

## UROTHELIAL CELLS ISOLATED FROM URINE OF FEMALE SUBJECTS RELEASE ATP IN RESPONSE TO ACID

### Hypothesis / aims of study

Previous studies have demonstrated release of mediators such as ATP from urothelial cells cultured from human bladder biopsies [1]. Recently, human urothelial cells have been successfully isolated from bladder washings collected at cystometry [2]. The aim of this study was to 1) develop a method to isolate urothelial cells from human urine samples and 2) to examine the ATP released from these isolated cells in response to a range of pH (pH 4.5 to 7.4). It was hypothesized that bladder urothelial cells will respond to low pH, which can be seen through their release of ATP.

### Study design, materials and methods

Urine was collected from female subjects aged 22 - 51 years, with normal bladder function. Samples were passed through a 100 µm mesh and decanted into 50 ml centrifuge tubes. They were then centrifuged 1500 rpm for 10 min at 4°C. The pellet was resuspended in physiological Krebs buffer and samples were re-centrifuged at 800 rpm for 10 min at 4°C. The final pellet was re-suspended in Krebs buffer and the viability of cells tested using Trypan Blue exclusion. This method stains dead cells blue, while viable cells exclude the dye. At the same time, the cell concentration was estimated in a hemocytometer. Viable cells were able to be prepared from 13 out of the 15 samples collected.

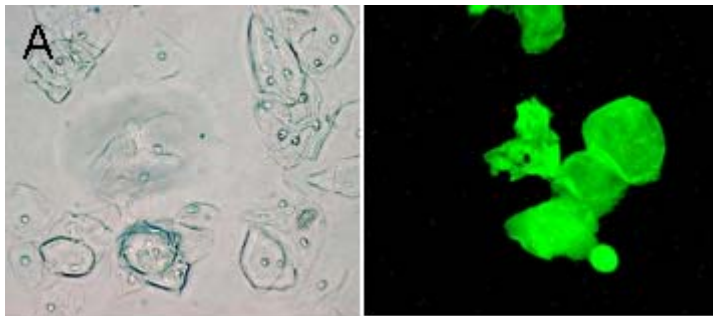
The morphology of cell preparations was examined by immunocytochemistry, performed using a fluorescent antibody specific for cytokeratins 8 and 18 (specific for transitional epithelial cells).

For examination of ATP release, stock solutions of Krebs at different pH were prepared. The total number of viable cells in the samples was calculated and samples were diluted to 25 000 cells/ 450 µl. Cells were aliquoted into separate eppendorf tubes (450 µl/ tube). Cells were allowed to rest for 15 min in a 37°C water bath before pH standard (50 µl) was added and cells incubated for another 15 min. For each urine sample, a minimum of 4 different pH concentrations were tested ranging from pH 4.5 - 7.4. At the conclusion of the experiment, cells were centrifuged for 20 sec and the supernatant removed and stored on ice, prior to ATP measurement.

ATP released was measured using the bioluminescence assay (Sigma) using a T20/20n luminometer. A standard curve ( $10^{-6}$  to  $10^{-11}$ M ATP) was prepared and the luminescence for each cell treatment calculated relative to the standard curve.

### Results

The morphology of cell preparations was found to be large and spreading. Preliminary cell characterisation showed that ninety percent of the cells present in the cell preparations showed positive staining for the specific antibody for cytokeratins CK8 and CK18 (Fig 1).

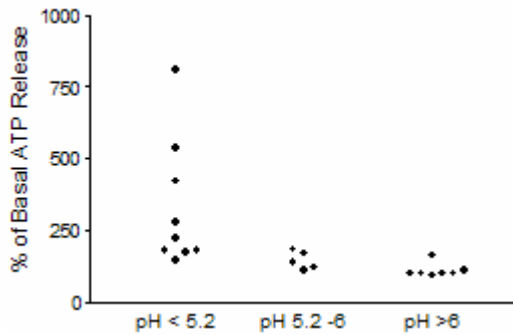


**Figure 1.** Morphology and cytokeratin staining of urine cell preparations.

Fluorescent staining with CK8/CK18 specific antibody.

Urine samples collected from normal subjects had a mean volume of 218 ml (range 70 ml to 490 ml) and a pH range between 5.29 and 6.83. Only 13 out of 15 urine samples yielded sufficient cells for ATP release studies and the mean cell yield was  $44.6 \times 10^4$  cells (range  $7 \times 10^4$  cells to  $140 \times 10^4$  cells). Trypan blue exclusion studies indicated that the mean percentage viability of isolated urothelial cells was 42% (range 23 to 75%) viable cells per sample.

Of the 13 cell preparations (from 6 subjects) which yielded sufficient cells for ATP release studies, only 8 preparations (from 4 subjects) showed a response to acid. Peak acid response occurred at a pH of less than 5.2 (Figure 2). There was a significant difference between ATP release at different pH values ( $P = 0.02$ , Kruskal-Wallis test).



**Figure 2.** ATP release in response to acid in five different cell preparations taken from a single subject.

Peak ATP release occurred in response to pH less than 5.2. Results are presented as % of basal ATP release (pH 6.8)

Whereas some subjects provided cells that responded to low pH each time a sample was tested, other subjects provided cells which showed a more variable response. In non-responders, basal release of ATP ranged from 2 nM to 20 nM.

#### Interpretation of results

This is a preliminary study that indicates that it is possible to isolate and prepare viable urothelial cells from urine samples. The size of the cells suggests that they represent umbrella cells. These cells respond to low pH solutions (<5) by releasing ATP into the medium. The nature of the mechanism has not yet been investigated although it is of interest that human superficial urothelial cells have been shown to express vanilloid receptors [3].

It was notable that there was variability between subjects and also for a single subject on different days. This requires further investigation: age, hormonal status, ethnicity and/or diet may play a role in the variable response properties of these cells.

#### Concluding message

This is a non-invasive technique to obtain viable umbrella cells from urine. Further validation of the technique is required and the properties of these cells merit further characterisation. So far, their response to acid has been investigated. These cells may also be able to respond to other chemicals such as capsaicin. In future studies, expression of mRNA and protein can be investigated, using patients with urodynamically characterised disorders as well as control subjects.

#### References

- [1] Am J Physiol (2006) 290: C27-34
- [2] Scand J Plast Surg Hand Surg (2003) 37: 41-45
- [3] Eur Urol (2004) 46 (6): 792-8

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**HUMAN SUBJECTS:** This study was approved by the The St. George Hospital Ethics Committee, Sydney and followed the Declaration of Helsinki Informed consent was obtained from the patients.