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THE THERAPEUTIC EFFECTS OF GENE THERAPY WITH REPLICATION-DEFICIENT HERPES SIMPLEX VIRUS (HSV) VECTORS ENCODING PORELESS TRPV1 OR PROTEIN PHOSPHATE 1A (PP1A) ON BLADDER OVERACTIVITY AND NOCICEPTION IN A RAT MODEL OF CYSTITIS INDUCED BY HYDROGEN PEROXIDE

Hypothesis / aims of study

Increased afferent excitability is considered to be an important pathophysiological basis of interstitial cystitis/bladder pain syndrome (IC/BPS) or overactive bladder (OAB). Previous studies reported that transient receptor potential vanilloid-1 (TRPV1) receptors greatly contribute to afferent sensitization. Animals with hydrogen peroxide (HP)-induced cystitis have recently been introduced as a model that mimics pathologic features of chronic inflammatory bladder condition [1, 2]. We have also previously shown that HSV vector-mediated gene delivery of poreless TRPV1, in which the segment in C terminus of TRPV1 receptor is deleted to suppress TRPV1 activation, or protein phosphate 1α (PP1α), which negatively modulates TRPV1 activation, had a therapeutic effect on TRPV1-mediated bladder overactivity and pain behavior in rats with acute chemical irritation [3]. Therefore, we investigated the effect of gene therapy with HSV vectors encoding poreless TRPV1 or PP1α using a rat model of chronic cystitis induced by HP treatment.

Figure1

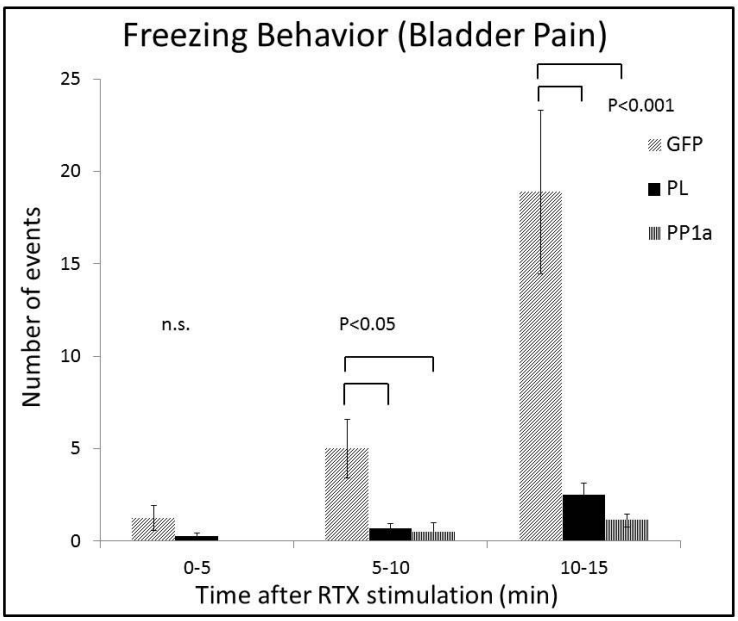
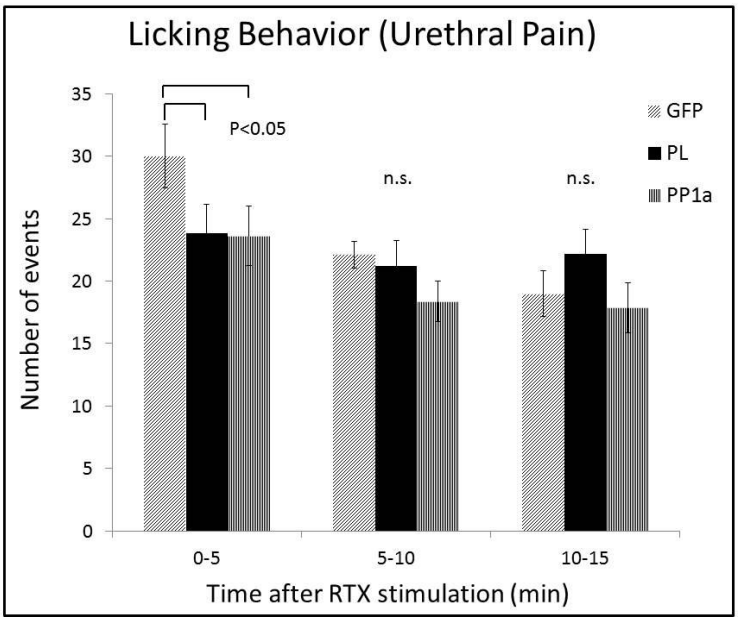


Figure2



Study design, materials and methods

Replication-deficient HSV vectors encoding green fluorescent protein (GFP), poreless TRPV1 or PP1α were injected into the bladder wall of adult female Sprague-Dawley (SD) rats. One week later, 1% HP or normal saline was administered into the bladder via a transurethral catheter. Two weeks after viral injection, awake cystometry (CMG) was performed, nociceptive behavior such as licking (urethral pain) and freezing (visceral pain) induced by intravesical instillation of resiniferatoxin (RTX; 3μM for 1 min) was observed, and the bladder mucosa, detrusor and L6/S1 dorsal root ganglia (DRG) were harvested. The mRNA expression of nerve growth factor (NGF) was measured by RT-PCR. GFP expression in the L6/S1 DRG and the bladder was also evaluated.

Results

GFP expression was seen in L6/S1 DRG and the bladder after HSV-GFP vector inoculation into the bladder wall. In CMG, the GFP + HP (GFP/HP) group showed a significant decrease in intercontraction intervals (ICIs) compared to the GFP + saline (GFP/NS) group. Then, the reduced ICIs in the GFP/HP group were significantly prolonged by 57.8% and 68.0% in poreless TRPV1 + HP (PL/HP) and PP1α + HP (PP1α/HP) groups ($p<0.01$), respectively. The number of freezing behavior was significantly lower in PL/HP and PP1α/HP groups by 86.1% and 93.5%, respectively, compared to the GFP/HP group (Figure 1). PL/HP and PP1α/HP groups also showed a significant decrease in licking behavior during the first 5 minutes (20.8% and 21.4%, respectively, $p<0.05$), but not during the following 5 to 15 minutes period after RTX stimulation (Figure 2). In RT-PCR, the GFP/HP group showed a significantly higher expression of NGF ($p<0.05$) in the bladder mucosa than GFP/NS group, which was significantly decreased in PL/HP and PP1α/HP groups ($p<0.05$). There was no significant difference in the expression of NGF in the detrusor among 4 groups.

Interpretation of results

Rats with HP-induced cystitis (7 days) exhibited bladder overactivity shown by reduced ICIs, which was ameliorated in HSV-poreless TRV1 or PP1α-treated rats. Freezing behavior representing bladder pain was almost completely blocked by both poreless TRPV1 and PP1α treatment. NGF expression in the mucosa was elevated in rats with HP, which was decreased in both treatment groups. These results indicate that HSV vectors-mediated gene delivery of PP1α or poreless TRPV1 significantly reduced bladder overactivity and pain sensation, by reducing bladder-derived freezing pain behavior more effectively than urethra-derived licking pain behavior, in HP cystitis rats. Also it seems likely that activation of TRPV1 expressing C-fiber bladder afferent pathways is involved in NGF overexpression in the bladder mucosa, which has been proposed as an important mechanism underlying IC/BPS and OAB symptoms.

Concluding message

HSV-mediated TRPV1-targeting gene therapy could be a novel modality for the treatment of IC/BPS and/or OAB.

References

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Disclosures

Funding: DOD W81XWH-12-1-0565 **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** University of Pittsburgh Institutional Animal Care and Use Committee