Shimizu S, Martin D, Trejo J, Wheeler M, Liu J, Saito M, Weiss R, Hittelman A. 1. Department of Pharmacology, Kochi Medical School, Kochi University, 2. Department of Urology, Yale University School of Medicine

THE INTRAVESICAL DELIVERY OF BOTULINUM TOXIN A/DIMETHYL SULFOXIDE IN A NORMAL RAT AND IN AN ACETIC ACID INDUCED BLADDER INFLAMMATORY MODEL

Hypothesis / aims of study
Intravesical therapy of drug is an effective alternative to systemic drug delivery. Injection of Botulinum Toxin A (BoNT) into bladder muscle has been used for treatment of refractory urination disorders such as overactive bladder, and bladder pain syndrome/interstitial cystitis (IC) [1]. The current approaches to BoNT delivery have some advantages like the uneven distribution and pain at injection sites [2]. Alternatively, less invasive approaches like intravesical instillations are being evaluated. However, BoNT which is a larger molecular weight protein, does not easily pass through the urothelium to reach the smooth muscle. DMSO is used as an intravesical therapy for the treatment of IC, and also has been shown to enhance bladder absorption of drugs [2]. In the present study, we investigated whether DMSO would enhance BoNT uptake across the urothelium, and reduce the inflammatory response in an AA model of inflammation through suppression of neurotransmitter release (i.e. calcitonin gene-related peptide (CGRP)).

Study design, materials and methods
Eight-week old female Sprague-Dawley rats were intravesically instilled (2 h) with 750 ul of the following treatments: Saline, 25% DMSO, 10U BoNT in saline, or 10U BoNT in 25% DMSO (BoNT+DMSO). One day prior to sacrifice, bladders were instilled with saline (control) or with 0.25% acetic acid (AA) for 1 h. Rats were sacrificed 7, 14, or 30 days following the initial intravesical instillation, and their bladder weight were measured. Synaptosomal associated protein of 25 kDa (SNAP-25) and CGRP expression were measured by western blot, and qRT-PCR, respectively and localized using immunohistochemistry. Histological evaluation was quantified using H&E staining. The bladder muscle contractility was measured using the organ bath method. Bladder maximum muscle contractility (Emax) was investigated in response to carbachol.

Results
Intravesical instillation of BoNT+DMSO, but not BoNT or DMSO alone, decreased SNAP-25 protein expression at 7 days (56.8±23.3%; P < 0.05) (Figure 1) and increased CGRP mRNA expression 4 fold, at 7 and 14 days post drug instillation, as compared to saline controls (P < 0.001). Intravesical BoNT+DMSO inhibited AA mediated inflammatory reaction in AA model at 7 days, but not at 30 days in the histological section (Table 1). Moreover, intravesical BoNT+DMSO decreased SNAP-25 immunoreactivity and increased CGRP immunoreactivity at 7 days in AA model, as compared to control AA treated rats. Contractility measurements of bladder muscle in response to carbachol (Emax) in the AA treated rats were significantly lower than in saline control bladders at 7 days. The Emax in rat bladders treated BoNT+DMSO followed by AA (BoNT+DMSO/AA) were increased compared to that of AA group. There was no difference between AA and BoNT+DMSO/AA groups in mean bladder weight at 7 days.

Interpretation of results
BoNT injection has been known to cleave the SNAP-25, and prevents the release of CGRP and other neurotransmitters at neuromuscular junctions [3]. In the present study, intravesical instillation of BoNT alone did not induce SNAP-25 cleavage. On the other hand, Intravesical instillation of BoNT+DMSO cleaved SNAP-25, inhibited CGRP release and suppressed AA mediated bladder inflammation and contractile impairment, 7 days after the initial instillation. The one of the possible mechanism is that intravesical instillation of BoNT+DMSO decreased the inflammatory response through the cleavage of SNAP-25, thus preventing release of CGRP, an immune modulator. These results suggest the benefit of DMSO as a delivery vehicle to enhance BoNT uptake.

Concluding message
These results indicate that DMSO as a delivery vehicle enhances bladder uptake of BoNT.
Intravesical instillation of BoNT+DMSO can suppress AA mediated inflammation and impairment of bladder contractility.
Figure 1. Effect of BoNT+DMSO pretreatment on normal rat SNAP-25 expression as assessed by western blot. (A) SNAP-25 expression normalized with actin and expressed as % control. (B) A representative western blot of SNAP-25 *Significantly different from control (P < 0.05).

Table 1. Histological evaluation, functional study and bladder weight at 7 days after the initial instillation.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BoNT+DMSO</th>
<th>AA</th>
<th>BoNT+DMSO/AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema</td>
<td>0.25 ± 0.16</td>
<td>0.43 ± 0.20</td>
<td>1.71 ± 0.29*</td>
<td>0.63 ± 0.18</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>0.29 ± 0.18</td>
<td>0.57 ± 0.20</td>
<td>1.86 ± 0.26*</td>
<td>0.75 ± 0.16</td>
</tr>
<tr>
<td>Emax (g/mm²)</td>
<td>2.05 ± 0.21</td>
<td>1.90 ± 0.25</td>
<td>0.74 ± 0.20*</td>
<td>1.41 ± 0.19</td>
</tr>
<tr>
<td>EC50 (×10⁻⁴M)</td>
<td>3.66 ± 1.15</td>
<td>3.44 ± 0.72</td>
<td>1.04 ± 0.22*</td>
<td>2.17 ± 0.42</td>
</tr>
<tr>
<td>Bladder weight (mg)</td>
<td>82.0 ± 5.6</td>
<td>102.6 ± 3.5*</td>
<td>115.1 ± 7.2*</td>
<td>114.0 ± 5.9*</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SEM of seven to eight separate determinations in each group. The inflammatory response of urinary bladder to AA was graded on a score of 0-3 as follows: 0, no evidence; 1, mild; 2, moderate; 3, severe. Histological scores were analyzed with the Mann-Whitney U test with Bonferroni’s correction for multiple comparison. Emax and EC50 values are for carbachol. Emax: maximal contraction in response to carbachol, EC50: concentration of carbachol to achieve half maximal contraction. ANOVA was used to establish the statistical significance of differences between more than two groups.

Control = saline control; BoNT+DMSO = saline pretreated with BoNT+DMSO group; AA = acetic acid pretreated with saline group; BoNT+DMSO/AA = acetic acid pretreated with BoNT+DMSO group.

* significantly different from control (P < 0.05)
* significantly different from other groups (P < 0.05)

References

Disclosures
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