An *in vitro* Human Primary Thick Ascending Limb Model For The Assessment of Compound Nephrotoxicity

Robert Liang[1], Keith Pye[1], Elena Tasinato[1,2], Judith Verdonk[1,3], Siannah Shuttleworth[1], Kathryn Garner[1] and Colin D. A. Brown[1]

[1]Newcells Biotech Ltd, Newcastle Helix, Newcastle Upon Tyne, UK. [2]Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK. [3]Utrecht University, Utrecht, Netherlands.

Abstract No.
4644

Introduction

The thick ascending limb (TALH), which forms part of the loop of Henle (Fig. 1), plays multiple key roles in renal physiology. These include sodium and potassium reabsorption, the formation of a cortico-medullar osmotic gradient, which is instrumental in urine concentration and dilution, and acid-base homeostasis. Additionally, close to 60% of filtered magnesium[1] and approximately 25% of filtered calcium[2] are reabsorbed paracellularly in this nephron site. The TALH is also the main site for uromodulin synthesis, the most abundant protein in the urine that is vital for ion transport regulation and the safeguarding against kidney stones and kidney injury[3].

Drug-induced nephrotoxicity in the TALH has been observed with various drugs such as loop diuretics. These include electrolyte imbalances (hypokalemia, hypomagnesemia, hypocalcemia), as well as acid/base disturbances (hypochloremic alkalosis). Recently, a few Antisense Oligonucleotides (ASO) undergoing clinical research have been associated with the impairment of renal function consistent with TALH dysfunction. We have developed an *in vitro* primary human TALH cell model that can be used to evaluate the interaction of drug molecules such as ASOs with the TALH.

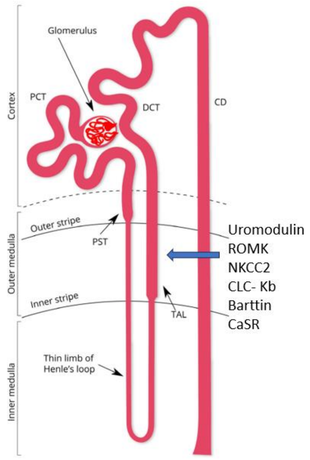

Figure 1: Localization of TALH in the nephron and main proteins, channels, transporters. ROMK, renal outer medulla K^+channels; NKCC2,Na-K-2Cl cotransporter; CLC- Kb, chloride channel Kb; CaSR, calcium sensing receptor. Adapted from Kumaran and Haukoglu[4].

Methods

- Primary TALH cells were isolated from human kidneys using Percoll density gradient (PG) and magnetic-activated cell sorting (MACS) using anti-human uromodulin antibodies. The presence of TALH markers uromodulin (UMOD) and Na^+-K^+-$2Cl^-$ cotransporter (NKCC-2), and de-enrichment of Aquaporin 1 (AQP-1) were assessed using immunofluorescence (IF) (**Fig. 2**). Cell polarity was evaluated using uromodulin quantification (ELISA).
- TALH cells were cultured until a trans-epithelial electrical resistance (TEER) of > 60 $\Omega.cm^2$ was achieved. Cells were then treated with two anti-sense oligonucleotides (ASO) at 30 and 100 µM for 3 days. TEER was measured to assess changes to barrier integrity, while lactate dehydrogenase (LDH) activity, a marker of cytotoxicity, was evaluated using the CyQUANT LDH Cytotoxicity Assay Kit. Paracellular permeability was determined by adding lucifer yellow (LY) to the apical cell membrane and sampling the basolateral compartment after 30 minutes.

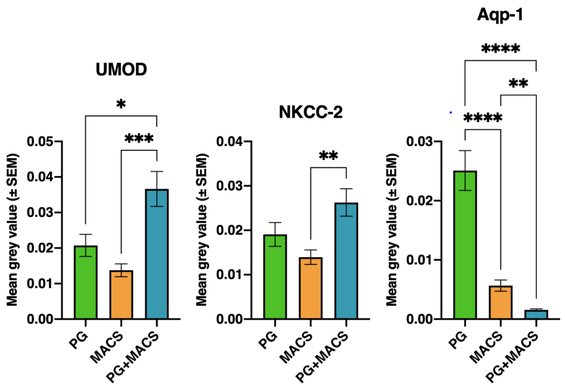

Figure 2: Isolated cell monolayers express uromodulin (UMOD) and Na^+-K^+-$2Cl^-$ cotransporter (NKCC-2). Immunofluorescence staining intensities were quantified and expressed as the average of the mean grey value (± SEM).

Results

TALH cells reached a TEER of > 60 $\Omega.cm^2$ in 6-9 days and formed a polarized monolayer (**Fig. 3,4**) before being administered with two ASOs. ASO-1 caused a significant drop in TEER (60.2% ± 19.1% decrease) and resulted in increased LY paracellular transport (16.3% ± 1.4%) after 30 minutes post-treatment (**Fig. 5**). Furthermore, ASO-1 significantly increased LDH release as compared to control (22.7% ± 6.3% vs 1.6% ± 0.4%) (**Fig. 6**). Conversely, ASO-2-treated cells showed no changes in TEER, minimal LDH release, and similar LY permeability in comparison to control (**Fig. 5, 6**).

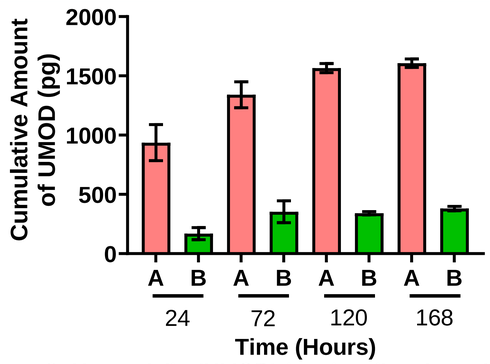

Figure 3: Uromodulin (UMOD) is primarily released from the apical membrane of isolated TALH cells. Apical (A) /basolateral (B) media were collected and assessed for UMOD protein. Error bars represent SD (n=2).

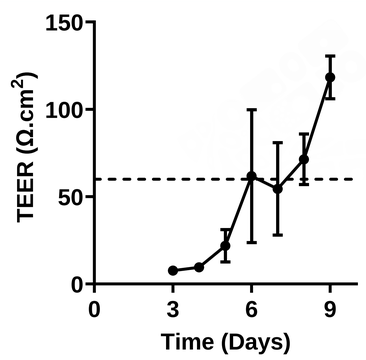

Figure 4: TEER of TALH cells over time. TEER was measured 3 days after seeding. Dashed line indicates 60 $\Omega.cm^2$. Error bars represent SEM (n=6).

TEER and LY Permeability

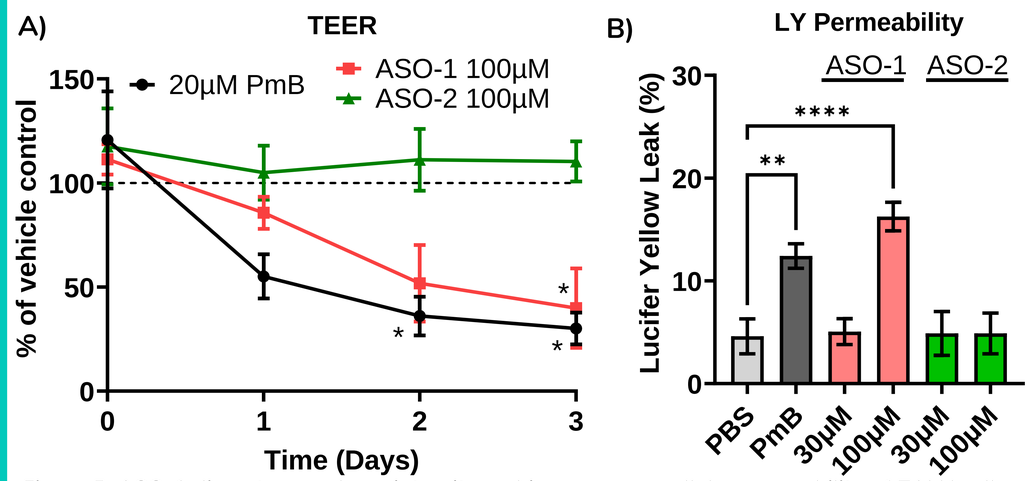

Figure 5: ASO-1 disrupts monolayer integrity and increase paracellular permeability of TALH cells. A) TEER of treated cells normalized to control wells; control wells denoted by dashed line (100%). B) Significant increase in LY leak seen with 100 µM ASO-1. Pre-treatment TEER indicated as 0, *,** and **** indicate p <0.05 and p <0.0001 respectively (RM One-Way ANOVA (A), One-Way ANOVA (B), Dunnett's test). Error bars represent SEM (n=6 (A), 3 (B)).

Cellular Damage

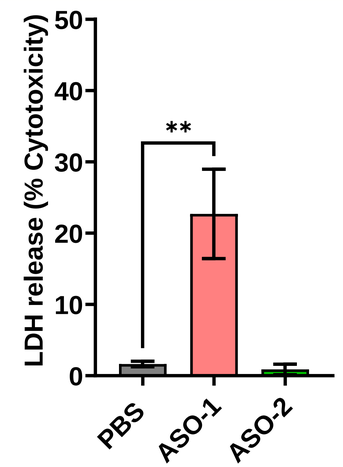

Figure 6: 100 µM ASO-1 causes cellular damage in TALH cells. ** indicates p < 0.05 (One-Way ANOVA, Dunnett's test). Error bars represent SEM (n=6).

Summary

- We have developed an *in vitro* model that is representative of TALH cells *in vivo*.
- This *in vitro* TALH model could be used to evaluate the effects and damage to TALH function by drug compounds such as ASOs.

References

1. Zacchia, M. et *al.* (2018) *Int J Nephrol Renovasc Dis*, *11*, 81–92.
2. Moor, M.B. and Bonny, O. (2016) *Am J Physiol Renal Physiol.*, 310, F1337-F1350.
3. Schaeffer et *al.* (2021) *Annu Rev Physiol.*, 83, 477-501
4. Kumaran, G.K. and Hanukoglu, I. (2020) *FEBS J.*, 287, 1176-1194

NEWCELLS
IN VITRO MODELS TO PREDICT IN VIVO OUTCOMES

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