307

Contreras-Sanz A[1], Kennedy-Lydon T[1], Bishara S[2], Lunawat R[2], Khasriya R[2], Taylor K M[3], Malone-Lee J[2], Wildman S S[1]

1. *Royal Veterinary College, London,* ***2.*** *Whittington Campus, UCL, London,* ***3.*** *School of Pharmacy, London*

ALTERED ATP SIGNALLING IN UROTHELIUM OF OAB PATIENTS WITH EXACERBATED SYMPTOMS

Hypothesis / aims of study

Overactive bladder (OAB) is characterized by frequency, urgency and urge-incontinence, in the absence of urinary infection. A previously unrecognized pyuria (≥10 WBC/µl urine) in 35% of catheter urine specimens from patients diagnosed as having OAB has been reported to be associated with more severe symptoms (1). It is well established that extracellular ATP signalling, originating from stretch-evoked ATP release from the urothelium and necessarily involving activation of a variety of P2 receptors throughout the bladder, is involved in bladder sensation(2). Furthermore, inflammation is associated with increased ATP release from epithelial cells (3). Taken together, we hypothesise that in pyuric OAB patients, there is increased ATP release and/or increased P2 receptor expression, caused by inflammation, which ultimately results in increased sensory nerve excitation and the exacerbation of OAB symptoms.

Study design, materials and methods

Bladder urothelium biopsies were obtained from *i*) asymptomatic patients, *ii*) pyuric OAB patients, and *iii*) non-pyuric OAB patients, using flexible cystoscopy. Smooth muscle tissue was carefully removed from the biopsy sample using fine forceps and a dissection microscope. Basal and hypotonicity-evoked (*i.e.* stretch-evoked; to mimic bladder filling) ATP release from urothelium was quantified using a luciferin/luciferase assay, P2 receptor mRNA levels were investigated using quantitative real time-PCR, and P2 receptor expression was investigated in snap-frozen sliced tissue using immunohistochemistry. Data are mean±s.e.m.

Results

Basal ATP release was 50-fold greater from the urothelium of pyuric OAB patients ($P<0.01$; n=10) than from non-pyuric OAB (n=9) or asymptomatic patients (n=9). In contrast, in all three patient groups, the concentration of ATP released following stretch was similar. Basal and stretch-evoked ATP release was abolished from the urothelium of OAB patients with pyuria by the addition of the P2 receptor antagonist suramin (1 mM; n=5), whereas, only stretch-evoked ATP release was abolished by botulinum toxin-A (20 units/ml; n=2). In asymptomatic patient urothelium, we detected significant levels of $P2X_{1\text{-}3,5\text{-}7}$ subunit and $P2Y_{1,2,6,11\text{-}14}$ receptor mRNA (n=6). Urothelium from pyuric OAB patients showed a significant increase in abundance of $P2X_5$ subunit and $P2Y_{2,11,12}$ receptor mRNA, and a significant decrease in abundance of $P2X_1$ subunit mRNA ($P<0.01$; n=3). Urothelium from non-pyuric OAB patients showed a significant increase in $P2Y_{11}$ mRNA ($P<0.01$, n=3). Immunohistochemisty confirmed our real time-PCR data (n=3).

Interpretation of results

In summary, these data demonstrate that in a subset of OAB patients (those with pyuria) there is increased basal ATP release from the urothelium, which is abolished by the P2 receptor antagonist suramin, and, altered P2 receptor expression. These data suggest that increased ATP release from the urothelium, involving activation of P2 receptors, may play a role in the heightened symptoms associated with pyuric OAB patients.

Concluding message

The suppression of urothelial ATP signalling may represent a therapeutic target to treat the exacerbated symptoms experienced by OAB patients with significant pyuria.

References

1. Urinary white cells and the symptoms of the overactive bladder. Neurourol Urodyn 2007;26(5):656-7
2. Purinergic signalling. Br J Pharmacol 2006 January;147 Suppl 1:S172-S181
3. Nucleotide-mediated inhibition of alveolar fluid clearance in BALB/c mice after respiratory syncytial virus infection. Am J Physiol Lung Cell Mol Physiol 2004 January;286(1):L112-L120

Specify source of funding or grant	**The Bloomsbury Consortium (University of London)**
Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**East London and the City Research Ethics Committee 1**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**