

IMPACT OF RHO-KINASE INHIBITOR HYDROXYFASUDIL IN PROTAMINE SULPHATE INDUCED CYSTITIS IN RATS.

Hypothesis / aims of study

To investigate the prevention of detrusor overactivity and bladder tissue damage with hydroxyfasudil in protamine sulfate (PS) induced cystitis rat model *in vivo* and *in vitro*.

Study design, materials and methods

Animals were divided into four groups. Chemical induced cystitis model was created by administrating PS intravesical with PE50 catheter by transurethral for three days. At the end of the third day, Rhokinase (ROCK) inhibitor hydroxyfasudil or a corresponding volume of saline was administrated intraperitoneally. There were eight animals in each group. Group 1: PS and hydroxyfasudil were administrated. Group 2: PS and saline were administrated. Group 3: Hydroxyfasudil was administrated intraperitoneally. Group 4 was the control Group, PS and hydroxyfasudil were not administered. *In vivo* micturition behaviors were recorded. *In vitro* contractions of bladder tissue strips were measured in tissue-bath by administering acetylcholine (Ach) and potassium chloride (KCl). Concentration response curves were obtained. Biochemical analyses were performed for oxidative stress and pathological evaluations were investigated.

Results

The mean weight of animal experiments was 280.2g ($\pm 30,1$). There was significantly higher frequency of micturition, lower volume and total urine output after PS administration in groups 1 and 2. *In vitro* contraction responses of bladder strips to KCl and Ach were statistically higher in Group 2 than other groups (Table 1 and Table 2). There were significantly lower LPO (lipid peroxidase) levels in Group 1 than Group 2 ($p=0.016$). Level of GSH (Glutathione) was higher in Group 1 than Group 2 ($p=0.001$). There were roughly the apparent more hyperemic blood vessels and transitional cells were spilled with severe degenerations under the microscope in Group 2 than other Groups.

Interpretation of results

PS induced cystitis, harmful substances in the urine are able to irritate the uretelium and help to stimulate the sensory for pain with the activation of C fibers. Therefore, PS can cause severe symptoms in rats that may be similar to the symptoms in patients with IC. In our study, there was higher micturition for groups 1 and 2 than for groups 3 and 4 during the first 3 days of PS administration that was significantly higher.

Hydroxyfasudil can be used in the asymptomatic period of IC for preventing OAB. Additionally, according to our findings, hydroxyfasudil seems to be a promising treatment for symptoms of OAB in patients with IC.

Decrease in LPO, increase of GSH activities and increase of SOD (superoxidase dismutase), CAT (catalase) enzyme activities were provided by hydroxyfasudil in PS induced cystitis rat model

Evidences of chemical cystitis were determined in groups 1 and 2 in pathological slides. Severe inflammation was observed in group 2, in which fibrosis, fibroblasts, macrophages and also mast cells were in the pathological findings. Group 1 also showed a reduction in inflammation (Figure 1).

Concluding message

Hydroxyfasudil decreased *in vitro* responses to contractions of bladder smooth muscle strips. Moreover, significant reduction of inflammation by affecting the anti-oxidant defence systems was provided with hydroxyfasudil which can be a potential new therapeutic option for OAB and inflammation in IC.

Table 1. Statistical values of rate of contractions (mean ± Standard deviations) with potassium chloride

Percent Contraction Values (Mean±SD)					p values for pairwise comparisons					
Study Groups					I			II		III
KCl	I	II	III	IV	II	III	IV	III	IV	IV
10mM	1.4±1.3	9.8±4.8	7.2±13.4	6.1±1.9	0.015	0.212	0.009	0.891	0.062	0.544
20mM	5.9±5.0	27.9±17.6	7.2±13.4	14.6±4.9	0.058	0.035	0.063	0.012	0.117	0.001
30mM	21.4±18.0	54.3±16.0	7.2±13.4	50.2±12.8	0.057	0.033	0.004	0.001	0.733	0.0001
40mM	36.9±25.1	83.9±11.3	12.7±26.6	75.2±9.5	0.012	0.016	0.012	0.001	0.344	0.0001
60mM	63.5±24.7	98.5±3.4	7.2±13.4	93.9±5.4	0.017	0.005	0.039	0.001	0.214	0.0001
80mM	99.0±2.2	100.0±0.0	7.2±13.4	100.0±0.0	0.363	0.001	0.363	N/A	N/A	N/A
100mM	98.6±1.6	100.0±0.0	7.2±13.4	99.2±1.1	0.107	0.028	0.299	0.025	0.162	0.026

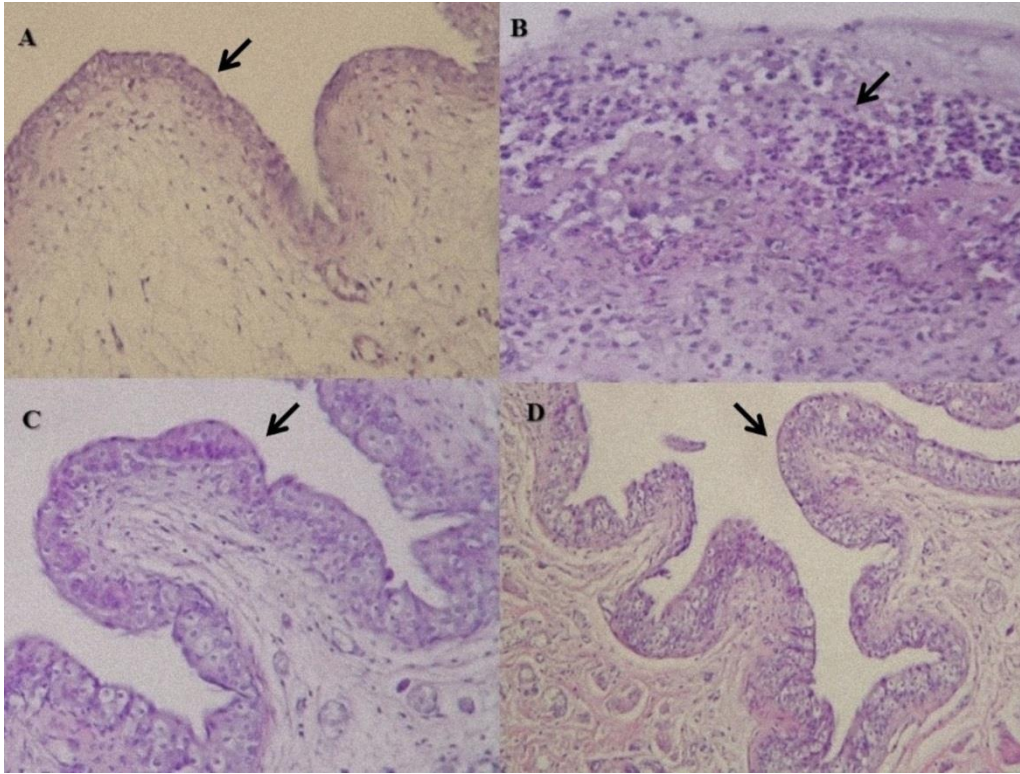
Abbreviations: **KCl**, potassium chloride, **SD**: Standard deviations, **N/A**, not assessed

Table 2. The rate of contractions with Acetylcholine.

Percent Contraction Values (Mean±SD)					p values for pairwise comparisons					
Study Groups					I			II		III
Ach	I	II	III	IV	II	III	IV	III	IV	IV
10 ⁻¹⁰	0.5±1.3	6.6±6.6	7.2±13.4	3.8±1.1	0.082	0.289	0.011	0.945	0.375	0.570
10 ⁻⁹	4.4±6.5	3.8±2.6	7.2±13.4	4.2±0.9	0.849	0.681	0.944	0.599	0.742	0.631
10 ⁻⁸	2.1±3.7	6.3±3.0	7.2±13.4	5.1±1.3	0.118	0.433	0.170	0.895	0.419	0.727
10 ⁻⁷	1.7±1.5	8.0±5.5	12.7±26.6	7.2±2.5	0.075	0.357	0.004	0.701	0.772	0.651
10 ⁻⁶	1.5±3.0	10.1±4.4	7.2±13.4	6.8±2.9	0.021	0.327	0.002	0.676	0.247	0.947
10 ⁻⁵	0.9±1.5	13.9±4.4	7.2±13.4	23.1±11.7	0.001	0.277	0.004	0.314	0.128	0.113
10 ⁻⁴	0.6±1.5	25.1±7.3	7.2±13.4	61.6±30.9	0.0001	0.281	0.004	0.013	0.047	0.017
10 ⁻²	100±0	100±0	33.3±51.6	100±0	N/A	0.025	N/A	0.025	N/A	0.025

Abbreviations: **ACh**, acetylcholine; **SD**, standard deviation; **N/A**, not assessed

Figure 1. Pathologic assessments of bladders are in figures. **A**. Decreased inflammation, degeneration. Arrow shows glycosaminoglycan.(x20) **B**. Severe inflammation reached to tunica muscularis and inflammatory cells can be observed everywhere. Arrow shows severe inflammation (x20) **C**. There is no inflammation, and glycosaminoglycan layer were on the transitional cells. Arrow shows the firm glycosaminoglycan layer (x50) **D**. Normal rat bladder, there is no inflammation with normal glycosaminoglycan layer. Arrow shows the normal rat bladder.(x100).



Disclosures

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