

Effects of antibiotic therapy on the appearance of the abnormal micturition reflex after spinal cord injury in rats

Broquères-You D¹, Behr-Roussel D¹, Oger S¹, Denys P², Alexandre L¹, Giuliano F²

¹ Pelvipharm, Orsay Parc Bat Cèdre, 86 rue de Paris, 91400 Orsay, France – www.pelvipharm.com

² AP-HP Raymond Poincaré hospital, Department of Neurological rehabilitation, Garches, France - giuliano@cyber-sante.org

OBJECTIVES

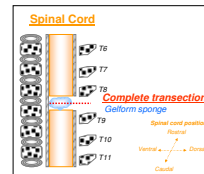
After spinal cord injury (SCI), the initial spinal shock phase where the bladder is areflexic is followed by the progressive emergence of an abnormal micturition reflex, characterized by neurogenic detrusor overactivity (NDO) associated to detrusor-sphincter-dyssynergia (DSD). Furthermore, SCI induces a significant bladder hypertrophy¹⁻⁴. This leads not only to urinary incontinence and inefficient bladder emptying but also to severe complications such as urinary tract infection (UTI). In SCI patients, the efficacy of antimicrobial use to prevent UTI has been well evidenced⁵⁻⁶, but the effects of antibiotic therapy on the recovery of the abnormal micturition reflex are unknown.

The aim of this study was to evaluate the effects of an antibiotic therapy on the time-course of emergence of abnormal micturition reflex and on bladder hypertrophy after SCI in rats.

MATERIALS & METHODS

Spinal cord transection

Female adult Sprague-Dawley rats (10 weeks-old, weighing 250-275 g, Elevage Janvier, France) were anesthetized with isoflurane. A complete spinal transection was then performed between 7th and 8th thoracic (T7-T8) vertebral levels. A sterile gelform sponge (Gelita-Spon®, Netherlands) was next placed between the cut ends of the spinal cord. The overlying muscle and skin were sutured. Rats were observed during 17 days after spinalization.



Antibiotic therapy after spinalization

Postoperatively, the animals were treated with antibiotics: cefovecin (Convenia®, 20 mg/kg, subcutaneous injection, a single injection at D0 with an extended-spectrum long-acting duration (10-14 days), Pfizer Animal Health, UK) and/or enrofloxacin (Baytril®, 20 mg/kg/day, subcutaneous injection, once a day, Bayer HealthCare AG, Germany). Rats were divided into three groups:

- (1) rats treated with cefovecin only (**Cefo**, n=27);
- (2) rats treated with cefovecin and manipulated with particular reinforced aseptic care (**Cefo+Care**, n=6, **particular reinforced aseptic care**: sterilization of the gloves for manipulating rats with Phagobiol®, Phagogene - Dec., France);
- (3) rats treated with cefovecin, manipulated with aseptic care, and receiving enrofloxacin from the day of appearance of nitrites in urine (between 4-10 days post-SCI) to complete recovery of micturition reflex (**Cefo+Care+Enro**, n=10).

Postoperative care of the animals and urinary collection

After complete spinal transection during the spinal shock phase, the bladder was manually emptied by Credé maneuver until the abnormal micturition reflex was totally established and the collected volume of urine was measured.

Urinary tract infection and bladder weight

At the end of study, animals were anesthetized with urethane. Urinary tract infection was detected macroscopically by the presence of white spots in the kidneys and/or in the ureters. Each bladder was collected and weighed to evaluate of chronic effects of antibiotic therapy on SCI induced-bladder hypertrophy.

Data Analysis

Statistical analysis was performed using a two-way ANOVA statistical analysis followed by Bonferroni post test to compare all groups for expressed urinary volume. The comparison of all other parameters was performed using one-way ANOVA tests followed by Newman-keuls complementary analysis. P values < 0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism® 4.03 software.

CONCLUSIONS

These results indicate that antibiotics may modulate or even restrain the appearance/occurrence of an abnormal micturition reflex and decrease bladder hypertrophy after SCI. Thus, the treatment of urinary infection with an adapted antibiotic therapy could alter the natural history of neuroplasticity, thereby modulating reflex incontinence and possibly, reducing bladder hyperactivity.

RESULTS

Effects of antibiotic therapy on mortality and urinary tract infection in SCI rats

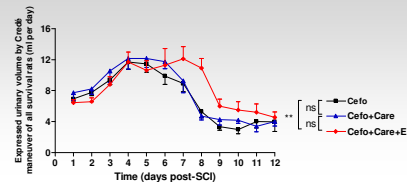
| Group | Treatment | Nb of rats undergoing SCI | Pre-mature death | | Urinary tract infection | |
|-------|----------------|---------------------------|------------------|----------------|-------------------------|----------|
| | | | Nb | Mortality rate | Nb | UTI rate |
| 1 | Cefo | 27 | 8 | 30% | 14 | 52% |
| 2 | Cefo+Care | 6 | 1 | 17% | 1 | 17% |
| 3 | Cefo+Care+Enro | 10 | 0 | 0% | 1 | 10% |

Mortality rate of SCI rats was significantly decreased in Cefo+Care group (17%) and was totally abolished in Cefo+Care+Enro group (0%) when compared to Cefo group (30%).

Percentage of SCI rats developed urinary tract infection was significantly decreased in Cefo+Care group (17%) and Cefo+Care+Enro group (10%) when compared to Cefo group (52%).

Effects of antibiotic therapy on the time-course of emergence of abnormal micturition reflex in SCI rats

Expressed urinary volume curve



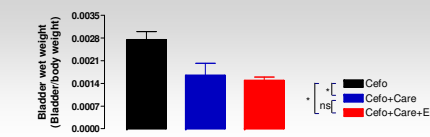
The urinary volume curve following Credé maneuver was unchanged in Cefo+Care group (p=0.25) whereas it was shifted to the right in Cefo+Care+Enro group (p<0.01) when compared to Cefo group reflecting that enrofloxacin associated with particular reinforced aseptic care delayed the emergence of the abnormal micturition reflex after SCI.

Number of SCI rats which did not recover the abnormal micturition reflex

| Group | Treatment | Nb of survival rats undergoing SCI | Rats without recovery of micturition reflex | |
|-------|----------------|------------------------------------|---------------------------------------------|------|
| | | | Nb | Rate |
| 1 | Cefo | 19 | 1 | 5% |
| 2 | Cefo+Care | 5 | 1 | 20% |
| 3 | Cefo+Care+Enro | 10 | 4 | 40% |

Moreover, the number of rats which did not recover their micturition reflex at the end of the observation period was 4/10 rats in the Cefo+Care+Enro group versus only 1/19 rat in the Cefo group and 1/5 rats in the Cefo+Care group indicating that enrofloxacin associated with particular reinforced aseptic care prevented the recovery of the abnormal micturition reflex.

Effects of antibiotic therapy on bladder weight in SCI rats



The bladder weight of SCI rats was significantly decreased in Cefo+Care group (0.00164±0.00037, p<0.05) and Cefo+Care+Enro group (0.00149±0.00011, p<0.05) when compared to Cefo group (0.0027±0.00026) suggesting that enrofloxacin associated with particular reinforced aseptic care decreased SCI induced-bladder hypertrophy.