

## F16357, A NOVEL PAR-1 ANTAGONIST IMPROVES URODYNAMIC PARAMETERS AFTER INFLAMMATION IN A NEW MODEL OF RAT CYSTOMETRY BASED ON BLADDER SENSATION.

### Hypothesis / aims of study

The main roles of urinary bladder are the storage of urine and its voiding at a socially convenient moment. Storage properties can be disturbed in many pathological situations, among them cystitis and painful bladder syndrome. Proteases released during bladder inflammation play a key role in the urinary system and hyperalgesia, and the deficiency of protease-activated receptor-1 reduced bladder in inflammation. The aim of this study was (i) to characterize a new model of cystometry by telemetry in conscious rats, (ii) to determine whether the animal decision plays a role in the initiation of voiding in physiological, inflammatory and painful conditions, (iii) to evaluate bladder effects of a PAR-1 antagonist under physiological and inflammatory conditions.

### Study design, materials and methods

14 adult female rats were anaesthetized and a small laparotomy was carried out. A telemetric transmitter was placed in the abdominal cavity and sutured on the abdominal wall. The pressure transducer was inserted and secured in the dome of the urinary bladder.

For the characterisation of the model, animals received an oral treatment of Vehicle, or Tolterodine (10 mg/kg). One hour after, a subcutaneous injection of Furosemide (10 mg/kg) was performed and bladder pressure and voided volumes were continuously recorded for 60 min.

After an appropriate wash out period, F16357 30µM, a PAR-1 selective antagonist, or its vehicle was administered intravesically for a one hour under anaesthesia. Urodynamic evaluation under Furosemide was carried out 3H30 after the end of intravesical administration.

Upon bladder inflammation, urodynamics and voiding patterns were evaluated on same animals. An acute intraperitoneal injection of Cyclophosphamide (CYP, 150 mg/kg) was performed and voiding patterns were analyzed 7H and 24H after injection.

All cystometric evaluations were performed on conscious freely moving rats and recorded parameters (mean ± SEM) were Micturition Pressure (MP), Basal Pressure (BP), Inter Contraction Interval (ICI) and Voided volume (V).

### Results

Quality of traces and quantitative parameters recorded telemetrically were comparable to those with conventional cystometry. Furosemide induced a reproducible increase of urine production (total voided volume) and frequency of voiding vs naive rats, which reversed after 60 minutes.

Tolterodine did not modify the number of micturitions ( $8 \pm 1$  for vehicle) but significantly ( $p < 0.05$ ) reduced their amplitude ( $23.5 \pm 3.4$  mmHg,  $n=12$ ), when compared to vehicle ( $40.8 \pm 3.1$  mmHg,  $n=19$ ). Neither mean voided volume per micturition ( $1.4 \text{ mL} \pm 0.1 \text{ mL}$ ), nor total voided volume ( $9.9 \pm 0.5 \text{ mL}$ ) was significantly modified by Tolterodine within 60 minutes after the pulse of Furosemide.

In physiological conditions, F16357 intravesical administration did not modify the amplitude of micturitions ( $49.0 \pm 13.6$  mmHg), number of micturitions ( $6 \pm 1$ ), mean voided volumes per micturition ( $1.8 \pm 0.5 \text{ mL}$ ) or total voided volumes ( $9.8 \pm 0.5 \text{ mL}$ ) when compared to vehicle.

7H after injection, CYP evoked a significant ( $p < 0.05$ ) increase of number of micturitions ( $18 \pm 5$  vs.  $5 \pm 1$  for vehicle) without modification of amplitude of bladder contraction ( $39.9 \pm 9.3$  mmHg vs.  $44.9 \pm 8.6$  mmHg for Vehicle). CYP decreased the mean volume of individual micturitions ( $0.7 \pm 0.2 \text{ mL}$  vs.  $2.1 \pm 0.5 \text{ mL}$  for Vehicle,  $p < 0.05$ ) and total voided volume ( $7.6 \pm 0.4 \text{ mL}$  for CYP-treated group vs  $9.6 \pm 0.6 \text{ mL}$ ,  $p < 0.05$ ) within the 60 minutes recording.

F16357 at 30 µM blocked urodynamics modifications induced by CYP: number of micturitions ( $7 \pm 2$ ), mean voided volume per void ( $1.5 \pm 0.3 \text{ mL}$ ) and total voided volume ( $8.1 \pm 0.3 \text{ mL}$ ) returned to physiological levels.

After a second pulse of Furosemide 24H after the CYP injection, the large increase of the number of micturitions returned to the level of vehicle group and the volume of each micturition was still lower in CYP-treated group and comparable with the first pulse. Consequently, the total voided volume in 60 min was further decreased 24H after CYP injection.

In contrast, F16357 30µM still significantly reduced the number of micturitions ( $2 \pm 0$  vs  $4 \pm 1$  micturitions in vehicle group,  $p < 0.05$ ) and improved the bladder capacity ( $1.7 \pm 0.2 \text{ mL}$  vs  $0.8 \pm 0.1 \text{ mL}$  in vehicle group,  $p < 0.05$ ) but had no effect on the diuresis ( $2.8 \pm 0.4 \text{ mL}$  vs  $2.9 \pm 0.4 \text{ mL}$  for total voided volume in vehicle group).

### Interpretation of results

Furosemide was used to stimulate the intrinsic diuresis and the number of micturitions (about 7-fold). The selected dose (10 mg/kg) ensured a good reproducibility of the voiding pattern during one hour and did not modify it even after many pulses. Tolterodine decreased the micturition amplitude without modification of micturition interval and voided volumes, suggesting that micturition pressure and voided volumes were not correlated. F16357 was devoid of any impact on voiding pattern in physiological conditions but highly improved it in a situation of experimental model of interstitial cystitis.

This severe model is mainly characterized by an increase of micturitions during the acute phase of bladder inflammation compared to the respective physiological situation in same animals. Moreover, the voiding pattern after CYP treatment was dramatically modified with a decrease of bladder capacity. These results lead to a correlation between the increase in number of voids and bladder pain/sensation. Furthermore, results obtained during the chronic phase of inflammation suggested that CYP induced a major modification/reduction of the diuresis.

After an acute and local administration, F16357 at 30µM sustainably abolished the deleterious effects of Cyclophosphamide and restored the physiological voiding pattern even after many micturition cycles.

Moreover, in a model assessing in parallel bladder function and sensation, F16357 results demonstrated the interest of the PAR-1 antagonism in rat interstitial cystitis elicited by CYP.

#### Concluding message

This telemetric model is likely to be more accurate than previously described conscious conventional cystometry, and allows the evaluation of compounds which could modulate the voiding pattern by targeting the detrusor contractility and the sensitive afferences. Furthermore, this model addresses some key criterias of human Interstitial Cystitis and Bladder Pain Syndrome such as urgency, pain or pressure. In these conditions, the novel PAR-1 antagonist F16357 seems to be a good candidate for IC/PBS treatment.

#### References

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#### Disclosures

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