

ANTISENSE OLIGONUCLEOTIDE TARGETING NGF ATTENUATES BLADDER PAIN BEHAVIOR IN RAT WITH EXPERIMENTAL COLITIS

Hypothesis / aims of study

Pelvic organ “cross sensitization” is suspected to contribute to clinically overlapping symptoms in patients with chronic pelvic pain syndrome (CPPS) such as irritable bowel syndrome (IBS) and bladder pain syndrome/interstitial cystitis (BPS/IC). Previous animal studies demonstrated that experimental colitis evoked bladder overactivity associated with hyperexcitability of afferent neurons innervating the bladder [1] although it has not been investigated whether this colitis model exhibits bladder pain. On the other hand, overexpression of nerve growth factor (NGF) in the bladder has been shown to play an important role 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. Therefore, this study was performed to explore whether pain behaviour induced by bladder irritation and NGF expression in the bladder are increased after colitis and whether instillation of liposomal-OND conjugates into the bladder can suppress pain behaviour and NGF expression in a rat model of experimental colitis.

Study design, materials and methods

Female Sprague-Dawley rats were used and divided into five groups; (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). NGF antisense-liposome solution for intravesical application was made by dissolving 2 μ l of anti-NGF ODN (2mM) in 0.5ml of liposome (7mM). Under isoflurane anesthesia, 0.5ml of either liposomal-OND or saline was instilled to the bladder through an inserted urethral catheter. 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 pain behaviour testing or bladder tissue removal.

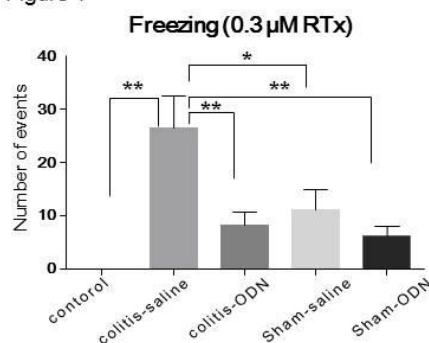
(1) Nociceptive behaviour testing: Licking and freezing behaviour in response to 1-min intravesical administration of resiniferatoxin (RTx), a TRPV1 receptor agonist was examined. After 2 hours acclimation in a metabolic cage, RTx (0.3 μ M, 0.3ml) was instilled through the inserted urethral catheter 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) Quantification of messenger RNA (mRNA) and protein of NGF: The harvested bladder was microdissected to divide to 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=5).

Results

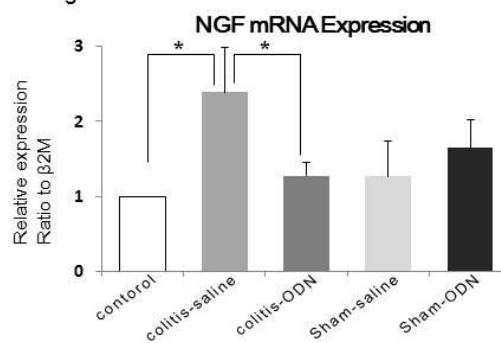
(1) In the colitis-saline group, the score of freezing behaviour was significantly higher than that of all other groups including the colitis-OND group (Figure 1). The licking score in the colitis-saline group was significantly higher than in the control group and tended to higher compared to other 3 groups without significant differences. (2) The mRNA expression of NGF in the colitis-saline group was significantly increased in the mucosa compared to control and colitis-OND groups (Figure 2). In addition the protein level of NGF in the mucosa was also higher in the colitis-saline group compared to other groups.

Figure 1



(Figure 1) Freezing was significantly increased in colitis-saline group (* p <0.05, ** p <0.01).

Figure 2



(Figure 2) Expression of NGF mRNA was significantly increased in colitis-saline group compared to control and colitis-OND group (* p <0.05).

Interpretation of results

Colitis evoked by TNBS enhanced the freezing behaviour, which corresponds to bladder pain [2], and increased NGF expression at both mRNA and protein levels in the bladder mucosa. These results indicate that bowel inflammation facilitates the nociceptive responses derived from the bladder in association with the increased expression of NGF in the bladder and that the intravesical instillation of NGF antisense with liposome reduces the bladder pain behaviour and the mucosal expression of NGF. Thus, it seems likely that NGF overexpression in the bladder has an important role in the colon-to-bladder cross-

sensitization to induce bladder pain after colitis and that intravesical application of liposomal ODN targeting NGF is effective to reduce NGF production in the bladder and bladder pain sensation induced by colitis.

Concluding message

This study shows that the rat model of experimental colitis is useful to study the mechanism inducing bladder pain behaviour in addition to bladder overactivity that has previously been shown [2]. The liposomal antisense treatment targeting NGF in the bladder could be a new, effective modality for the treatment of bladder pain in CPPS patients including those with BPS/IC and IBS, in whom the cross-sensitization mechanism is involved in the emergence of overlapping symptoms from different pelvic organs.

References

1. Gastroenterology. 2005 Jun;128(7):1953-64
2. Neurourol Urodyn. 2010 Mar;29(3):501-5

Disclosures

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