

## ANTICHOLINERGIC DRUGS CAN SUPPRESS NON-NEURONAL ATP RELEASE FROM HUMAN BLADDER UROTHELIUM

### Hypothesis / aims of study

It has been reported that the important role of acetylcholine and adenosine triphosphate (ATP) released from non-neuronal source, especially from urothelium, on pathogenesis of overactive bladder. Recent reports demonstrated abundant muscarinic receptors in human bladder urothelium and suburothelial cells (1, 2). However, the functional role of urothelial muscarinic receptors has not been fully evaluated. To clarify the contribution of urothelial and/or suburothelial muscarinic receptors to bladder function, we investigated the effects of various anticholinergic drugs on the stretch-induced non-neuronal ATP release in human bladder.

### Study design, materials and methods

Human bladders were obtained from 12 patients (10 men and 2 women, 59 to 73 years old), who were undergoing cystectomy due to bladder carcinoma. Smooth muscle strips with and without urothelium were suspended in organ baths filled with Krebs-Henseleit solution. To obtain bladder strips without urothelium, the urothelium was carefully removed using scissors under microscope. Microdialysis probe was inserted into the strip, and Ringer solution was perfused into the probe at a constant flow rate of 2.0  $\mu$ l/min, and dialysate was collected every 10 min in polyethylene tube, under 40 mN resting tension with TTX pre-treatment. The amount of ATP released in the dialysate was measured by luciferine-luciferase assay. Effects of various anticholinergic drugs on stretch-induced non-neuronal ATP release were evaluated.

### Results

Stretch-induced non-neuronal ATP release from bladder strip without urothelium was about 10 % of those from strips with urothelium. Non-neuronal ATP release was significantly inhibited (67% inhibition) by pretreatment with nifedipine ( $10^{-6}$  M) or in  $Ca^{2+}$  free medium. Atropine caused 60 % reduction in stretch-induced non-neuronal ATP releases. Both methoctramine ( $M_2$  receptor selective drug) and 4-DAMP ( $M_3$  receptor selective drug) significantly inhibited the release. However,  $M_1$  receptor selective antagonist (pirenzepine) did not have effect on the release. Propiverine, oxybutynin and tolterodine showed dose-dependent reduction in non-neuronal ATP release. The rank order of the maximum inhibition rate was atropine ( $59.6 \pm 5.8\%$ ) = propiverine ( $55.1 \pm 6.2\%$ ) > tolterodine ( $38.0 \pm 5.0\%$ ) = oxybutynin ( $36.3 \pm 5.4\%$ ). The inhibition rates of atropine and propiverine were significantly ( $P < 0.05$ ) higher than that of tolterodine and oxybutynin.

### Interpretation of results

Removal of urothelium caused significant reduction of non-neuronal ATP release. The data implied that human bladder urothelium/suburothelium was a main source of stretch-induced non-neuronal ATP release in human bladder. Non-neuronal ATP release was significantly inhibited in the treatment with nifedipine or in  $Ca^{2+}$ -free medium, suggesting that intra- and/or extra-cellular  $Ca^{2+}$ -dependent mechanism contribute to non-neuronal ATP release. Furthermore, the present data suggested that the stimulations of  $M_2$  and  $M_3$  receptor subtypes were related to non-neuronal ATP release, and that muscarinic receptor subtype selectivity of anticholinergic drugs might partly contribute to the mechanism of non-neuronal ATP release in human bladder

### Concluding message

It is suggested that non neuronal ATP release is partly regulated by stimulation of urothelial and/or suburothelial muscarinic receptors. It may be possible that one of action mechanisms of anticholinergic drugs is the inhibitory effect of non-neuronal ATP release via blocking of urothelial/suburothelial muscarinic receptors. Furthermore, various anticholinergic drugs may have different inhibitory effects on non-neuronal ATP release in human bladder.

### References

1. J Urol (2006) 176; 367-373.
2. J Urol (2006) 176; 1673-1678.

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