

Majima T¹, Tyagi P², Dogishi K³, Kadekawa K⁴, Kashyap M², Wada N⁵, Takai S⁶, Shimizu T⁷, Gotoh M⁸, Chancellor M⁹, Yoshimura N²

1. University of Pittsburgh, Nagoya University Graduate School of Medicine, 2. University of Pittsburgh, 3. Graduate School of Pharmaceutical Sciences, Kyoto University,, 4. University of Pittsburgh, Southern Knights' Laboratory LLP, 5. University of Pittsburgh, Asahikawa Medical University, 6. University of Pittsburgh. Nagoya University Graduate School of Medicine, 7. University of Pittsburgh, Kochi Medical School, Kochi University, 8. Nagoya University Graduate School of Medicine, 9. William Beaumont Oakland University School of Medicine

THE EFFECT OF INTRAVESICAL LIPOSOME-BASED NGF ANTISENSE THERAPY ON BLADDER OVERACTIVITY AND NOCICEPTION IN A RAT MODEL OF CYSTITIS INDUCED BY HYDROGEN PEROXIDE

Hypothesis / aims of study

Nerve growth factor (NGF) has been proposed to be an important mediator for inducing hyperexcitability of afferent pathways that contributes to pain and storage symptoms of interstitial cystitis/bladder pain syndrome (IC/BPS). A previous clinical study indicated that systemic application of NGF monoclonal antibody significantly reduced pain/urgency in IC/BPS patients, but its systemic adverse events such as paresthesia and hyperesthesia were a critical issue [1]. Therefore, the site-specific reduction of NGF would be desirable to reduce the intrinsic toxicity from systemic blockade of NGF. In this regard, our previous study demonstrated that intravesical liposome-based NGF antisense therapy significantly improved bladder overactivity in a rat model of acute cystitis induced by intravesical application of acetic acid [2]. However, it still remains unclear whether local NGF antisense therapy has a chronic effect. Therefore, we investigated the effect of intravesical liposome-based NGF antisense therapy on bladder overactivity and nociceptive behaviour in a rat model of chronic cystitis induced by hydrogen peroxide (HP).

Study design, materials and methods

Adult Sprague-Dawley female rats were used according to the experimental protocol approved by the Institutional Animal Care and Use Committee.

- 1) 1.5% HP was administered into the bladder on day 0. Liposomes conjugated with NGF antisense tagged with TYE563 fluorescent protein was given into the bladder on day 2. The expression of TYE563 was observed under a fluorescent microscope on day 3.
- 2) In another set of experiments, rats were divided into 4 groups: a) saline + vehicle (SV) group, b) saline + liposome-NGF antisense (SN) group, c) 1.5% HP + vehicle (HV) group, d) 1.5% HP + liposome-NGF antisense (HN) group. Saline or 1.5% HP was administered into the bladder on day 0. Each rat was treated with intravesical vehicle or NGF antisense administration on day 2. Continuous cystometry (CMG) was performed in an awake condition on day 7.
- 3) Nociceptive behaviours induced by 1-min intravesical instillation of resiniferatoxin (TRPV1 agonist, RTx) such as licking behaviour (lower abdominal licking) and freezing behaviour (motionless head-turning towards lower abdomen) was observed on day 7.
- 4) After rats were intracardially perfused with cold heparinised-saline, followed by 4% paraformaldehyde, the bladder and L6 DRG were removed on day 7. Haematoxylin-Eosin (HE) staining as well as immunofluorescence staining for NGF were performed.
- 5) Bladder tissue was harvested on day 7. The mRNA expression of NGF, TRPV1 and brain-derived neurotrophic factor (BDNF) was measured by RT-PCR.

Results

- 1) TYE563 expression was observed in the bladder urothelium 1 day after intravesical application of TYE563- tagged liposome-NGF antisense conjugates.
- 2) In CMG, the HV group showed significantly ($p=0.001$) shorter intercontraction intervals (ICI) than the SV group (ICI: 442 ± 64 and 889 ± 73 sec, respectively). The HN group showed significantly ($p=0.007$) longer ICI than the HV group (ICI: 711 ± 46 and 442 ± 64 , respectively). There was no significant difference in ICI between SV and SN groups ($p=0.56$, Fig.1.)
- 3) There were no significant differences in licking behaviour among 4 groups. However, the number of freezing events was significantly ($p=0.002$) higher in the HV group than in the SV group (18 ± 2 and 6 ± 1 events for 15 min after RTx treatment, respectively). The HN group showed the significantly ($p=0.04$) less number of freezing events than the HV group (9 ± 3 and 18 ± 2 events, respectively.) There was no significant difference in the number of freezing events between the SV and SN group (Fig.2.)
- 4) HE staining showed that there were substantial infiltration of the inflammatory cells, submucosal bleeding, and detrusor hypertrophy in the bladder wall in the HV group compared with SV group, which were alleviated in the HN group. Immunofluorescence staining indicated that the expression of NGF protein in the mucosa ($p=0.02$) and L6 DRG ($p=0.01$) was significantly higher in the HV group than in the SV group. On the other hand, the HN group indicated significantly lower expression of NGF protein in the mucosa ($p=0.002$) and L6 DRG ($p=0.01$) than the HV group (Fig.3 and 4.) In the detrusor, there was no significant difference in the NGF expression among 4 groups.
- 5) In RT-PCR, the HV group showed the significantly higher expression of NGF ($p=0.001$) and TRPV1 ($p=0.03$) mRNA in the mucosa than the SV group ($p=0.001$), whereas the HN group showed the significantly lower expression of NGF ($p=0.007$) and TRPV1 ($p=0.02$) than the HV group. There was no significant difference in BDNF expression in the mucosa among 4 groups. In the detrusor, the HV group showed the significantly higher expression of NGF ($p=0.03$) and BDNF ($p=0.02$) mRNA than the SV group while HN group did not show significant differences compared to the HV group. There was no significant difference in the expression of TRPV1 in the detrusor among 4 groups.

Fig.1

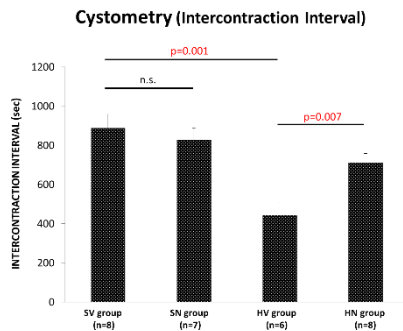


Fig.2

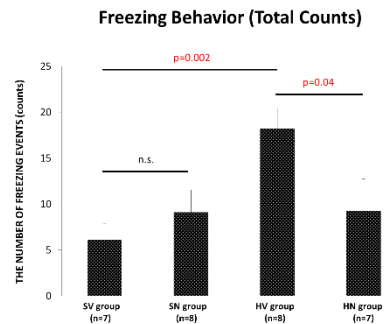


Fig.3

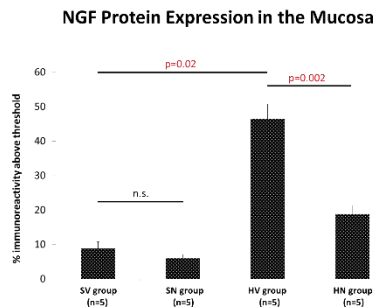
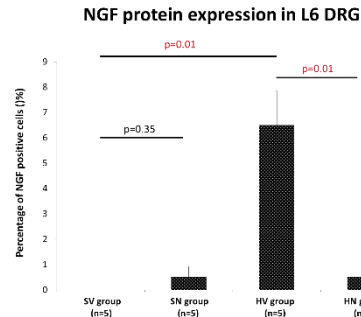


Fig.4



Interpretation of results

These results indicate that; (1) intravesical hydrogen peroxide instillation, which induces bladder inflammation up to 7 days after the instillation, elicit frequent urination shown by reduced ICI, and enhanced bladder pain sensitivity shown by increased freezing behaviour, which are associated with increased expression of NGF mRNA and protein as well as TRPV1 mRNA in the bladder mucosa and bladder afferent pathways and (2) intravesical liposome-based NGF antisense therapy induces a reduction in the NGF mRNA and protein expression in the bladder mucosa and bladder afferent pathways, which results in the improvement of bladder pain behaviour and frequent urination induced by hydrogen peroxide-induced chronic cystitis.

Concluding message

Intravesical liposome-based NGF antisense therapy could be a novel treatment that can avoid systemic adverse events for hypersensitive bladder disorders such as IC/BPS, in which NGF has been implicated as an important mediator for inducing afferent sensitization.

References

- Evans RJ, Moldwin RM, Cossons N, Darekar A, Mills IW, Scholfield D. Proof of concept trial of tanezumab for the treatment of symptoms associated with interstitial cystitis. *The Journal of urology* 2011 May;185(5):1716-21.
- Kashyap M, Kawamorita N, Tyagi V, Sugino Y, Chancellor M, Yoshimura N, et al. Down-regulation of nerve growth factor expression in the bladder by antisense oligonucleotides as new treatment for overactive bladder. *The Journal of urology* 2013 Aug;190(2):757-64.

Disclosures

Funding: Department of Defense (W81XWH-12-1-0565), National Institutes of Health (DK088836 and P01DK093424), **Clinical Trial:** No **Subjects:** ANIMAL **Species:** rat **Ethics Committee:** the Institutional Animal Care and Use Committee