414

Kiuchi H[1], Yamamoto K[1], Nakayama J[1], Takao T[1], Fukuhara S[1], Hirai T[1], Ueda T[1], Komori K[1], Matsuoka Y[1], Miyagawa Y[1], Tsujimura A[1], Okuyama A[1]

1. *Osaka Universty Graduate school of Medicine*

SESQUITERPENE LACTONE PARTHENOLIDE AMELIORATES BLADDER HYPERACTIVITY AND INFLAMMATION IN A CYCLOPHOSPHAMIDE-INDUCED CYSTITIS MODEL BY INHIBITING THE ACTIVATION OF NF-KAPPA B

Hypothesis / aims of study

Nuclear factor-kappa B (NF-kappaB) has been implicated in chronic inflammatory disease, and is thought to plays a central role in regulating inflammatory mediators. The activation of NF-kappaB is increased predominantly in bladder urothelial cells in the patients with interstitial cystitis/ painful syndrome, suggesting that inflammatory responses in interstitial cystitis can be exacerbated possibly by persistent activation of NF-kappaB. We investigated the therapeutic potential of a sesquiterpene lactone parthenolide (PTN), an inhibitor of NF-kappa B, both in vitro and vivo experiments.

Study design, materials and methods

We studied the effect of PTN on suppression of NF-kappaB activation and the synthesis of IL-6 by HUC-1 cells, human benign urothelial cell lines. HUC-1 cells were preincubated with PTN, then stimulated with tumor necrosis factor-alpha (TNF-alpha) or IL-1 beta. The nuclear translocation of NF-kappaB p65 was determined by immunofluorostaining. The levels of NF-kappaB p65, inhibitory kappaB-alpha (IkappaB-alpha) and the phospholilation of these proteins in the cells and IL-6 release in the cell culture media were measured.

In vivo study, female Sprague-Dawley rats (220–250 g) were used as follows: sham+vehicle (n =5), cyclophosphamide (CYP)+vehicle (n = 7), CYP+PTN(n = 8). In a separate group of rats, PTN (a once-daily subcutaneous administration of 3 mg/kg) or vehicle were administered 3 days before a single intraperitoneal injection of CYP (150 mg/kg). At 24 hours after CYP injection, cystometry was performed under urethane anaesthesia. Urodynamics were evaluated to quantify intercontractile interval (ICI), maximum voiding pressure and postvoidal urine volume. The bladder from CYP-induced cystitis rats was also assessed histopathologically, bladder edema by bladder weight, and the NF-kappaB activation by Western blotting.

Results

Stimulation of HUC-1 cells by TNF-alpha or IL-1 beta increased nuclear translocation of NF-kappaB and phospholilation of NF-kappaB, which was suppressed by the addition of PTN in dose dependent manner. TNF-alpha or IL-1 beta stimulation also increased the degradation of IkappaB-alpha and increased phospholilation of IkappaB-alpha, which was restored with the addition of PTN. Increased activation of NF-kappaB was also associated with increased levels of IL-6. PTN significantly reduced the IL-6 production.

On cystometrography, there was frequent voiding in CYP cystitis rats. Administration of PTN had a significant decrease in ICI ($p<0.05$). Histopathologicaly, PTN significantly reduced the bladder damage which was induced by CYP. Increased bladder weight in CYP rats was significantly reduced by PTN administration. Moreover, Western blot analysis revealed that CYP activated NF-kappaB in the rat bladder, and PTN significantly reduced NF-kappaB activation in the bladders.

Interpretation of results

PTN therapy is an effective treatment in reducing bladder inflammation and prevention of pollakisuria in well-established cystitis rat models. Our data suggest that PTN therapy may be a novel approach for the treatment of interstitial cystitis/ painful syndrome,.

Concluding message

Sesquiterpene lactone parthenolide ameliorates bladder hyperactivity and inflammation in a cyclophosphamide-induced cystitis model by inhibiting the activation of NF-kappa B

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 Comittee of Experimental Animal Sciences Osaka University Medical School**