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

Okada H1, Tyagi P1, Kawamaruta N1, Majima T1, Chancellor MB2, Yoshimura N1
1. Department of Urology, University of Pittsburgh, 2. Department of Urology, William Beaumont Hospital

Background

- The basic research of painful bladder syndrome/interstitial cystitis (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, which mimic some aspects of urothelial dysfunction in human PBS/IC.1
- It has also been reported that protaglandin E2 (PGE2) is a mediator in processing of pain hypersensitivity via the EP1 receptor.2

Hypothesis

Changes in urothelial permeability can initiate a cascade of inflammatory events in the bladder, leading to mast cell activation, C-fiber activation, and peripheral sensitization, and then increased sensory input can induce wind up phenomenon in the spinal cord and CNS, resulting in central sensitization. PS is overproduced by inflammation or nociceptor stimuli in sensory pathways. PGE2 is a key mediator in processing of pain hypersensitivity via the EP1 receptor.

Objectives

- To develop a novel animal model of chronic urothelial injury with bladder pain hypersensitivity using intermittent PS infusion into the bladder in rats.
- To investigate the effects of EP1 receptor inhibition using ONO-8539, a selective EP1 receptor antagonist, on bladder pain hypersensitivity in this model.

Methods

**Animal:**

Female Wistar 7W Rats

**Treatment:**

Urothelial injury was induced by intravesical perfusion (5.7mL/h) of protamine sulfate (Sigma, 10mg/mL) for 3 hours once a week.

**Measurement:**

1. Awake CMG (detection of time-dependent bladder dysfunction)
2. Histopathological changes
3. Pain behaviors
4. PGE2 levels and EP1 mRNA expressions in different tissues (bladder, urethra, L6-S1 dorsal root ganglia [DRG] and spinal dorsal horn)

**Experimental procedure**

- Pain behaviors were measured at 7days after the 4th protamine treatment in order to avoid an evaluation in acute epithelial defect and acute inflammatory conditions.
- The pain behaviors (licking and freezing) were assessed in accordance with the validating methods in our laboratory.
- Vehicle, ONS-8539 (0.1, 1 and 10mg/kg) or antiinflammatory drug (indomethacin 10mg/kg) was administrated orally, that was followed by water loading, and then the rats were injected into the bladder. Pain behaviors were evaluated for 15 minutes.

**Results**

- Repeated protamine treatments produced bladder overactivity shown by multiple bladder contractions during the storage phase.
- The maximum contraction frequency was observed at day28 (at 7 days after the 4th protamine treatment).
- Pain behaviors were measured at 7days after the 4th protamine treatment in order to avoid an evaluation in acute epithelial defect and acute inflammatory conditions.
- The pain behaviors (licking and freezing) were assessed in accordance with the validating methods in our laboratory.

**Interpretation of results**

- PS-induced intermittent urothelial injury for 4 weeks resulted in bladder overactivity evidenced by spontaneous bladder contractions during the storage phase and increased bladder pain sensitivity shown by enhanced pain behaviors induced by nociceptor stimuli in the bladder, which were observed even after the remission of inflammatory changes in the bladder.
- Also, EP1 receptor activation by increased levels of PGE2 in the bladder, arterial 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.

**Conclusion**

- 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.
- EP1 receptor blockade may be a novel therapeutic option for controlling PBS/IC symptoms related to bladder pain hypersensitivity.