523

Elkelini M[1], Pravdivyi I[1], Ghazwani Y[1], Fehlings M[1], Hassouna M[1]
1. *Toronto Western Research Institute, University of Toronto*

INTRAVESICAL INSTILLATION OF BOTULINUM TOXIN A (BTX-A) INHIBITED THORACIC AND LUMBAR C-FIBRE ACTIVITY IN HIGH THORACIC SPINAL CORD INJURY RAT MODEL.

Hypothesis / aims of study

Bladder dysfunction and autonomic dysreflexia (AD) are a common outcome following cervical and high thoracic spinal cord injury (SCI). Bladder dysfunction after SCI is characterized by a brief spinal shock phase, followed by return of bladder activity after 2-12 weeks, which includes detrusor hyperreflexia (DH) and detrusor-sphincter-dyssynergia, leading to a high bladder pressure and impaired renal integrity. Bladder distention may cause initiation of AD, which involves life-threatening episodes of paroxysmal hypertension and bradycardia. AD and DH after SCI develop in a time-dependant manner, suggesting that neuroplasticity contributes significantly to both conditions. Following SCI, the bladder afferent pathways are reformed inducing C-fibre afferents containing the neuropeptide calcitonin-gene-related-peptide (CGRP) [1]. CGRP primary afferents have been used as a marker for sprouting after SCI and also linked to the development of AD and DH.
BTX-A has been successfully used recently in SCI patients because it reduces the detrusor contractility via inhibiting acetylcholine release from efferent nerve endings. More recently however, there is increasing evidence that BTX-A may also affect sensory nerve fibers and afferent signaling mechanisms [2]. It is still however unknown, if BTX-A may affect primary C-afferent fibres sprouting and putative spinal neurons involved in DH and AD following SCI. In this study, we examined the changes in CGRP concentration in T4, L5 and L6 dorsal root ganglia in spinalized rats and in intravesically BTX-A treated spinalized rats.

Study design, materials and methods

In the present study, female Sprague Dawley rats were stratified into three groups: normal controls (C); spinally transected at T4 (S); and spinally transected with intravesically BTX-A treatment at 48 hours before sacrifice (B). Under general anesthesia with a combination of xylazine (5mg/kg) and ketamine (50 mg/kg), a midline incision was made through the skin overlying the spinal column. After laminectomy, complete spinal cord transection was performed at T4 vertebra level and the incision was closed in layers. The body temperature was monitored and controlled during the operation using a heating pad. Supplementary subcutaneous lactated ringer solution (20 ml/kg) was administered during the procedure and in the first postoperative day. Perioperative antibiotics in the form of Bactrim (2.2 mg/kg) were injected subcutaneously. The rats were kept in low height cages for easy access to food and water in a 29^{o}C warm room. The rats' bladders were evacuated by manual expression in the first 2 weeks following the spinal cord transection 3 times a day. In BTX-A treated group, 48 hours before animal sacrifice, PE-50 tubing (Clay-Adams, Parsippany, New Jersey) was inserted under anesthesia into the bladder through the urethra. The bladder was emptied of urine and slowly filled with BTX-A (1 ml, 20 U/ml in saline) (Allergan, Irvine, California). 3 weeks after spinal transection, CGRP was extracted from the dorsal root ganglia (DRG) of the T4, L5 and L6 roots of all study groups and quantified by radioimmunoassay. Univariate ANOVA was used to analyse the data and statistical significance was set at $P < 0.05$.

Results

Spinal cord transection significantly increased T4 CGRP concentration (59.00± 5.29 fmol/ml) than the control group (31.0± 2.64 fmol/ml; $P < 0.001$); BTX-A significantly reduced CGRP concentration in T4 (21.0 ± 1.0 fmol/ml; $P < 0.001$). Furthermore, BTX-A significantly reduced CGRP concentration in L5 (24.33 ± 10.78 fmol/ml) than the spinally transacted group (50.0 ± 7.9 fmol/ml; $P = 0.03$) (Table 1), Figure1A, 1B.

Table 1: CGRP concentration fmol/ml (mean ± SD)

Group	T4	L5	L6
Control	31.0±2.6	48.6±6.1	48.0±8.5
Spinal transection	59.0±5.2	50.0±7.9	49.0±2.6
SCI + BTX-A	21.0±1.0	24.3±10.7	41.3±8.3

Interpretation of results

In recent studies, BTX-A has been shown to affect sensory fibres and signalling mechanisms. In particular, one study showed that BTX-A significantly reduced pain and inhibited CGRP release in a rat model [3]. In this study, CGRP, a neuropeptide known to reflect C-fibres activity, was significantly increased in T4 DRG following spinal cord injury which supports current evidence of the involvement of C-fibres in AD pathogenesis. Intravesical instillation of BTX-A significantly reduced CGRP concentration in T4 DRG which supports a modulatory effect of BTX-A on thoracic C-fibre activity. This may also suggest that intravesical BTX-A could play a role in AD control following SCI. Furthermore, the significant reduction in CGRP concentration in L5 by BTX-A reflects another modulatory effect on the C-fibre activity of the urinary bladder following SCI.

Concluding message

Intravesical BTX-A administration seems to inhibit C-fibre activity at the thoracic and lumber levels in rats following spinal cord injury. This provides the first evidence that intravesical BTX-A could affect AD in a preclinical SCI animal model.

Figure 1.A: T4 CGRP concentration (fmol/ml)

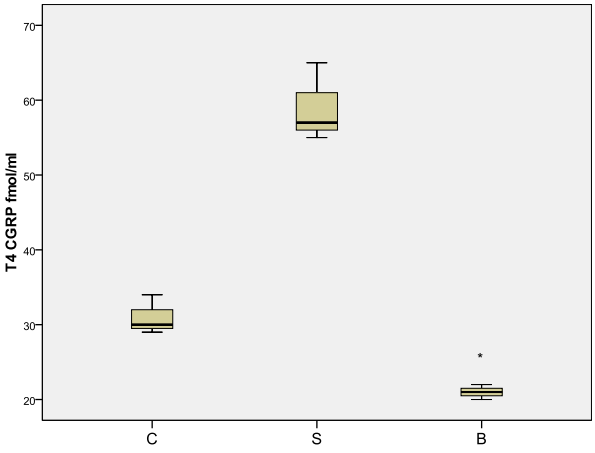
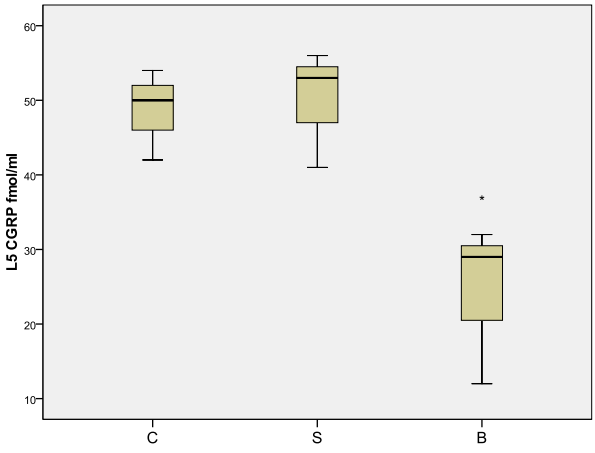


Figure 1.B: L5 CGRP concentration



References

1. Yoshimora N. Bladder afferent pathway and spinal cord injury: possible mechanisms including hyperreflexia of the urinary bladder. Prog Neurobiolo 1999; 57: 583-606.
2. Chancellor MB, Fowler CJ, Apostolidis A et al. Drug Insight: biological effects of botulinum toxin A in the lower urinary tract. Nat Clin Pract Urol. 2008 Jun;5(6):319-28.
3. Chuang YC, Yoshimura N, Huang CC et al. Intravesical botulinum toxin a administration produces analgesia against acetic acid induced bladder pain responses in rats. J Urol. 2004 Oct;172(4 Pt 1):1529-32.

Specify source of funding or grant	**Canadian Institute of Health Research (CIHR): Postodoctoral Fellowship to Mohamed Elkelini.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University Health Network Animal Care Committee in accordance with the policies established in the Guide to the Care and Use of Experimental Animals prepared by the Canadian Council on Animal Care.**