# 371 - Botulinum toxin A in the treatment of visceral dysfunction after spinal cord injury: mechanisms beyond the cleavage of SNAP-25

R. Oliveira[1,2,3], S. Chambel[1,2,3], H. Cavaleiro[1,2,3], F. Cruz[2,3,4], C.D. Cruz[1,2,3]
[1] Dept. of Biomedicine, Faculty of Medicine of the University of Porto, Porto, Portugal
[2] Translational NeuroUrology Group, IBMC - Instituto de Biologia Molecular e Celular, Porto, Portugal
[3] i3S - Instituto de Investigação e Inovação em Saúde, Porto, Portugal
[4] Dept. of Urology, Hospital São João, Porto, Portugal

## STATE OF THE ART

Bladder wall injections of botulinum toxin A (BoNT/A) is the gold-standard treatment for neurogenic detrusor overactivity (NDO). BoNT/A acts on motor, autonomic and sensory bladder fibres, cleaving SNAP-25 and impairing vesicle-mediated neurotransmission. Urodynamic improvements are long-lasting but fade over time, requiring re-injection. Understading the fine mechanisms of action of BoNT/A opperating at the intracellular level could help improve treatment and potentiate the duration of the effects.

## AIM

To investigate intracellular events occurring in bladder afferents after BoNT/A injections in NDO rats. We hypothesize that the durability of urodynamic improvements might reflect toxin-induced neuronal injury and cellular stress.

## EXPERIMENTAL DESIGN

Female rats were submitted to T8/T9 largely incomplete spinal cord transection (SCT) or sham surgery. Awake cystometries were performed at baseline, 1 week and 4 weeks after SCT to evaluate the development of urinary dysfunction. At 4 weeks post-SCT, when NDO is typically present, rats received 10 bladder-wall injections of BoNT/A 10U diluted in 50uL of saline. Control rats received only saline.

Three days after bladder injections, awake cystometries were performed and rats were euthanized, followed by bladder and DRG L5-S1 collection. Bladders were processed for immunohistochemistry to evaluate the catalytic activity of BoNT/A and DRG were processed for western blotting (n=4/group) to measure levels of cell stress markers and primary cell culture (n=3/group) to evaluate neuronal growth and dendritic branching.

## RESULTS

### I –Effects of bladder-wall injections of Botulinum toxin A in urodynamics and bladder tissue

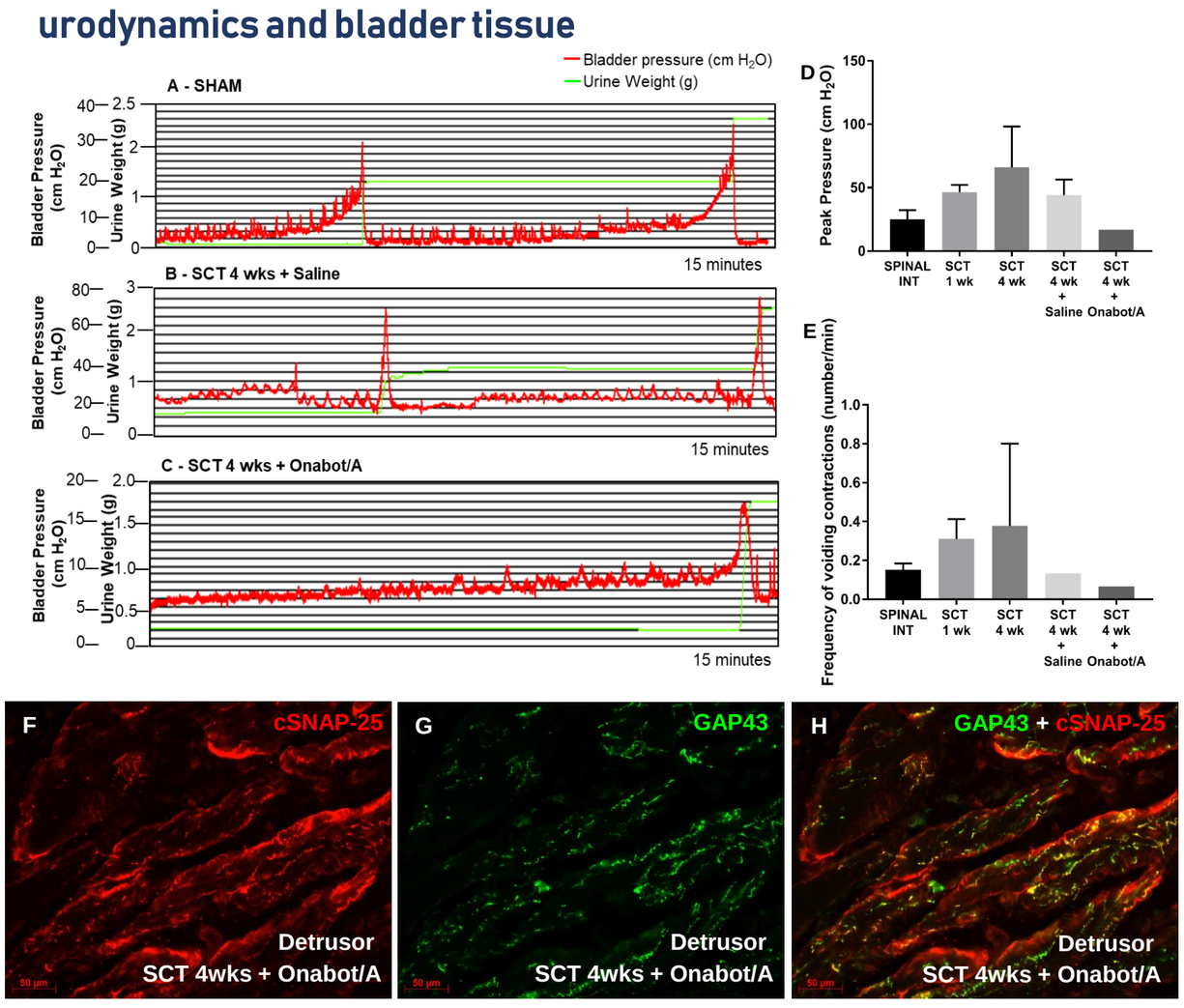


**Figure 1:. Effects of bladder-wall injections of OnabotulinumtoxinA in urodynamics and bladder tissue.** Representative cystometric recordings, obtained in awake animals, show normal bladder reflex activity in SHAM-operated rats (**A**), which completely changes at 4 weeks post-SCI (**B**), with a strong increase in bladder pressures. Treatment with Onabot/A seems to improve urological function in 4 weeks SCT rats (**C**) by reducing peak pressure (**D**) and frequency (**E**) of bladder contractions. Pictures depicting the immunohistochemistry reaction against the end-product of Onabot/A effect, cSNAP-25 (red; **F**,**H**), and the neuronal growth marker GAP43 (green; **G**,**H**) suggest some degree of colocalization (yellow; **H**) indicating that some but not all GAP43 expressing fibres were affected by the treatment. Error bars show standard deviation.

### II –Effects of bladder-wall injections of Botulinum toxin A in lumbosacral dorsal root ganlgia

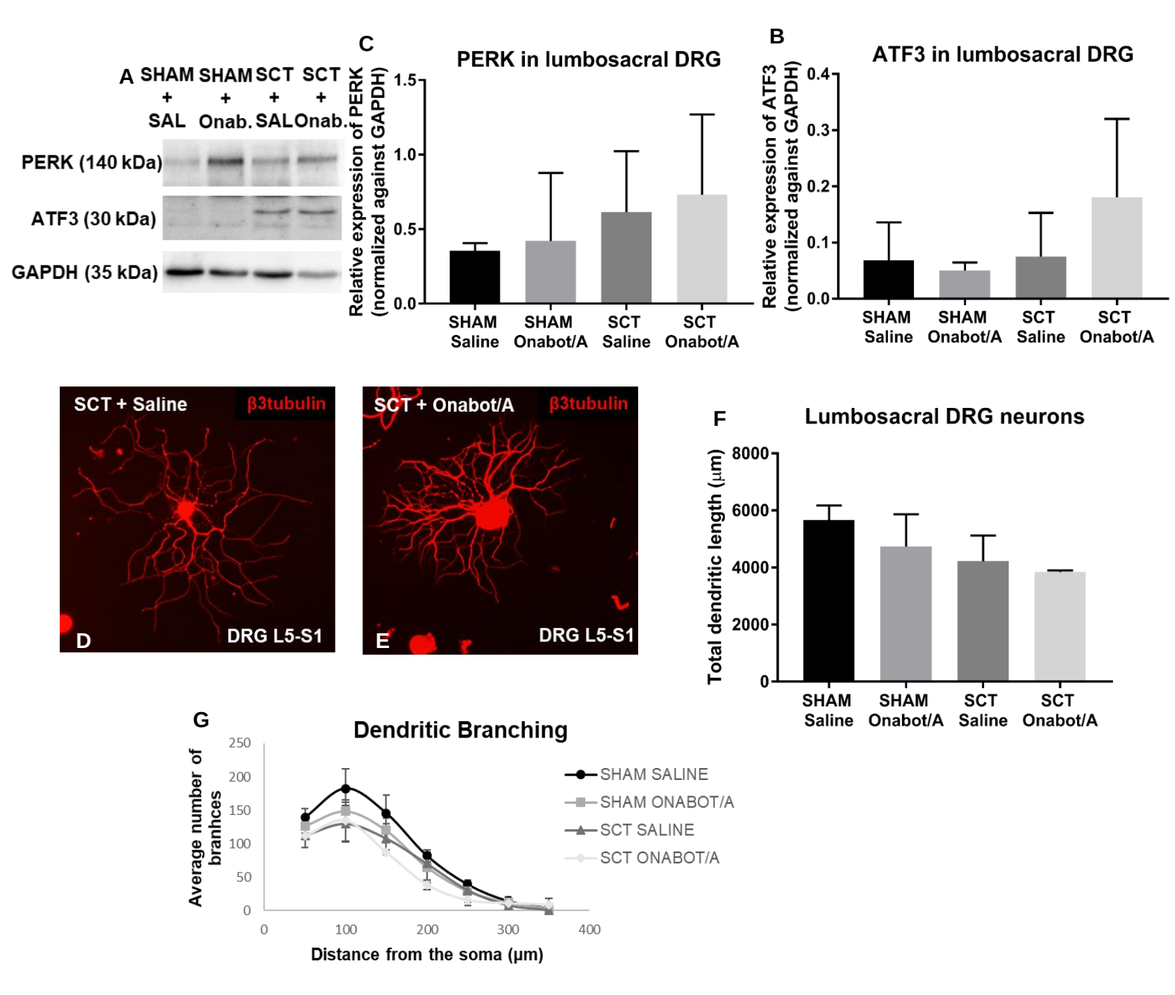


**Figure 2**: **Effects of OnabotulinumtoxinA bladder treatment in lumbosacral DRG**. Representative Western immunoblotting bands (**A**) corresponding to the expression of the RE stress marker PERK (upper lane), the neuronal stress marker ATF3 (middle lane) and the loading control protein GAPDH (lower lane) of all experimental groups. Averaged relative expression of PERK (**B**) and ATF3 (**C**) in the bladder of SHAM-operated and 4 weeks SCT rats treated with Saline and Onabot/A. Preliminary results suggest an increase in cellular stress after treatment of 4 weeks SCT rats with the toxin. Cultured neurons from SCT rats treated with Saline (**D**) or Onabot/A (**E**) were able to expand their branches in vitro. However, treatment with Onabot/A seems to result in the decrease of total length of the neuronal dendrites (**F**) but not in the number of neurites (**G**).

## CONCLUSIONS

A better understanding of the fine molecular mechanisms of action of BoNT/A is crucial to explain the long duration of effects of treatment. Identification of cellular events induced by BoNT/A could contribute to modification of the toxin to improve its efficacy and expand and potentiate its therapeutic use in neuro-urology.

## FUTURE WORK

- Increase the number of animals in protocols of awake cystometries to validate our preliminary findings;
- Target bladder afferents with neuro-tracers and focus the analysis of toxin effects exclusively on those neurons;
- Evaluate additional neuronal stress mechanisms that might be induced by the treatment;
- Analyse effects of the toxin treatment on major pelvic ganglia.

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