Liposome-based Intravesical Therapy targeting Nerve Growth Factor (NGF) ameliorates Bladder Hypersensitivity in Rats with Experimental Colitis

Naoki Kawamorita1, Satoru Yoshikawa1, Pradeep Tiyagi1, Michael B. Chancellor2, Naoki Yoshimura1

1. University of Pittsburgh, 2. Oakland University, USA

AIM of STUDY

The complex pathophysiology of chronic pelvic pain syndrome (CPPS) and bladder pain syndrome/interstitial cystitis (BPS/IC) could be interrelated. It has recently been proposed that pelvic organ “cross sensitization” contributes to clinically overlapping symptoms in CPPS such as irritable bowel syndrome (IBS) and BPS/IC. Previous animal studies also demonstrated that experimental colitis evokes bladder overactivity evidenced by frequent urination in association with hyperexcitability of afferent neurons innervating the bladder [1] although it has not been investigated whether this colitis model exhibits an increase in bladder pain sensation. Meanwhile, overexpression of nerve growth factor (NGF) in the bladder is thought to be one of the key factors in the symptom development in BPS/IC patients. We recently reported that instillation of liposome conjugated with antisense oligonucleotide (OND) targeting NGF into the bladder suppressed bladder overactivity in a rat model of acute cystitis [2]. Therefore, this study explored whether bladder hypersensitivity and NGF overexpression in the bladder are induced after colitis and whether intravesical liposomal-OND treatment can suppress bladder hypersensitivity and NGF expression in a rat model with experimental colitis.

Study Design

Study Design

METHODS

TNBS* or Vehicle

• Intracolonic injection

- TNBS: 2,4,6,trinitrobenzen sulfonic acid

- Intravesical

NGF antisense ODN+ Liposome solution or Saline

• Intravesical injection

- Saline pre-injection

- ODN pre-injection

Groups

- Colitis-saline group

- Sham-saline group

- Control group (No treatment)

- Colitis-OND group

- Sham-OND group

- Vehicle injection

- Saline pre-injection

- ODN pre-injection

(1) Nociceptive behaviour testing: Licking and freezing behaviours were counted for 15 minutes in response to intravesical administration of resiniferatoxin (RTx, 0.3μM, 0.3ml) in a metabolic cage.

(2) Awake cystometry: An intravesical catheter was implanted under urethane anaesthesia 3 days before cystometry. A PE-50 catheter with the end flared by heat was inserted into the bladder dome, ligated and placed subcutaneously. Saline followed by 0.1% acetic acid (AA) were continuously infused to evaluate changes in intracystonic intervals (ICIs) in conscious rats.

(3) Immunohistochemistry: The frozen section of the bladder was DAB stained with NGF antibody (1:250 dilution). The positive staining was visualized with a DAB kit.

(4) Quantification of messenger RNA (mRNA) and protein of NGF: The bladder was dissected to divide into mucosal and detrusor layers. Quantitative polymerase chain reaction (qPCR) and Enzyme-Linked ImmunoSorbent Assay (ELISA) were used to measure the mRNA and protein expression of NGF, respectively.

RESULTS

(1) In the colitis-saline group, the score of freezing behaviour was significantly higher than that of all other groups including the colitis-OND group (Figure 1). The licking score in the colitis-saline group was significantly higher than in the control group and tended to higher compared to other 3 groups without significant differences.

(2) ICIs before intravesical AA stimulation were not different among groups; however, the ICI reduction rate after AA instillation into the bladder was significantly higher in the colitis-saline group than that in the colitis-OND group (Figure 2).

(3) There was increased immunoreactivity of NGF in the bladder mucosa in the colitis-saline group, whereas there was only faint staining in the control and colitis-OND groups (Figure 3).

(4) The mRNA and protein expression of NGF in the colitis-saline group was significantly increased in the mucosa compared to control and colitis-OND groups (Figure 4).

CONCLUSION

- The rat model of experimental colitis is useful to study the mechanism inducing bladder hypersensitivity such as pain behaviour in addition to changes in bladder activity.

- The liposome-based antisense treatment targeting NGF in the bladder could be a new, effective modality for the treatment of bladder pain and overactivity in CPPS patients including those with BPS/IC and IBS, in whom the pelvic organ “cross sensitization” mechanism is involved in overlapping symptoms from different pelvic organs.

Mechanism of Action

- Bowel inflammation facilitates the nociceptive responses derived from the bladder in association with the increased expression of NGF in the bladder.

- Intravesical instillation of NGF antisense with liposome reduces bladder pain behaviour and the mucosal expression of NGF.