Development of predictive in vitro cross-species proximal tubule models for nephrotoxicity studies

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Introduction

- Nephrotoxicity is a major reason for drugs failing during clinical development.
- Currently there is no in vitro platform that enables cross-species comparisons of drug transport or nephrotoxicity.
- Our innovative solution is to develop highly differentiated assay platforms using primary renal proximal tubule cells(PTCs) derived from key animal species to measure both drug transport and drug induced kidney injury a range of biomarkers across species
- Here we showcase a highly differentiated Human proximal tubule model

Methods

- PTCs were isolated from either NHP or canine kidneys as described in Figure 1, and cultured onto Transwell inserts.
- Transepithelial fluxes of labelled probes were measured to assess functional polarity and functional expression of key drug transporters in NHP and canine PTC monolayers.
- Toxicity using relevant biomarkers – TEERs, cell viability, KIM-1, and clusterin – was also measured on NHP PTC monolayers to assess their utility as nephrotoxicity model.

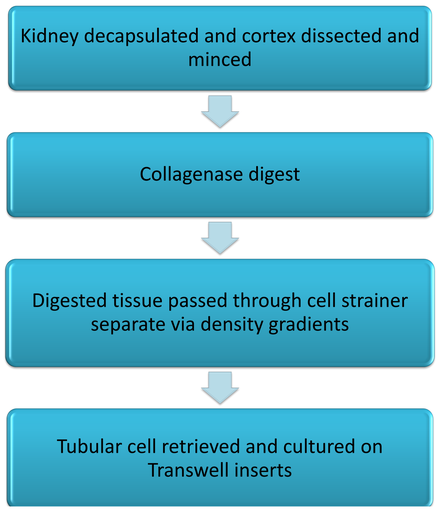


Figure 1: PTC isolation and culture.

Transporter expression in NHP PTC monolayers

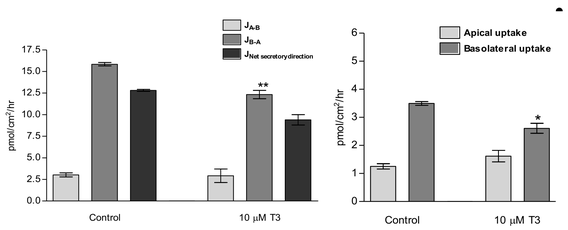


Figure 2: Digoxin flux and uptake by NHP PTC monolayers

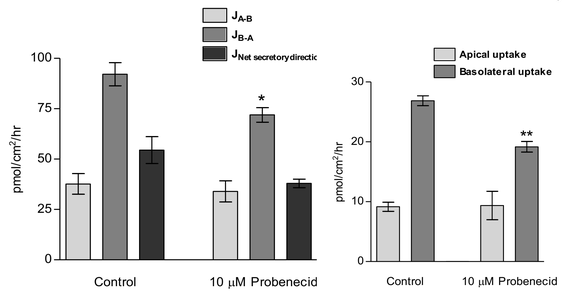


Figure 3: PAH flux and uptake by NHP PTC monolayers

- At 10 μM digoxin, the apical to basolateral flux (J_{ab}) was 3.3 ± 0.2 pmol/cm²/h, significantly smaller than the basolateral to apical flux (J_{ba}) of 15.8 ± 0.2 pmol/cm²/h (Figure 2). Net secretion of digoxin was inhibited by addition of triiodothyronine (T3) to the apical membrane.
- Net secretion of PAH was also observed in NHP PTC monolayers (Figure 3), with its magnitude decreased in the presence of probenecid.

These results were consistent with with OATP4C1-mediated uptake of digoxin, and functional expression of OAT1 responsible for PAH flux.

Transporter expression in canine PTC monolayers

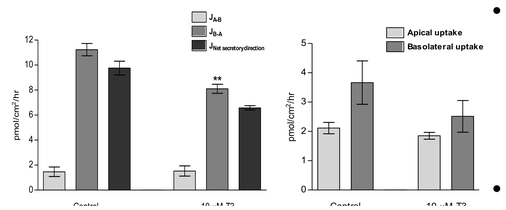


Figure 4: Digoxin flux and uptake by canine PTC monolayers

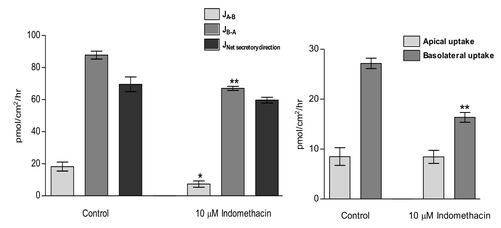
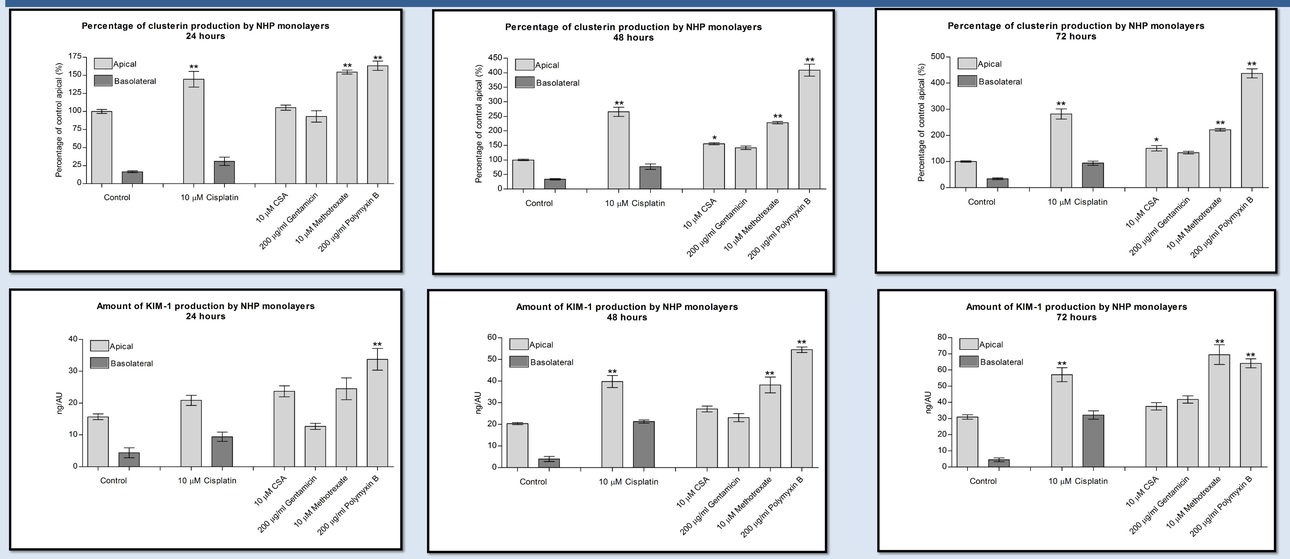


Figure 5: PAH flux and uptake by canine PTC monolayers

- Similar to the NHP PTC monolayers, the canine model also exhibited significantly more digoxin J_{ba}, which gave a net secretion of digoxin (Figure 4).
- Digoxin flux was also sensitive to T3, which saw a 30 % decrease in magnitude in its presence.
- PAH flux by canine PTC was also predominantly in the secretory direction, and was sensitive to indomethacin (Figure 5).

These results demonstrated digoxin handling by OAPT4C1 and MDR1, whereas MRPs and OAT1 were responsible for the transport of PAH in canine PTC monolayers.

Release of Clinical relevant Clusterin and KIM-1 biomarkers in Response to Nephrotoxic Challenge



- Levels of clinically relevant biomarkers KIM-1 and clusterin increased sigmificantly in response to challenge with a range nephrotoxicants.
- Biomarker release was predominately across the apical membrane than across the basolateral membrane

- Increase in KIM-1 and clusterin with time demonstrate the cumulative effect of exposure to nephrotoxin challenge.
- Data is from Rhesus Monkey. Initial observations show identical responses in Cynomolgus Monkey

Figure 6:Release of clinically relevant biomarkers to nephrotoxic drug challenge in aProximate™ NHP proximal tubule cell monolayers

Conclusions

Human primary proximal tubule cell monolayers retain a remarkable degree of differentiation and express a range of functional transporters and clinically relevant biomarkers of nephrotoxicity that are sensitive to nephrotoxin challenge over time. Human PTC monolayers show excellent potential as an in vitro predictive screening platform