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Influence of intermedin/adrenomedullin-2 on nephrotoxic drug-related injury to human renal mesangial and tubular epithelial cells

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Introduction: Intermedin/adrenomedullin-2 (IMD/AM2) is a peptide closely related to adrenomedullin (AM) and calcitonin gene-related peptide, with which it shares a family of receptors (1). IMD/AM2 protects the mammalian heart and vasculature from oxidative stress and ischaemia-reperfusion injury (2). Less is known about the distribution of IMD/AM2 and its receptor components and potential for a similar protective role in the mammalian kidney, particularly in human (1, 3). The aim was to study expression of the peptide and its receptor components in human renal mesangial pericytes (HRMC) and in tubular epithelial cells (HREpiC) and determine protective effects of IMD/AM2 on cells subjected to nephrotoxic drugs.

Method: Expression of IMD and receptor components was determined at mRNA level by quantitative RT-PCR, expressed relative to AM, and at protein level by immunofluorescence staining of cells attached to coverslips, expressed relative to actin. Nephrotoxic drugs were applied to confluent cells for 48 h +/- IMD (1 nM). Cellular injury was determined qualitatively by microscopy and quantitatively by trypan blue exclusion. Data are given as mean+SEM (n cell sources) and analysis was performed using two factor ANOVA followed by unpaired Student's T-test, as applicable.

Results: IMD/AM2 and the receptor components (CLR, RAMP1-3) were detected at mRNA level in HREpiC and HRMC (n=6). Expression was confirmed at protein level by immunofluorescence (n=10).

Nephrotoxic drugs induced injury ($p<0.05$) in HRMC and HREpiC at 48 h as evidenced by microscopy. IMD/AM2 did not influence cell viability in the absence of nephrotoxic drugs but did attenuate nephrotoxic drug injury (**Table 1**).

Table 1 Protection by IMD/AM2 (1 nM) from nephrotoxic drug injury; viabilities at 48 h are expressed relative to control in the absence of IMD/AM and nephrotoxic drugs and are mean+SEM, n=5 sources each run in quadruplicate. *$p<0.05$ nephrotoxic drug vs. untreated cells; #$p<0.05$ with vs. without IMD.

	Untreated	Lithium 1.5 mM	Indomethacin 7 $mg.L^{-1}$	Epirubicin 2$\mu g.ml^{-1}$
HRMC control	100.0+3.97	54.78+3.38*	39.29+2.35*	47.69+5.27*
HRMC + IMD (1 nM)	98.44+3.36	79.74+2.24#	61.13+1.06#	60.60+6.56
HREpiC control	100.0+6.34	66.50+5.01*	45.03+2.52*	51.82+4.83*
HREpiC + IMD (1 nM)	102.7+3.44	83.48+2.75#	72.61+4.30#	68.52+7.03

Conclusions: In summary, IMD/AM2 is present in human renal cells and is protective of both mesangial pericytes and tubular epithelial cells against injury induced by a range of nephrotoxic drugs.

References:

(1) Holmes D *et al.* (2013). *Curr Prot Pept Sci* **14:** 294-329.

(2) Bell D *et al.* (2012). *J Physiol* **590:** 1181-1197.

(3) Qiao X *et al.* (2013). *Am J Physiol-Renal Physiol* **304:** F112-F119.