Abstract # 100

Rho-kinase inhibition impacts neurogenic detrusor overactivity in chronic spinalized rats

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OBJECTIVES

- Spinal cord injury (SCI) severely disrupts normal bladder function by inducing neurogenic detrusor overactivity (NDO). First line SCI-induced NDO treatments i.e. antimuscarinics often associated with intermittent catheterization are somewhat limited by a mild to moderate clinical efficacy and a significant incidence of side effects. Thus the development of new effective drugs for the treatment of NDO is of crucial importance.
- Rho-kinase has a central role in the regulation of detrusor smooth muscle contraction since components of the rhoA/rho-kinase signalling pathway are involved in the Ca^{2+}-sensitization of the smooth muscle [1]. Moreover, in vitro and in vivo data from animal models of overactive bladder (OAB) indicate that rho-kinases are involved in pathophysiological mechanisms responsible for OAB [2,3].

Thus, we aimed to evaluate the effects of a rho-kinase inhibitor (Y-27632) on urodynamic parameters in rats with chronic SCI.

1. Teixeira et al, Biochem Pharmacol. 2007; 2. Rajasekaran et al, Neurourol Urodyn 2005; 3. Kim et al, American Urological Association Atlanta, USA. 2006.

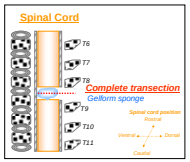
MATERIALS & METHODS

Animals

A total of 17 female adult Sprague-Dawley rats weighing 250-275 g were used in this study.

Complete spinal cord transection

Rats were anesthetized using isoflurane (2.0-2.5 %). A dorsal midline incision was first made to expose dorsally between the 6th and 10th thoracic (T6, T10) vertebrae processes. Tissue and the muscle in front of T7-T8 were then cleared away. To visualize the whole width of the spinal cord, a T7-T8 laminectomy was then performed. Complete spinal cord transection was performed using fine dissecting scissors. A sterile gelform sponge (Gelita® Medical) was next placed between the cut ends of the spinal cord. The overlying muscle and skin were sutured.



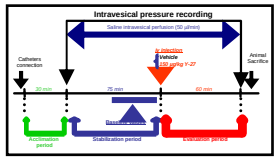
In order to prevent urinary tract infection (UTI), the animals were treated with antibiotics. Postoperatively, the animals were given a single subcutaneous injection of cefovecin (20 mg/kg). From 1 day post-SCI, enrofloxacin (20 mg/kg/day) and sulfamethoxazole/trimethoprim (50 and 10 mg/kg/day respectively) in drinking water were then alternately delivered every week. Urinary bladders were manually expressed by Credé maneuver first 3 times daily then 2 times daily until an abnormal micturition reflex was totally established.

Catheter implantation

At 3-4 weeks post-SCI, the rats are anesthetized with isoflurane (1.5-2.0%). For intravenous (iv) administration, a polyethylene catheter (PE-10) was placed into the jugular vein. The bladder dome was then exposed via a midline abdominal incision. A polyethylene catheter (PE-50) was then inserted within the bladder through the apex of bladder dome and secured in place. The free ends of the bladder and venous catheters were tunnelled subcutaneously, exteriorized at the back of the neck and sutured between the scapula. Postoperatively, the animals were treated with netilmicin (20 mg/kg, intramuscular injection, a single injection) to prevent UTI.

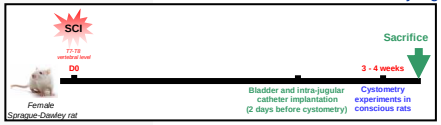
Cystometric investigation

Cystometry experiment on conscious rats was performed in metabolic cage at 48 hours after catheter implantation. The free tip of the bladder catheter was connected to a pressure transducer (Elcomatic EM 750) for bladder pressure recording and a syringe-pump KDS-200 (Phymep) allowing continuous bladder perfusion (50 µl/min) with room temperature sterile saline. In addition, voided volume was continuously collected and directly measured by means of a weighing device (Sartorius BP2215). Three reproducible micturition cycles were recorded before any drug administration used as baseline values. Then, the effects of Y-27632 (150 µg/kg, intravenous injection, iv, n=7) and vehicle (saline, iv, n=10) were evaluated during a treatment period of 60 minutes.



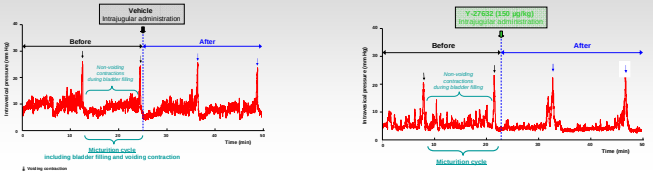
Data Analysis

Urodynamic parameters were analysed: maximal amplitude of micturition pressure (MP); baseline intravesical pressure (BP); delta pressure threshold for inducing micturition (delta PT); intercontraction interval, (ICI); voided volume; amplitude of non-voiding contractions (NVC), frequency of NVC and volume threshold necessary to elicit NVC. Urodynamic parameters were expressed in percentage of baseline value. All the data were expressed as mean±SEM for N experiments corresponding to N animals. Statistical comparisons of urodynamic parameters were performed with a two-way analysis of variance (anova) statistic analysis test followed by Bonferroni post-test with GraphPad Prism®4.03 software. $P < 0.05$ was considered statistically significant.

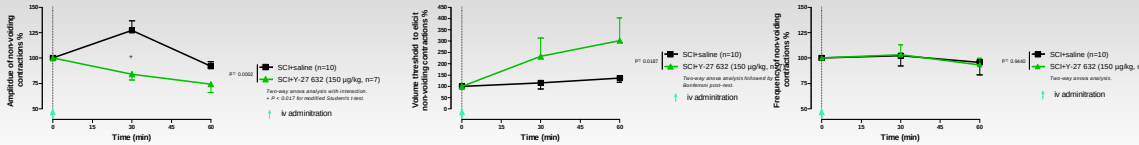


RESULTS

Effect of Y-27632 or vehicle on the micturition reflex in conscious SCI rats

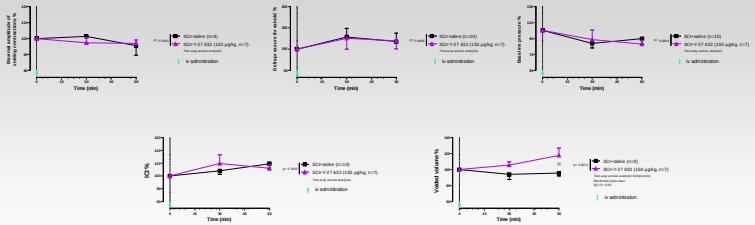


Effect of Y-27632 on urodynamic parameters related to non-voiding contractions in conscious SCI rats



Y-27632 (150 µg/kg) decreased the amplitude of non-voiding contractions and increase the volume threshold necessary to elicit non-voiding contractions. It did not modify the frequency of non-voiding contractions.

Effect of Y-27632 on urodynamic parameters in conscious SCI rats



Y-27632 (150 µg/kg) did not modify maximal pressure, baseline pressure, pressure threshold to elicit voiding contractions and the intercontraction interval between two micturitions. In contrast, it increased the voided volume.

CONCLUSIONS

- The present study demonstrates that inhibition of rho-kinase alters the urodynamic parameters related to non-voiding contractions and enables a better bladder emptying in the rat model of SCI-induced neurogenic detrusor overactivity
- Rho-kinases may be involved in the regulation of bladder afferent nerve activity since the initiation of non-voiding contractions is closely linked to bladder afferent nerve activity
- Rho-kinase inhibitors thus appear as a putative but very attractive therapeutic possibility for the treatment of OAB