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Minimal effective dose (MED) of onabotulinumtoxinA (onaBoNT-A) and abobotulinumtoxinA (aboBoNT-A) in a rat model of neurogenic detrusor overactivity (NDO)

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OBJECTIVES

- Spinal cord injury (SCI) induces plasticity within neural pathways innervating the lower urinary tract (LUT), with the recruitment of nociceptive "silent" C-fibers leading to the development of an abnormal automatic micturition reflex and emergence of neurogenic detrusor overactivity (NDO), thereby greatly compromising bladder filling during the micturition cycle.
- In SCI patients, intradetrusor injections of both onaBoNT-A (BOTOX®) and aboBoNT-A (DYSPORT®) have been evaluated for the treatment of refractory NDO and have been reported to decrease urinary incontinence frequency and maximum intravesical pressure while increasing bladder capacity and compliance. However, these two distinct commercialized products have different potency units and are therefore not interchangeable.

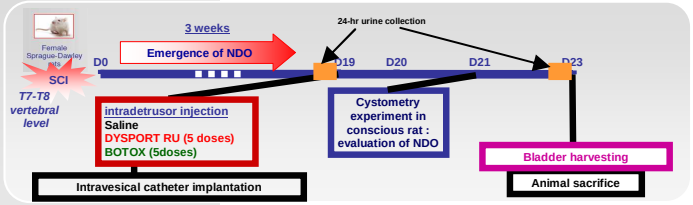
The main objective of this study was to determine the dose-response and the minimal effective dose (MED) of each product in the SCI rat, a relevant experimental model for human NDO after SCI.

MATERIALS & METHODS

Experimental design

A total of 98 female adult Sprague-Dawley rats (weighing 250-275 g; Elevage Janvier, France) were used. The protocol for this study complied with the animal protection legislation for animal studies in experimentation and all other applicable laws and regulations in force in France (authorization from competent French Ministry of Agriculture - Agreement No. A91-471-109, May 2009).

Eleven experimental groups were considered: vehicle (V, n=9), aboBoNTA 2U (n=11), 5U (n=9), 7.5U (n=8), 10U (n=9), 12.5U (n=9), onaBoNTA 0.8U (n=9), 2U (n=8), 5U (n=8), 7.5U (n=10), 10U (n=8). Each rat was weighed daily with a careful monitoring of health status and 24-hour urine collection was performed before intradetrusor injections and 4 days post-treatment.



✓ **Animal preparation**

Spinalization

After 1-week acclimation, rats were anesthetized with isoflurane (1.5-2.0%, Centravet, France). A dorsal midline incision was first made to expose dorsally between the 6th and 10th thoracic (T6, T10) vertebrae. The tissue and the muscle in front of T7-T8 were cleared away and a T7-T8 laminectomy was then performed. The dura and spinal cord were cut with fine scissors using a microscope to ensure that the transection was complete. A sterile gelform sponge (3 x 2 x 2 mm, Gelita-Spon®, Netherlands) was next placed between the cut ends of the spinal cord. The overlying muscle and skin were sutured.

Postoperatively, the animals were treated with antibiotics to prevent urinary tract infection and bladder was manually emptied by Credé maneuver until the abnormal micturition reflex was totally established.

Intradetrusor injection and catheterization

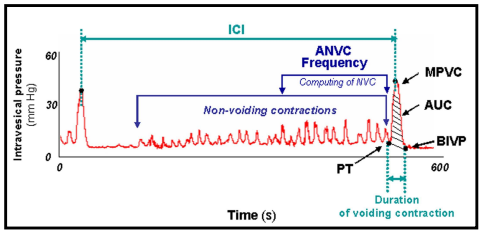
At 19 days post-spinalization, bladders were exposed under isoflurane anesthesia (1.5-2.0%) and emptied by catheterization through the urethra. Using a microscope, aboBoNTA, onaBoNTA or V (25µl) was injected into the detrusor in 8 divided sites sparing the trigone with a 30-gauge needle connected to a microdialysis pump at 1µl/min (CMA/102, Microdialysis AB, Sweden). Then, a PE-50 catheter was inserted within the bladder dome, tunnelled subcutaneously, exteriorized at the back of the neck and sutured between the scapulas. Postoperatively, rats were treated with gentamicine (Gentalline®, 10 mg/kg, Schering-Plough, US).

✓ **Cystometry experiments**

Forty-eight hours after intradetrusor injections, cystometry was performed in conscious rats. Bladder pressure was recorded using a pressure transducer (Elcomatic EM 750, UK) and direct measurements of micturition volumes were performed by means of a weighing device (Sartorius BP221S, France). After 30-minute acclimation, the bladder was continuously perfused (50 µl/min) with saline until 2-3 reproducible micturition cycles were obtained. Then, an evaluation period was recorded (60 min) in order to determine the effect of aboBoNTA, onaBoNTA or V on the micturition reflex.

✓ **Data and statistical analysis**

The following parameters were analyzed: maximal pressure (MP, mmHg) of voiding contraction (VC), duration of VC (s), baseline intravesical pressure (BP, mmHg), delta pressure threshold (ΔPT, mmHg, corresponding to the difference between the intravesical pressure at which voiding is initiated and BP), intercontraction interval (ICI, s), voided volume (VV, µl), amplitude of non-voiding contractions (NVC) (mmHg), frequency of NVC (number per min) and volume threshold to elicit NVC (% of total bladder filling volume). All data were expressed as mean ± SEM and averaged per treatment group. A Grubbs test was used for exclusion of outliers. A one-way ANOVA test followed by Dunnett's post-test was used for physiological parameters (GraphPad Prism® 5.02). An ANOVA Step Down Trend Test (ASDTT) was performed for each cystometry parameter (SAS®), thus taking into account the increase in dose range to determine the MED for a single parameter. P values < 0.05 were considered significant.



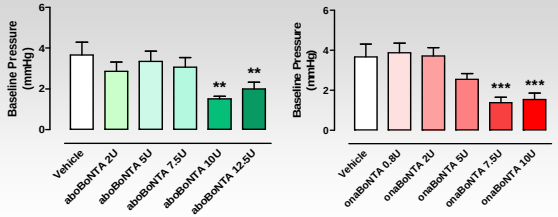
Representative cystometrogram in a conscious rat illustrating the parameters analyzed from such experiment.

✓ **Drugs and chemicals**

AboBoNTA (DYSPORT; Ipsen, Ltd., Slough, UK), onaBoNTA (Allergan, Inc., CA, USA) and V were provided by Ipsen. Antibiotics (except Gentalline®) and anaesthetics were purchased from Centravet (France) and Roche (France). All other drugs and chemicals were purchased from Sigma (France).

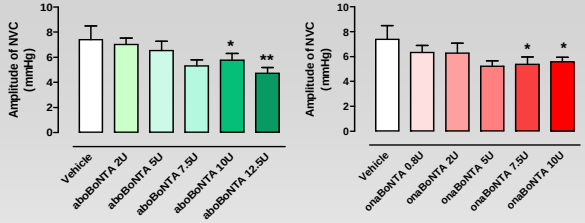
RESULTS

Effect on baseline pressure



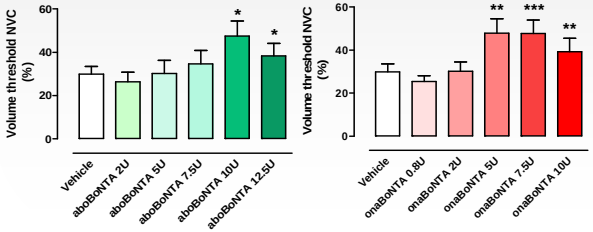
p<0.01**, p<0.001***, ANOVA Step Down Trend Test versus Vehicle.

Effect on amplitude of NVC



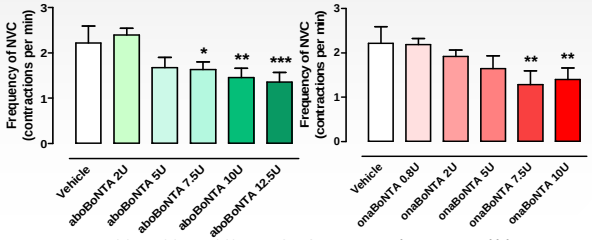
p< 0.05*, p<0.01**, ANOVA Step Down Trend Test versus Vehicle.

Effect on volume threshold to elicit NVC



p< 0.05*, p<0.01**, p<0.001***, ANOVA Step Down Trend Test versus Vehicle.

Effect on frequency of NVC



p< 0.05*, p<0.01**, p<0.001***, ANOVA Step Down Trend Test versus Vehicle.

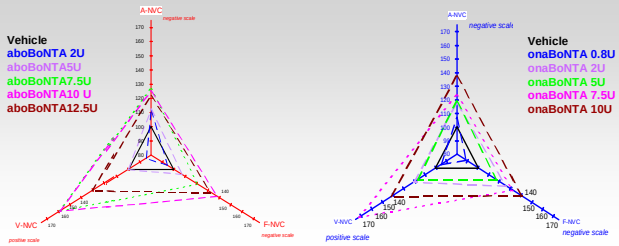
All the other urodynamic parameters evaluated were not modified whatever the treatment and the dose

Effect on physiological parameters

- Before intradetrusor injections, body weight and diuresis of SCI rats were similar among rats.
- Four days post-intradetrusor injections, body weight was significantly reduced in aboBoNTA 7.5U, 10U and 12.5U groups (258.7±4.3g, 263.1±4.7g and 244.5±4.0g respectively) and in onaBoNTA 7.5U and 10U groups (244.0±7.2g & 244.9±5.8g respectively), compared to V-treated rats (278.2±3.7g).
- Four days post-intradetrusor injections, aboBoNTA 12.5U (8.8±0.9ml/24h), onaBoNTA 7.5U (8.5±1.2ml/24h) and 10U (8.3±1.1ml/24h) significantly decreased diuresis versus V-treated rats (12.8±0.7ml/24h).
- Neither treatment had any effect on bladder wet weight normalized to absolute body weight.

CONCLUSIONS

- The MED which decreased significantly intravesical BP was 10U for aboBoNT-A and 7.5U for onaBoNT-A (*vs* Vehicle). Moreover, both aboBoNT-A 10U and onaBONT-A 7.5U, modulated significantly all NVC parameters *vs* Vehicle by decreasing i) amplitude and ii) frequency of NVC, and iii) by increasing the volume threshold to elicit NVC.
- This is the first preclinical dose-ranging study with aboBoNTA and onaBoNTA in standardized conditions showing similar inhibiting effects on NDO, albeit at different MED.



RADAR representations of aboBoNT-A and onaBoNTA on NVC parameters (expressed as % of vehicle)

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