

Analysis of Four Hepatotoxicants Using the xCELLigence Analytical System and HepaRG Cells (030514)

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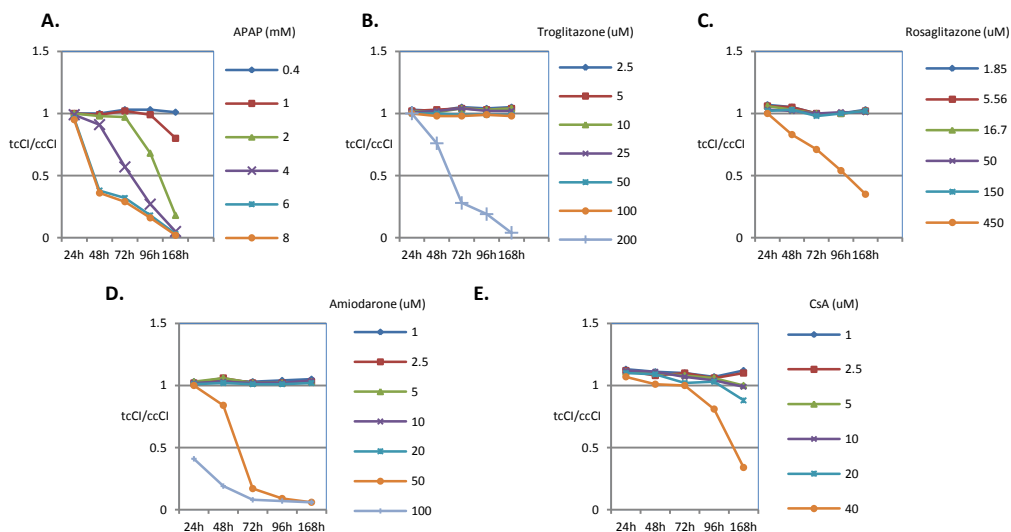
ABSTRACT

Hepatotoxicants act through different mechanisms and kinetics, and we reasoned that an analytical system allowing for convenient, kinetic monitoring of relevant effects combined with metabolically competent human hepatic cells, should facilitate recognition of the risk of hepatotoxicity. Our protocol involved culturing cryopreserved, differentiated HepaRG cells onto the xCELLigence 96-well cell culture plate, and after a four-day adaptation period, treating them with different concentrations of four compounds with known risk of hepatotoxicity APAP, amiodarone, CsA, and troglitazone-and the PPAR agonist rosiglitazone. The Cell Index (CI), an expression of the electrical impedance of adherent cells as measured on sensors in culture plate wells by the xCELLigence Cell Analyzer, of treated cells (tcCI) was compared to the CI from HepaRG cells exposed to a 0.5% DMSO control (ccCI) after exposure times of 24, 48, 72, 96, and 168 hrs, during which time the medium was replenished every 48 hours. The tcCI/ccCI was also assessed at 2, 5, 10, and 20 hours after treatment with eight APAP concentrations to determine if damage to the cells could be detected rapidly, since McGill et al¹ reported significant glutathione depletion within three hours after exposing HepaRG to APAP.

INTRODUCTION

- Kinetic measurement of electrical impedance of charged, adherent cells has been shown to detect changes in morphology, cell contact and conductivity, and lipid bilayer²
 -DILI is a serious concern and is often difficult to predict in preclinical testing³
 -- APAP, amiodarone, CsA, and troglitazone are known to cause DILI in either an exposure-related or idiosyncratic fashion and through somewhat different mechanisms^{3 4 5}
 -- Primary human hepatocytes have been used for in-vitro assessment of toxicity risk but have many limitations which the human hepatic cell lines HepaRG has been shown to improve upon.⁶

1. RESULTS CONCENTRATION AND TIME-DEPENDENT EFFECTS



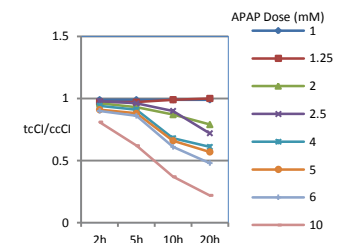
MATERIALS AND METHODS

Cryopreserved, differentiated HepaRG cells were thawed and seeded into 96 well E-Plates containing microsensors at the bottom of each well to measure electrical impedance using Real-Time Cell Analyzers from ACEA. Following a five-day adaptation period during which the media was replenished twice, HepaRG cells were exposed to test articles including 0.5% DMSO as a control for a period of seven days during which time the medium + TA or 0.5% DMSO was changed every two days. During the entire period of exposure the CI values from both DMSO control and TA-treated HepaRG cells were monitored and tcCI/ccCI was determined.

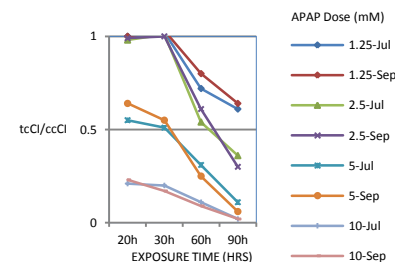
CONCLUSIONS

- ✓ Combined use of the HepaRG cells and the xCELLigence analytical system demonstrated a distinct, concentration and exposure-time dependent effect from different hepatotoxicants when the Cell Index of treated cells was compared with that of control (0.5% DMSO) cells.
- ✓ Kinetic measurement allowed for detection of hepatotoxicant effect on HepaRG cells' impedance as it occurred (results not shown).
- ✓ Different mechanisms of toxicity (i.e., metabolism-dependent, steatosis, oxidative stress, idiosyncratic necrosis, glutathione depletion) quantitatively affected the HepaRG cells' impedance using the same study design.
- ✓ Mechanisms requiring extended exposure time for clinical toxicity to develop (steatosis) demonstrated significant change in HepaRG cells' impedance within 48hrs at higher concentrations.
- ✓ Between the glitazones with significantly different clinical risk of hepatotoxicity, a distinct concentration effect was demonstrated with the "safter" rosiglitazone requiring a concentration of 450uM and exposure time of 72 hr to demonstrate approximately equivalent effect of 200uM of troglitazone for 48hrs on HepaRG cells' impedance.
- ✓ The system essentially duplicated results derived in July with APAP when the same conditions were used in a September study.

2. RESULTS, ACUTE DOSING MONITORING tcCI/ccCI after exposure intervals



3. RESULTS, REPRODUCIBILITY OF tcCI/ccCI (studies in July and Sept)



July Experiment

1.25 mM APAP (1.0; 1.02; 0.72; 0.60)
 2.50 mM APAP (0.98; 1.01; 0.54; 0.36)
 5.0 mM APAP (0.55; 0.51; 0.31; 0.11)
 10.0 mM APAP (0.21; 0.20; 0.11; 0.02)

Sept Experiment

1.25 mM APAP (1.0; 1.03; 0.80; 0.64)
 2.50 mM APAP (0.98; 1.00; 0.61; 0.30)
 5.0 mM APAP (0.64; 0.55; 0.25; 0.05)
 10.0 mM APAP (0.23; 0.17; 0.09; 0.02)

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