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A NOVEL ANIMAL MODEL OF CHRONIC UROTHELIAL INJURY AND BLADDER PAIN HYPERSENSITIVITY INDUCED BY INTERMITTENT INFUSION OF PROTAMINE SULPHATE INTO THE BLADDER IN RATS: THE INVOLVEMENT OF PROSTAGLANDIN E2 AND EP1 RECEPTOR ACTIVATION

Hypothesis / aims of study

Painful bladder syndrome/interstitial cystitis (PBS/IC) is chronic pain condition characterized by suprapubic pain related to the bladder. Although the aetiology of PBS/IC is multifactorial, urothelial dysfunction with increased urothelial permeability are considered to be a major pathogenesis of the disease. However, the basic research of PBS/IC is hampered because there are no appropriate animal models of chronic bladder injury associated with increased bladder pain sensitivity. Previous studies showed that intravesical infusion of protamine sulfate (PS) in rats causes the urothelial damage, especially in the surface glycosaminoglycan layer and apical umbrella cells of the urothelium, leading to decreased transepithelial resistance and bladder inflammation, which mimic some aspects of urothelial dysfunction in human PBS/IC [1]. Therefore, we first attempted to develop a novel animal model of chronic urothelial injury with bladder pain hypersensitivity using intermittent PS infusion into the bladder in rats. It has also been reported that prostaglandin E_2 (PGE_2) is a key mediator in processing of pain hypersensitivity via the EP1 receptor [2]. Therefore, we also investigated the effects of EP1 receptor inhibition using ONO-8539, a selective EP1 receptor antagonist, on bladder pain hypersensitivity in rats with PS-induced urothelial injury.

Study design, materials and methods

Experiments were performed on female Wistar rats.

(1) Cystometry and PS infusion: Seven days prior to the 1st PS treatment, a catheter was implanted into the bladder from the bladder dome under isoflurane anaesthesia. At 7 days after the catheter implantation, saline was continuously infused into the bladder at a rate of 0.1 ml/min over 1hours to evaluate bladder activity under an awake condition. After a saline infusion period, PS (10 mg/mL) was infused into the bladder at the same rate for 3 hours. These bladder activity measurement and PS infusion were repeated at day 0 (before 1st PS treatment), day 7 (7days after 1st PS treatment), day 14 (7days after 2nd PS treatment), day 21 (7days after 3rd PS treatment) and day 28 (7days after 4th PS treatment). (2) Urothelial damage and bladder histopathological changes were assessed by periodic acid-schiff (PAS) and hematoxylin and eosin staining (HE), respectively. The number of mast cells in the bladder was evaluated by toluidine blue staining. (3) Behavioural assessment: Nociceptive behaviours were measured at 7days after 4th PS treatment (=day 28). The nociceptive behaviours such as lower abdominal licking (licking) and motionless head-turning (freezing) induced by intravesical application (0.3mL for 1min) of resiniferatoxin (RTx, a TRPV1 agonist: 0, 0.3 or 3μM) through a temporally-inserted urethral catheter were scored every 5 sec for 15 min. (4) Effects of EP1 receptor inhibition: In a separate group of rats, vehicle (control), ONO-8539 (0.1, 1 and 10mg/kg) or anticholinergic drug tolterodine (10mg/kg) was administered orally 60 min before application of RTx to evaluate the effects of EP1 receptor inhibition on RTx-induced pain behaviours at 7days after 4th PS treatment (=day 28). (5) Prostaglandin E_2 (PGE_2) levels and COX-1, COX-2, EP1, EP2 EP3 and EP4 receptors mRNA levels in the bladder, dorsal root ganglia (DRG), spinal dorsal horn, and the pons were determined by quantitative ELISA and RT-PCR, respectively.

T-tests and one-way ANOVA were used to test for differences among groups, and $p<0.05$ was considered significant.

Results

(1) Repeated PS treatments produced spontaneous bladder contractions during the storage phase during intravesical saline infusion (figure 1). The amplitude and frequency of spontaneous contractions increased as the number of PS treatments was increased, with the maximal frequency at day 28 (7 days after 4th PS treatment). There were no significant changes in other cystometric parameters such as bladder capacity or maximum intravesical pressure. (2) An irregular surface of the urothelial layer was observed at day 22 (at 1 day after 4th PS treatment), and this PS-induced urothelial damage was almost completely restored at day 28 (7days after 4th PS treatment). Similarly, severe inflammatory changes in the bladder such as edema, hemorrhage or vascular ecstasia were observed at day22, but not at day28, and TB-positive mast cells were significantly increased at day 22, but not at day28. (3 & 4) The licking and freezing behaviours induced by 0.3μM RTx were significantly increased at day 28, compared to sham rats treated with 0.3μM or 3μM RTx (figure 2). In PS-treated rats at 28 days, ONO-8539 dose-dependently and significantly suppressed the RTx-induced pain behaviours induced by RTX; however, tolterodine did not affect them even at the effective dose that reduced maximum voiding pressure (figure 2). (5) PGE_2 and COX-2 levels were significantly higher in L6-S1 DRG, L6-S1 spinal dorsal horn and the pons as well as in the bladder at day 28 as compared to the sham group. COX-1, and EP1-EP4 receptors mRNA expression were detected in the L6-S1 DRG, L6-S1 spinal dorsal horn, the pons and the bladder, but the mRNA levels of these molecules were not changed after PS treatment.

Interpretation of results

PS-induced intermittent urothelial injury for 4 weeks resulted in bladder hyperactivity evidenced by spontaneous bladder contractions during the storage phase and increased bladder pain sensitivity shown by enhanced pain behaviours induced by nociceptive stimuli in the bladder, which were observed even after the remission of acute inflammatory changes in the bladder. Also, EP1 receptor activation by increased levels of PGE2 due to COX-2 upregulation in the bladder, afferent pathways, the spinal cord and/or the pons is likely to contribute at least in part to peripheral and central sensitization leading to increased bladder pain sensitivity in this model.

Concluding message

Rats with intermittent PS infusion are suitable for a chronic animal model of urothelial injury that exhibits bladder hypersensitivity, which could be used for the research identifying the mechanisms underlying bladder pain and dysfunction induced by urothelial damage that are often seen in PBS/IC patients. Moreover, EP1 receptor blockade may be a novel therapeutic option for controlling PBS/IC symptoms related to bladder pain hypersensitivity.

Figure 1: Effects of intermittent PS treatments on bladder activity

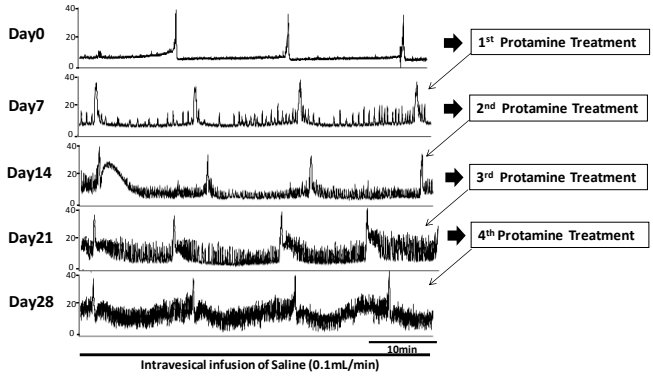
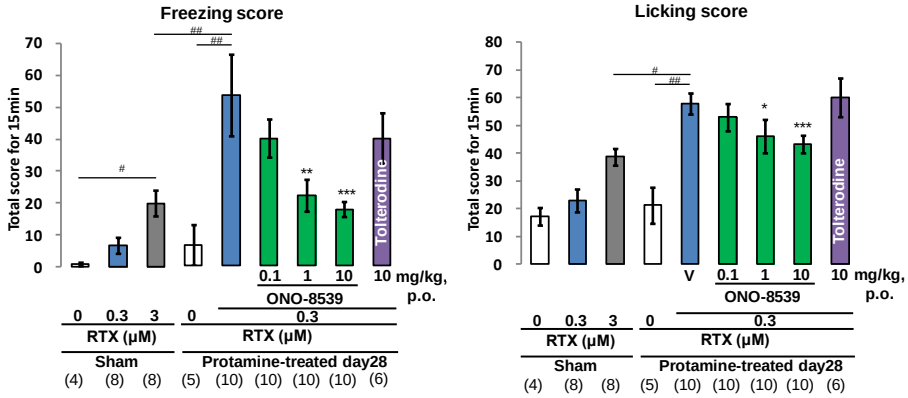


Figure 2: Effects of intermittent PS treatments and EP1 receptor inhibition on pain behaviour



References

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2. Sarkar S et al., Gastroenterology, 124: 18-25, 2003

Disclosures

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