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INHIBITION OF BLADDER HYPERSENSITIVITY BY INTERLEUKIN 4 (IL-4) GENE THERAPY USING HERPES SIMPLEX VIRUS (HSV) VECTORS IN RATS WITH CYCLOPHOSPHAMIDE INDUCED CYSTITIS

Hypothesis / aims of study

Painful bladder syndrome/interstitial cystitis (PBS/IC) is a serious disease whose main symptoms are bladder pain and frequent urination. We first examined whether chemical cystitis induced by cyclophosphamide (CYP) enhances pain behaviour elicited by bladder irritation using resiniferatoxin (RTx). Furthermore, we investigated effects of gene therapy using replication-deficient HSV vectors expressing anti-inflammatory cytokine IL-4 (S4IL4) on pain behaviour and bladder overactivity induced by intravesical application of RTx in this cystitis rat model.

Study design, materials and methods

(1) Saline or CYP200mg/kg was injected to female SD rats intraperitoneally. Two days later, in an awake condition, 0.3uM RTx (0.3ml, 1 min) was injected to the bladder through a urethral catheter to evaluate nociceptive behaviours such as licking (lower abdominal licking) and freezing (motionless head-turning) were counted and recorded every 5 seconds for 15 minutes [1]. Urine volume and frequency were recorded simultaneously in metabolic cages (n=5 each).

(2) The replication-deficient HSV vector expressing LacZ, but not IL-4, was used as a control (SHZ). Two weeks prior to study, 20 μl of viral suspension (3.9×10^9 pfu/ml S4IL4 or 5×10^8 pfu/ml SHZ) were injected to the bladder wall. CYP200mg/kg was injected to HSV infected rats 2 days before the study. In an awake condition, 0.3uM RTx (0.3ml, 1 min) was injected to the bladder to evaluate nociceptive behaviours (licking and freezing). Urinary frequency was also compared (n=6 each).

Results

(1) Freezing behaviour, which corresponds to bladder-derived pain [1], was significantly increased in CYP cystitis rats compared to saline-treated control rats (72 ± 17 vs. 13 ± 5times, p<0.001) (Fig.1). Bladder volume (urine volume/ micturition) was significantly decreased in CYP cystitis rats compared to controls (0.2 ± 0.03 vs. 0.61 ± 0.08 ml, p<0.01) (Fig.2).

(2) Freezing behaviour induced by 0.3ul RTx was significantly decreased in S4IL4 rats by 72% compared to SHZ rats (24 ± 3 vs. 87 ± 10 times, p<0.01) (Fig.3). Also, bladder volume after RTx stimulation was significantly increased in S4IL4 rats compared to SHZ rats (0.55+-0.14 vs. 0.28+-0.08 ml, p<0.05) (Fig.4).

Figure 1

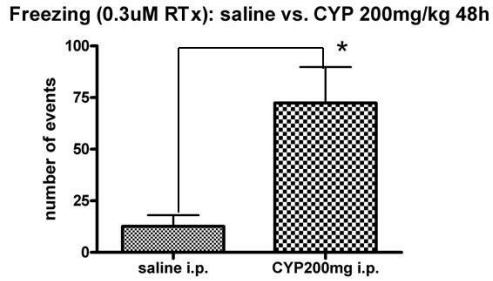


Figure 2

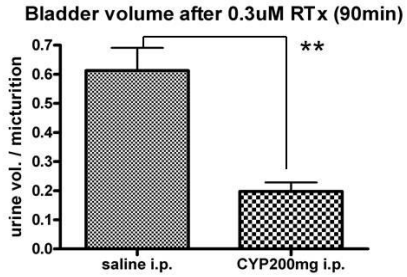


Figure 3

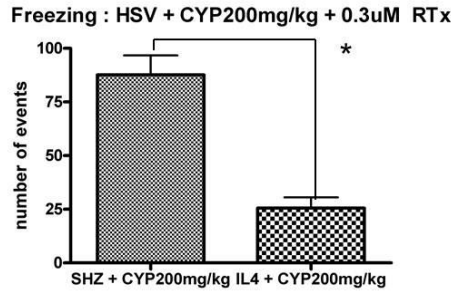
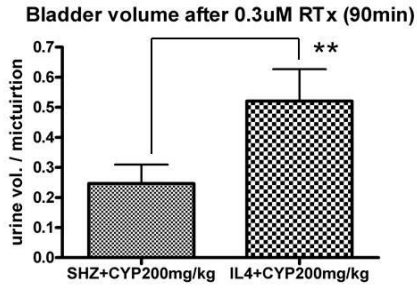


Figure 4



Interpretation of results

(1) In subacute (2 days) cystitis induced by CYP, a low concentration of RTx, which induces minimal behavioural changes in normal rats, was enough to elicit bladder pain behaviour and reduce bladder volume, indicative of bladder hypersensitivity after CYP-induced bladder inflammation.

(2) HSV vector-mediated IL-4 gene therapy suppressed bladder overactivity and enhanced pain behaviour in the subacute CYP cystitis rat model. Since we previously showed that the HSV-IL4 treatment induces IL-4 expression in the bladder and lumbosacral dorsal root ganglia (DRG) and reduces inflammatory cytokines in the bladder in an acute cystitis rat model [2], anti-inflammatory IL-4 gene therapy seems to be also effective to suppress inflammation in bladder and DRG to reduce bladder pain and overactivity in this subacute cystitis condition.

Concluding message

Bladder inflammation evokes bladder hypersensitivity and overactivity, which are suppressed by anti-inflammatory cytokine therapy mediated by replication-deficient HSV vectors. Thus, IL-4 gene therapy could be a new strategy for treating bladder pain and/or urinary frequency in patients with PBS/IC.

References

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2. Oguchi T, Funahashi Y, Yokoyama H et al: Effect of herpes simplex virus vector-mediated interleukin-4 gene therapy on bladder overactivity and nociception.Gene Ther. 2013 Feb;20(2):194-200.

Disclosures

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