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Masunaga K[1], Yoshida M[2], Nagata T[3], Tsukui W[1], Homma Y[4], Kasuya Y[1]

1. *Department of Urology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan,* ***2.*** *Department of Urology, Kumamoto Hospital of Japan Labor Health and Welfare Organization, Kumamoto, Japan,* ***3.*** *Department of Urology, Toshiba General Hospital, Tokyo, Japan,* ***4.*** *Department of Urology, University of Tokyo, Tokyo, Japan*

NON-NEURONAL ADENOSINE TRIPHOSPHATE (ATP) RELEASE AND IMMUNOHISTOCHEMICAL EXAMINATION OF BLADDER IN HYDROCHLORIC ACID (HCL)-INDUCED CYSTITIS RATS

Hypothesis / aims of study

Interstitial cystitis (IC) is one of chronic diseases of the urinary bladder characterized by urinary frequency, urgency, and pelvic pain associated with bladder distention. Although the symptoms and specific findings during cystoscopy are obvious, the etiology remains unclear. Recently, it has been suggested that adenosine triphosphate (ATP) releases from the bladder mucosa are related to the pathophysiology of IC. And, it has been reported that the histological characteristics of the bladder induced by hydrochloric acid (HCl) instillation is similar to that of clinical IC. Therefore, the present study was performed to investigate bladder function, non-neuronal ATP release from bladder mucosa, and immunohistological change of bladder in HCl-induced cystitis rats.

Study design, materials and methods

In female SD rats, 0.2 ml of 0.4N HCl (HCl group) or physiologic saline (sham group) were instillated into the bladder through a transurethral catheter, under anesthesia. After 1 week, cystometry was performed under urethane anesthesia in each rat. Smooth muscle strips were dissected from the bladder body, and were suspended in a 20-ml organ bath filled with Krebs-Henseleit solution. Microdialysis probe was inserted into the strip, and Ringer solution was perfused into the probe at a constant flow rate of 2 µl/min. For measurement of non-neuronal ATP release from mucosa, the dialysate was collected under resting tension (0 to 40 mN) with TTX pre-treatment, and the amount of ATP releases was measured by luciferine-luciferase assay. In the separate experiments, isolated bladder specimens were stained immunohistochemically using polyclonal rabbit P2X3, TRPV1, and TRPV4 receptor antibodies.

Results

In the cystometric findings, urinary frequency was significant increased in HCl group, as compared to sham group (micturition interval (min): 5.0±1.1 vs 8.3±0.8). Non-voiding contractions were observed in HCl group. In both groups, non-neuronal ATP releases from mucosa increased resting tension-dependently. And, the amount of non-neuronal ATP releases was significantly higher in HCl group than that in sham group (Figure). In immunohistochemical staining of HCl group, P2X3 positive cells significantly increased in the submucosal and smooth muscle layers, and TRPV4 immunoreactivity significantly increased in the mucosa, as compared to sham group. However, there were not significant differences in the characteristics of immunohistochemical staining for TRPV1 between two groups.

Interpretation of results

The present study suggested that the increases of non-neuronal ATP release, up-regulation of P2X3, and TRPV4 receptors may contribute to the urinary frequency and non-voiding contractions in HCl-induced cystitis rats.

Concluding message

In the present study, histological findings also showed that HCl instillation led to be similar conditions to IC. Therefore, these findings seem to be useful to clarify the pathophysiology of IC.

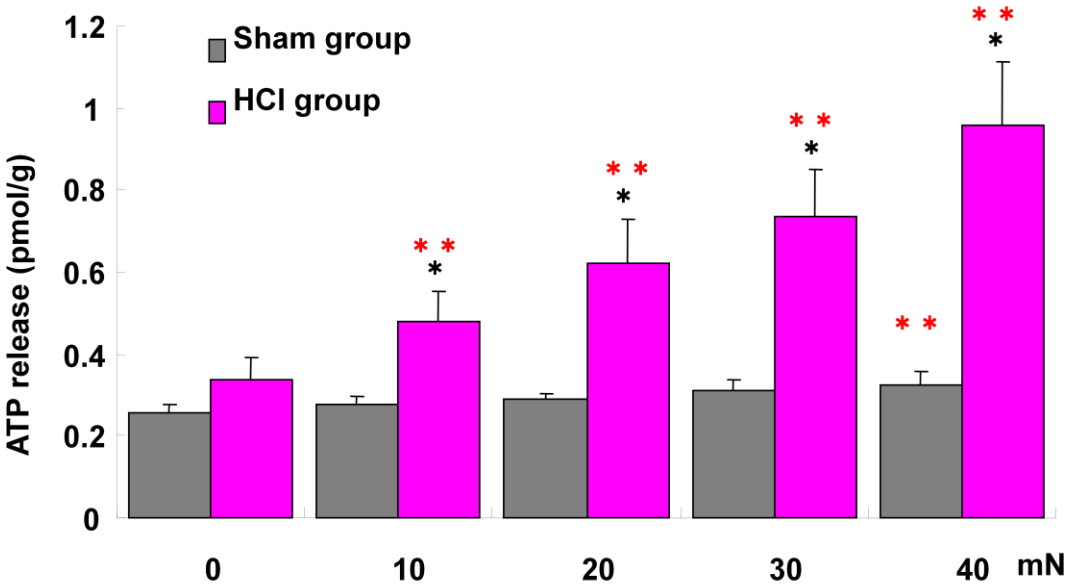

Fig. The effects of stretch of bladder strips on non-neuronal ATP releases in sham and HCl groups. Resting tension was changed from 0 to 40 mN.
Each bar shows mean±S.E.M..
＊Significantly different from ATP releases in sham rats (n=12) (p<0.05)
＊＊Significantly different from ATP releases when the tension is 0 mN (n=12) (p<0.05)

Specify source of funding or grant	None
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	The ethics committe of Kumamoto University