Human primary proximal tubule cell monolayers as a predictive model of nephrotoxicity

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By current estimates around 50% of preclinical in vivo rodent toxicity screens failing to predict subsequent human toxicity, representing a significant challenge in drug development. As a result, regulatory agencies have led the call for improved in vitro screening assays to better predict human toxicity. Here we demonstrate the use of aProximate™ human proximal tubule cell (hPTC) monolayers as an in vitro tool to investigate nephrotoxicity using the clinically relevant biomarkers NGAL, KIM-1, and Clusterin. Freshly isolated hPTCs were seeded onto Transwell filters, and grown to confluence over 3 days before addition of nephrotoxicants. Biomarker generation was assessed by ELISA using samples removed from the apical chamber at various time points. In addition, measurements of trans-epithelial electrical resistance (TEER) were taken to determine monolayer integrity. Exposure of the hPTCs to gentamycin (200 μg/ml), cyclosporine A (10 μM), cisplatin (10 μM), or methotrexate (10 μM) for periods of up to 120 hours resulted in significant (P < 0.001) increases in biomarker production. Furthermore, we observed that for hPTC exposed to cyclosporine A, cisplatin, or methotrexate, the elevation in biomarkers at 120 hours was also accompanied by a significant decrease in TEER, suggesting that the monolayer integrity was compromised. In contrast, TEER values were unchanged upon exposure to gentamycin. In summary, these data suggest that aProximate™ hPTC monolayers express clinically relevant biomarkers of nephrotoxicity, and so have excellent potential for in vitro predictive human toxicology screening during drug development.