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GENE THERAPY WITH REPLICATION-DEFICIENT HERPES SIMPLEX VIRUS VECTORS ENCODING PORELESS TRPV1 OR PROTEIN PHOSPHATASE 1A (PP1A) REDUCES BLADDER OVERACTIVITY AND NOCICEPTION IN RATS

Hypothesis / aims of study

Increased afferent excitability has been proposed as an important pathophysiological basis of bladder pain syndrome/interstitial cystitis (BPS/IC). It has also been reported that transient receptor potential vanilloid-1 (TRPV1) receptors predominantly expressed in C-fibre afferent pathways greatly contribute to afferent sensitization in chronic pain conditions including BPS/IC. However, the clinical application of TRPV1 antagonists for chronic pain has been hampered partly due to their adverse events (AEs) such as hyperthermia and impaired noxious heat sensation [ref 1]. Hence, the development of local therapies that can target TRPV1 receptors expressed in the affected organs and their afferent pathways without inducing systemic AEs would be useful for the treatment of chronic pelvic pain conditions such as BPS/IC.

Herpes simplex virus (HSV) has a natural property that it is transported from primary infection sites into the afferent neurons, which could offer an organ-specific treatment for nerve-related diseases. A previous study indicated that deletion of the channel pore-forming domain of TRPV1 receptor inhibits the assembly including an aqueous pore, which blocked the channel function [ref 2]. In addition, we recently found that protein phosphatase 1α (PP1α) as a modulator of TRPV1 receptor activity using an HSV vector cDNA library screen method to identify genes that inhibit TRPV1 activation. Therefore, this study investigated the effect of HSV vectors-mediated gene delivery of non-functional, poreless TRPV1 or PP1α on TRPV1-mediated bladder overactivity and pain-related behaviour in rats.

Study design, materials and methods

Replication-deficient HSV vectors encoding porelss TRPV1 or PP1α were injected into the bladder wall of adult female Sprague-Dawley rats. vHG or DAP-green fluorescent protein (GFP) vectors, which have the same mutant background as poreless TRPV1 or PP1α vectors, respectively, except that ICP4 genes were replaced with GFP transgene, were used as control vectors. Cystometry (CMG) under urethane anaesthesia was performed 1 week after viral inoculation to evaluate the effects on bladder overactivity induced by resiniferatoxin (RTX, a TRPV1 agonist). In another group of animals, nociceptive behaviour such as licking (lower abdominal licking) and freezing (motionless head-turning) was observed for 15 min after intravesical RTX application (3μM for 1 min) 2 weeks after viral inoculation. Using immunohistochemistry, GFP expression in L6-S1 dorsal root ganglia (DRG) and the bladder as well as c-Fos positive cells in response to intravesical RTX application in the L6 spinal cord dorsal horn were also evaluated.

Results

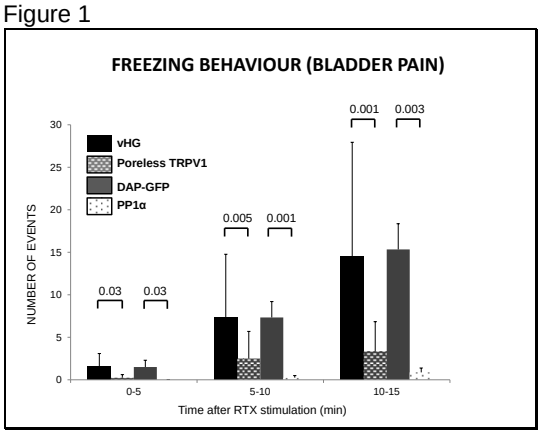
GFP expression was observed in the bladder and L6-S1 DRG neurons 2 weeks after the vHG or DAP-GFP control vectors. In CMG, the poreless TRPV1 group showed a significantly (p=0.03) smaller reduction (55±3%, n=6) in intercontraction intervals (ICI) after RTX infusion than the vHG group (68±4%, n=6). Similarly, the PP1α group showed a significantly (p=0.03) smaller reduction (46±1%, n=5) in intercontraction intervals (ICI) after RTX infusion than the DAP-GFP group (64±2%, n=6) (Table1). In pain behavioural studies, there was no significant difference of the number of RTx-induced licking events, which represent urethral pain, between two groups. However, the number of the RTX-induced freezing events, which are correlated with bladder pain sensation, was significantly (p<0.05) decreased in the porelss TRPV1 group than the vHG group. Also, the PP1α group showed significantly (p=<0.05) less number of freezing events than the DAP-GFP group (Fig. 1). The number of c-Fos positive cells in the dorsal commissure (DCM) and spinal parasympathetic nucleus (SPN) regions of the L6 spinal dorsal horn was significantly (p<0.05) smaller in the poreless TRPV1 group (7±2 and 3±0.9 per section, respectively, n=7) than in the vHG control group (24±3 and 9±1 cells per section, respectively, n=6). Similarly, the number of c-Fos positive cells in the DCM and SPN regions of the L6 spinal dorsal horn was significantly (p<0.05) smaller in the PP1α group (9±2 and 3±1 cells per section, respectively, n=8) than in DAP-GFP group (24±3 and 9±1 cells per section, respectively, n=6).

Table.1

CMG (intercontraction intervals)

	n	Saline infusion (sec)	RTx infusion (sec)	Reduction (%)
vHG	6	1112±220	339±58	68±4
Poreless TRPV1	6	975±164	412±61	55±3
p value		0.62	0.40	**0.03**
DAP-GFP	6	1099±80	374±21	64±2
PP1α	5	925±48	536±42	46±1
p value		0.41	0.32	**0.03**

Figure 1



Interpretation of results

These results indicate that; (1) replication-deficient HSV vectors injected into the bladder wall are transported to L6-S1 DRG neurons through bladder afferent pathways, (2) The vectors expressing non-functional, poreless TRPV1 inhibited bladder overactivity and bladder pain behaviour induced by TRPV1 receptor activation in the bladder and (3) HSV vector-mediated PP1α gene delivery is similarly effective to reduce TRPV1 receptor-induced bladder overactivity and bladder pain behaviour.

Concluding message

Gene therapy with replication-deficient herpes simplex virus vectors encoding poreless TRPV1 or PP1α, which can suppress TRPV1 receptor activation in the bladder and bladder afferent pathways, could be a novel treatment that can avoid systemic adverse events for hypersensitive bladder disorders such as BPS/IC.

References

1. Szallasi A et al., Expert Opin Investig Drugs 21, 1351-69, 2012
2. Srinivasan R et al., Nat Methods 4, 733-9, 2007

Disclosures

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