

LIPOSOME-BASED INTRAVESICAL THERAPY TARGETING NERVE GROWTH FACTOR (NGF) AMELIORATES BLADDER HYPERSENSITIVITY IN RATS WITH EXPERIMENTAL COLITIS

Hypothesis / aims 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 the 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 was planned to explore whether bladder hypersensitivity induced by experimental colitis 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, materials and methods

- Animal groups: Adult female Sprague-Dawley rats were used; (a) control group (no treatment), (b) colitis-OND group (intracolonic 2,4,6-trinitrobenzen sulfonic acid [TNBS] enema and intravesical liposomal OND were given), (c) colitis-saline group (intracolonic TNBS and intravesical saline were given), (d) sham-OND group (intravesical liposomal OND was given without colitis) and (e) sham-saline group (intravesical saline was given without colitis).
- Intravesical administration of NGF antisense-liposome solution (liposomal-OND): Under isoflurane anesthesia, 0.2ml of either liposomal-OND or saline was instilled to the bladder through an inserted urethral catheter.
- Experimental colitis model: Twenty-four hours after instillation of liposomal-OND or saline and fasting, colitis was induced by the enema of 30mg TNBS dissolved in 50% ethanol through a polyethylene catheter inserted 8 cm proximal to the anus in a head-down position. Ten days after liposomal-OND or saline injection, animals were subjected to either in vivo studies or bladder tissue removal.

(1) Nociceptive behaviour testing: Licking (lower abdominal licking) and freezing behaviours (motionless head-turning towards lower abdomen) in response to 1-min intravesical administration of resiniferatoxin (RTX), a TRPV1 receptor agonist, were examined. After 2 hours acclimation in a metabolic cage, RTX (0.3µM, 0.3ml) was instilled through an inserted urethral catheter for 1 min, and the catheter was then removed. Thereafter, both licking and freezing behaviours were scored during 5-s intervals for 15 minutes in the cage (n=4-6).

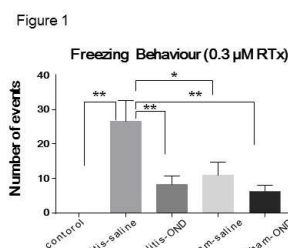
(2) Awake cystometry: Intravesical catheters were implanted under urethane anaesthesia 3 days before cystometry. PE-50 catheter with the end flared by heat was inserted into the bladder dome, ligated and placed subcutaneously. Saline followed by 0.1% acetic acid (AA) were continuously infused to evaluate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

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

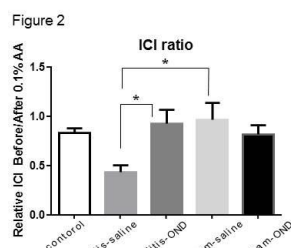
(4) Molecular analysis of NGF: The harvested bladder was micro-dissected to divide into mucosal and detrusor layers. Quantitative polymerase chain reaction (qPCR) and Enzyme-Linked ImmunoSorbent Assay (ELISA) were used to measure the mRNA and protein expression of NGF, respectively (n=3-5).

Results

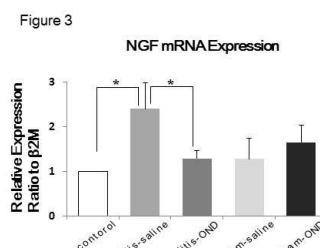
(1) In the colitis-saline group, the score of freezing behaviour was significantly higher than that of all other groups including the colitis-OND group (Figure 1). The score of licking behaviour in the colitis-saline group was significantly higher than in the control group and tended to higher compared 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. (4) The mRNA expression of NGF in the colitis-saline group was significantly increased in the mucosa compared to control and colitis-OND groups (Figure 3). In addition the protein level of NGF in the mucosa was also higher in the colitis-saline group compared to other groups.



(Figure 1) Freezing behaviour was significantly increased in the colitis-saline group and decreased in the colitis-OND group (* $p < 0.05$, ** $p < 0.01$).



(Figure 2) The ICI reduction rate after intravesical infusion of acetic acid (AA) was significantly higher in the colitis-saline group than that in the colitis-OND group (* $p < 0.05$).



(Figure 3) Expression of NGF mRNA was significantly increased in the colitis-saline group compared to control and colitis-OND groups (* $p < 0.05$).

Interpretation of results

These results indicate that: (1) colitis evoked by TNBS induced bladder hypersensitivity shown by increased freezing behaviour, which represents bladder pain sensation, and enhanced bladder overactivity in response to nociceptive bladder stimuli such as RTX or AA and (2) colitis-induced bladder hypersensitivity is associated with an increase of NGF expression at both mRNA and protein levels in the bladder mucosa. Furthermore, the intravesical liposome-based treatment with NGF antisense, which reduced the mucosal expression of NGF, had a therapeutic effect on colitis-induced bladder hypersensitivity as evidenced by reductions in RTX-induced freezing behaviour and AA-induced bladder overactivity. Taken together, it is likely that NGF overexpression in the bladder mucosa plays an important role in the colon-to-bladder cross-sensitization to induce bladder hypersensitivity after colitis and that intravesical application of liposomal OND targeting NGF is effective to reduce bladder pain and overactivity induced by colitis.

Concluding message

This study shows that 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.

References

1. Michael A. Pezzone, et al. *Gastroenterology*. 2005 Jun;128(7):1953-64
2. M. Kashyap, et al. *J of Urol*. 2013 Aug;192(2):157-64

Disclosures

Funding: NIH (DK088836 and P01 DK093424) and DOD (W81XWH-11-1-0763 and W81XWH-12-1-0565) **Clinical Trial:** No
Subjects: ANIMAL **Species:** Rat **Ethics Committee:** the University of Pittsburgh Institutional Animal Care and Use Committee