0.075</td>
</tr>
<tr>
<td>1 mo</td>
<td>24.5 (22.3–29.0)</td>
<td>27.0 (22.8–34.3)</td>
<td>0.792</td>
</tr>
<tr>
<td>Urgency, 3 d</td>
<td>32 (23.3–42.0)</td>
<td>27.5 (20–30.8)</td>
<td>0.097</td>
</tr>
<tr>
<td>1 mo</td>
<td>22 (15.8–26.3)</td>
<td>24.5 (16.8–28.8)</td>
<td>0.196</td>
</tr>
<tr>
<td>UUI*, 3 d</td>
<td>0.5 (0.0–8.25)</td>
<td>5.5 (2.0–14.0)</td>
<td>0.136</td>
</tr>
<tr>
<td>1 mo</td>
<td>0.0 (0–4.75)</td>
<td>3.5 (0.25–17.3)</td>
<td>0.797</td>
</tr>
<tr>
<td>OABSS</td>
<td>4.0 (8.0–12.8)</td>
<td>12.0 (8.75–12.8)</td>
<td>0.278</td>
</tr>
<tr>
<td>1 mo</td>
<td>8.5 (4.75–8.0)</td>
<td>4.5 (2.75–12.5)</td>
<td>0.011</td>
</tr>
<tr>
<td>FBC, ml</td>
<td>300 (243–370)</td>
<td>200 (200, 310)</td>
<td>0.568</td>
</tr>
<tr>
<td>1 mo</td>
<td>265 (225–340)</td>
<td>200 (200, 353)</td>
<td>0.018</td>
</tr>
<tr>
<td>Q_{max}, ml/s</td>
<td>12.6 (9.48–19.9)</td>
<td>11.0 (6.8–19.3)</td>
<td>0.381</td>
</tr>
<tr>
<td>1 mo</td>
<td>14.5 (8.25–19.8)</td>
<td>10.5 (6.0–15.5)</td>
<td>0.291</td>
</tr>
<tr>
<td>Volume, ml</td>
<td>180 (136–261)</td>
<td>134 (88.5–263)</td>
<td>0.454</td>
</tr>
<tr>
<td>1 mo</td>
<td>153 (132–225)</td>
<td>150 (86.3–191)</td>
<td>0.586</td>
</tr>
<tr>
<td>PVR, ml</td>
<td>25.5 (2.25–65.5)</td>
<td>21.0 (7.5–66.0)</td>
<td>0.985</td>
</tr>
<tr>
<td>1 mo</td>
<td>33 (19.3–59.3)</td>
<td>24.5 (9.0–50.8)</td>
<td>0.521</td>
</tr>
</tbody>
</table>

BL = baseline; FBC = functional bladder capacity; OABSS = Overactive Bladder Symptom Score; N/S = normal saline; PVR = postvoid residual volume; Q_{max} = maximum flow rate; UUI = urgency urinary incontinence.

Data are shown as medians with interquartile ranges (Q1–Q3). The p values at BL indicate the statistical analysis of variables between groups.

* UUI results are based on the total study population.
SV2A protein detected in the bladder mucosa

Fig. 3 - Synaptic vesicle glycoprotein 2A (SV2A) expression in the bladder mucosa of a representative control subject and an overactive bladder (OAB) patient. (A) Immunohistochemical staining; (B) Western blotting.
SNAP25 detected in the bladder mucosa

Fig. 4 – Immunohistochemistry of synaptosomal-associated protein, 25 kDa (SNAP25) in the bladder mucosa of a representative overactive bladder patient. (A) Negative control; (B) bladder mucosa at baseline; (C) bladder mucosa at 3 mo after Lipotoxin treatment.
Bladder Instillation of Liposomal Botulinum Toxin Type A Improves Overactive Bladder Symptoms- A Prospective Multi-center Double Blind Randomized Trial

Yao-Chi Chuang, Jonathan H Kaufmann, David Chancellor, Michael Chancellor, Hann-Chorng Kuo*

J Urol, 2014
• Double-blind, randomized, parallel, placebo-controlled trial

• Inclusion criteria included symptoms of OAB with a mean frequency >8/day and urgency or urge incontinence episode >1/day) and an urgency severity scale of at least 2 based on a 3-day voiding diary

• All subjects, male and female, were patients that had taken antimuscarinic agents for at least 4 weeks without effect or with intolerable adverse effects

• Major exclusion criteria included bladder outlet obstruction; neurogenic bladder dysfunction; PVR volume >150 ml; incontinence where stress was the predominant factor
Formulation of Lipotoxin and Instillation into Bladder

- Botox (Allergan, Inc, Irvine, CA, USA) 200U/10 ml saline, and 80mg sphingomyelin liposomes (Lipella Pharmaceuticals, Inc, Pittsburgh, PA, USA) /40ml sterile water

- Lipotoxin or saline was administered via a 6 Fr Nelaton tube inserted into the bladder via urethra and remained for 60 minutes.

- Subjects were eligible for open-label retreatment with lipotoxin as early as four weeks post-treatment for either group, if the subject did not show significant effect at the primary end-point.
Baseline demographics and disease characteristics of the intention-to treat population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Details</th>
<th>Lipo-BoNT, n=31</th>
<th>Placebo, n=31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>Mean</td>
<td>64.43</td>
<td>65.81</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Associated Medical</td>
<td>DM</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Conitions</td>
<td>Hypertension</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Lower Urinary Tract</td>
<td>Frequency</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Urgency</td>
<td>25</td>
<td>28</td>
</tr>
</tbody>
</table>
Results

- At four weeks post-treatment, Lipo-BoNT was associated with a statistically significantly decrease in micturition events per three-days (-4.64 for Lipo-BoNT instillation versus -0.19 for placebo; p = 0.0252)

- Drug instillation was also associated with a statistically significant decrease in urinary urgency event with respect to baseline but not placebo (-7.43 for Lipo-BoNT instillation versus -3.43 for placebo)

- Lipo-BoNT instillation was associated with a statistically significant decrease in USS scores versus those of placebo (p = 0.0181)

- No increase in residual urine volume or cases of urinary retention

- Effects of lipo-BoNT on UUI were inconclusive, due to a relatively small number of subjects having baseline incontinence.
Conclusions

- A single intravesical instillation of lipo-BoNT was associated with significant decreases of micturition frequencies and urgency severity scores.
- No increase in post void residual urine volume or cases of urinary retention
- Botulinum toxin may exerts its beneficial effects, at least in part, at the levels of the urothelium and/or afferent nerves raises the possibility that more direct targeting of these sites may maintain efficacy but have improved tolerability
- Intravesical instillation of liposomal botulinum toxin may be a promising approach for OAB
Potential study

- Lipotoxin for IC (waiting for approval)
- Lipotarcolimus for inflammatory cystitis and hemorrhagic cystitis - Chuang* et al, Neurourol Urodyn, 2011; Nirmal & Chuang* et al., J Urol, 2013)
Figure 3. Possible mechanisms of action of radiation and cyclophosphamide (CYP) induced inflammation leading to hemorrhagic cystitis and possible role of tacrolimus liposomal formulation. Chemotherapy agent cyclophosphamide is converted to acrolein, which activates urothelial transient receptor potential cation channel (TRPV1 and TRPA1) receptors, causing calcium influx and cytokine gene transcription via calcineurin dependent nuclear factor of activated T cells (NF-AT) pathway. Radiation can have similar effect by causing membrane lipid peroxidation, leading to acrolein formation and activating inflammatory pathways. Radiation can induce cytoplasmic free radical formation, leading to oxidative damage and inflammation. Tacrolimus-RK506 binding protein (FKBP) complex inhibits dephosphorylation of nuclear factor of activated T cells (NF-AT), preventing formation of nuclear factor of activated T cells complex and suppressing transcription of several cytokine genes. NOS, nitric oxide synthase. NO + •O2, free radicals. P, phosphate.
Acknowledgement

- Grant: National Science Council, Taiwan; Chang Gung Memorial Hospital
- Liposomes are free gift from Lipella Pharmaceutical company (Pittsburgh, USA)
- Teams from Kaohsiung CGMH
- UPMC and Beaumont Hospital with Professor Chancellor, Tyagi, and Yoshimura
Thank you for your attention.