

AMELIORATION OF DETRUSOR-SPHINCTER DYSSYNERGIA FOLLOWING SPINAL CORD INJURY WITH LM11A-31, A SMALL MOLECULE P75 NEUROTROPHIN RECEPTOR ANTAGONIST

Hypothesis / aims of study

One of the most debilitating long term consequences of spinal cord injury (SCI) is the inability to empty the bladder due to simultaneous contraction of the bladder and external urethral sphincter (EUS) referred to as detrusor-sphincter-dyssynergia (DSD) for which there is currently no effective treatment. This can lead to complications including urolithiasis, vesicoureteral reflux, hydronephrosis, obstructive uropathy and renal failure. The p75 neurotrophin receptor (p75^{NTR}) binds uncleaved pro-nerve growth factor (proNGF) and pro-brain derived neurotrophic factor (pro-BDNF) triggering apoptosis, and later the mature active proteins promote cell growth. It is hypothesized that SCI rapidly increases p75^{NTR} expression and pro-neurotrophin levels in the bladder, as has been demonstrated in the spinal cord [1]. Activation of p75^{NTR} leads to neuronal and urothelial apoptosis that is believed to contribute to DSD. Accordingly, our goal was to determine the benefits of the small molecule selective p75^{NTR} antagonist, LM11A-31, in ameliorating DSD.

Study design, materials and methods

Female four to six week old C57Bl/6 mice had their spinal cords transected between the T8-T9 vertebrae. Following surgery, they were treated with an analgesic and prophylactic antibiotic and their bladders manually expressed twice a day. Expressed urine volumes were measured to estimate changes in bladder capacity. Seven days following SCI and daily thereafter, animals were gavaged with LM11A-31 (100 mg/kg in 100 μ l of water). Fourteen days after transection, (seven days post treatment initiation), animals were decerebrated for anesthetic-free cystometrograms (CMG) and EUS electromyogram (EMG) recordings, after which their bladders were isolated for histology using hematoxylin and eosin (H&E) staining. All experiments were carried out using $n \geq 4$ mice.

Results

Control mouse CMG and EUS-EMG recordings demonstrate a guarding reflex that prevents leaking as bladder pressure approaches voiding threshold. This is followed by bursting with decreased tonic activity during which voiding occurs and bladder pressure returns to baseline (figure 1A). Two weeks post SCI, there is increased EUS tonic activity as the bladder contracts (DSD) resulting in non-voiding contractions (indicative of detrusor overactivity) and eventually overflow incontinence (figure 1B). In untreated SCI animals, bladder weights and urine volumes were dramatically increased: 78 ± 5 mg *versus* 24 ± 2 mg; and up to 500 μ l of urine *versus* 90 ± 9 μ l in non-transected controls, respectively. Treatment with LM11A-31 decreases bladder weights (to 41 ± 6 mg, $p < 0.05$) and urine volumes (to less than 100 μ l) which contributed to much improved voiding function (figure 1C). Importantly, our results demonstrate that daily LM11A-31 treatment, starting seven days post injury, alleviated DSD and non-voiding contractions permitting a return towards normal voiding function (figure 1C). Histological analysis of bladders from untreated SCI mice showed urothelial hyperplasia and detrusor hypertrophy (figure 2B) as a consequence of DSD and bladder overdistention. In animals treated with LM11A-31, the urothelial layer was preserved and the detrusor was not hypertrophied (figure 2C) as in controls (figure 2A).

Interpretation of results

Our results demonstrate that selective inhibition of p75^{NTR} and pro-neurotrophins interactions with small molecule LM11A-31 is remarkably effective in maintaining the structural integrity of the bladder wall and treating DSD permitting voiding. The mechanisms may include apoptosis inhibition of both neurons in spinal cord (preventing neuronal degeneration centrally) and urothelium in the bladder (reducing inflammation peripherally).

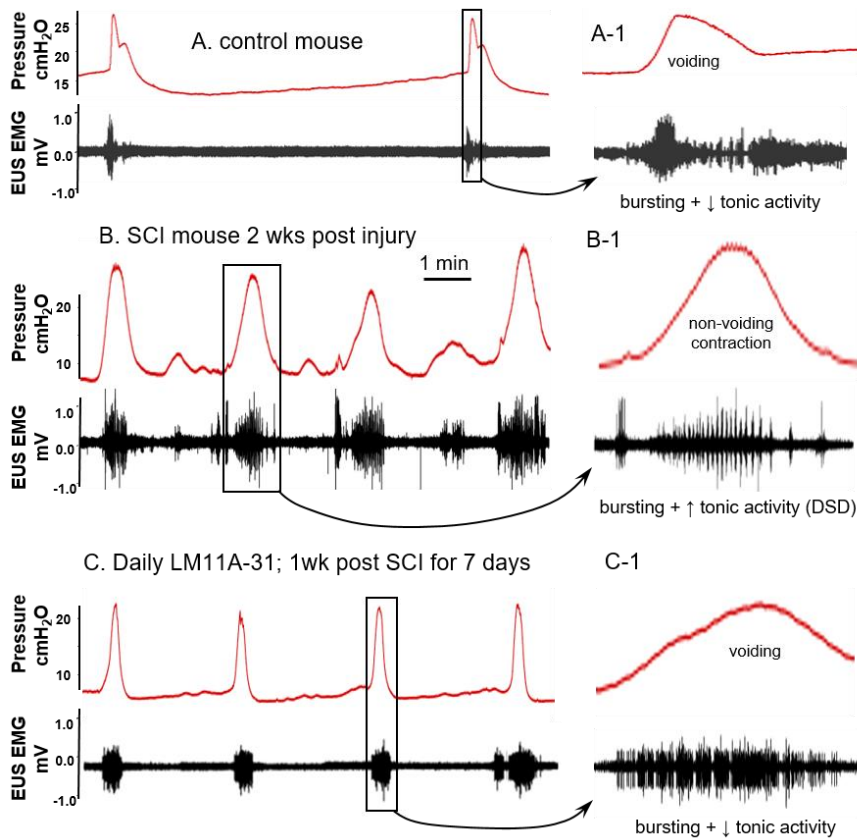


Figure 1. CMG and EUS-EMG recordings from T8-T9 SCI mice with and without LM11A-31 therapy. CMG (red traces) over EUS-EMG (black traces) recordings are shown from a spinal cord intact mouse (A), SCI mouse 2 weeks post injury (B), and SCI mouse with daily LM11A-31 treatment starting 1 week after injury and continued for seven days (C). A-1 shows bursting activity with decreased tonic activity during which voiding occurs and pressure returns to baseline. Following SCI and the development of DSD (B-1), when the bladder contracts, sphincter tonic activity increases, resulting in non-voiding contractions and eventually overflow incontinence. Gavage with LM11A-31 (100 mg/kg/day) caused a reduction in EUS tonic activity during bladder contractions facilitating urine outflow.

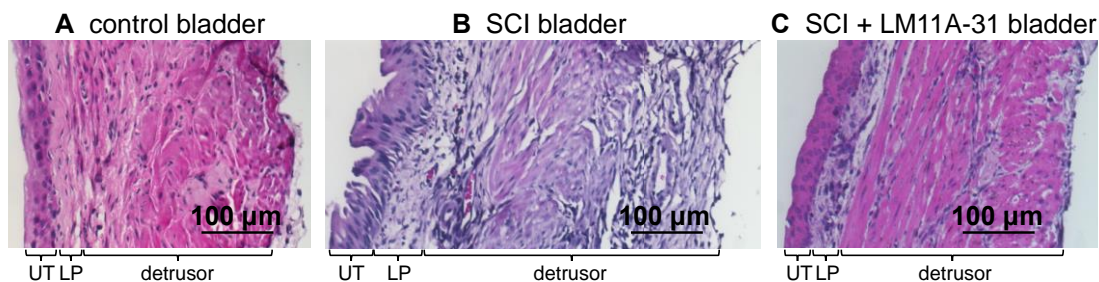


Figure 2. Histological sections of the bladder wall with and without LM11A-31 therapy. A: Control mouse bladder with an intact urothelium (UT) and characteristic lamina propria (LP) and detrusor architecture. B: Bladder from an untreated SCI mouse, 14 days post injury, showing hyperplasia of the UT, disruption of the LP and detrusor hypertrophy. C: Bladder from a SCI mouse 14 days post injury with seven days of LM11A-31 therapy showing an intact UT, LP and non-hypertrophied detrusor, similar to controls.

Concluding message

LM11A-31 has been demonstrated to improve motor coordination in mice with spinal cord contusion [2] and is safe and translational as evidenced by the drug entering phase II clinical trials to treat Alzheimer's disease. Our results clearly demonstrate that it is therapeutic and reverses DSD to treat lower urinary tract dysfunction due to SCI.

References

1. Beattie, MS et al. *Neuron*, 36:375, 2002
2. Tep, C et al. *Journal of Neuroscience*, 33:397, 2013

Disclosures

Funding: NIH P01 DK093424 NIH R01 DK071085 **Clinical Trial:** No **Subjects:** ANIMAL **Species:** mouse **Ethics Committee:** University of Pittsburgh Institutional Animal Care and Use Committee