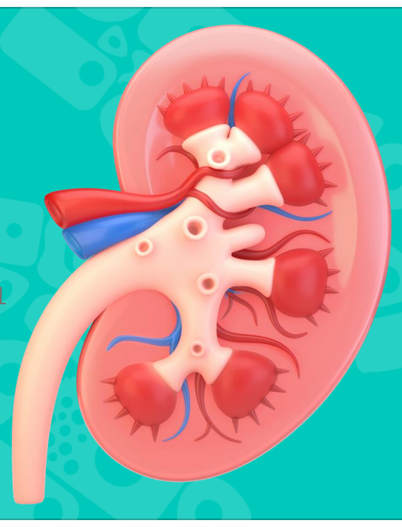
A high-throughput, *in vitro* assessment of antibiotic induced podocyte injury

Kirsten Ramsay[1], Kate Gilroy[1], Nick Brittain[1], Ben Coker, Cindy Carr[1], Claire Devlin[1], Keith Pye[1], Kathryn Garner[1]

Newcells Biotech Limited, The Biosphere, Draymans Way, Newcastle Helix,
Newcastle upon Tyne. UK NE4 5BX



Introduction

The glomerulus is the structure in the kidney that filters small molecules and waste products from the blood into the urine. Within the glomerulus, specialised epithelial cells, known as podocytes, form a size-selective sieve from their foot-like processes that surround blood vessels. Thus, forming the Glomerular Filtration Barrier (GFB). Prolonged exposure to compounds in the bloodstream can result in drug-induced injury, disrupting the GFB. Disruption of the GFB allows for passage of larger proteins from the blood to the filtrate, resulting in proteinuria. A key indication of proteinuria is the presence of Albumin in urine (Albuminuria).

The emergence of antimicrobial resistance has created an urgent need to develop new antimicrobial agents. Assessing drug-induced podocyte injury will play a key role in this process as many existing antimicrobial compounds, such as Adriamycin, are known to cause podocyte injury.

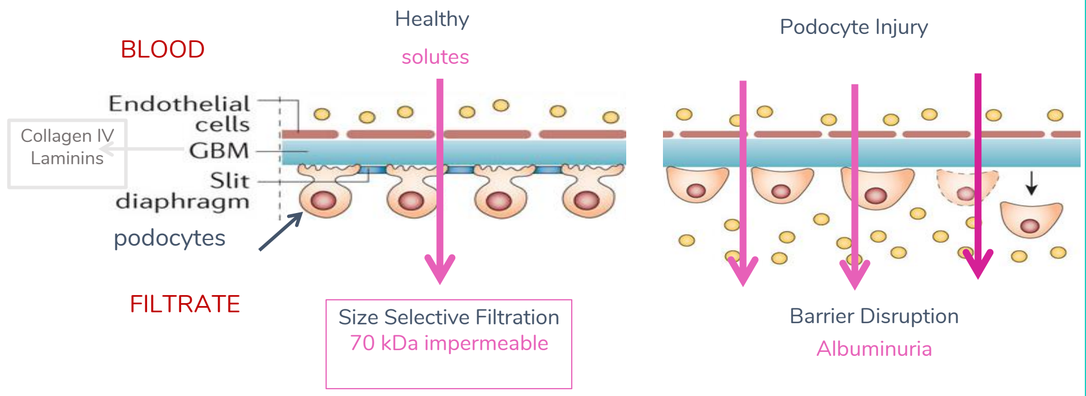


Figure 1: GFB structure. A healthy GFB exhibits size and charge selective filtration. Podocyte injury causes selectivity to be altered, allowing passage of larger molecules such as Albumin (~69kDa) into the filtrate, causing Albuminuria.

Methods

Primary podocytes were isolated from human kidneys and seeded onto 96-well Corning Transwell inserts. The cells were monitored daily for the formation of Transepithelial Electrical Resistance (TEER) using an EVOM2 Voltohmeter. Once TEER reached a threshold of 100Ω.cm^2, podocytes were treated with a range of antimicrobial compounds for 72 hours. Podocytes were also treated with 3µM Adriamycin for 72 hours as a positive control.

After 72 hours compound exposure, podocyte injury was assessed via three readouts. TEER was measured to determine the effect of compounds on monolayer integrity. To assess barrier disruption, triplicate wells were administered with 70 kDa FITC-Dextran on the apical side of the membrane, and the filtration rate was determined by sampling of the basolateral compartment after 30, 60 and 90 minutes. For assessment of podocyte viability, the CellTiterGLO (Promega) ATP luminescence assay was used.

Results

Gentamicin, Daunorubicin and Valrubicin were administered to podocytes for 72 hours, alongside a 3µM Adriamycin positive control. Podocyte injury was then assessed via TEER measurement (monolayer integrity), permeability to 70 kDa FITC-Dextran (filtration barrier integrity) and cell viability by measurement of ATP content. Results show varying sensitivities to the range of compounds used. Daunorubicin, a known glomerular toxin, is shown to be highly toxic to podocytes, by markedly reducing TEER and cell viability, and increasing 70kDa FITC-Dextran filtration rate compared to Gentamicin and Valrubicin. However, Valrubicin appears to have a greater impact on cell viability than Gentamicin, despite them having similar effects on both TEER and 70 kDa FITC-Dextran filtration.

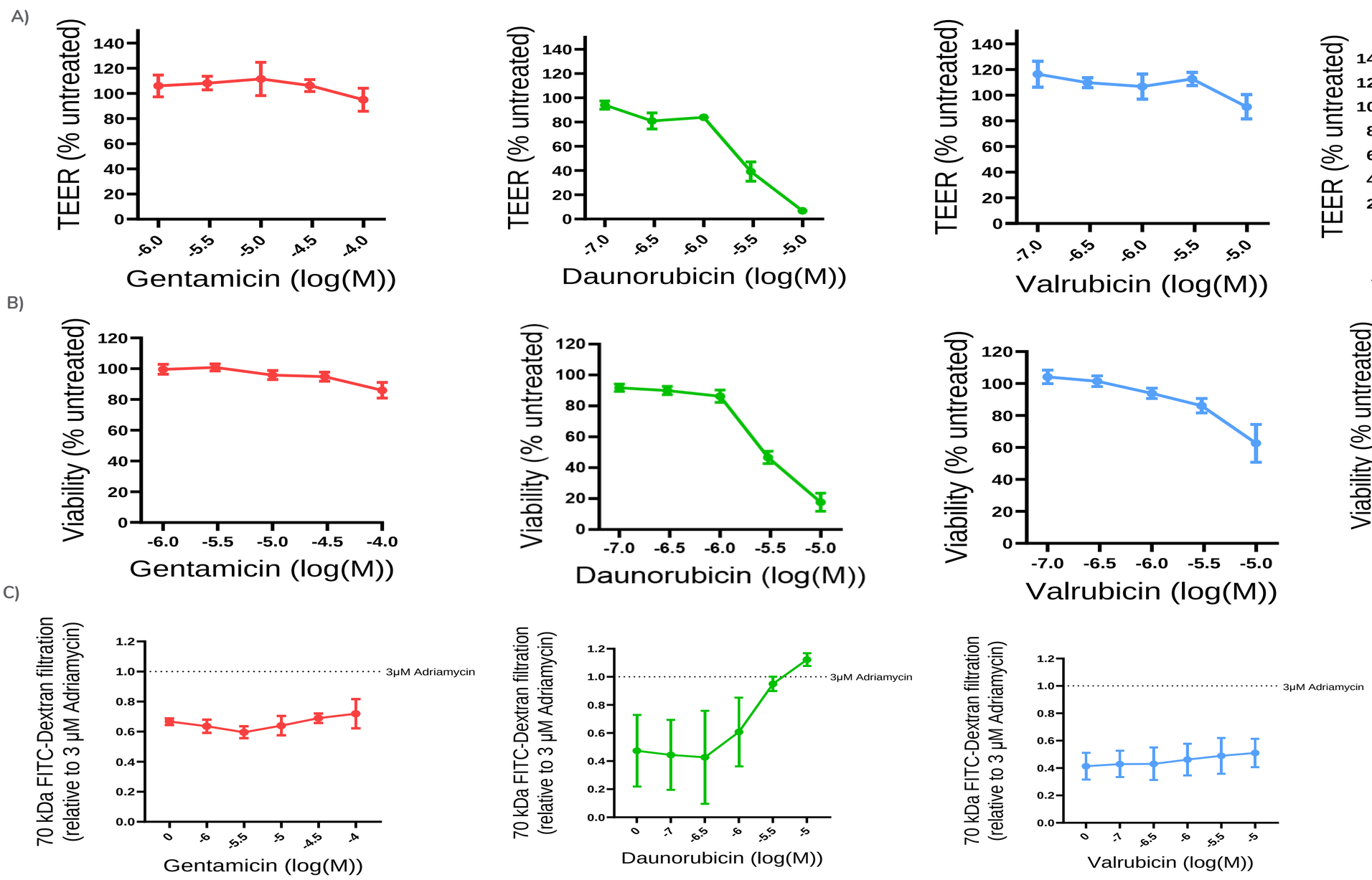


Figure 2: Antibiotic panel tested on primary podocytes. Gentamicin N=3, Daunorubicin N=2, Valrubicin N=3 and 3µM Adriamycin (positive control). A) The effect of compounds on monolayer integrity. TEER presented as a percentage of untreated podocytes. **B) The effect of compounds on primary podocyte viability.** Viability presented as a percentage of untreated podocytes. **C) The effect of compounds on podocyte filtration barrier integrity.** 70kDa FITC-Dextran Filtration presented relative to 3µM Adriamycin filtration.

Further details available online or contact us at
enquiries@newcellsbiotech.co.uk

References

1. Santos, Maria Luísa Cordeiro et al. "Nephrotoxicity in cancer treatment: An overview." *World journal of clinical oncology* vol. 11,4 (2020): 190-204.

Summary

- Daunorubicin is known to cause focal segmental glomerular sclerosis (1), in line with our findings that daunorubicin causes severe podocyte injury.
- Gentamicin and Valrubicin have less of a toxic effect on podocytes than 3µM Adriamycin.
- Daunorubicin, Valrubicin and Adriamycin are closely related anthracyclines and show differential toxicity to primary podocytes.
- Newcells' primary podocyte model allows for a sensitive, high-throughput, *in vitro* assessment of drug-induced podocyte injury.

NEWCELLS
IN VITRO MODELS TO PREDICT IN VIVO OUTCOMES