

IMPROVEMENT BY EVIPROSTAT TREATMENT OF BLADDER DYSFUNCTION AND ALTERED LEVELS OF PHARMACOLOGICAL RECEPTORS AND URINARY CYTOKINES IN RATS WITH CYCLOPHOSPHAMIDE-INDUCED CYSTITIS

Hypothesis / aims of study

Interstitial cystitis (IC) is a chronic, abacterial inflammatory disease of the bladder characterized by urinary frequency, urgency and suprapubic pain associated with bladder filling and relieved by voiding, but its exact etiology and pathogenesis remain unclear and effective treatment is not established. Currently, there are increasing evidences to suggest the idea that the abnormality of bladder receptors and urinary cytokines may implicate in the development of cystitis in rats [1,2]. The present study aimed to characterize the pharmacological effects of a phytotherapeutic agent, Eviprostat (EVI), by measuring urodynamic parameters, bladder muscarinic and purinergic receptors and urinary cytokines (interleukin-IL-6, IL-1 β and IL-17) in rats with cystitis induced by cyclophosphamide (CYP).

Study design, materials and methods

Cystitis model was induced by injecting CYP (150 mg/kg, i.p.) in female Sprague-Dawley rats (9 weeks old). Rats were divided into sham group, CYP-treated group, CYP+EVI-treated group. EVI (36 mg/kg) was orally administered for 7 days. On the last day of treatment, the mechanical responses of bladder in CYP-treated rats were monitored by the cystometric method under an anaesthesia. The muscarinic and purinergic receptors in rat tissues were measured by radioreceptor assays using (N-methyl-³H) scopolamine methyl chloride (³H]NMS) and $\alpha\beta$ -methylene-ATP (2,8-³H) tetrasodium salt (³H] $\alpha\beta$ -MeATP), respectively, and binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) were estimated by nonlinear regression analysis using Graph Pad Prism. Urinary cytokines were measured by ELISA kits.

Results

In the cystometry of CYP-treated rats compared with sham rats, the micturition interval and micturition volume were significantly decreased and the frequency of micturition, basal pressure, and residual urine volume were significantly increased. The repeated administration of Eviprostat significantly increased the micturition interval and micturition volume and decreased the frequency of micturition, basal pressure, and residual urine volume compared with CYP-treated rats which are in agreement with previous study [3]. As shown in Table 1, the B_{max} for specific binding of ³H]NMS and ³H] $\alpha\beta$ -MeATP was significantly decreased in the bladder of CYP treated rats compared with sham rats. Thus, CYP treatment was shown to cause down-regulation of muscarinic and purinergic receptors in the bladder of rats. There was significant increase in the B_{max} for ³H]NMS and ³H] $\alpha\beta$ -MeATP in the bladder of rats treated with CYP+EVI, compared with CYP-treated rat bladder. The elevation in urinary cytokine level was seen in CYP-treated rats, and this alteration was effectively attenuated by repeated treatment with Eviprostat (Fig. 1).

Interpretation of results

The present results revealed down-regulation of muscarinic and purinergic receptors in the bladder and elevation of cytokines in urine of rats with chemically induced cystitis, suggesting significant involvement of bladder receptors and urinary cytokines in the pathophysiology of cystitis. Moreover, the alteration of urodynamic parameters, pharmacologically relevant receptors and urinary cytokines in CYP-treated rats was attenuated by the repeated treatment with EVI at pharmacological doses.

Concluding message

Alteration of bladder muscarinic and purinergic receptors and urinary cytokines in CYP-treated rats may be implicated in the pathophysiology of cystitis. EVI may be useful in the pharmacological therapy of cystitis.

Table 1. K_d and B_{max} for specific binding of ³H]NMS and ³H] $\alpha\beta$ -MeATP in sham, cyclophosphamide (CYP) alone and CYP + EVI-treated rats

Variable	K_d (pM)	B_{max}
[³H]NMS		(fmol/mg protein)
Bladder		
Sham	194 \pm 14	160 \pm 15
CYP	171 \pm 10	76.4 \pm 4.3***
CYP + EVI	197 \pm 14	139 \pm 15 [†]
Submaxillary gland		
Sham	154 \pm 12	176 \pm 19
CYP	144 \pm 12	188 \pm 13
CYP + EVI	144 \pm 18	147 \pm 10
[³H]$\alpha\beta$-MeATP		(pmol/mg protein)
Bladder		
Sham	906 \pm 68	12.5 \pm 0.9
CYP	817 \pm 91	5.2 \pm 0.5***
CYP + EVI	810 \pm 52	7.9 \pm 0.2*** [†]

Values are expressed as mean \pm S.E. (n=7–10). Asterisk shows the significant difference from the values in sham, ***P<0.001. Dagger shows the significant difference from the values in CYP-treated rats, [†]P<0.05.

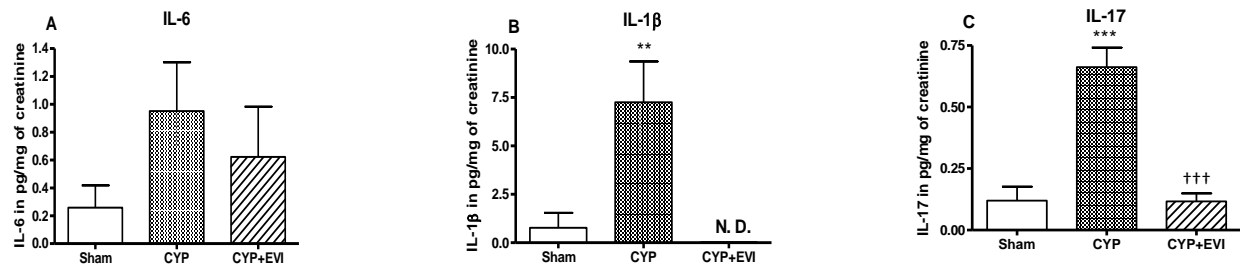


Figure 1. Effects of Eviprostat (EVI) on urinary cytokines {IL-6 (A), IL-1 β (B) and IL-17 (C)} level in cyclophosphamide (CYP)-induced cystitis rats.

Bars represent the mean \pm SEM (n=6). Asterisk shows the significant difference from the values in sham, **P<0.01 ***P<0.001. Dagger shows the significant difference from the values in CYP-treated rats, †††P<0.001. N.D.-Not detected.

References

1. Neurosci Lett 436: 81-84, 2008
2. J Urol 73 (2): 421-426, 2009
3. Int J Urol 15: 356-360, 2008

Disclosures

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