CHARACTERIZATION OF CISPLATIN TOXICITY IN aPROXIMATE™ HUMAN PROXIMAL TUBULE CELL MONOLAYERS

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Introduction

- Around 50 % of preclinical toxicity screens fail to predict subsequent toxicity *in vivo*.
- This leads to significant attrition of drug molecules during drug development.
- Understanding nephrotoxicity has been hampered by the lack of good renal model.
- Here we demonstrate the utility of the aProximate human proximal tubule cell (PTC) monolayers as an *in vitro* tool to investigate nephrotoxicity using clinically relevant biomarkers NGAL, KIM-1 and clusterin.

Methods

Kidney decapsulated and cortex dissected and minced

↓

Collagenase digest

↓

Digested tissue passed through cell strainer separate via density gradients

↓

Tubular cell retrieved and cultured on Transwell inserts

Figure 1: PTC isolation and culture.

- PTCs were isolated from human kidneys as described in Figure 1, and cultured onto Transwell inserts.
- Confluent monolayers were treated with nephrotoxins.
- Transepithelial electrical resistance (TEERs) were measured, in addition to cell viability using an Real time Glo™™ assay
- Culture media was collected from both apical and basolateral compartments and analysed for biomarkers using an ELISA approach.

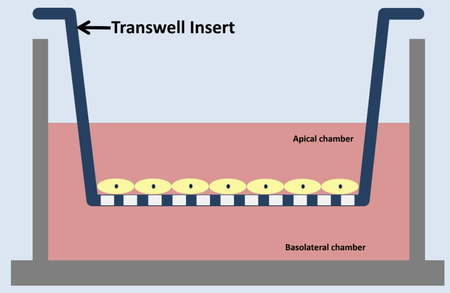


Figure 2: PTCs cultured on Transwell insert to recapitulate proximal tubule epithelial formation.

Biomarkers are released in to the apical media in a concentration dependent manner

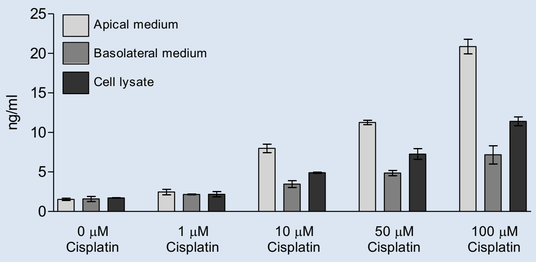


Figure 3: KIM-1 levels by aProximate monolayers after 24 hour treatment with cisplatin.

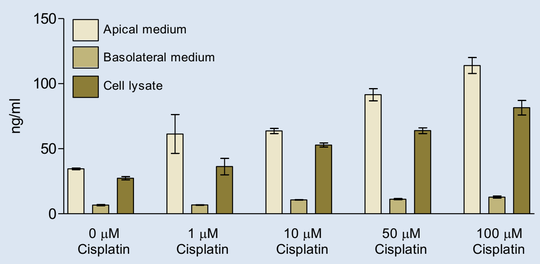


Figure 4: NGAL levels by aProximate monolayers after 24 hour treatment with cisplatin.

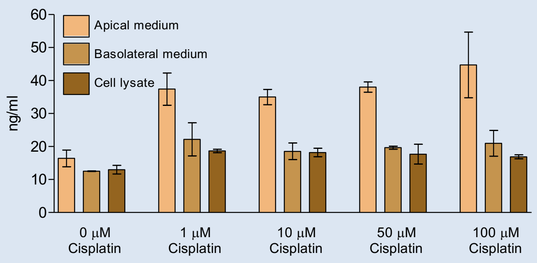


Figure 5: Clusterin levels by aProximate monolayers after 24 hour treatment with cisplatin.

- Levels of all 3 clinically relevant biomarkers KIM-1, NGAL, and clusterin increased within response to challenge with a range cisplatin concentrations.
- Importantly, as in vivo, biomarker release was predominately across the apical membrane than across the basolateral membrane (Figure 3, 4 & 5).

Time-dependent increase in biomarker release in response to cisplatin challenge

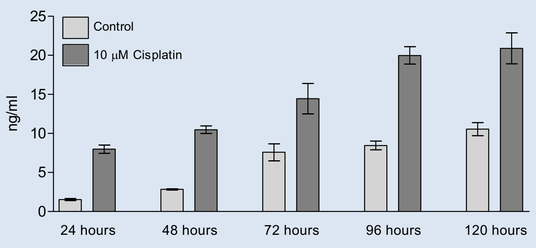


Figure 6: KIM-1 levels by aProximate monolayers at different periods of exposure to 10 µM cisplatin.

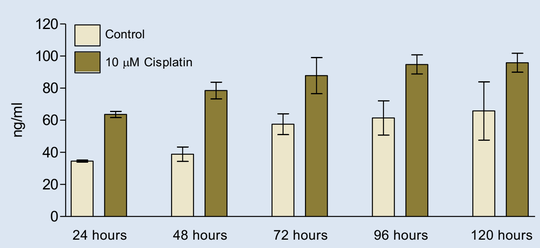


Figure 7: NGAL levels by aProximate monolayers at different periods of exposure to 10 µM cisplatin.

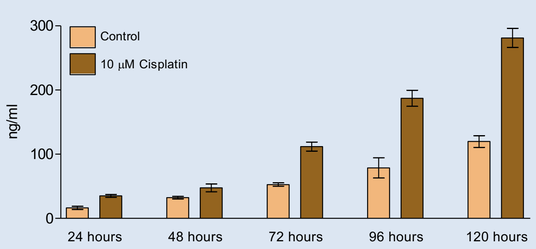
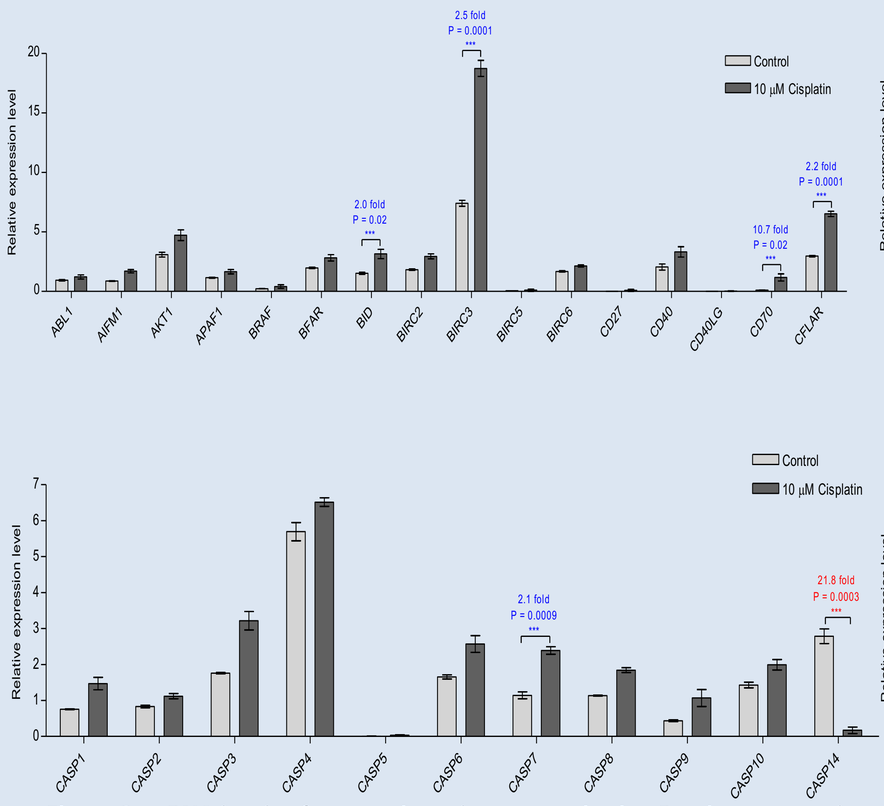
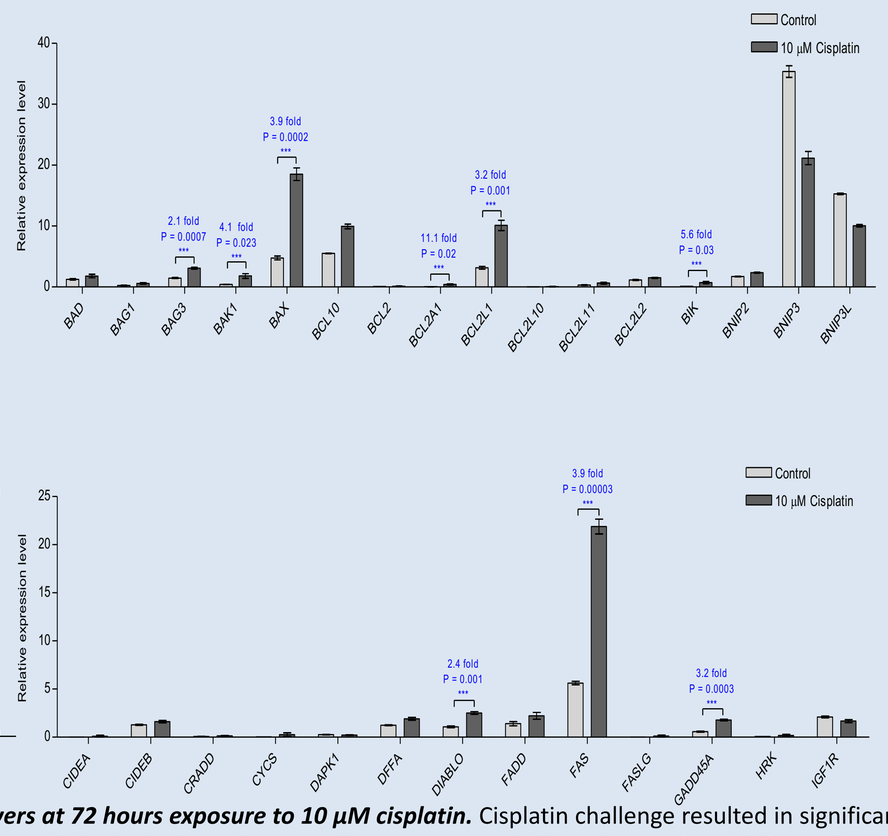


Figure 8: Clusterin levels by aProximate monolayers at different periods of exposure to 10 µM cisplatin.

- Levels of KIM-1, NGAL and clusterin in control and treated monolayers increased over a period of 5 days (Figure 6, 7 & 8).
- Increase in KIM-1, NGAL and clusterin with time demonstrate the cumulative effect of exposure to nephrotoxin challenge.

Response of Apoptosis Signalling Pathways in Human PTCs to Cisplatin Challenge





Gene symbol	Description	Fold regulation	p-value
BAG3	BCL2-associated athanogene 3	2.1	0.0007
BAK1	BCL2-antagonist/killer 1	4.1	0.02
BAX	BCL2-associated X protein	3.9	0.0002
BCL2A1	BCL2-related protein A1	11.1	0.02
BCL2L1	BCL2-like 1	3.2	0.001
BID	BH3 interacting domain death agonist	2	0.02
BIK	BCL2-interacting killer (apoptosis-inducing)	5.6	0.03
BIRC3	Baculoviral IAP repeat containing 3	2.5	0.0001
CASP14	Caspase 14, apoptosis-related cysteine peptidase	21.8	0.0003
CASP7	Caspase 7, apoptosis-related cysteine peptidase	2.1	0.0009
CD70	CD70 molecule	10.7	0.02
CFLAR	CASP8 and FADD-like apoptosis regulator	2.2	0.0001
DIABLO	Diablo, IAP-binding mitochondrial protein	2.4	0.001
FAS	Fas (TNF receptor superfamily, member 6)	3.9	0.00003
GADD45A	Growth arrest and DNA-damage-inducible, alpha	3.2	0.0003
LTBR	Lymphotoxin beta receptor (TNFR superfamily, member 3)	2.2	0.006
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	2.3	0.001
TNF	Tumor necrosis factor	7.4	0.0006
TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a	3.8	0.0001
TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b	2.9	0.0001
TRAF3	TNF receptor-associated factor 3	2.1	0.002

Figure 9: mRNA levels of apoptotic pathway proteins in aProximate monolayers at 72 hours exposure to 10 µM cisplatin. Cisplatin challenge resulted in significant increases in genes associated with apoptosis at the qPCR level. These included the death receptors; Fas (3.9 fold) CD70 (11 fold) and TNFR (3.8 fold), intrinsic pathway mediators BAX (3.9 fold) and BAK1 (4.1 fold) together with AIF (1.9 fold) and DIABLO (2.35 fold) and ER stress pathway markers resulting in significant increases in downstream caspase production leading to apoptosis. Importantly cisplatin also stimulated mRNA levels of the anti-apoptotic proteins; caspase 14 (21.8 fold) and BCL2A1 (11.1 fold).

Conclusion

In summary, aProximate™ human proximal tubule cell monolayers retain a remarkable degree of differentiation, express clinically relevant biomarkers of nephrotoxicity and signalling pathways that translate to an appropriate response to cisplatin challenge aProximate™ human proximal tubule cell monolayers show excellent potential as an in vitro predictive human toxicology screening platform during the drug development process