Pilot Study of Liposome-encapsulated OnabotulinumtoxinA for Patients with Overactive Bladder: A Single-center Study

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**Abstract**

**Background:** Intradetrusor onabotulinumtoxinA (BoNT-A) injection benefits overactive bladder (OAB) patients, but increased postvoid residual (PVR) urine volume and urinary tract infection (UTI) remain risks. Intravesical instillation of liposomal BoNT-A instead of injection could prevent such adverse events.

**Objective:** To evaluate instillation of liquid liposomal BoNT-A (Lipotoxin) for the treatment of OAB and to determine its mechanism of action.

**Design, setting, and participants:** A double-blind randomized parallel controlled pilot trial in 24 OAB patients at a single tertiary center.

**Intervention:** Patients were randomly assigned to intravesical instillation of Lipotoxin containing 80 mg liposomes and 200 U BoNT-A or normal saline (N/S). Patients were retreated with Lipotoxin 1 mo later if they failed the first treatment.

**Outcome measurement and statistical analysis:** Voiding diaries, OAB symptom scores, urodynamic studies, and adverse events were monitored. The primary end point was change of total urinary frequency per 3 d at 1 mo after treatment. Immunohistochemistry and Western blotting for synaptic vesicle glycoprotein 2A (SV2A) and synaptosomal-associated protein, 25 kDa (SNAP25) were performed at baseline and 3 mo after treatment. The Wilcoxon rank sum test and Wilcoxon signed rank test were used for statistical analysis.

**Results and limitations:** At 1 mo after treatment, the change of urinary frequency per 3 d significantly improved in the Lipotoxin group (n = 12; median: −6.50; interquartile range [IQR]: −18.3 to −0.25; p = 0.008) but not in the N/S group (n = 12.0; IQR: −7.75 to 8.0; p = 0.792). Urgency episodes also showed a significant decrease in the Lipotinic group (n = 12.0; IQR: −20.3 to −2.75; p = 0.012) but not in the N/S group (n = 1.0; IQR: −11.0 to 2.5; p = 0.196). SV2A and SNAP25 were expressed in urothelial cells and suburothelial tissues. However, the protein expression did not significantly differ between responders and nonresponders at 3 mo after treatment.

**Conclusions:** Intravesical Lipotoxin instillation effectively reduced frequency episodes 1 mo after treatment in OAB patients without any increase in PVR or risk of UTI.

**Patient summary:** We demonstrated that intravesical Lipotoxin instillation reduced frequency episodes at 1 mo in overactive bladder patients. This procedure is safe, without an increase in postvoid residual or the risk of urinary tract infection.

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1. Introduction

Management of overactive bladder (OAB) starts with nonpharmacologic means [1,2]. The traditional medication for OAB is an antimuscarinic agent that targets the muscarinic receptors in the bladder. However, intolerable adverse effects of antimuscarinic drugs such as dry mouth, constipation, and blurred vision often limit its long-term use, especially in elderly patients [3].

 Intravesical onabotulinumtoxinA (BoNT-A) injection relieves OAB symptoms [4,5] as it enters bladder neurons binding to synaptic vesicle glycoprotein 2A (SV2; also known as SV2) [6], causing cleavage of synaptosomal-associated protein, 25 kDa (SNAP25). The release of acetylcholine from the vesicles is inhibited causing muscle paralysis [7]. Expressions of purinergic receptor P2X3 and transient receptor potential vanilloid receptor subfamily 1 on suburothelial sensory fibers are also reduced in patients receiving detrusor BoNT-A injections for detrusor overactivity (DO), which is associated with a reduction in urgency in patients responding to BoNT-A therapy [8]. However, increased postvoid residual (PVR) urine volume and urinary tract infection (UTI) remain risks yet to be resolved [9,10].

 Because BoNT-A injection requires sedation or anesthesia and the high rate of adverse events 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.

 BoNT-A is a neurotoxin with a high molecular weight of 150 kDa that makes accessing the submucosal nerve plexus difficult when it is dissolved in saline without a direct injection to pass the urothelial barrier. Since 2002, Tyagi et al. have used an intravesical liposome formulation that coats the pharmacologic ingredient (BoNT-A) for instillation into the bladder [11]. Fraser et al. reported a physiologic effect for intravesical liposomes alone in a hyperactive bladder model that involved the use of protease and potassium chloride (KCl) [12]. Another study demonstrated that liposomal encapsulation of BoNT-A improved acetic acid–induced bladder hyperactivity. It showed less of an inflammatory reaction and SNAP25 expression than in liposome- or BoNT-A-pretreated rats [13]. These studies used the same liposome preparation, and the effect of BoNT-A was found in the rat bladder muscle layer [13]. We hypothesized that large amounts of BoNT-A could be delivered into the bladder through the urothelial barrier and avoid physical or chemical trauma. Although previous studies showed the benefit of liposomes in a cystitis model, it has not been demonstrated for OAB.

In this proof-of-concept study, we tried 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. With limited penetration depth in the human bladder, Lipotoxin might have a similar effect as BoNT-A on the urothelium without affecting detrusor contractility.

2. Materials and methods

This pilot study was designed as a randomized double-blind parallel controlled trial. Patients with confirmed OAB were randomly assigned to receive intravesical instillation of either Lipotoxin (treatment group) or normal saline (N/S; control group). Both groups were evaluated at the treatment visit and primary end-point evaluation 1 mo after treatment by recording symptom scores, adverse events, and overall satisfaction assessments. This study was approved by the research ethics committee of the hospital. Informed consent was obtained from every patient, after advising them of potential adverse events related to BoNT-A and intravesical instillation.

2.1. Patients

Adults ≥20 yr of age with symptoms of urgency frequency and/or urgency incontinence (UUI), and an urgency severity scale (USS) of at least 2 confirmed by a 3-d voiding diary, with or without urodynamically proven DO, were consecutively enrolled at a single tertiary center. The patients were asked to record a 3-d voiding diary for frequency, urgency, UUI, and functional bladder capacity (FBC). The patients must have been treated with an antimuscarinic agent for ≥3 mo without a response. Patients who had a previous BoNT-A treatment were not included in this study. The appendix lists the inclusion and exclusion criteria.

2.2. Lipotoxin preparation and treatment

Sphingomyelin mixed with N/S creates a liposomal dispersion of sphingomyelin. 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. Patients were randomly assigned to either group using permuted randomization coding. Before intravesical instillation, patients were asked to void completely. 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. Patients were allowed to drink water and distend their bladders for another 30 min.

Antibiotics were given for 3 d after treatment to prevent a possible UTI. Patients were then regularly monitored at 2 wk, 1 mo, and 3 mo after the treatment. If patients were not satisfied with the treatment result at 1 mo, repeated instillation with Lipotoxin was performed regardless of patient allocation. Patients who received a second treatment were monitored as for the first treatment. Patients were not allowed to take any medication during the study period including anticholinergics, tricyclic antidepressants, or calcium channel blockers.

2.3. Measurements and follow-up

The primary end point of the study was the net change in total frequency per 3 d from baseline to 1 mo after Lipotoxin or N/S treatment. The
response to treatment was defined as $\geq$25% decrease in the frequency per 3 d at the 1 mo follow-up visit. The secondary end points were the net change of variables from baseline to 1 mo after treatment including urgency episodes over 3 d, UUI over 3 d, Overactive Bladder Symptom Score, USS, FBC, maximum flow rate ($Q_{\text{max}}$), PVR, and Global Response Assessment (GRA).

Adverse events (hematuria, micturition pain, UTI, PVR >150 mL, and urinary retention) occurring after intravesical instillation were recorded. Urinalysis was routinely done before treatment and at follow-up visits if patients had UTI symptoms. Transabdominal sonography was also performed to measure PVR at each visit. Subjects were asked to report systemic adverse event symptoms such as general weakness, respiratory distress, gastrointestinal upset, and dizziness.

Video urodynamic studies were performed upon enrollment into the study to exclude the possibility of bladder outlet obstruction and to confirm the presence of DO. Cystoscopy was also performed, and a bladder biopsy was obtained to exclude the possibility of carcinoma. At 3 mo after treatment, a repeat cystoscopic biopsy was performed in some of the study patients who agreed to provide comparative tissues before and after treatment. The bladder biopsy specimens were sent to the pathology department and embedded in OCT medium and stored at $-80^\circ$C in a refrigerator or liquid nitrogen tank until further investigation.

2.4. Immunohistochemistry and Western blots of the urothelium

Urinary bladder specimens were fixed and processed for immunohistochemistry as previously described [14]. Sections were incubated overnight at 4 $^\circ$C with antibodies for antihuman SV2A (HPA007863, Sigma Life Sciences, St. Louis, MO, USA) or antihuman SNAP25 (ab41455, Abcam, Cambridge, UK). For SNAP25 and SV2A immunofluorescence staining, after incubation with primary antibody, the slides were then washed in phosphate-buffered saline with Tween; immunoglobulin and fluorescein isothiocyanate (1:500 Dako Cytomation Denmark, Copenhagen, Denmark) were then applied to the sections and incubated for 1 h at room temperature. The sections were then counterstained with 4,6-diamidino-2-phenylindole (1:10000, Invitrogen Molecular Probes, Eugene, OR, USA). The slides were examined under fluorescence microscopy and processed using a digital imaging system (Carl Zeiss, Oberkochen, Germany). Total protein extracted from bladder tissue of control subjects, OAB patients, and normal human urothelial cells lysate (ScienCell Research Laboratories, Carlsbad, CA, USA) were also investigated using Western blotting for SV2A expression.

The bladder specimens were prepared for Western blot analyses of SV2A and SNAP25 expression according to the standard protocol (Amersham Biosciences). The antibodies used were rabbit anti-SV2A polyclonal antibody (ab22942, Abcam); goat anti-SNAP25 polyclonal antibody (ab41455, Abcam), and mouse anti-glyceraldehyde phosphate dehydrogenase (GAPDH) monoclonal antibody (SC-32333, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Western blots were visualized using an enhanced chemiluminescence detection system (Millipore, Temecula, CA, USA). The amount of GAPDH was also detected as the internal control. Quantitative analyses were done using Image J software (National Institutes of Health, Bethesda, MD, USA). The Western blots and immunohistochemistry studies were performed with blinding as to patient allocation and treatment.

2.5. Statistical analysis

Continuous variables are expressed as medians with interquartile ranges (IQRs). The Wilcoxon rank sum test was used for statistical comparisons of continuous variables between groups. The Wilcoxon signed rank test was used to evaluate the significant difference of variables at baseline and after treatment. The ordinal logistic regression method was used to analyze changes in the USS and GRA after treatment. All statistical assessments were two sided and considered significant at $p < 0.05$. Statistical analyses were performed using SPSS v.15.0 statistical software (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Clinical therapeutic results of Lipotoxin treatment

A total of 24 patients were eligible for the treatment including 10 men and 14 women with a mean age of 67 yr (range: 38–82). The study profile is shown in Figure 1. Among the patients, 18 had OAB wet, and 6 had OAB dry. There was no significant difference in gender distribution or average age between the groups. At baseline, there was no significant difference in any measured variable between the Lipotoxin and N/S groups (Table 1).

Table 1 shows the medians of variables measured at baseline and 1 mo after treatment in the Lipotoxin and control groups. The median change of frequency per 3 d from baseline to 1 mo was significantly decreased in the Lipotoxin group ($-6.50$; IQR: $-18.3$ to $-0.25$; $p = 0.008$) but not in the N/S group ($0.0$; IQR: $-7.75$ to $8.0$; $p = 0.792$). Post hoc power calculation revealed the power was 0.875 based on the changes of frequency episodes between groups. The urgency episodes also significantly decreased in the Lipotoxin group ($-12.0$; IQR: $-20.3$ to $-2.75$; $p = 0.012$) but not in the N/S group ($-1.0$; IQR: $-11.0$ to $2.5$; $p = 0.196$). However, the UUI episodes did not change after Lipotoxin treatment. USS decreased in 6 and 5 patients, and GRA increased in 9 and 8 patients of Lipotoxin and N/S group, respectively ($p = 0.682$ and 1.000). There were no significant differences in the change of uroflow parameters from baseline to 1 mo in either the Lipotoxin or N/S group (Fig. 2).

No adverse events such as large PVR, urinary retention, or UTI were reported by the patients during the follow-up period.

As shown in Figure 1, at 1 mo, six patients (50%) in the Lipotoxin group and one (8.3%) in the control group had responses to treatment. Six Lipotoxin nonresponders and six N/S nonresponders received a second treatment with Lipotoxin at 1 mo. All nonresponders to the first Lipotoxin treatment also failed the second Lipotoxin treatment; only one patient in the N/S group who received Lipotoxin treatment at 1 mo remained a responder at the 3 mo assessment. The cumulative efficacy of Lipotoxin at 1 mo was 50% (6 of 12 patients) but was only 28% (5 of 18 patients) at 3 mo.

3.2. Expression of synaptic vesicle protein 2A and synaptosomal-associated protein 25 in the urothelium

SV2A expression was demonstrated in apical cells, urothelial cells, and suburothelial tissues of OAB and control patients by immunohistochemical staining (Fig. 3A). Expression of SV2A was observed in normal urothelial cell lysate, mucosa of control subjects, and OAB patients by Western blotting (Fig. 3B). SNAP25 expression in the suburothelial area was detected by immunohistochemical
staining at baseline and could still be detected in the suburothelial tissue 3 mo after Lipotoxin treatment (Fig. 4).

Among all patients, 17 had bladder biopsies at baseline and 3 mo after treatment; expressions of SV2A and SNAP25 were investigated. For SV2A, this antibody detects bands approximately between 82 and 92 kDa; and for SNAP25, this antibody detects band at 26 kDa. Thus the Western blot bands are present at the expected range per what was

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></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</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></td>
<td>p = 0.008</td>
<td>p = 0.792</td>
<td></td>
</tr>
<tr>
<td>Urgency, 3 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</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>0.496</td>
</tr>
<tr>
<td></td>
<td>p = 0.012</td>
<td>p = 0.196</td>
<td></td>
</tr>
<tr>
<td>UUI, 3 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</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 (0.0–2.75)</td>
<td>3.5 (0.25–17.3)</td>
<td>0.781</td>
</tr>
<tr>
<td></td>
<td>p = 0.797</td>
<td>p = 0.018</td>
<td></td>
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<tr>
<td>OABSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>9.0 (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 (4.75–0.8)</td>
<td>9.0 (7.25–12.5)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>p = 0.041</td>
<td>p = 0.011</td>
<td></td>
</tr>
<tr>
<td>FBC, ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>300 (243–370)</td>
<td>250 (200, 413)</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></td>
<td>p = 0.106</td>
<td>p = 0.018</td>
<td></td>
</tr>
<tr>
<td>Qmax, ml/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</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></td>
<td>p = 0.934</td>
<td>p = 0.291</td>
<td></td>
</tr>
<tr>
<td>Volume, ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>180 (136–261)</td>
<td>134 (68.5–263)</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></td>
<td>p = 0.567</td>
<td>p = 0.586</td>
<td></td>
</tr>
<tr>
<td>PVR, ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>25.5 (22.5–65.5)</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>
<tr>
<td></td>
<td>p = 0.890</td>
<td>p = 0.521</td>
<td></td>
</tr>
</tbody>
</table>

BL = baseline; FBC = functional bladder capacity; OABSS = Overactive Bladder Symptom Score; N/S = normal saline; PVR = postvoid residual volume; Qmax = 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.

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Fig. 1 – Study profile. Asterisks indicate bladder biopsies were performed. N/S = normal saline.
Fig. 2 – Median changes of voiding diary and uroflow variables from baseline to 1 mo between the Lipotoxin and normal saline groups.
FBC = functional bladder capacity; N/S = normal saline; OABSS = Overactive Bladder Symptom Score; PVR = postvoid residual volume; Q_max = maximum flow rate; UUI = urgency urinary incontinence.

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.
quoted by the producer. At 3 mo after Lipotoxin treatment, five patients were responders to Lipotoxin; seven were Lipotoxin nonresponders. Protein expression did not differ significantly in Lipotoxin responders, nonresponders, and controls at 3 mo compared with the baseline level (Fig. 5).

4. Discussion

The pilot study results revealed that liposomes can be a vehicle for delivering BoNT-A into the urothelium of patients with OAB without the need for injection. Both frequency and urgency episodes significantly decreased after treatment in the Lipotoxin group, but UUI did not improve significantly. We also showed that SV2A receptors were present in human urothelial cell lysate. Three months after Lipotoxin treatment, however, SNAP25 did not show a significant decrease in all responders and nonresponders.

Intravesical administration of drug solutions provides excellent local drug concentrations in the bladder that may decrease the risk of systemic side effects [15,16]. However, an important obstacle in the success of intravesical drug delivery arises from the low permeability of the bladder urothelium. The watertight barrier is usually located in the umbrella cells, which are the superficial layer of bladder urothelium augmented by glycosaminoglycans and uroplakins [15]. Liposomes are vesicles composed of concentric phospholipid bilayers separated by aqueous compartments [17]. Because liposomes adsorb to cell surfaces and fuse with cells, they are used as vehicles for drug delivery and gene therapy [15,16]. Intravesical administration of liposomes into the wounded urothelium may improve the dysfunctional urothelium and provide an alternative treatment for interstitial cystitis/bladder pain syndrome [18]. Lipotoxin-pretreated rats had decreased inflammatory reactions and SNAP25 expression and decreased bladder hyperactivity. These results support liposomes as an efficient vehicle for delivering BoNT-A without the need for injection [13].

In this pilot human clinical trial, Lipotoxin was effective in reducing a median frequency by 6.5 episodes and a median urgency by 12.0 episodes per 3 d at 1 mo after treatment. Although the frequency and urgency episodes decreased at 1 mo after Lipotoxin treatment, the reduction of UUI episodes did not differ significantly when compared with the baseline. This result was not as beneficial as that of BoNT-A injection for OAB treatment [5,10,19]. Nonetheless, this clinical effect is similar to that of recently published data for mirabegron, the β3-adrenoceptor agonist [20,21]. OAB is a syndrome with urothelial, neurogenic, and musculogenic disease components. The penetration of BoNT-A delivered by liposomes might not reach a sufficient depth to have an effect on the detrusor and nerves and achieve a significant improvement of UUI.

![Fig. 4](image1.png) **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.

![Fig. 5](image2.png) **Fig. 5** – Expressions of synaptic vesicle glycoprotein 2A (SV2A) and synaptosomal-associated protein, 25 kDa (SNAP25) at baseline and 3 mo after Lipotoxin treatment. There was no significant difference in any proteins in the Lipotoxin responders and nonresponders. GAPDH = glyceraldehyde phosphate dehydrogenase; Pt = patient.
In our study, $Q_{\text{max}}$ and PVR did not differ significantly from the controls after Lipotoxin treatment, and no adverse events occurred that were related to the treatment drug or procedure, indicating the safety of Lipotoxin treatment.

We found that in urothelial cross sections, SV2 receptors and SNAP25 protein were expressed and highly localized within the human bladder urothelium. Previous studies using bladder urothelium and cultured urothelial cells also showed positive expression of soluble N-ethylmaleimide-sensitive factor activating protein receptor proteins [22]. The presence of SV2 receptors on the urothelial apical cells indicates that BoNT-A protein can be delivered with liposome encapsulation and enter the urothelial cells through endocytosis. Nonetheless, we did not find that SNAP25 protein decreased 3 mo after Lipotoxin treatment. Although the post-treatment assessment was performed 3 mo after treatment, it is possible that the SNAP25 proteins had recovered by 3 mo after treatment.

Although we provided physiologic evidence for the efficacy of Lipotoxin, the mechanism of its action remains to be determined. Through encapsulation of BoNT-A in the liposomes, the activity of Lipotoxin seemed to be restricted to the urothelium and blocked the urothelial release of sensory neurotransmitters. Whether Lipotoxin can be further transported into the suburothelial space in the human bladder remains to be explored. It is intriguing that fluorescent-tagged liposomes bound to the plasma membranes of cultured urothelial cells internalized as temperature increased, suggesting an endocytotic mechanism [23].

Limitations of this pilot study are the small number of patients, lack of prospective power calculation, and lack of urodynamic data after Lipotoxin treatment at 1 mo. Biopsy taken at 3 mo after treatment might not reflect the bladder condition of responders at maximum efficacy. A full randomized placebo-controlled multicenter trial had been conducted to elucidate the mechanism of action and transport of the liposome encapsulated into the urothelium.

5. Conclusions

This pilot study demonstrated that intravesical Lipotoxin instillation can effectively reduce frequency and urgency episodes 1 mo after treatment in OAB patients. The PVR did not increase, and all patients were free of UTI after the treatment.

Financial disclosures: Hann-Chorng Kuo certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Hann-Chorng Kuo is a consultant for Lipella Pharmaceuticals and an investigator for Allergan, Pfizer, Astellas, and GlaxoSmithKline. Yao-Chi Chuang is a consultant for Lipella Pharmaceuticals and an investigator for Allergan, Pfizer, Astellas, and GlaxoSmithKline. Michael B. Chancellor is a founder, corporate board member, stockholder, and the chief scientific officer of Lipella Pharmaceuticals and has received grants from Allergan, Astellas, Medtronic, Pfizer, and Targacept. Hsin-Tzu Liu and Lori Birder have nothing to disclose.

Funding/Support and role of the sponsor: Buddhist Tzu Chi General Hospital, Grant TCRD-I9901-02, helped collect the data for the study.

Appendix – Inclusion and exclusion criteria of the study

Inclusion criteria:

1. Adults $\geq 20$ yr of age
2. Patients with symptoms of urgency frequency and/or urge incontinence and an Urgency Severity Scale of at least 2, with or without urodynamically proven detrusor overactivity
3. Free of active urinary tract infection
4. Free of bladder outlet obstruction on enrollment
5. Free of overt neurogenic bladder dysfunction
6. Had been treated with antimuscarinic agents for at least 4 wk without effect or with intolerable adverse effects
7. Patient had not been treated with bladder surgery for overactive bladder, such as enterocystoplasty that might affect the therapeutic effect of the test drug
8. Patient can record a voiding diary for urinary frequency and urgency
9. Patient or his or her legally acceptable representative has signed the written informed consent form

Exclusion criteria:

1. Use of an antimuscarinic agent effective in the treatment of lower urinary tract symptoms
2. Patients with severe cardiopulmonary disease such as congestive heart failure, arrhythmia, poorly controlled hypertension, inability to receive regular follow-up
3. Patients with bladder outlet obstruction on enrollment
4. Patients with postvoid residual volume $> 150$ ml
5. Patients with uncontrolled confirmed diagnosis of acute urinary tract infection
6. Patients with laboratory abnormalities at screening including alanine aminotransferase more than three times the upper limit of the normal range, aspartate aminotransferase more than three times the upper limit of the normal range, and abnormal serum creatinine levels more than two times the upper limit of the normal range
7. Patients with any contraindication for urethral catheterization during treatment
8. Female patients who are pregnant, lactating, or of childbearing potential who are not using contraception
9. Patients with myasthenia gravis or Eaton-Lambert syndrome.
10. Patients with any other serious disease considered by the investigator unsuitable for general anesthesia or to enter the trial
11. Patients having participated in an investigational drug trial within 1 mo before entering this study

References