Developing a Comprehensive Biomarker Package for Detecting Drug-Induced Injury in Podocytes

KIDNEY

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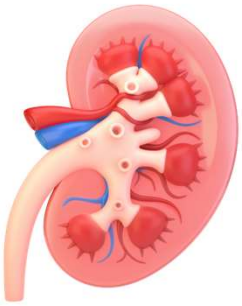

Introduction

Drug-induced damage to the kidneys is a key contributor to the development of multiple kidney pathologies, including chronic kidney disease (CKD) which is characterised by the progressive loss of kidney function. A main mechanism of drug-induced toxicity is through damage to kidney cells known as podocytes. Podocytes are specialised epithelial cells that form cytoplasmic projections called foot processes which interdigitate to form a sieve-like barrier for the size-selective filtration of blood. Due to continuous contact with the bloodstream, podocytes are especially susceptible to drug toxicity via prolonged exposure to xenobiotics, and this damage can lead to impaired barrier integrity, causing issues such as proteinuria (Fig. 1).

Biomarkers are molecules produced by cells under specific environmental conditions, such as damage and stress, that can be used as measures of adverse drug effects. For example, changes in IL-6 levels have previously been implicated in nephrotoxin-induced kidney injury. Therefore, to aid in the drug discovery process, we are developing a high throughput method to identify and validate biomarkers that are influenced by drug-induced injury in isolated primary human podocytes.

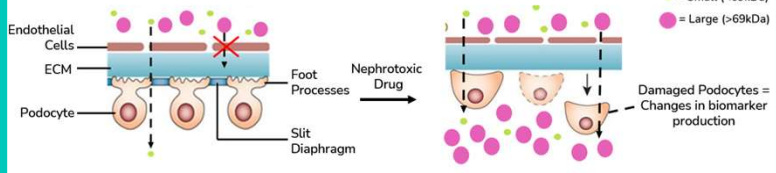

Figure 1: Effects of Nephrotoxic Drugs on Glomerular Filtration Barrier. Damage caused by nephrotoxic drugs disrupts the selective filtration and triggers changes in biomarker production.

Methods

Primary podocytes isolated from human kidneys were seeded onto 96-well Corning black-walled plates optimised for imaging. Dose response curves were set up in duplicate.

Following 72-hour incubation, Interleukin-6 (IL-6), Vascular Endothelial Growth Factor (VEGF), Osteopontin (Opn), and Monocyte Chemoattractant protein 1 (MCP-1) biomarkers were quantified using Duo-Set ELISA (Biotechne Ltd.), and normalised relative to cell count (cells stained with Hoechst and imaged and quantified live using the ImageXpress Pico and CellReporter Xpress (CRX) software) or ATP (CellTiter-Glo® Cell Viability Assay (Promega)).

Experimental Workflow

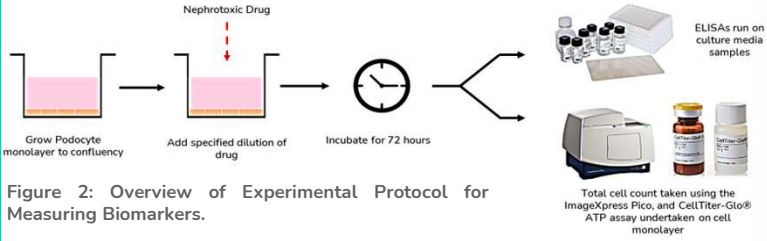

Figure 2: Overview of Experimental Protocol for Measuring Biomarkers.

Results

Quantifying Cell Health

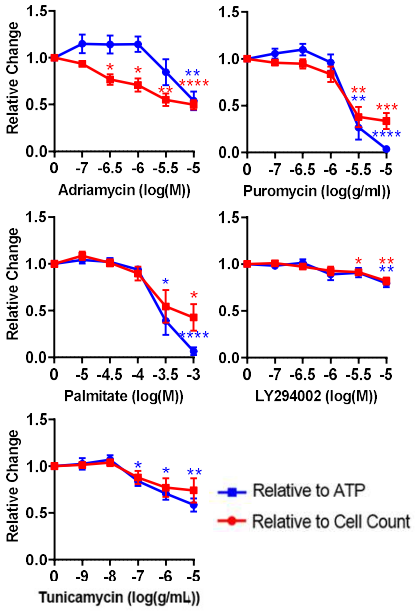

Figure 3: The drug panel have varying levels of impact on cell health. Cell health measured by the change in ATP (cell viability) and total cell count of human podocyte monolayers treated with nephrotoxins (n=4). ANOVA statistical analysis was undertaken, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

Biomarker Validation Screen

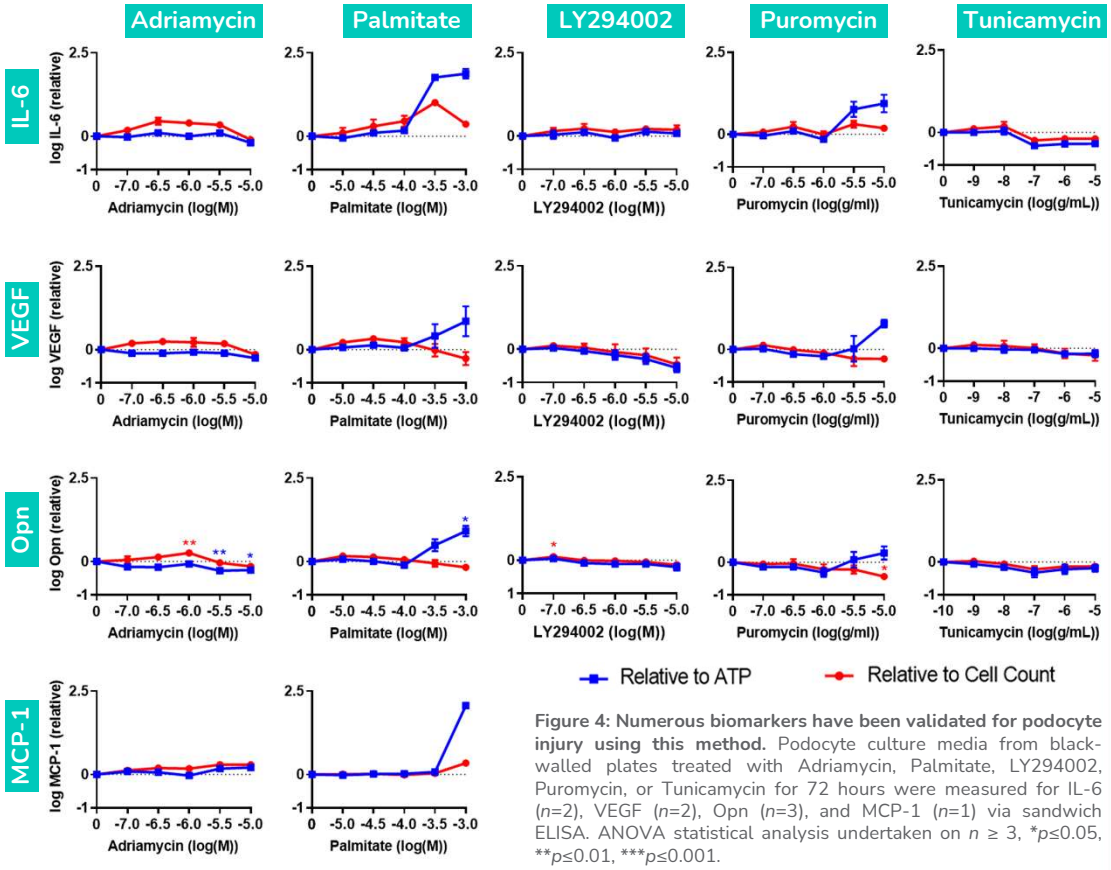

Figure 4: Numerous biomarkers have been validated for podocyte injury using this method. Podocyte culture media from black-walled plates treated with Adriamycin, Palmitate, LY294002, Puromycin, or Tunicamycin for 72 hours were measured for IL-6 (n=2), VEGF (n=2), Opn (n=3), and MCP-1 (n=1) via sandwich ELISA. ANOVA statistical analysis undertaken on $n \geq 3$, *p≤0.05, **p≤0.01, ***p≤0.001.

Summary

- A high throughput method has been developed to validate potential biomarkers for drug-induced human podocyte injury.
- We have identified methods to account for cell health and its effect on biomarker production, accounting for total cell number and cell viability.
- Three biomarkers have been validated for measuring nephrotoxic drug effects on podocytes: IL-6, VEGF, and Opn.

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