236

Oguchi T[1], Yokoyama H[1], Nishizawa O[2], Goins W F[3], Goss J R[3], Glorioso J C[3], Yoshimura N[1]
1. *Urology, University of Pittsburgh,* ***2.*** *Urology, Shinshu University,* ***3.*** *Microbiology and Molecular Genetics, University of Pittsburgh*

EFFECT OF HERPES SIMPLEX VIRUS (HSV) VECTOR-MEDIATED INTERLEUKIN-4 (IL-4) GENE THERAPY ON BLADDER OVERACTIVITY AND NOCICEPTION

Hypothesis / aims of study

Although the etiology of bladder pain syndrome/interstitial cystitis (BPS/IC) is not fully understood, bladder inflammation associated with production of inflammatory cytokines or chemokines has been proposed as potential pathophysiology of the disease. For example, IL-2, IL-6, TNFα or other chemokine levels in urine or bladder tissues are significantly higher in IC patients than controls [1, 2]. On the other hands, IL-4 is a prototypical anti-inflammatory cytokine, which can inhibit secretion of cytokines such as IL-1β, TNFα, and IL-6 [3]. However, because of its pleiotropic effects on immune system, systemic treatment with IL-4 might cause various side effects. Therefore, we examined the effects of localized and targeted gene therapy using replication-deficient HSV vectors expressing murine IL-4 (S4IL4), on bladder overactivity and pain behaviour induced by intravesical application of resiniferatoxin (RTx) in rats.

Study design, materials and methods

The HSV vector expressing β-galactosidase (LacZ) was used as control (SHZ). Twenty μl of viral suspension (3.9×10^9 pfu/ml S4IL4 or 5×10^8 pfu/ml SHZ) was injected into the bladder wall of female SD rats under pentobarbital anesthesia. (1)One week later, cystometry was performed under urethane anesthesia. After 2-hour saline infusion (0.04 ml/min), RTx solution (10nM) was continuously administered into the bladder to induce bladder overactivity. (2)Two weeks after HSV injection, in an awake condition, 3μM RTx (0.3ml for 1 min) was administrated into the bladder through a temporary indwelled urethral catheter to induce bladder pain. Nociceptive behaviours such as licking (lower abdominal licking) and freezing (motionless head turning) were recorded ever 5 sec for 15 min. (3) Murine IL4 mRNA in L6 dorsal root ganglia (DRG) and the bladder was examined using RT-PCR.

Results

(1) There was no significant difference in intercontraction interval (ICI) between S4IL4 (n=9) and SHZ (n=8) groups during 2-hour saline infusion (p=0.15). After RTx administration, SHZ-treated rats showed a significant reduction in ICI (p<0.05) while ICI was not significantly altered (p=0.47) after RTx administration in S4IL4-treated rats (Figs.1-2).

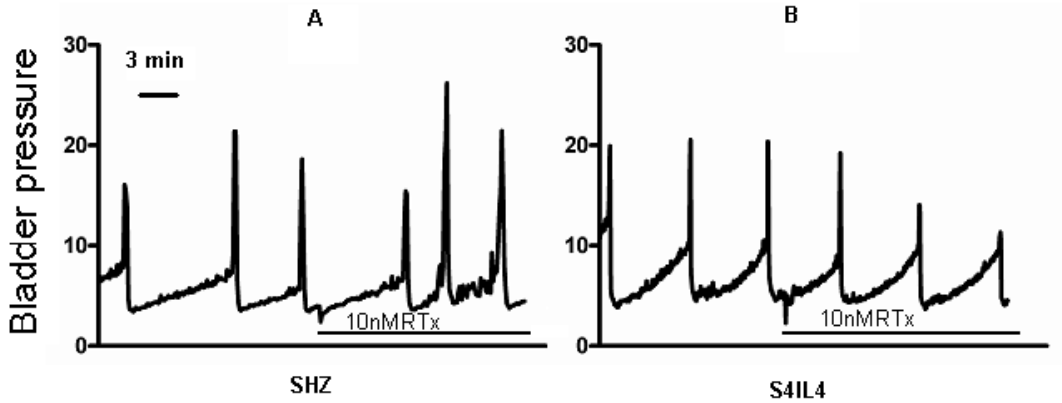

Fig. 1. A. Representative cystometrograms before and after intravesical application of 10nM RTx. A. SHZ-injected rat. After 10nM RTx intravesical administration, bladder overactivity evidenced by a reduction in ICI was induced. B. S4IL4-treaetd rat. No apparent reduction in ICI was observed after intravesical RTx administration. Bars underneath the traces indicate the period of RTx administration into the bladder.

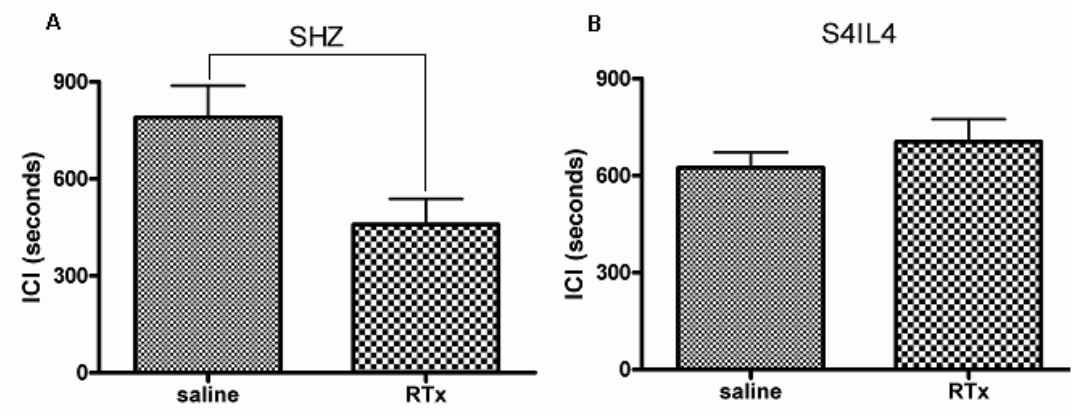

Fig. 2. Changes in ICI (intercontraction interval) during cystometry after intravescial RTx administration. A. SHZ-treated rats (n=8). B. S4IL4-treated rats (n=9). Following RTx administration, ICI of SHZ rats was significantly reduced ($p<0.05$) while no significant reduction in ICI was found in S4IL4 rats ($p=0.47$)

(2) Freezing behaviour, which is mainly dependent on pelvic nerve activation, was suppressed in the S4IL4 group (n=8) by 27% ($p<0.05$) compared to the SHZ group (n=7) (Fig. 3). Licking behaviour, which is predominantly pudendal nerve-dependent, was not different between S4IL4 and SHZ groups.

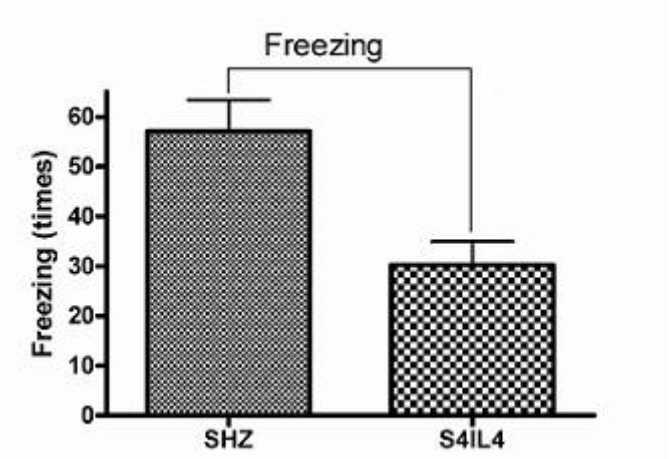

Fig. 3. Freezing behaviour induced by intravescial RTx administration. In S4IL4 rats (n=8), the number of freezing behaviour counted during 15 min after RTx treatment was significantly ($p<0.05$) smaller compared with SHZ-treated rats (n=7).

(3) Murine IL4 mRNA was detected in L6 DRG and the bladder of S4IL4-treated rats.

Interpretation of results

These results indicate that HSV vectors expressing anti-inflammatory IL-4 gene are transferred to bladder afferent pathways after bladder wall injection and that IL-4 gene delivery into the bladder and bladder afferent pathways reduces bladder overactivity and nociceptive behaviour induced by chemical bladder irritation in rats.

Concluding message

HSV vector-mediated IL-4 gene therapy could be a new treatment modality for urinary frequency and/or bladder pain in patients with BPS/IC.

References

1. Urology 1999; 54(3) : 450-453
2. J Urol 2010; 183(3) : 1206-1212
3. Blood 1990; 76(7) : 1392-1397

Specify source of funding or grant	**NIH DK57267, DK68557 and P01DK44935**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee of University of Pittsburgh**