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EFFECT OF UROPATHOGENIC ESCHERICHIA COLI ON ATP RELEASE IN HELA CELLS.

Hypothesis / aims of study

ATP release can be induced from many cells under conditions of cellular stress and molecular signals. In the bladder, ATP plays an important sensory role, signalling urgency through activation of purinergic receptors. Urinary tract infection (UTI) also causes similar symptoms of urgency; it is possible that ATP release may be involved in sensory signalling of urgency following UTI. Intracellular bacterial colonisation (IBC) by uropathogenic *E. coli* (UPEC) has been discovered in bladder epithelial cells in animal model and in human [1], IBC may be related to overactive bladder and detrusor overactivity.

The adhesive property of HeLa epithelial cells to Escherichia coli (*E. coli*) has made HeLa cells become widely used as an *in vitro* model for studying interaction between *E. coli* and eukaryotic cells. The adhesion and internalisation of *E. coli* in HeLa cells may have the similar properties, such as ATP release, to IBC by UPEC in bladder epithelial cells.

In this study, HeLa cells were used to investigate the effect of *E. coli* infection on ATP release.

Study design, materials and methods

HeLa cells were cultured in 48-well plate in RPMI1640 culture media supplemented with 10% fetal bovine serum. At confluence (after 2 days in culture), HeLa cells were inoculated with *E. coli* or culture medium (as control) for 1 hour. After treatment cells were washed with PBS, and incubated with culture medium for further 1 hour or 24 hours, before ATP release measurement was undertaken. For ATP release measurement cells were first equilibrated with Kreb's solution for 1 hour, then Kreb's (for basal ATP release) or hypotonic 50% Kreb's (for stretch-induced ATP release) was applied for 10 minutes before samples were collected. The ATP concentration (pM) in each sample was determined immediately using a bioluminescence assay (Sigma). Data were expressed as medium, IQR.

Results

Basal ATP release: There was not a significantly difference of basal ATP release between controls and *E. coli* inoculated cells at 1 hour post-infection (Fig 1A), but a significant difference was found between controls and *E. coli* inoculated cells at 24 hours post-infection (Fig 1A).

Stretched-induced ATP release: There was not a significant difference of stretch-induced ATP release between controls and *E. coli* inoculated cells at 1 hour post-infection (Fig 1B), but a significant difference of ATP by stretch was found between controls and *E. coli* inoculated cells at 24 hours post-infection (Fig 1B).

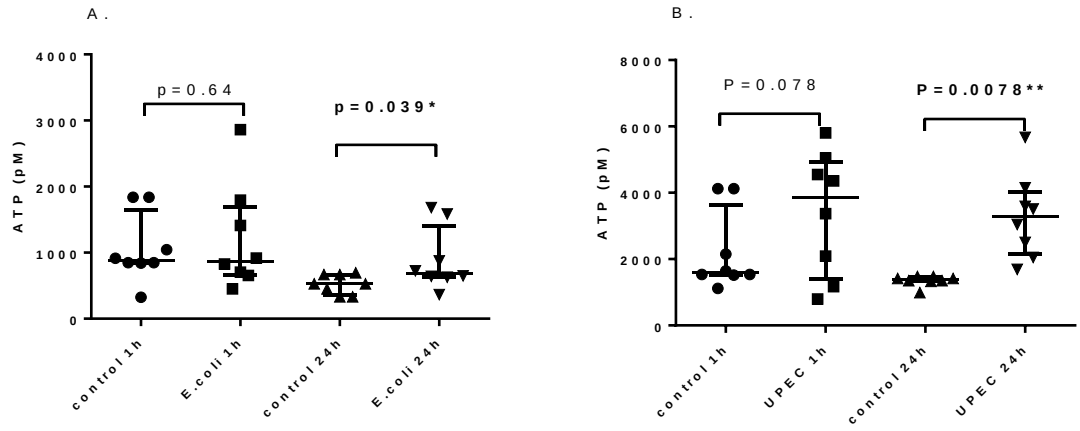
Interpretation of results

Both basal and stretch-induced ATP release was not altered by *E. coli* infection at 1 hour post-infection in HeLa cells. However, both basal and stretch-induced ATP release was induced by *E. coli* infection at 24 hours post-infection in HeLa cells. These indicate that the induction of both basal and stretch-induced ATP release by *E. coli* in HeLa cells might be a chronic effect.

Concluding message

The induction of ATP release by UPEC infection may lead to the activation of purinergic receptors and resulting urgency in patients with UTI.

Figure 1. The effect of UPEC on basal ATP (A) and stretch-induced ATP (B) in HeLa cells



References

1. PLoS Med. 2007 Dec;4(12):e329

Disclosures

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