Altered urothelial ATP signalling in a major subset of human overactive bladder patients with pyuria

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Introduction

Overactive bladder (OAB) is an episodic condition characterized by urinary frequency, urgency, incontinence and pain; in the absence of routinely traceable urinary infection or other underlying pathology.

Its prevalence increases in the ageing population, affects their quality of life, and costs the NHS in excess of ~£700 billion (in 2000). 1

We have described a previously unrecognized chronic low-grade inflammatory response (i.e. pyuria ≥100 wbc μl−1) in the urine of the majority of patients diagnosed as having OAB; associated with more severe symptoms. 2

It is established that extracellular ATP signalling (originating from stretch-evoked ATP release from urothelial cells and probably involving activation of urothelial ATP-activated P2 receptors (P2R)) to facilitate further ATP release, and culminating in activation of P2R on sensory afferents) is involved in bladder sensation.1

It is also established that inflammation is associated with increased ATP release from epithelial cells. 3 Nucleotide signalling in cat and human urothelium is also altered in interstitial cystitis, a similar condition to OAB but with an inflammatory aetiology component.

Studies on humans with acute cystitis demonstrate that E. coli can thrive in the urothelium as intracellular bacterial colonies (IBC), resisting host immunity. 4

Taken together we hypothesize that in a subset of OAB patients (i.e. those with pyuria) there is increased ATP release and/or P2R expression, caused by an intracellular bacteria driven low-inflammation, which ultimately results in increased sensory nerve excitation and the exacerbation of OAB symptoms.

Methods

Bladder urothelium biopsies were obtained from a) asymptomatic patients, b) OAB patients with pyuria, and c) OAB patients without pyuria, using flexible cystoscopy. Any smooth muscle tissue was carefully removed from the biopsy samples using fine forceps and a scalpel.

ATP release from the urothelium was quantified using a luciferin/luciferase assay. ATP release was measured at a) rest (basal), and b) following addition of hypotonic solution to mimic bladder stretch during filling (stimulated). The effect of several drugs known to interfere with ATP signalling was tested in both basal and stimulated conditions (Figure 1).

Real time RT-qPCR was used to quantify P2R mRNA levels in the urothelium of the three patient groups.

Immunohistochemistry on wax-embedded tissue slices was used to investigate tissue viability, vesicular release of ATP (results not shown), and to search for IBC. Fresh live cells isolated from human urine samples were stained with acridine orange to assess for IBC in shed urothelial cells of the urine.

Results 1 (histology)

Bright field image showing full-thickness urothelium is exhibited on human bladder samples.

Results 2 (luciferin/luciferase assay)

Basal ATP release is significantly greater from the urothelium of OAB patients with pyuria, but stretch-evoked ATP release (by hypotonic solution) is not significantly different in all three patient groups

Figure 2. In basal conditions (A), urothelium from OAB patients with pyuria (n=10) releases significantly greater amounts of ATP than urothelium from OAB without pyuria (n=4) or asymptomatic patients (n=4). Following stimulation (B), all three groups release significantly more ATP than at basal conditions, yet this amount of ATP is not statistically significantly different between the three sample groups. * P<0.05

Basal ATP release mechanism(s) differ from stimulated ATP release mechanism(s) in the urothelium of OAB patients with pyuria

Figure 3. Urothelium was pre-treated with the stated drugs for 5 min prior to measuring ATP release. Basal (A) ATP release was significantly attenuated by both suramin (1 μM, n=3), and carbon monoxide (CBX, 50 μM) and significantly potentiated by UTP (1 μM). The potentiating effects of UTP were attenuated by co-treatment of either CBX or suramin. Botulinum toxin A (BTX-A, 20 units/mg), brevidoxin (BFA, 20 μM), capsaicin (3 μM, 4,4’-disothiocyanato-2,2’-stilbenedisulphonic acid (DDS), 100 μM) did not have any significant effect on basal ATP release vs basal control. Stimulated (B) ATP release from urothelium was attenuated by suramin, BTX-A, and BFA. Co-treatment of BFA or suramin with UTP had no effect. No differences were observed for capsaicin, CBX, BFA, DDS, UTP, n=3-5 for all samples. * P<0.05

Results 3 (real-time-qPCR)

Metabotropic P2Y11 and 13 receptor mRNA expression is significantly altered in the urothelium of OAB patients

Figure 4. P2Y1 and P2Y13 mRNA expression is shown in respect to a constitutively expressed housekeeping gene (GAPDH), Isometric P2X2 levels (A) were similar in the three experimental groups. Metabotropic P2Y2 levels (B) in urothelium from OAB patients without pyuria showed increased mRNA levels of P2Y2 and as opposed to urothelium from OAB with pyuria showed greater mRNA levels of P2Y2 and n=4 for each patient group. * P<0.05

Results 4 (Intracellular bacteria localisation)

Intracellular bacteria were identified in shed urothelial cells from 81% of OAB patients with pyuria but not in OAB without pyuria or non-OAB patients

Figure 5. Fresh urine samples (n=16) were spun onto slides, stained with acidine orange (which fluoresces green in the presence of viable DNA), and imaged with an upright fluorescence microscope. A crystal violet counterstain quenched the fluorescence of extracellular organisms. Representative microphotographs of: (A) non-OAB, (B) OAB without pyuria and (C) OAB with pyuria presenting with IBC (white areas). In (D) further confirmation of the intracellular localisation and cell type was obtained by treatment with anti-uroplakin III (marker of urothelial cells, in red) and DAPI (marker of DNA, in blue) of shed cells from urine of an OAB patient with pyuria. Images were acquired using a confocal microscope. (D) shows a 2D reconstruction of the Z-series, while (C) and (D) show the side projections indicated by the dashed lines and white arrows in (D). Scale bars equal 10 μm.

Conclusions

A subset of OAB patients have a previously unrecognised low-grade pyuria (≥100 wbc μl−1)

Bladder urothelial morphology is similar in all patient groups

Basal ATP release is increased in OAB patients with pyuria compared to non-OAB or OAB without pyuria patients.

Mechanisms of ATP release from urothelium of OAB patients with pyuria are notably different in basal vs. stimulated conditions:

- Basal ATP release is possibly mediated by hemichannels and downstream P2-receptors (i.e. P2Y13).
- Stimulated ATP release is likely to be driven by vesicles and P2-receptors (immunohistochemistry results, not shown here, also show less vesicular staining following hypotonic stimulation).
- mRNA P2Y2 expression in the urothelium of OAB patients with pyuria is different compared with OAB without pyuria or non-OAB controls.

- Shed urothelial cells from OAB patients with pyuria present with IBC, indicating a subclinical UTI component, contrary to common belief.
- The altered mechanisms of ATP release from urothelium of OAB patients with pyuria may play a role in the heightened symptoms, and could be used to provide a new therapeutic target.
- These results suggest that OAB with pyuria patients should be clinically re-classified as different from OAB without pyuria and therefore treatments may need to be tailored accordingly.
- Larger, multi-centre investigations are now needed.

References

2 Malone-Lee et al. Urodyn. (ICS; Rotterdam), abstract number 42, 2007