Bladder Instillation of Liposome Encapsulated OnabotulinumtoxinA Improves Overactive Bladder Symptoms: A Prospective, Multicenter, Double-Blind, Randomized Trial

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Purpose: Cystoscopic intradetrusor injection of botulinum toxin has helped patients with refractory overactive bladder but with the increased risks of urinary tract infection and urinary retention. We assessed whether catheter instillation of 200 U onabotulinumtoxinA formulated with liposomes is safe and effective for the treatment of overactive bladder.

Materials and Methods: This 2-center, double-blind, randomized, placebo controlled study enrolled patients with overactive bladder inadequately managed with antimuscarinics. Patients were assigned to intravesical instillation of lipo-botulinum toxin (31) or normal saline (31). The primary end point was the mean change in micturition events per 3 days at 4 weeks after treatment. Additional end points included mean changes in urgency events, frequency and urinary urge incontinence, as well as changes in overactive bladder symptom scores and urgency severity scores.

Results: At 4 weeks after treatment lipo-botulinum toxin instillation was associated with a statistically significant decrease in micturition events per 3 days (-4.64 for lipo-botulinum toxin vs -0.19 for placebo, $p = 0.0252$). Lipo-botulinum toxin instillation was also associated with a statistically significant decrease in urinary urgency events with respect to baseline but not placebo. However, lipo-botulinum toxin instillation was associated with a statistically significant decrease in urgency severity scores compared to placebo ($p = 0.0181$). These observed benefits of lipo-botulinum toxin instillation were not accompanied by an increased risk of urinary retention. The effects of lipo-botulinum toxin on urinary urge incontinence were inconclusive.

Conclusions: A single intravesical instillation of lipo-botulinum toxin was associated with decreases in overactive bladder symptoms without side effects. Intravesical instillation of liposomal botulinum toxin may be a promising approach to treat refractory overactive bladder.

Key Words: onabotulinumtoxinA; liposomes; urinary bladder, overactive

Abbreviations and Acronyms

AE = adverse event
BoNT = botulinum toxin
GRA = global response assessment
OAB = overactive bladder
OABSS = OAB symptom scores
onaBoNTA = onabotulinumtoxinA
PVR = post-void residual
Qmax = maximum flow rate
TEAE = treatment related adverse event
USS = urgency severity scores
UTI = urinary tract infection
UUI = urinary urge incontinence

Accepted for publication July 10, 2014.

Study received institutional review board approval.

Supported by Kaohsiung Chang Gung Memorial Hospital CMRPG8A0171, Buddhist Tzu Chi General Hospital TCRD-I9901-02 and Lipella Pharmaceuticals.

* Financial interest and/or other relationship with Lipella, Allergan, Pfizer, Astellas and GlaxoSmithKline.

† Financial interest and/or other relationship with Lipella Pharmaceuticals Inc.

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‖ Financial interest and/or other relationship with Lipella, Allergan, Astellas and GlaxoSmithKline.

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Overactive bladder syndrome, characterized by urgency and frequency with or without urinary urge incontinence, is a common medical condition with a significant impact on quality of life across the world.[1–3] Antimuscarinics are the mainstay of pharmacotherapy for OAB.[4] However, systemic side effects often limit their tolerability and long-term use.[5]

0022-5347/14/1926-1743/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2014.07.008
Vol. 192, 1743-1749, December 2014
Printed in U.S.A.

Intradetrusor injection of onaBoNTA is an effective option for patients with OAB unresponsive to antimuscarinics.[4] Botulinum toxin A is known for its ability to induce chemical denervation by using the synaptic vesicle protein SV2 as its protein receptor,[6] to cleave cytosolic translocation protein SNAP-25, and to inhibit the process of exocytosis and reduce the release of various neurotransmitters.[7] BoNT may confer effects beyond the inhibition of muscle overactivity by inhibiting acetylcholine release. BoNT has also been shown to modulate pain and inhibit afferent neurotransmission including substance P, glutamate, nerve growth factor, calcitonin gene related peptide and adenosine triphosphate.[8–11]

Bladder application of BoNT requires a cystoscopic procedure with intradetrusor needle injection into 20 to 30 sites with the patient under local anesthesia or sedation. Topical application of BoNT to visceral cavities may be a viable alternative if the BoNT can be effectively delivered without needle injections. The avoidance of intradetrusor BoNT needle injections may potentially decrease the risk of hematuria, pain, large PVR and UTI.[12,13] Because of the limited permeability of the bladder wall, onaBoNTA, being a large molecule (approximately 150 kDa), has limited access to the submucosal nerve plexus when not injected with a needle. Recently Chuang et al demonstrated that bladder uptake of BoNT improved when the toxin was formulated with liposomes in a hyperactive bladder model in rats.[14] Therefore, we hypothesized that instillation of onaBoNTA formulated with liposomes can effectively reduce symptoms of OAB without an increased risk of urinary retention.

MATERIALS AND METHODS

This phase 2, proof of concept study was designed as a double-blind, randomized, parallel, placebo controlled trial conducted at the Buddhist Tzu Chi General Hospital, and the Kaohsiung Chang Gung Memorial Hospital between January 2011 to July 2013 after approval by the institutional review board, and was in compliance with the ethical principles of Good Clinical Practice guidelines and the Declaration of Helsinki. Informed consent was obtained from patients before any study procedures were performed.

Men and women 20 years old or older were enrolled in the study. Inclusion criteria were symptoms of OAB with a mean frequency greater than 8 per day, and urgency or urge incontinence episode greater than 1 per day) and an urgency severity score of at least 2 based on a 3-day voiding diary. All subjects had taken antimuscarinic agents for at least 4 weeks without effect or with intolerable adverse effects. The washout period for antimuscarinics was 2 weeks or more. Major exclusion criteria were clinically significant bladder outlet obstruction (with Qmax less than 10 ml per second or pressure flow multichannel urodynamically confirmed); overt neurogenic bladder dysfunction such as spinal cord injury, multiple sclerosis, cerebral vascular accident, Parkinson disease, radiculopathy and head injury; significant PVR volume (greater than 150 ml); incontinence where stress was the predominant factor; bladder surgery, previous pelvic radiation therapy or malignant disease of the pelvic organs; and any other serious disease making the patient unsuitable for the study as considered by the investigator.

The permuted block randomization method was applied to generate randomization codes. Each randomization number was assigned to individual patients according to the time sequence for the screened patient to become eligible. Subjects were randomized 1:1 to placebo (normal saline) or lipo-BoNT. This formulation contained premixed onaBoNTA (Allergan Inc., Irvine, California) 200 U/10 ml saline and 80 mg sphingomyelin liposomes (Lipella Pharmaceuticals, Inc., Pittsburgh, Pennsylvania) per 40 ml sterile water. It was administered via a 6Fr Nelaton tube inserted into the bladder via urethra and left for 60 minutes with the catheter clamped in place. The lipo-BoNT and saline for instillation were obtained without label (blind) from the hospital pharmacy. Antibiotics were given after procedures for 3 days to prevent urinary tract infection. Followup visits were scheduled at 2 weeks, 4 weeks, 3 months and 6 months after the treatment visit. Subjects were eligible for open label re-treatment with lipo-BoNT as early as 4 weeks after treatment in either group if the subject did not show a significant effect (defined as decrease in total frequency within 3 days by 25% or more and an improved GRA by 2 scales) at the primary end point. Efficacy analysis of 12-week posttreatment evaluation visits excluded subjects who underwent re-treatment.

Both groups were evaluated at baseline screening and with at least 2 study required visits (treatment visit and primary end point evaluation visit) by symptom score recording, urodynamic studies, adverse event recording and global satisfaction assessments. Patients were not allowed to take certain medications during the study period, including anticholinergics, tricyclic antidepressants and calcium channel blockers.

The primary end point was the net change in total micturition events per 3 days from baseline to 4 weeks after the treatment day based on the 3-day voiding diary (3 consecutive days within 7 days before the visit). The secondary end points were changes in several measures from baseline to 4 weeks after treatment, including urgency episodes and UUI episodes per 3 days, OABSS, USS, functional bladder capacity (the maximal voided volume that appears in the 3-day voiding diary), Qmax, PVR and GRA, categorized into −3, −2, −1, 0, 1, 2 and 3, indicating markedly worse, moderately worse, mildly worse, no change, mildly improved, moderately improved and markedly improved patient perception of bladder condition, respectively. Safety measurements included local TEAEs such as hematuria, micturition pain, UTI, urinary retention and any systemic TEAE that occured after treatment.

The frequencies of overactive bladder symptoms (micturition episodes, urgency episodes and urinary incontinence episodes in a 3-day period) were treated as continuous variables. Changes in these frequencies

associated with treatment were measured on a paired basis at each visit less baseline. The Wilcoxon matched pairs signed rank test was used to test the statistical significance of treatment associated changes in each of these frequencies. Analysis of variance was used to test the significance of intergroup differences with treatment as the factor and frequency change the variate. PVR volume was also treated as a continuous variable, and its measurements were similarly subjected to the same significance tests.[15]

The treatment associated changes in USS, OABSS and GRA were treated as ordinal variables. The sign test was used to test the statistical significance of their changes from baseline and the Wilcoxon rank sum test was used for intergroup (ie lipo-BoNT vs saline) comparisons.[15]

Efficacy analyses of posttreatment evaluation visits beyond the primary end point (ie beyond 4 weeks after treatment) excluded all subjects who received re-treatment. Subject attrition precluded efficacy analyses beyond 12 weeks after treatment. The power calculation for the primary outcome measure was based on an expected effect size of −2.5 episodes per day for the lipo-BoNT group, −0.5 episodes per day for the placebo group and a 2.5 episodes per day standard deviation for both groups. Assuming a 2-tailed type I error rate of 0.05, a sample size of 60 (30 subjects per group) was expected to be sufficient to detect this anticipated effect with 85% power.

RESULTS

The baseline characteristics were balanced between the treatment groups (table 1). The intent to treat population consisted of 62 randomized subjects, of whom 57 (92%) completed the primary end point visit (ie 4 weeks after treatment) (fig. 1). Re-treatment was conducted in a similar number of subjects in both groups, in 15 of 31 (48%) for the lipo-BoNT group and 16 of 31 (52%) for the saline group. By 12 weeks 23 subjects (37%) continued the study without re-treatment. Eleven subjects (18%) completed the study through 6 months after treatment evaluation.

Table 1. *Baseline demographics and disease characteristics of the intent to treat population*

	Lipo-BoNT	Placebo
Mean pt age	64	66
No. gender:		
M	13	16
F	18	15
No. associated medical conditions:		
Diabetes mellitus	3	9
Hypertension	5	12
Pelvic surgery	0	1
Other disease	1	1
No. lower urinary tract symptoms:		
Frequency	24	21
Urgency	25	28
Urge incontinence	27	24
Stress incontinence	4	5
Dysuria	10	9
Bladder pain	0	0

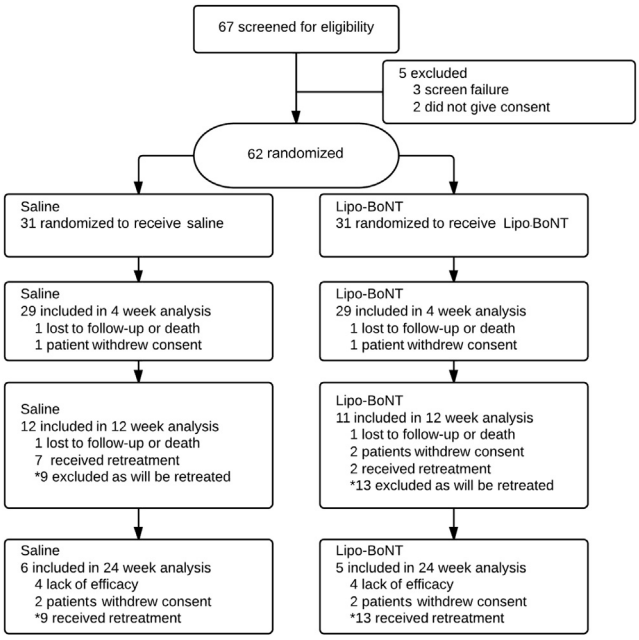

Figure 1. Patient allocation and flow chart of study

The changes in OAB measures and voiding diary parameters in the lipo-BoNT and placebo groups from baseline to 1 month after intravesical treatment are shown in table 2. The primary end point, mean micturition frequency reduction at 4 weeks after treatment, was −4.64 events per 3 days for the lipo-BoNT group and −0.19 events per 3 days for the saline group (table 3, fig. 2). The difference in micturition frequency reduction associated with lipo-BoNT vs that of saline was statistically significant. Also, micturition frequency reductions from baseline were statistically significant at all 3 points for the lipo-BoNT group but not for the saline group.

The lipo-BoNT associated urgency event frequency reductions from baseline were statistically significant at 2 of the 3 points (2 weeks and 4 weeks after treatment). None of the saline associated reductions in urgency event frequency was statistically significant. The differences in urgency event frequency reduction associated with lipo-BoNT vs that of saline were not statistically significant.

Table 2. *Changes in voiding diary parameters from baseline to 1 month after intravesical treatment*

	Baseline				1-Mo			
Episode/3 Days	Mean	SD	Lower	Upper	Mean	SD	Lower	Upper
Lipo-BoNT:								
Frequency	38	14	33	43	32	11	27	36
Urgency event	28	16	21	31	20	21	11	23
UUI	2	4	0	4	3	10	1	5
Placebo:								
Frequency	34	10	30	38	33	13	28	38
Urgency event	29	14	21	32	25	16	16	27
UUI	3	5	−1	6	3	6	1	7

Table 3. *Summary of OAB symptom results*

	Mean Change from Baseline, Episodes per 3 Days (95%CI)		Mean % Change from Baseline	
	Lipo-BoNT	Normal Saline	Lipo-BoNT	Normal Saline
Micturition episodes:				
Wk 2 (in 22, 19)	−2.64 (−5.10, −0.15)*	−0.63 (−3.86, 2.59)	−7	0
Wk 4 (in 28, 27)	−4.64 (−7.46, −1.82)†	−0.19 (−2.98, 2.61)	−10	−1
Wk 12 (in 9, 9)	−7.77 (−14.31, −1.83)*	−0.78 (−6.34, 4.79)	−18	−3
Urgency episodes:				
Wk 2 (in 22, 19)	−4.91 (−9.21, 0.61)*	−2.71 (−6.80, 1.38)	−31	−13
Wk 4 (in 28, 27)	−7.43 (−11.68, −3.18)†	−3.43 (−7.30, 0.45)	−35	−17
Wk 12 (in 9, 9)	−10.77 (−22.80, 5.20)	−6.78 (−17.00, 3.44)	−22	−32
Urinary incontinence episodes:				
Wk 2 (in 8, 12)	−1.25 (−7.09, 4.59)	−0.17 (−4.48, 4.15)	−72	2
Wk 4 (in 11, 13)	−1.11 (−2.13, −0.06)	−0.92 (−3.18, 1.33)	−61	−10
Wk 12 (in 7, 10)	−1.29 (−3.40, 0.83)	0.50 (−1.84, 2.84)	−11	−13

* $p < 0.05$.
† $p < 0.005$.

The measurements of UUI frequency, shown in table 2, had less statistical power than those associated with the other 2 overactive bladder symptoms measured, given that the sample set for urinary incontinence was approximately half the size based on the 3-day voiding diary, not the baseline characteristics seen in table 1. None of the means of the paired changes from baseline in UUI frequency were statistically significant, nor were the differences of the change distributions comparing the lipo-BoNT and saline groups. Also, the urodynamic parameters including Qmax, PVR and functional bladder capacity did not show significant changes in either group (fig. 3).

Histograms of treatment associated changes in USS, OABSS and GRA at 4 weeks after treatment are provided in figure 4. The lipo-BoNT group had a statistically significant shift in USS at 4 weeks and 12 weeks after treatment. The significance tests associated with the saline group yielded no measurable shift in USS. The lipo-BoNT associated USS reduction at 4 weeks after treatment was significantly larger than that of saline ($p = 0.0052$).

The lipo-BoNT group had a statistically significant shift in OABSS at 4 and 12 weeks after treatment. The significance tests associated with the saline group yielded no measurable shift in OABSS. The OABSS reductions associated with lipo-BoNT were larger than those with saline. However, the 2 groups were not statistically distinct.

GRA scores improved by a statistically significant amount for the lipo-BoNT and saline groups. At 2 weeks after treatment the GRA scores for the

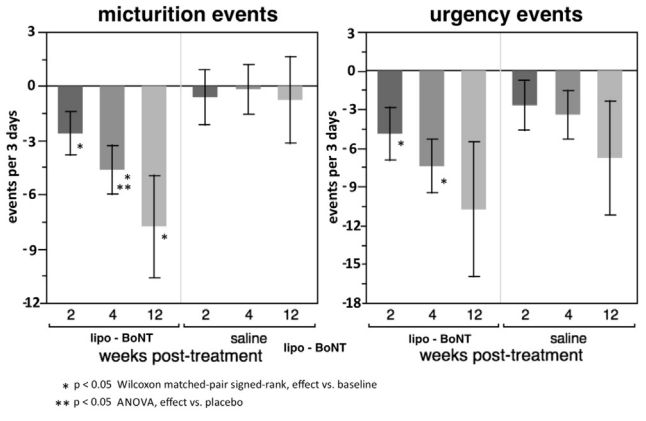

Figure 2. Decreases in micturition and urgency event frequency. Bars indicate mean paired change from baseline and standard errors of means are also indicated. Larger confidence intervals at 12 weeks after baseline are result of reduced analysis population size (from subject attrition and re-treatment).

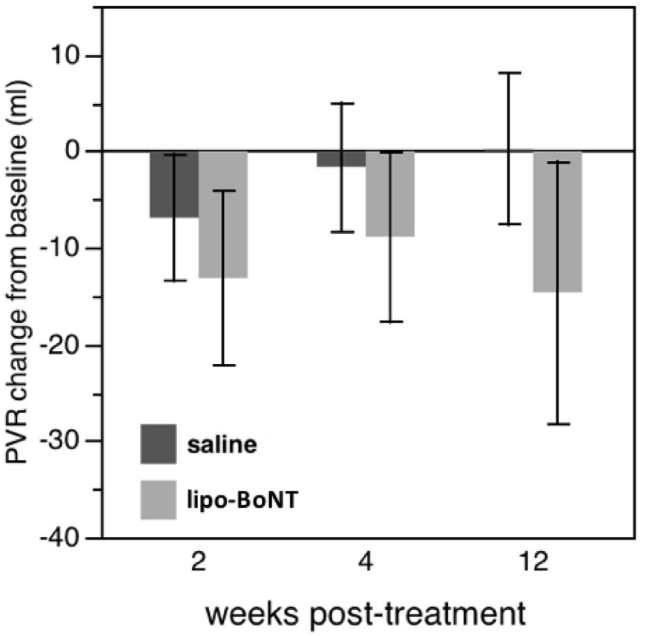

Figure 3. Change in PVR from baseline for both groups at 2, 4 and 12 weeks after treatment. Error bars show standard error of mean. There was no significant change in PVR for lipo-BoNT or saline group at any point.

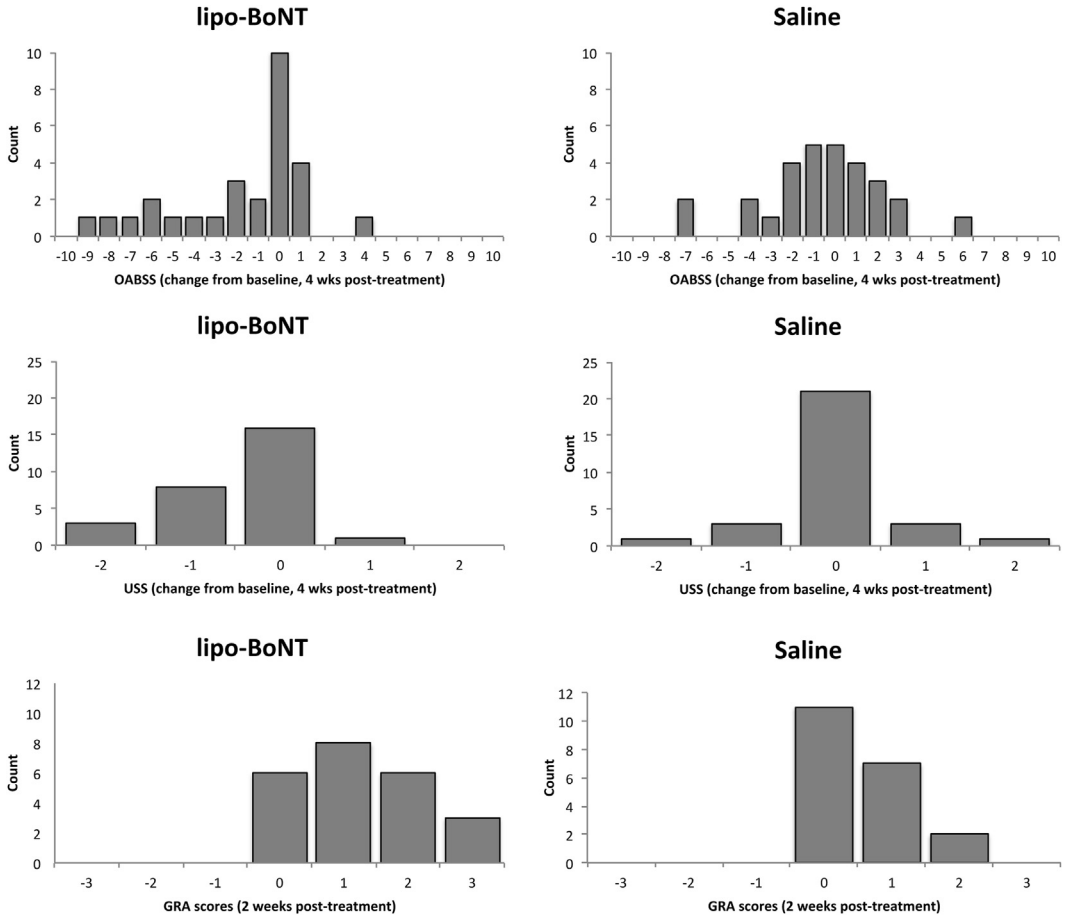

Figure 4. Histograms of OABSS, USS and GRA outcomes. OABSS and USS are shown as paired changes from baseline at 4 weeks after treatment. GRA is reported as survey value at 2 weeks after treatment. Lipo-BoNT group treatment related OABSS paired reduction from baseline is statistically significant ($p = 0.0064$). However, comparison with saline was not statistically significant. Saline group USS change histogram is symmetric, indicating no measurable effect. However, USS change histogram associated with lipo-BoNT treatment had significant reduction from baseline ($p = 0.0049$) and compared to that of saline ($p = 0.0181$). GRA scores showed significant improvement for both groups. However, only at 2 weeks after treatment did GRA scores for lipo-BoNT group show more improvement than those for saline group ($p = 0.0169$).

lipo-BoNT group showed more improvement than those for the saline group ($p = 0.0169$). However, there was no statistically significant difference between the groups for the remaining visits.

No local AEs were reported in any subject in the lipo-BoNT group. Hematuria was reported at 3 months in 1 subject (3.57%) and UTI in 1 subject in the saline group. A total of 48 AEs occurred in 17 (27.41%) subjects during the study, including 20 events with lipo-BoNT in 25.81% of subjects and 28 events with saline in 29.03% of subjects. Except for 2 events of chest pain (1 subject from each group), all other AEs occurred as a single event in the lipo-BoNT or saline group. Most of the AEs were mild in intensity (92.66%). There were 3 severe TEAEs, all occurring in a single subject in the lipo-BoNT group (table 4). None of the TEAEs was considered related to treatments with lipo-BoNT or saline.

DISCUSSION

Intradetrusor BoNT injection is a newly approved treatment for refractory OAB but the procedure has the potential increased dose related risks of urinary retention and UTI.[13] We report the first double-blind, placebo controlled, multicenter trial that suggests intravesical instillation of liquid liposome

Table 4. *Treatment emergent and serious adverse events*

	No. Events/No. Subjects (%)	
	Lipo-BoNT (28)	Placebo (27)
Nondrug related TEAEs	3/1 (3.57)*	0/0 (0.00)
Drug related TEAEs	0/0 (0.00)	0/0 (0.00)
Serious AEs	1/1 (3.57)†	2/2 (7.40)‡
Drug related serious AEs	0/0 (0.00)	0/0 (0.00)

* Abdominal fullness, pain, cough and upper respiratory tract infection.
† Right knee osteoarthritis.
‡ Hyponatremia and C-spine whiplash injury with radiculopathy.

BoNT formulation is safe and effective in patients with OAB refractory to antimuscarinics.

The urothelium serves as a bladder permeability barrier to prevent urine and waste solutes from penetrating into the submucosal layer.[16] The urothelial barrier restricts the movement of drugs after intravesical administration and also restricts the action of the active drug fraction in the urine. Thus, many drugs fail to reach the desired therapeutic levels in bladder tissue.[17] The bladder urothelium also has a neuron-like activity. Bladder epithelium and sensory axons have an important role in the afferent transduction mechanisms modulating micturition, particularly in conditions of increased sensory nerve transmission following bladder inflammation, bladder hypersensitivity and detrusor overactivity.[18] The release of acetylcholine from the urothelium during the storage phase of micturition can activate muscarinic receptors in the urothelium and involve them in the pathophysiology of OAB. Activated muscarinic receptors trigger the release of urothelial adenosine triphosphate leading to activation of the afferent pathway.[19] Thus, blockade of urothelial muscarinic receptors could indirectly act to reduce afferent nerve activation and decrease OAB symptoms.

Instillation of this high molecular weight toxin is restricted from passing the urothelium barrier and is also degraded by urine. Pretreatment of the urothelium with protamine sulfate improved the permeability to BoNT in a rat model.[10] However, physical or chemical trauma to break the urothelial barrier in order to deliver BoNT would be associated with bladder injury, infection and risk of systemic spread of toxin. Liposomes are lipid vesicles composed of phospholipid bilayers surrounding an aqueous core.[20] They can incorporate various sizes of drug molecules, hydrophilic and hydrophobic, and even plasmids, and show greater uptake into cells through endocytosis.[21] Intravesical empty liposomes have therapeutic benefits in patients with interstitial cystitis/bladder pain syndrome which may be attributed to liposomes coating the bladder urothelium that may improve epithelial healing and barrier function.[22] Lipo-BoNT has been shown to split the SNAP-25 in the bladder urothelium.[14]

In the current study lipo-BoNT was effective in reducing OABSS, micturition frequency and urgency episodes but not urge incontinence at 1 month after treatment. Lipo-BoNT effects may involve effect of the BoNT on the urothelium and afferent nerves to reduce inflammatory signaling and pain processing without directly affecting the detrusor. After lipo-BoNT instillation there was no urinary retention or increase in PVR. In a recent single institution pilot study of lipo-BoNT,[23] the onaBoNTA receptor SV2 and SNAP-25 were found in the human bladder urothelium at baseline but did not show a decrease in SNAP-25 protein level 3 months after lipo-BoNT treatment. It is possible that the SNAP-25 proteins may have recovered by 3 months after treatment and future studies assessing for the cleaved SNAP-25 may be helpful.

The limitations of this study include the small number of subjects and limited duration of followup that was not optimized to obtain a significant change in micturition events beyond 4 weeks after treatment, given that subjects in both groups had the option of open label treatment at that point. This attrition is likely the source of the increase of the error bars in figures 2 and 3. In addition, it is possible that the study was not sufficiently large to measure change in incontinence, given that baseline incontinence was not required for subject entry. The proportion of subjects with baseline incontinence, being approximately 50%, may not have been high enough to adequately power statistical hypothesis testing on the effect of treatment on incontinence.

CONCLUSIONS

A single intravesical instillation of liposome-onabotulinumtoxinA decreased several OAB symptoms 4 weeks after treatment, and was well tolerated with a safety profile similar to that of placebo, with no treatment associated increase in PVR or urinary retention. Intravesical instillation of liposomal botulinum toxin may be a promising approach for the treatment of refractory OAB without the need for cystoscopic needle injection.

ACKNOWLEDGMENTS

Data were collected by the investigators at the study center, and were monitored and analyzed by Choice Pharma.

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