The Role of Chloride Channels on Cyclophosphamide-induced Overactive Bladder in Rats.

Yuh-Chen Kuo[1], Hong-Chiang Chang[2], Ju-Ton Hseih[2]*

[1]*Department of Urology, Taipei City Hospital, Taipei, Taiwan*

[2]*Department of Urology, School of Medicine, National Taiwan University, Taipei, Taiwan*

Aims of study

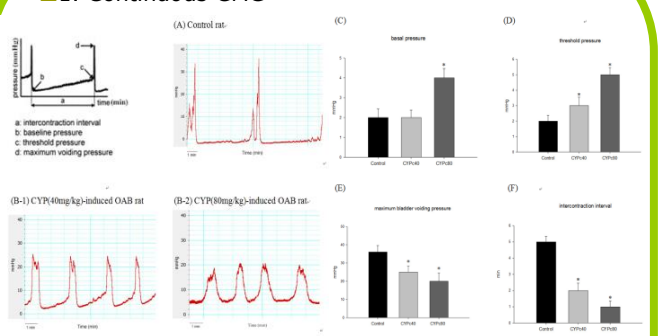
- Overactive bladder (OAB) is mostly caused by detrusor uninhibited contraction.
- Our previous study has demonstrated the important role of chloride channels on the regulation of urinary bladder smooth muscle tone.
- We investigated the alteration of distribution and functional expression of CLC-3 and CLCA4 chloride channel on bladder tissue (urothelium and muscle) in rats with cyclophosphamide (CYP)-induced OAB.

Materials and Methods

- A total of 96 adult male Wister rats (10–12 weeks) were divided into three groups (CYPc40, CYPc80 and control).
- CYP-induced OAB was provoked by four i.p injections on day 0, 2, 4, 6 (CYPc40: 40 mg/kg and CYPc80: 80 mg/kg).
- Control rat received saline injections.
- The experiments were performed on day 7.
- We conducted a strategy involving:
  - (1) Continuous infusion cystometry (CMG) under anaesthesia to record the basal pressure (BP), maximum bladder voiding pressure (MBVP), intercontraction interval (ICI) and threshold pressure (TP).
  - (2) Urinary nerve growth factor (NGF) detection before CMG.
  - (3) Western blot analysis and immunohistochemistry (IHC) of CLC-3 and CLCA4 protein on rat bladder tissues.
  - (4) Reverse transcription-polymerase chain reaction (RT-PCR) of the mRNA for CLC-3 and CLCA4 channels in normal and CYP-OAB bladder tissue.
  - (5) The CMG parameters, urine NGF level, molecular expressions of chloride channels are compared between rats in control, CYPc40 and CYPc80 groups.

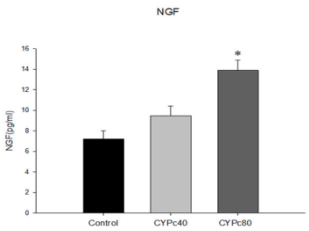
Results

1. Continuous CMG



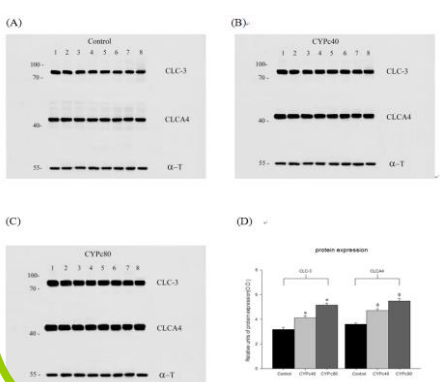
After CYP treatment, CMG showed the BP and TP increased significantly, and MBVP and ICI decreased significantly. There was a dose dependent manner.

2. Urinary nerve growth factor (NGF)



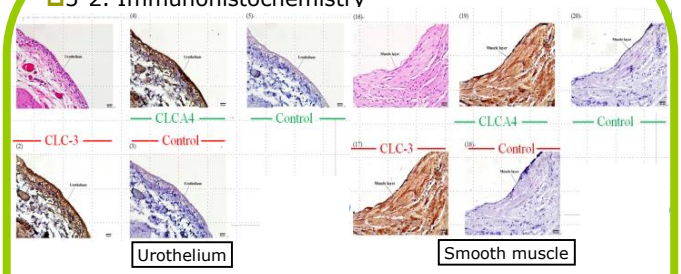
The urine NGF significantly increased in the CYPc80 group when compared with the control group. The urine NGF in CYPc40 also increased. However, there was no significant difference when compared with control group.

3-1. Western Blot Analysis of ClC-3 or CLCA4



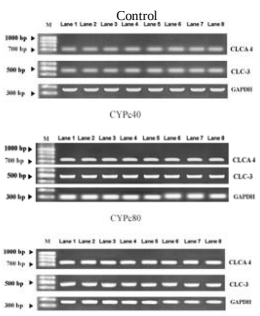
Western blotting technique showed that both the CLC-3 and CLCA4 proteins on rat bladder tissues were significantly up-regulated in the CYP-treated groups in a dose dependent manner.

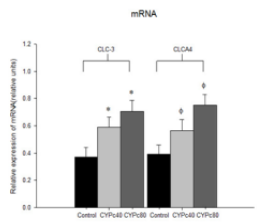
3-2. Immunohistochemistry



After CYP injection, mild hyperplasia of the urothelium and edema of the suburohelial space and muscle layer were found. IHC demonstrated the CLC-3 and CLCA4 could be expressed on both urothelium and muscle layer.

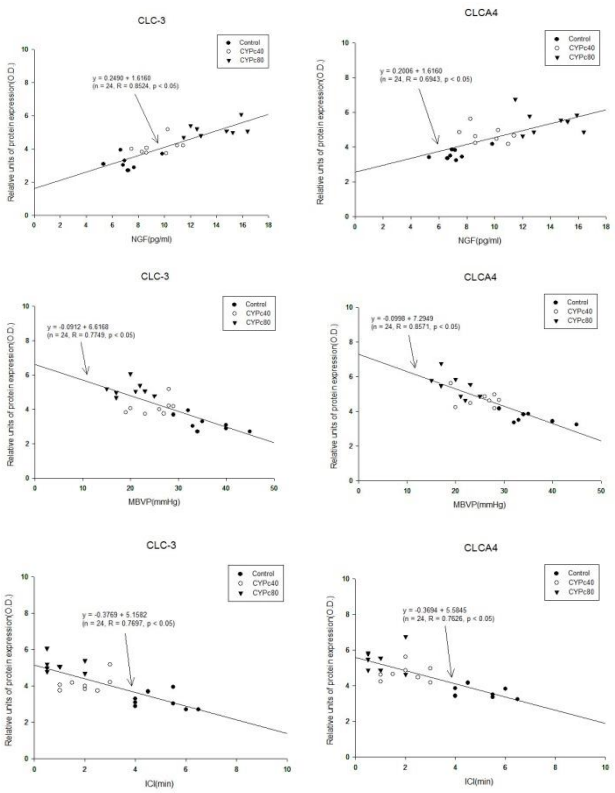
4. Quantitative RT-PCR Analysis of ClC-3 or CLCA4 mRNA





As compared to the control group, the mRNA expressions of ClC-3 and CLCA4 were significantly increased in the CYP-treated group in a dose dependent manner.

5. Correlations of protein and mRNA expressions of chloride channels with urinary NGF and CMG parameters.



The protein and mRNA expressions of CLC-3 and CLCA4 chloride channels were positively correlated with the NGF levels and negatively correlated with the MBVP and ICI (all $p<0.05$).

Conclusions

- This study demonstrated that a model using repeated injection of low dose CYP (40 or 80 mg/kg) could successfully induce OAB like status in rats which was illustrated by CMG.
- The above results suggest that both the CLC-3 and CLCA4 chloride channels may play important roles in the pathogenesis of OAB and provide possible new therapeutic targets in treating OAB.

Disclosures Statement: None