Oestrogen and the Innate Epithelial Defences of the Uro-genital Tract

Anna Stanton, Professor Rob Pickard, and Dr Judith Hall
Epithelial Research Group
Institute of Cellular and Molecular Biosciences

Introduction

- Urinary tract infections (UTIs) are common bacterial infections in women.
- UTIs are predominantly caused by uropathogenic Escherichia coli (UPEC).
- 40% of women will suffer at least one UTI in their lifetime.
- 50% of post-menopausal women will experience recurrence.
- Recurrent UTIs in post-menopausal women are linked to decreased oestrogen levels.
- Classically, UTIs are treated with antibiotics, but vaginal oestrogen treatment is being explored as an alternative due to antibiotic resistance.
- The efficacy of oestrogen therapy is compromised as the role of oestrogen in the innate defence against UPECs is not known.

Aims

- To investigate the effects of oestrogen on the expression of antimicrobial peptides, which are key factors of the innate epithelial defences of the uro-genital tract, using the vaginal epithelial cell line VK2 E6/E7.

Methods

- Grow VK2 cells
- Challenged VK2 cells with:
  1) Oestrogen (1mg/ml)
  2) Flagellin (50ng/ml)
  3) Oestrogen + Flagellin

  - UPEC
  - Flagellin: models bacterial infection

  1) End-point PCR
     - Oestrogen receptors
  2) Fluorescence microscopy
     - Oestrogen receptor
  3) Quantitative-PCR
     - Oestrogen receptors
     - Antimicrobial peptides

Results: Oestrogen Receptor Regulation

- Both flagellin, and oestrogen with flagellin treatment caused a significant reduction in GPER expression after 8 and 12 hours (3i).
- The α-receptor expression was significantly down-regulated by both flagellin, and oestrogen with flagellin at 8 hours (3ii).
- Significance was tested by one-way ANOVA and Dunnett’s test (n=3, *P<0.05, **P<0.01). Error bars = standard error.

Results: Antimicrobial Peptide Regulation

- The expression of two antimicrobial peptides, hBD2 and hBD3, was analysed.
- Flagellin treatment caused a significant up-regulation in hBD2 expression at 8 hours (4i).
- It is inconclusive if oestrogen with flagellin had a positive effect on hBD2 expression.
- Oestrogen with flagellin caused a significant 48-fold increase in hBD3 expression after 24 hours (4ii).
- Oestrogen treatment enhanced the expression of hBD3 in the presence of flagellin.
- Significance was tested by one-way ANOVA and Dunnett’s test (n=3, * P<0.05, **P<0.01). Error bars = standard error.

Conclusion

- The α-, β-, and GPER oestrogen receptors were all expressed in vaginal epithelial cells.
- Flagellin, with and without oestrogen, caused significant down-regulation of expression of the α- and GPER oestrogen receptors.
- The results reveal that oestrogen treatment during infection may increase expression of the antimicrobial peptide hBD3.
- The results also suggest that oestrogen treatment of infected cells may increase hBD2 expression.

Future Work

- Examine effect of oestrogen, flagellin, and oestrogen with flagellin on expression of the β-receptor and the cellular localisation of the GPER.
- Investigate pathways involved in regulation of expression of the oestrogen receptors and antimicrobial peptides using PCR arrays, as knowledge of these pathways may direct the development of future therapeutics.

References