An All-Human Hepatic System, TruVivo® Distinguishes Between Marketed Human Hepatotoxicants and Non-Hepatotoxicants

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ABSTRACT

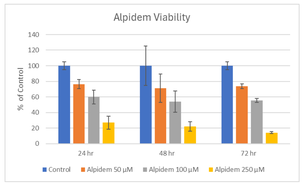
Background and Purpose:
Many in vitro models for hepatotoxicity have been explored over the past decade including novel alternative methods (NAMs) such as hepatic spheroids, organoids and liver-on-chip models. While these platforms provide improved physiological models of the tissue, they are often limited by throughput cost and the need for specialized equipment. While initially used for drug metabolism and PK modeling, hepatocyte co-cultures have reemerged as useful models for studying pharmacological pathways, generation of in vitro disease models, and investigative tools for hepatotoxicity. In this study we utilized TruVivo®, an all-human hepatocyte co-culture system, to see if it could distinguish between approved drugs that were removed from the market for hepatotoxicity and their non-hepatotoxic analogs.

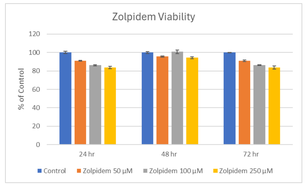
Methods: Cells and media were provided by LifeNet Health. Cells were seeded and cultured according to instructions provided with the kits. Stromal cells were seeded first followed by hepatocytes at 30,000 cells per well in 96-well collagen-coated plates. Following seven days of culture, test compounds were added at three different concentrations and cells were further incubated for up to 24, 48 and 72 hours. DMSO (0.5% final concentration) served as the vehicle control. Four different endpoints were assessed at each time point. The endpoints included cell viability, glutathione (GSH) depletion, generation of reactive oxygen species (ROS) and mitochondrial membrane potential (MMP). MMP was assessed by fluorescence using the cationic dye JC-10 (Abcam) while the other endpoints were assessed using chemiluminescent kits from Promega. All cell-based measurements were made on a Cytation 5 Multimode plate reader (BioTek). In addition, media was changed daily with replenishment of test compounds. At the end of each time point, media samples were collected and analyzed for parent compound, major metabolite and GSH conjugate formation by LC-MS/MS.

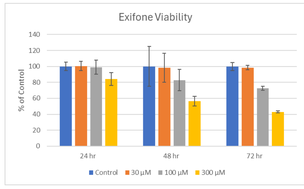
Results: Alpidem and zolpidem were each tested at 50, 100 and 250 µM while exifone was tested at 30, 100 and 300 µM. Alpidem caused a time- and concentration-dependent loss of cell viability and intracellular GSH. In addition, alpidem stimulated the formation of ROS and led to a loss of MMP. Metabolism data showed active formation of its known major metabolite and also a GSH conjugate, suggesting the formation of a reactive intermediate. In contrast, zolpidem did not cause a loss of cell viability at any time point or test concentration nor did it deplete intracellular GSH. Zolpidem treatment resulted in a modest stimulation of ROS but had no effect on MMP. Analysis of zolpidem metabolism indicated the formation of its known major metabolite; however, no GSH conjugate was detected. Similar to alpidem, exifone caused a time- and concentration-dependent loss of cell viability and intracellular GSH. At the highest concentration (300 µM) exifone caused almost complete depletion of GSH. Additionally, exifone caused a robust stimulation of ROS. Addition of N-acetylcysteine or 1-aminobenzotriazole (ABT) did not prevent the cytotoxic effects of alpidem or exifone.

RESULTS

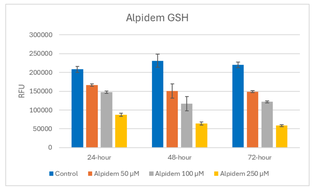
Cell Viability

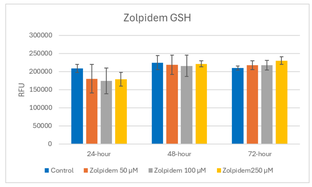


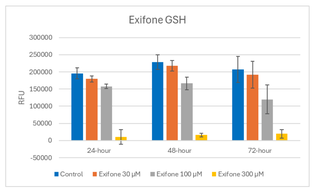




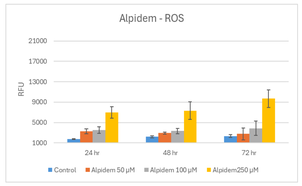
Glutathione

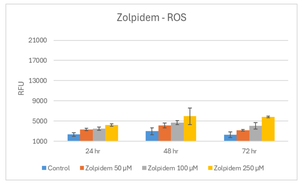


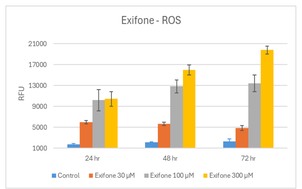




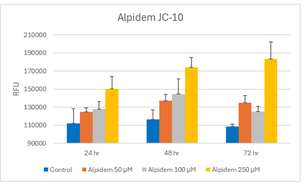
ROS

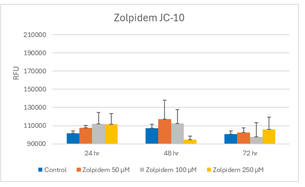


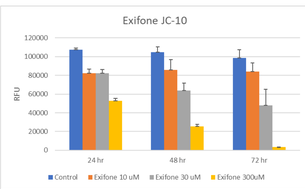




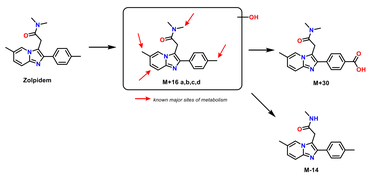
MMP

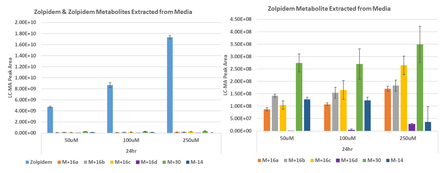




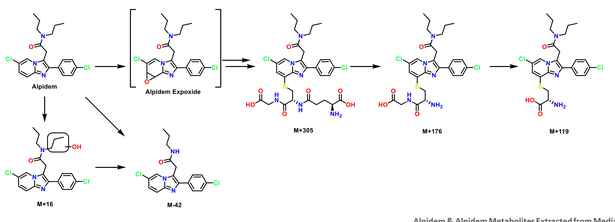


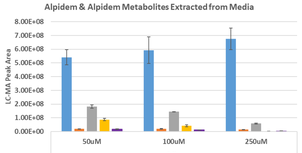
Zolpidem Metabolism

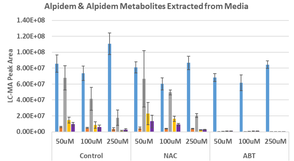




Alpidem Metabolism

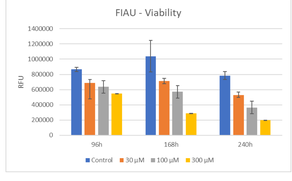


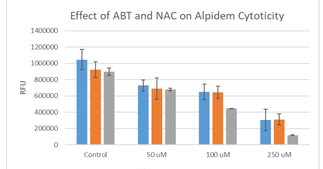


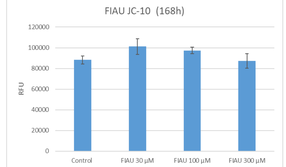


Fialuridine (FIAU)

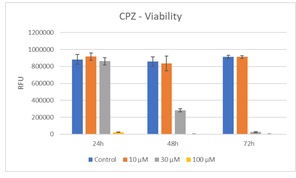
FIAU is a nucleoside antiviral compound that caused severe hepatotoxicity and death in a phase II clinical trial in the 1990s. FIAU inhibits mitochondrial γ DNA polymerase resulting in lactic acidosis. Toxicity takes multiple days of dosing to appear

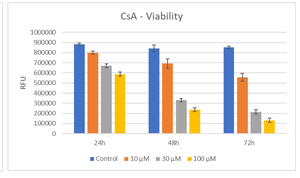




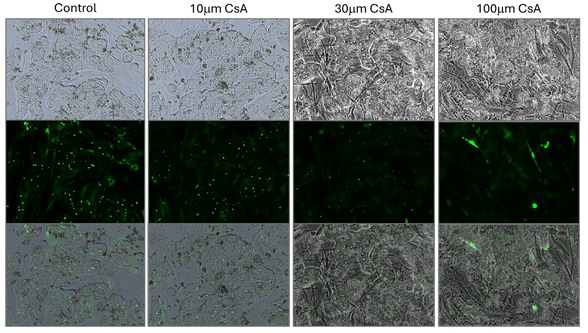


Cholestatic Compounds: Chlorpromazine and Cyclosporin A





Effect of 24hr Cyclosporin A (CsA) treatment on CDFDA accumulation in bile canaliculi of hepatocytes in TruVivo 2D+ System



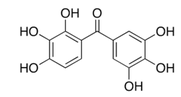
Images generated with a 10X phase objective and ECHO Revolve Microscope using transmitted light for bright field images (top) and fluorescence with FITC filter set for green CDFDA images (center). Bright Field and fluorescent images are overlayed to show position of green fluorescent bile canaliculi (bottom)

TEST COMPOUNDS

Alpidem (Ananxyl) is a nonsedating anxiolytic (GABA receptor agonist) launched in 1991 in France and withdrawn in 1993 due to liver injury (104 cases). Alpidem also binds to the peripheral benzodiazepine receptor (TSPO) located on the outer mitochondrial membrane.

Zolpidem (Ambien) is a benzodiazepine receptor agonist that was approved for use in the United States in 1992 for the short-term treatment of insomnia. Although a close structural analog of alpidem, zolpidem has not been reported to cause clinically apparent liver injury.

Exifone (Adlone) is a nootropic that was developed to treat cognitive memory problems in the aged. It was launched in April 1988 in France but was withdrawn in May 1989 due to several cases of hepatic injury. A tentative estimate of the prevalence of hepatic reactions was 1 in 10,000-15,000 treated patients The metabolism of exifone is complex and no metabolites were recovered from media following cell incubations. Additional work is required to identify and quantify the metabolites. ABT did not inhibit cytotoxicity



Conclusions

The TruVivo® hepatocyte co-culture system was able to detect hepatotoxicity from two drugs that passed through pre-clinical and clinical studies with an acceptable safety profile. Once marketed they were soon removed for serious cases of hepatotoxicity. In addition, the in vitro assay did not detect signs of hepatotoxicity with zolpidem, a close structural analog of alpidem with no known liver toxicity. In addition, two compounds associated with cholestasis (chlorpromazine and cyclosporin A) and one known inhibitor of mitochondrial DNA synthesis (FIAU) also showed adverse effects in this system.

Results provided insight into possible mechanisms of toxicity for both alpidem and exifone such as reactive intermediate formation with GSH depletion, mitochondrial dysfunction and ROS generation. The extended culture time of the co-cultures afforded an opportunity for repeat dosing out to 10 days and potentially longer. In summary, TruVivo® appears to be a useful tool to study the hepatotoxicity of problematic drugs.