Intermedin protects human renal mesangial and tubular epithelial cells against nephrotoxic drugs.

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The BPS vacation studentship provided me with an excellent opportunity to carry out valuable research within Professor Paul Spiers’ lab based in the Department of Pharmacology and Therapeutics, Trinity College Dublin.

Introduction
Intermedin (Adrenomedullin-2), is a vasodilator peptide with similar properties to calcitonin gene-related peptide (CGRP) and adrenomedullin, all of which work through the same family of receptors. The receptor family consists of a CGRP-prefering receptor (CGRP) and two adrenomedullin preferring receptors (AM1, AM2), depending on which one of three possible transmembrane receptor activity modifying proteins (RAMPs 1-3) is coupled with the calcitonin-like receptor protein (which is a G-protein coupled receptor) (1). Currently, there is limited knowledge on the distribution of Intermedin and its associated receptors, especially in human, although the peptide has been shown to provide a protective effect upon the human heart and vasculature when subjected to oxidative stress and ischaemia-reperfusion injury (2). Less is known about the distribution and actions of Intermedin in the mammalian kidney. The aim of the summer studentship programme was to first investigate the expression of Intermedin and its receptor components at mRNA and protein level within human renal mesangial pericytes (HRMC) and human renal tubular epithelial cells (HREpic). Secondly, to provide evidence for a protective role of Intermedin on renal cells subjected to nephrotoxic injury by a series of drugs, namely lithium, indomethacin and epirubicin.

Methods
Cell Culture
Human renal epithelial cells (HREpic) and Human renal mesangial cells were cultured in cell-type specific media supplemented with specific growth factors. Cells were grown in T75 flasks at 37°C in 5% CO₂ in a humidified incubator. Cells were sub-cultured every 3-4 days at ~80% confluence.

Cell Viability
After trypsinisation then neutralisation with foetal bovine serum (50% vol.vol⁻¹ in normal medium), 100µl of suspension was taken and further suspended in 100µl of phosphate-buffered saline (PBS) containing 0.2% Trypan blue by gentle pipetting and introduced at the edge of a haemocytometer chamber. Cells were counted in triplicate aliquots over 4 corners of the haemocytometer grid.

RNA Extraction and Quantitative Real Time PCR (qRT-PCR)
RNA extraction was performed on confluent T25 flasks and quantified using a ND-100 Spectrophotometer. Complementary DNA (cDNA) was synthesised through reverse transcription. SYBR green was used to evaluate results in real time.

Indirect Immunofluorescence (IIF) and Quantification in HREpic and HRMC
Cells were first permeabilised before staining. Coverslips were rinsed x3 with PBS, then incubated with PBS containing 1% wt/vol BSA and 0.5% v/v Triton X-100. Quantification was performed using SPOT advanced imaging. Image gain and shutter speed were consistent throughout. Images were analysed with green (peptide or receptor component) and red (actin counterstain) signal being measured.
Application of nephrotoxic drugs
Several drugs with known nephrotoxic properties were given to confluent flasks of HREpic and HRMC, incubated in unchanged medium, at the following concentrations: Lithium 1.5mmolL⁻¹; Indomethacin 7mg.L⁻¹; Epirubicin 2µg.ml⁻¹. Cells were maintained at 37°C in 5% CO₂ in a humidified incubator for 48h in the absence and presence of Intermedin (1nmol.L⁻¹) prior to determination of cell viability.

Results
CGRP family and receptor components distribution in HREpic and HRMC
IMD/AM2 and the receptor components (CLR, RAMP1-3) were detected at mRNA level in HREpiC and HRMC, and normalised to the relative expression of AM. Both cell lines demonstrated a similar pattern in their expression profiles as seen in Figure 1a and 1b.

Indirect Immunofluorescence quantification showed that IMD, AM, CLR and RAMP1-3 were all present at protein level within both cell types (See Fig 2a and 2b). RAMP3 was more abundantly expressed in HRMC than RAMP1 or 2, while all components were expressed at similar levels in HREpiC. IMD was more abundant than AM in both cell types.

Western blots; IMD (Fig 3a) and RAMP2 (Fig 3b) were both shown to be significantly more abundant within the tubular cells than the mesangial cells (p<0.05).
Merged image: IIF staining for intermedin (green), actin (red). IMD was not localised to a particular region of the cell.

Scale bar 100µm

Merged image: IIF staining for RAMP2 (green), actin (red). RAMP2 was localised to the perinuclear region.

Scale bar 100µm
Lithium, Indomethacin and Epirubicin were all shown to attenuate the viability of HRMC and HREpic, with Indomethacin causing the greatest effect and Lithium showing the least nephrotoxic action. Varying amounts of protection was seen in HRMC and HREpiC by exogenous IMD against the deleterious effects of the drugs. There was a significant protection of cell viability seen in both cell lines against the detrimental effects of Lithium and Indomethacin with IMD. IMD also tended to be protective against the detrimental effects of Epirubicin although differences were not statistically significant (See Fig 5a and 5b). N=5 cell sources run in quadruplicate. ***p<0.001 versus control; ###p<0.001; #0.01<p<0.05 versus similar conditions without IMD.

![Graphs showing cell viability with and without IMD](image)

**Conclusion and final comments**

Results from this study has created a strong foundation for further research into the protective effects of IMD/AM2 in human renal pathophysiology. Intermedin, Adrenomedullin and their receptor components were present within the tubular and mesangial renal cells examined. IMD has been seen to have a significant protective role against certain nephrotoxic drugs and has potential for therapeutic use in prevention or management of drug-induced nephrotoxicity.

During the 10 week research programme I was met with multiple challenges, e.g. unexpected cell death during culture, pharmacology calculations and time-management with experiments. However, with the help of the researchers in the laboratory at TCD, I was shown how to effectively manage and solve these issues. I would like to thank the BPS for this invaluable opportunity where I have been able to successfully build upon my previous research skills and knowledge which I will now take forth and utilise within my PhD this year.

**References**