

Using an impedance based assay in the investigation of drug induced liver injury comprising the gut/liver axis



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INTRODUCTION

Drug induced liver injury (DILI) accounts for approximately one half of all cases of acute liver failure [1]. Before entering the liver, drugs pass through the gut. Determining the dynamics of the gut/liver axis would contribute to a better understanding of how drugs are transported, prior to metabolism by the liver. APAP overdose is a leading cause of DILI [2]. Previously, using ECIS technology and the hepatic HepaRG cell line, we showed a dose dependent loss of impedance with specific disruption to tight junctions (TJs) at sub-therapeutic, therapeutic and toxic concentrations of APAP [3].

Here we investigate the effect of APAP on the Caco2 intestinal cell line to determine if any dose dependent changes in impedance can be detected. Understanding the mechanisms whereby APAP and its metabolites contribute to DILI could improve therapy thus reducing the number of fatalities per year.

Using model hepatotoxin Paracetamol (APAP), we highlight the use of impedance based assays in identifying possible mechanisms of damage in both hepatocyte and intestinal cell lines.

AIM

To investigate the effect of APAP on human intestinal Caco2 cell line using an ECIS Z0 impedance based assay.

MATERIALS AND METHODS

Caco2 cells were seeded on collagen coated 8W10+E ibidi arrays and monitored using the ECIS Z0 platform. Previously established concentrations of APAP [3] were administered at day 14, when the cells were confluent, and impedance was monitored for 24 hours. Cells were stained with nuclear stain Hoescht. Phase contrast/Hoescht microscopy was taken on EVOS fl auto x20 magnification after 24 hours exposure to APAP.

RESULTS

Phase contrast microscopy shows a monolayer of cells after 24 hrs exposure to APAP (0-20mM). (Figure 2)

Measurements of total impedance after application of APAP showed a different profile of impedance on Caco2 cells compared to that of hepatocytes. Hepatocytes showed dose dependent damage at a sub therapeutic, therapeutic and toxic concentrations (Figure 3A) while, Caco2 intestinal cells showed no loss of impedance at any concentration (Figure 3B) which indicated the enteric cells are an effective barrier with regards to APAP toxicity.

CONCLUSIONS

Impedance based assays can provide useful insight into the effect of APAP on the gut/liver axis. Here we show no loss of impedance in Caco2 intestinal cells challenged with sub therapeutic, therapeutic and toxic doses of APAP comprising overall membrane integrity and no loss of adhesion or tight junctions. This differs from a dose dependent toxic response seen in hepatocytes using the same concentrations. These results suggest that the enteric cells form a barrier in regards to APAP toxicity and may inform further investigation into the effects of APAP on the gut/liver axis. However, pharmaceutical excipients used alongside APAP preparations may have different effects on the enteric barrier and merit further investigation with ECIS.

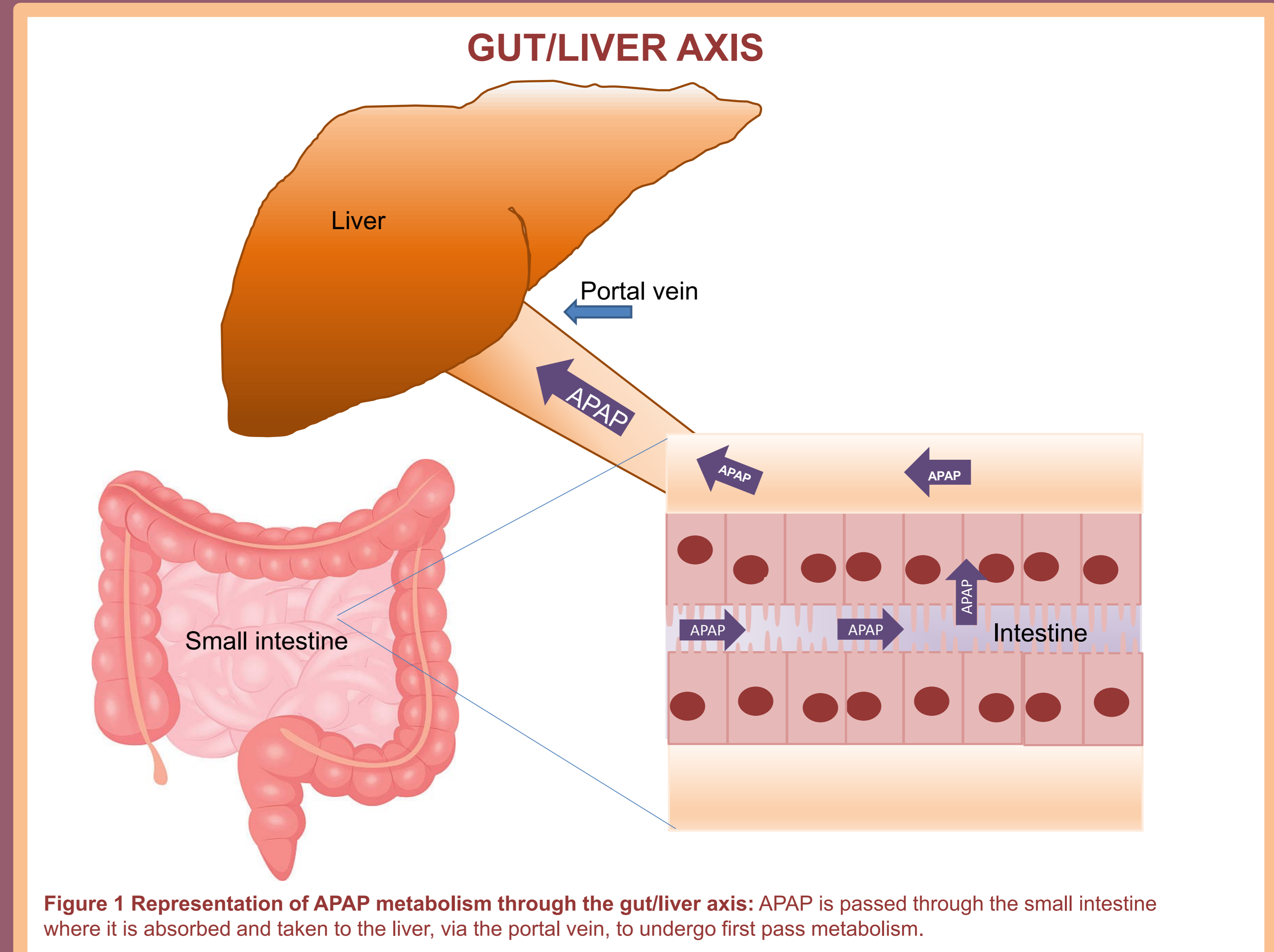


Figure 1 Representation of APAP metabolism through the gut/liver axis: APAP is passed through the small intestine where it is absorbed and taken to the liver, via the portal vein, to undergo first pass metabolism.

PHASE CONTRAST MICROSCOPY

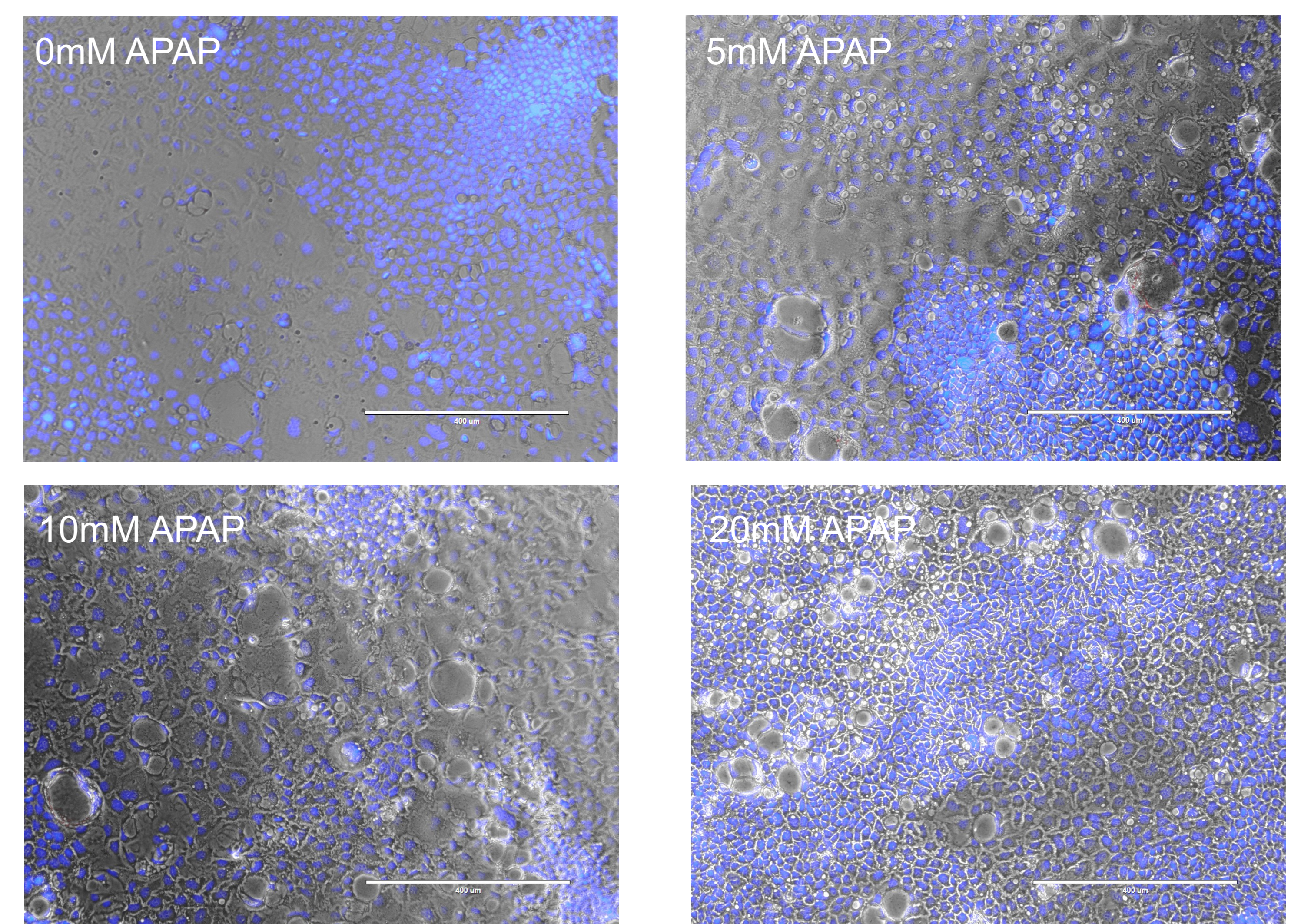


Figure 2 Phase contrast microscopy: Phase contrast and Hoescht overlay of day 8 Caco2 cells after 24 hours exposure to 0, 5, 10 and 20mM of APAP

ECIS BASED TOXICITY ASSAY

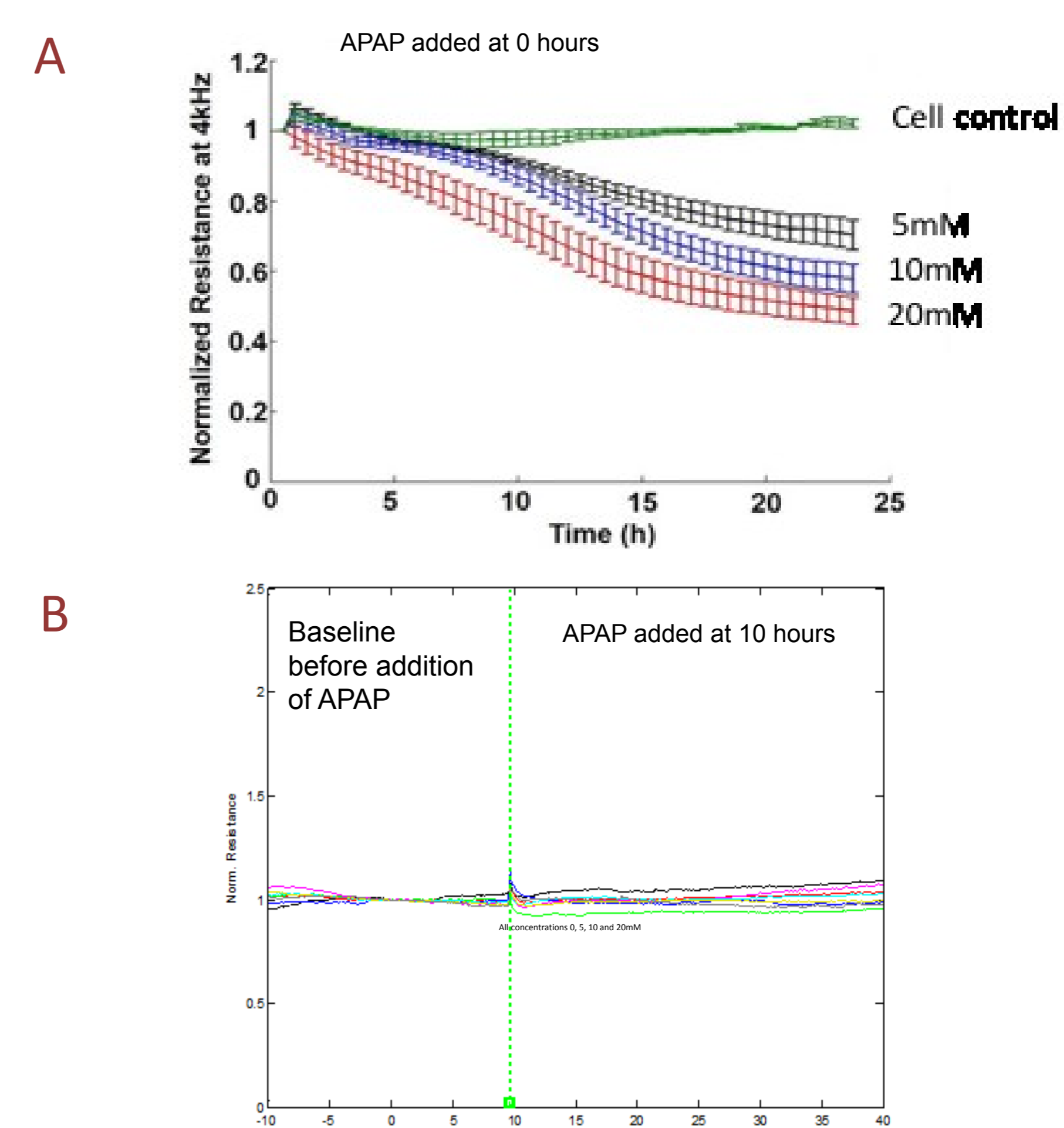


Figure 3 Impedance based assay: Comparison of A) Human hepatic cell line HepaRG's exposed to sub therapeutic, therapeutic and toxic concentrations of APAP vs B) human intestinal cell line Caco2 exposed to the same concentrations. HepaRG cells show a dose dependent loss of impedance for the parameter showing tight junctions while there is no change in impedance of tight junctions on the Caco2 cell line.

References

- Neil Kaplowitz; Drug-Induced Liver Injury, Clinical Infectious Diseases, Volume 38, Issue Supplement_2, 1 March 2004, Pages S44–S48, <https://doi.org/10.1086/381446>
- Sheen, C.L., Dillon, J.F., Bateman, D.N., Simpson, K.J., MacDonald, T.M. (2002) Paracetamol-related deaths in Scotland, 1994-2000. Br J Clin Pharmacol. 54(4):430-2
- Gamal, W., Treskes, P., Samuel, K., Sullivan, G. J., Siller, R., Srsen, V., ... Nelson, L. J. (2017). Low-dose acetaminophen induces early disruption of cell-cell tight junctions in human hepatic cells and mouse liver. Scientific Reports, 7, 37541. <http://doi.org/10.1038/srep37541>

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