ABSTRACT

In vitro methods capable of describing systemic effects of chemicals require use of multiple tissue types connected with a common perfusate. This arrangement allows integration of absorption, metabolism and toxicity data over extended times in vitro and provides a novel, animal-free tool for chemical, cosmetic, and pharmacological testing. In order to test this, a study on the uptake and distribution of acetaminophen (APAP) in a human dynamic multi-organ plate (HuDMOP™) with three tissue compartments was conducted in series: first absorption across the human 3D intestine (Epithelial, Mattek Corp), then on to a liver surrogate with human primary hepatocytes in sandwich culture and then to a kidney preparation (human renal proximal tubule cells) was developed. A common perfusate with human albumin connected the three compartments. APAP was placed on the apical side of the intestinal surrogate at 0 and 24 hr. Samples were collected from all three compartments over time and analyzed for APAP by LC/MS/MS and cytopoietic cytotoxicity by LDH leakage. The APAP in the apical reservoir peaked to 60.7µM at around 4 hours with a total uptake of 72% of the applied dose entering the first reservoir. A simple PK model was developed to describe the three cellular platforms and their physical arrangement. Mass balance equations were fit to experimental data to estimate uptake and transport characteristics. The inter-chamber flow rates and fitted experimental absorption rate constant, 0.073/hr, were consistent with a Cmax of 42.0µM and time of maximum concentration between 3 and 4 hr in the intestine compartment. With the current platform flow rates, much lower concentrations were present in the subsequent two compartments (liver and kidney) with maximum observed concentrations of 4.5 and 2.5 versus 3.1 and 0.9 µM predicted. The interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to expected in-life behavior of various chemicals.

METHODS – MODEL DEVELOPMENTS

In order to better understand the in vitro system a pharmacokinetic model was developed. Absorption across the HIE into the intestine compartment was simulated as a first-order absorption process. Intestine, kidney, and liver compartments were described by a volume, flow rate, and clearance rate. The final collection compartment was simulated as a sink accumulating any compound not retained or removed by the previous compartments. Compartment volumes were from 2.5 to 3 mL, and the flow through the system was 5 µL/min. The compartments are assumed to be well-mixed and in equilibrium with the semipermeable tubing perfusing the system. APAP and cycloheximide experiments were used to fit the absorption and clearance rates:

1. Concentration in compartment 1 (Intestine) → First-order absorption rate constant (ka)
2. Concentration in compartment 2 (Liver) → Michaelis-Menten metabolism (Vinax and Kin), Partition between media and tissue (PA)
3. Concentration in compartment 3 (Kidney) → First order elimination constant in the kidney (ka)

The model outputs for all compartments and the model output in the collection were exported and plotted using Microsoft Excel.

RESULTS

- Preparation of Plates: HuDMOP™ 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.
- Cell Culture
  - Intestinal Compartment: The Epithelial™ 3D human tissue from Mattek Corp. was used for the intestinal chamber. The tissue was cultured under standard conditions on transwell inserts. Tight junctions were assessed by transepithelial electrical resistance (TEER). The Epithelial™ tissues were placed into the HuDMOP™ plates (Figure 1) and connected to the liver compartment via simulated systemic system (Figure A).
  - Liver Compartment: The liver compartment was simulated with Transporter Certified™ human primary hepatocytes from BIOIVT in sandwich culture. The cells were added to the HuDMOP™ cup in culture media at a density of 500,000 cells/well and incubated at 37°C, 5% CO2 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 HuDMOP cup in culture media at a density of 1 x 106 cells/well and incubated at 37°C, 5% CO2 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 300 µM from a 2500 µM stock, while for cycloheximide (CyHex) the dose applied was 100 µM 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.

CONCLUSIONS

In vitro methods capable of describing systemic effects of chemicals require use of multiple tissue types connected with a common perfusate. This arrangement allows integration of absorption, metabolism and toxicity data over extended times in vitro and provides a novel, animal-free tool for chemical, cosmetic, and pharmacological testing. Integration with computational modeling is key to transforming these unique data to in vivo application, and the interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to expected in-life behavior of various chemicals.

The current data provide a basis for in silico modeling of the in vitro system. The computational model predicts represent the data well, though there appears to be a more abrupt appearance in the final perfusate collection for both chemicals, and the cause is under investigation. Current thoughts for computational probing include the possibility for nonspecific binding to plastic in the system, and rate-limiting uptake into and out of the semipermeable membrane perfusing the compartments.

The computational modeling approach is increasingly used as a way of mathematically representing, interpreting, and extrapolating experimental data from in vitro (and in vivo) systems. The development and model-based interrogation of such novel in vitro systems to better inform chemical kinetics and toxicity in future testing of chemicals holds significant promise for reducing animal use, time, and money, with the ultimate goal of predicting human kinetics and toxicity without animal testing.

FUTURE DIRECTIONS

The ultimate goal of this partnership of a novel in vitro system and computational modeling is to predict the human health effects of chemical exposure. Through iterative computational and laboratory innovation, we can achieve this goal.