Kidney *in vitro* models for the improved prediction of chemical-induced nephrotoxicity

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INTRODUCTION

Drug induced nephrotoxicity

- Chemical/Drug induced nephrotoxicity leads to 25% of renal failure in clinic.
- Understanding a chemical has the ability to cause nephrotoxicity is important in an exposure led safety risk assessment for developing a mechanistic level understand of potential toxicity
- Glutathione depletion, accumulation of phospholipids, mitochondrial disruption and cellular ATP depletion are key responses to predict chemical induced nephrotoxicity.
- *In vitro* three-dimensional (3D) cell cultures resemble the complex microenvironment of *in vivo* models better than traditional 2D monolayers.
- High content screening (HCS) allows the simultaneous detection of each cell health parameter in combination with a measure of cellular ATP content.

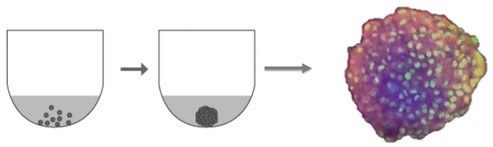

Figure 1. Microtissue formation in ultra-low attachment (ULA) plates

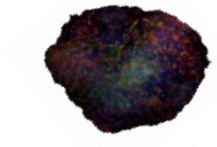

Figure 2. Kidney microtissue stained for F-actin, tight junctions and nuclei

AIMS

- Develop 2D and 3D models to accurately predict *in vivo* nephrotoxicants using primary kidney cells in mono and co-culture approaches.
- Develop a high content screening (HCS) assay for 2D and 3D to predict the potential of chemicals to cause nephrotoxicity.
- Screen a set of known nephrotoxicants.

METHODS/RESULTS

Cell models

- Human primary kidney cells (renal proximal tubule epithelial cells, renal cortical epithelial cells and renal fibroblasts) were used in this study.
- For 2D approaches renal proximal tubule epithelial cells (RPTEC) were seeded on collagen coated 96-well plates and grown to a confluent monolayer.
- For 3D renal proximal tubule epithelial cells alone or in co-culture with renal cortical epithelial cells and renal fibroblasts were seeded in ULA plates and allowed to form spheroids or microtissues over a course of 10 days (Figure 1 and 2).

High content screening (HCS) assay design

- Cell models were treated with test compounds for either 72hr, 216hr or 336hr. Solvent concentration was limited to 0.1% in the kidney models. The aminoglycosides gentamicin and tobramycin had a water solvent concentration of 10%.
- Compound doses were based on 100x C_{max} value or solubility limit.
- For time points 216hr or 336hr the cells were re-dosed every 3-4 days.
- Phospholipid dye (LipidTOX™ Red) was added 72hr before the assay.
- Following exposure to test compounds for 72hr, 216hr or 336hr the cells/spheroids/microtissues were stained with Syto11 (DNA structure), monochlorobimane (mBCl) (GSH content), and MitoTracker deep red (Mitochondrial function) for 30 minutes.
- Fluorescent images were acquired using a HCS reader ArrayScan™ VTI for 2D imaging and ArrayScan™ XTI in confocal mode for 3D (ThermoScientific).
- After image acquisition cellular ATP content was measured using either as appropriate: 2D CellTiter-Glo (Promega) or 3D CellTiter-Glo (Promega) and luminescence was read using a Biotek Synergy2 plate reader.

Comparison of cell models using gentamicin as a model nephrotoxicant

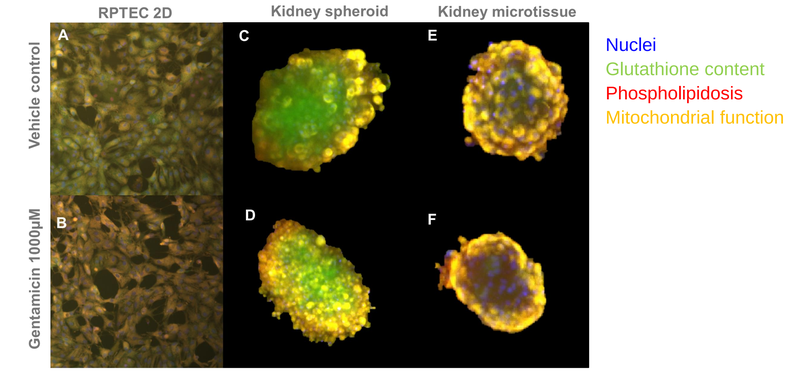

Figure 3. Example images of the different kidney models exposed to vehicle control (10% water; A, C, E) or 1000µM gentamicin (B, D, F) for 216hr. RPTEC grown in a 2D monolayer (A, B), RPTEC grown in kidney spheroids (C,D), and a multi cell kidney microtissue (E,F).

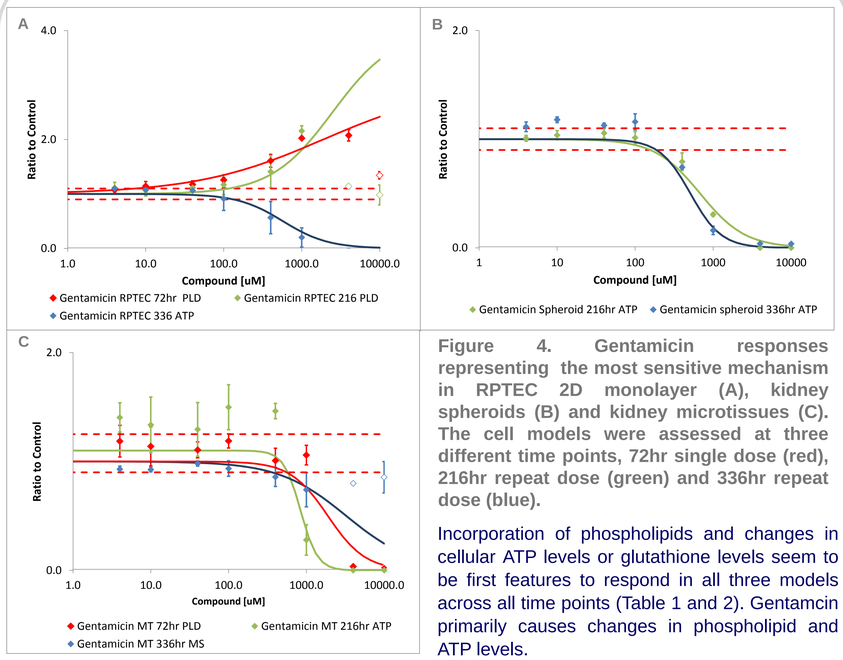

Figure 4. Gentamicin responses representing the most sensitive mechanism in RPTEC 2D monolayer (A), kidney spheroids (B) and kidney microtissues (C). The cell models were assessed at three different time points, 72hr single dose (red), 216hr repeat dose (green) and 336hr repeat dose (blue).

Incorporation of phospholipids and changes in cellular ATP levels or glutathione levels seem to be first features to respond in all three models across all time points (Table 1 and 2). Gentamcin primarily causes changes in phospholipid and ATP levels.

Table 1: Comparison of MEC in µM of the most sensitive mechanism of gentamicin exposed RPTEC, kidney spheroids or microtissues.

	72hr		216hr		336hr	
RPTEC 2D	249	PLD	189	PLD	219	ATP
Kidney spheroid	N/A	N/A	385	ATP	335	ATP
Kidney microtissue	971	ATP	255	ATP	239	MS

<1x Cmax
<10 Cmax
<30x Cmax
30-50
>50

MEC = minimum effective concentration, N/A = no data available, PLD = phospholipidosis, ATP = cellular ATP level, MS = Microtissue size.

2D and 3D kidney models accurately predict nephrotoxicity

Table 2: Nephrotoxicity prediction of 10 reference compounds based on MEC in µM and most sensitive mechanism

	Cmax [µM]*	RPTEC 72hr		RPTEC 216hr		MT 72hr		MT 216hr	
(S)-(+)camptothecin	0.083	0.102	PLD	0.18	GSH	0.263	MM	0.00746	MMP
acetaminophen	165.4	NR		1830	ATP	NR		1880	ATP
cisplatin	2	0.513	GSH	0.479	GSH	7.51	DNA	62.2	ATP
cyclosporin A	11	0.665	PLD	0.798	PLD	7.09	MMP	0.379	MMP
diclofenac	10.1	32.5	PLD	136	ATP	96.5	ATP	24.7	ATP
gentamycin	13	249	PLD	189	PLD	971	ATP	255	ATP
tobramycin	16	1020	PLD	128	PLD	13.9	MMP	768	DNA
buspirone	0.009	NR		NR		NR		NR	
piroxicam	12.79	NR		NR		NR		NR	
tacrine	0.077	19	PLD	25.7	GSH	NR		1.19	MM

Sensitivity	71.4%	100.0%	71.4%	100.0%
specificity	100.0%	100.0%	100.0%	66.7%
accuracy	80.0%	100.0%	80.0%	90.0%

MEC = minimum effective concentration, NR = no response, PLD = phospholipidosis, GSH = glutathione content, ATP = cellular ATP level, MM = mitochondrial mass, MMP = mitochondrial membrane potential, DNA = DNA structure. * human, non-tissue specific C_{max} values were taken from literature

SUMMARY/CONCLUSIONS

- Suitable cell models for screening approaches were developed to accurately predict *in vivo* nephrotoxicity. A 2D monolayer model using RPTEC, a kidney spheroid model using RPTEC and a kidney microtissues using RPTEC and renal cortical epithelial cells and renal fibroblasts were selected for further experiments.
- Primary human kidney cells were used in this study to increase relevance to human *in vivo* data.
- Multicellular 3D models resemble the complex microenvironment of *in vivo* models better than traditional 2D monolayer models.
- Known nephrotoxicants such as gentamicin were correctly predicted in all models based on a 30x C_{max} value following a 216hr exposure.
- The increased compound set indicated that the extended incubation time and repeat dosing improved the accuracy of *in vitro* to *in vivo* extrapolation for both 2D and 3D approaches by at least 10%. Test concentrations were based on a 100x Cmax or solubilitlimits.
- *In vivo* nephrotoxicity can be predicted with an accuracy of 100% (2D) and 90% (3D) based on this small reference set of compounds (within 50x C_{max}).
- Increased phospholipids appears frequently within the first 72hr of exposure, with other parameters responding more frequently during later time points including ATP and glutathione content.
- Future evaluation requires the reference chemical set to be further expanded with a broader range of compounds.
- Further *in vitro* to *in vivo* extrapolation could be increased by considering free concentration and also tissue specific C_{max} values.
- Both 2D and 3D models will be further characterised by assessing other determinants of kidney toxicity.

REFERENCES

Aschauer et al. (2015) ToxicolInVitro :30(1Pt A)95-105, Naughton (2008) AmFamPhys 78(6):743-750, Pazhayattil et al. (2014) InJNephroRenodis 7:457-468, Prange et al. (2015) EurJPhysiol 468(4);739-750