A Multiple Organ Integrated *In Vitro* Model for Studying Repeated Dose Toxicity

JM McKim, Jr.[1], JA Willoughby, Sr.[1], N Hibbard[1], B Ebendick-Corpus[1] and JT Dever[2]

[1]IONTOX LLC, Kalamazoo, MI and [2]Nutrilite Health Institute, Amway Corporation, Buena Park, CA

ABSTRACT

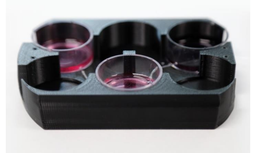
Repeated dose toxicity studies in animals provides information on cumulative toxicity of the test compound. These data may be both qualitative and quantitative, and provide information on organ toxicity, dose response, and no adverse effect levels. An *in vitro* integrated multiple organ model with simulated blood flow could provide many key parameters related to repeated dosing studies and ultimately lead to the replacement of these animal tests. Therefore, the aim of this study was to demonstrate how an *in vitro* approach can be used to evaluate repeated dose endpoints. Two well studied compounds Acetaminophen (APAP) and Cycloheximide (CYHex) were selected for this study. The compounds were evaluated in an *in vitro* integrated organ platform (HuDMOP™) that links human 3D intestine (EpiIntestinal™, MatTek), liver (transporter certified human primary hepatocytes in sandwich culture (HHSC)), and kidney (primary human proximal tubule epithelial cells (hRPTEC)) tissue models via a simulated blood system. Pharmacokinetic and pharmacodynamic effects can be measured over time. APAP (0.1mL of a 2500 µM stock) and CYHex (0.1 mL of a 100 µM stock) were applied to the apical side of the intestinal model (0.1mL of a 2500 µM stock) at time 0 and 24 hr. Samples for determining compound movement were collected from the intestinal basolateral chamber, liver media, kidney media, and perfusate at 0, 1, 2, 4, 6, 24, and 48 hr and analyzed by LC-MS/MS. Cytotoxicity was also determined at these times by lactate dehydrogenase release (LDH). Samples for gene expression and cytotoxicity (ATP and LDH) were collected at 48 hr. APAP showed a rapid increase in the intestinal basolateral chamber and in simulated blood. A Cmax of 1800 µM was achieved at 3-4 hr. Bioavailability was estimated to be 72%. Increases in APAP were time dependent in liver and kidney. Small increases in LDH were observed in the intestine at 24 hr (20% leakage) and 48 hr (35% leakage). No cytotoxicity was observed in the liver, but a small increase in LDH (10%) was observed in the kidney at 48 hr. APAP induced changes in CYP1A2 (2.5-fold), CYP3A4(3.5 fold) and Nrf2(2-fold)in the liver. Bcl2, CYP3A4, were induced in the kidney (3.5 fold). CYHex reached a Cmax of 16 µM at 5-6 hr with a bioavailability of 80%. LDH leakage was observed in the intestine at 24 and 48 hr, liver at 4 hr, and kidney at 48 hr only. In contrast to APAP, CYHex induced several genes (BAX 2-3 fold, CYP1A1/2 4-16 fold, glutathione peroxidase 2-4 fold, and Nrf2 5-10 fold) related to toxicity pathways in all three organs. This study demonstrates that a meso-scale three organ *in vitro* model can provide important data PK and PD for predicting repeated dose toxicity.

INTRODUCTION

The development of new technologies that mimic human organ structure and circulation is important for studying the interplay between body organs and for evaluating the effects of drugs and chemicals on systemic toxicity. The ability to perform repeated dose toxicity studies in human tissues would reduce the use of animals. In recent years the technologies used to develop human organotypic models has greatly improved. It is now possible to use 3D human lung, liver, skin and intestinal models to name only a few. Microfluidics and microfabrication has shown that tissues can survive longer in culture and maintain *in vivo* like properties. Although several groups are developing sophisticated and complex micro-models that combine bioengineering with functional anatomy of an organ, they are less likely to provide a realistic means of linking drug or chemical biological effects with actual human pharmacokinetics and pharmacodynamics. One reason for this is the miniature scale, which creates new challenges with regard to assay sensitivity and the balance between tissue mass and fluid volume. The aim of the present study was to evaluate a non-toxic (Acetaminophen) and a toxic (Cycloheximide) in an integrated three organ platform HuDMOP™.

FIGURE 1

Complete Hµ-DMOP Plate Design Showing Six and Three Organs



HµDMOP US Patent US9,631,167,B2

Diagrammatic representation of the full six organ plate layout (upper left) and three organ plate layout used for this study (upper right and lower).

METHODS

Preparation of Plates. Hµ-DMOP™ custom designed plates (Figure 1) were used and equipped with a simulated blood system. The simulated vascular system consisted of tubing connected to a semipermeable membrane. The section of semipermeable membrane was 3 cm in length. The tubing was custom fit into the plate, such that only the dialysis membrane was in contact with each organ compartment. A perfusion rate of 5 µl/min was used in each experiment.

Cell Culture

Intestinal Compartment. The EpiIntestinal™ 3D human tissue from MatTek, Corp. was used for the intestine chamber. Tissues were cultured under standard conditions on transwell inserts. Tight junctions were assessed by transepithelial electrical resistance (TEER). The EpiIntestinal™ tissues were placed into the Hµ-DMOP™ plates (Figure 1) and connected to the liver compartment via simulated blood system (Figure 2).

Liver Compartment. The liver compartment was simulated with Transporter Certified™ human primary hepatocytes from BIOIVT in sandwich culture. The cells were added to the Hµ-DMOP™ cup in culture media at a density of 500,000 cells/well and incubated at 37ºC, 5% CO_2 for 48 hr prior to beginning the experiments.

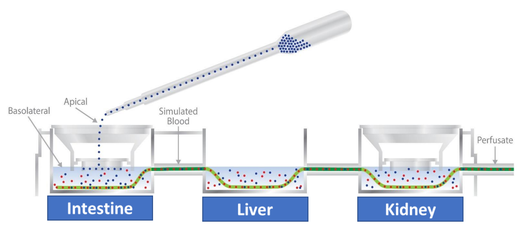
Kidney Compartment: To simulate a kidney human renal proximal tubule cells from Lonza were used. The cells were added to the Hµ-DMOP cup in culture media at a density of 1.1 x 10^6 cells/well and incubated at 37ºC, 5% CO_2 for ~5 days prior to beginning the experiments.

Dosing Regimen: After equilibration, the test material was added to the apical side of the intestinal chamber to simulate an oral exposure at time 0 and 24 hr. For acetaminophen (APAP) the dose was 100 µL from a 2500 µM stock, while for cycloheximide (CyHex) the dose applied was 100 µL from a 100 µM stock.

Analytical Procedures. APAP and CyHex were measured by LC-MS/MS. Standard curves and QC samples were prepared in PBS and compared to standard curves and QC samples in media with and without serum.

FIGURE 2

Diagrammatic Representation of a Three Chamber Plate System with Fluidics

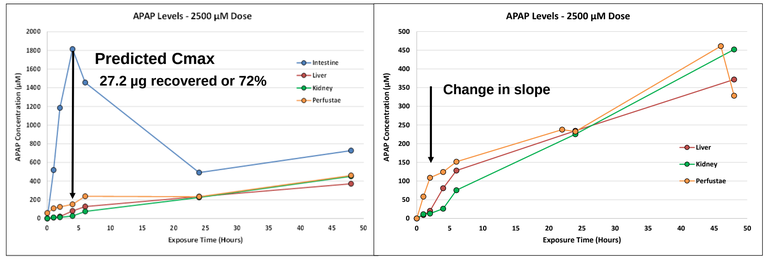


A simple three-compartment organ model was tested by adding APAP and CyHex to the apical side of the intestinal tissue at time 0 and 24 hr. Absorption and distribution of APAP and CyHex was determined by LC-MS/MS. Each simulated-organ compartment was isolated from the other compartments. Movement of APAP and CyHex between organ chambers occurred solely via movement across the semipermeable membrane of the simulated blood system.

RESULTS

FIGURE 3

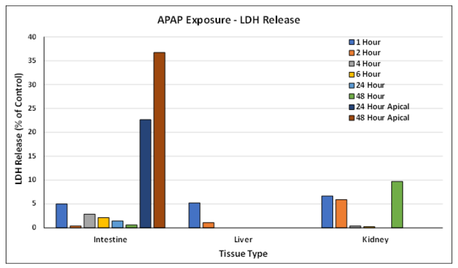
Time-Dependent Accumulation of Acetaminophen (APAP) in a Three Organ Configuration



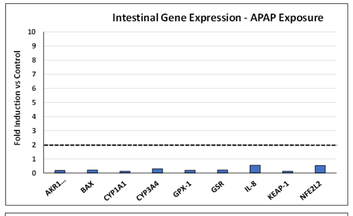
APAP was administered (100 µL of a 2500 µM stock = 38 µg mass at time 0) to the intestinal chamber at time 0 and at time 24 hr. The system was allowed to run for 48 hr. APAP kinetics was measured by removing aliquots of media in the basolateral compartment of the intestine, the media overlaying the hepatocytes, and the perfusate at time 0, 1, 2, 4, 6, 24, and 48 hr and analyzing for parent molecule by LC/MS/MS. At Cmax 27.2 ug had been recovered (72%). Both Cmas and Tmax determined by the system agreed well with values reported *in vivo*. For all chemicals tested the peak concentration in the basolateral compartment of the intestine represents systemic exposure and this correlates to a change in slope of perfusate (simulated blood (arrow on right)). Accumulation of parent in the liver and kidney could also be observed. Values represent the mean of 2 experiments.

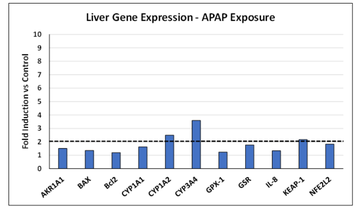
FIGURE 4

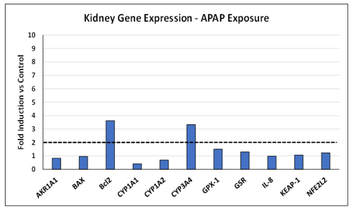
Effects of APAP on Cell Viability and Gene Expression



Lactate dehydrogenase (LDH) was used to measure toxicity. APAP at 2500 µM had a mild toxic effect on apical intestinal cell tissue, but not basolateral tissue. There were small effects in the liver, while the kidney showed a significant toxicity at 48hr. Values represent the mean of 3 samples each from 2 experiments.



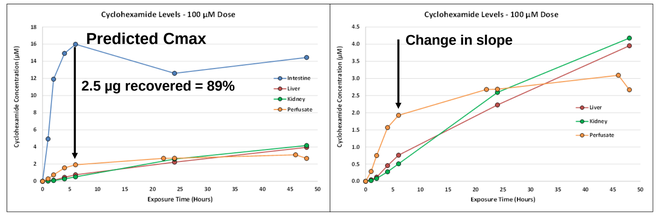




Key genes associated with cell health were monitored. APAP had no effect in the intestine, but induced CYP1A2 and CYP3A4 in liver, and Bcl2 and CYP3A4 in kidney**.** Values represent the mean of 3 samples from 2 experiments.

FIGURE 5

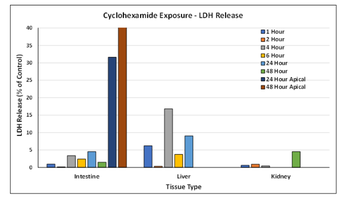
Time-Dependent Accumulation of Cycloheximide in a Three Organ Configuration



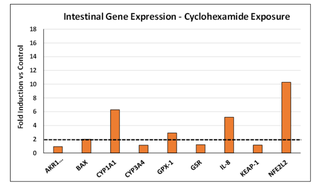
Cycloheximide (CyHex) is a potent inhibitor of protein synthesis and is considered to be cytotoxic with a rat acute oral toxicity of 2 mg/Kg. CyHex was added to the intestinal apical side at time 0 and 24 hr by adding a 100 µL aliquot of a 100 µM solution yielding a total mass of 2.8 µg. At Cmax, 2.25 µg had been recovered representing 89% of the initial dose. Like APAP, CyHex also produced a change in slope of the perfusate (right graph) that corresponded with Cmax. Both the Cmax and Tmax agreed with *in vivo* data.

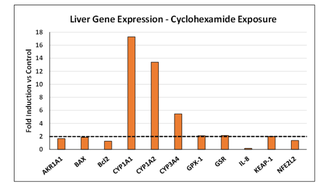
FIGURE 6

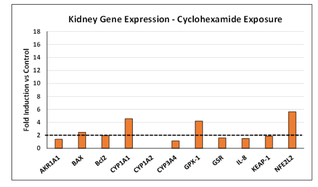
Effects of Cycloheximide on Cell Viability and Gene Expression



CyHex caused toxicity to the apical side of the intestine and mild toxicity to the basolateral side. there was significant toxicity in the liver with only a small effects measured in the kidney.







Key genes associated with cell health were monitored. CyHex increased the expression of several genes in all three organ compartments. The greatest effects were observed in the liver. these data are consistent with reported *in vivo* toxicity. Values represent mean of 3 samples from 2 experiments

SUMMARY

Repeated dose systemic toxicity in human tissues can be evaluated.

The IONTOX HuDMOP™ Integrated organ platform allows human tissues to be evaluated in an *in vitro* system. The meso-scale allows for repeated fluid and tissue samples.

This work has shown that the determination of pharmacokinetic parameters and toxicity without the use of animals is possible.