In Vitro Evaluation of the Pharmacokinetics of Phenoxyethanol in the Human Dynamic Multi-Organ Plate (HuDMOP™).

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ABSTRACT

The HuDMOP™ system is an integrated meso-scale multiple organ culture plate that may be used to predict human pharmacokinetics (PK) and pharmacodynamics (PD). Here we assessed 2-phenoxyethanol (PE) PK and PD in this system. PE is commonly used in cosmetics, personal care products, and pharmaceuticals. PE is well characterized and is metabolized to 2-phenoxyacetic acid (PAA) in the body. The first HuDMOP™ setup used consisted of human intestine (EpiIntestinal™, MatTek) and primary human hepatocytes in sandwich culture (HHSC) linked via simulated blood system. In the second setup, intestine was linked to HHSC and kidney cells (primary human proximal tubule epithelial cells (HRPTEC)) via a simulated blood system. PE solutions (100 µL of 0.5, 1, 5, and 50 mM) were applied to the apical side of the intestinal model. Samples were collected from the basolateral intestine media, liver media, kidney media, and the simulated blood (perfusate) at 0, 1, 2, 4, 6 and 24 hr. PE and PAA were measured in each sample by LC-MS/MS. Maximum concentration of PE in the intestinal basolateral media and perfusate was reached at 1-4 hr and was dose dependent. PE in the liver media was quantifiable at 2-4 hr, occurring more rapidly with higher doses. PE was also observed in the kidney media by 4 hr while PAA was detected at 24 hr in liver media and in the perfusate. The time to maximum PE and PAA concentration, and movement of PE and PAA through both setups, was dose and time dependent. Tissue health was determined by measuring LDH leakage. No substantial toxicity was measured over the 24 hr period. Finally, expression of interleukin-8 (IL-8), tumor necrosis factor alpha (TNFa), cytochrome P450s 1A1 (CYP1A1) and 3A4 (CYP3A4) were assessed by qPCR in all tissue types. No changes in gene expression were observed in the liver, and kidney, however a slight induction of CYP3A4 at the highest dose (2.4-fold) and IL-8 at the two highest doses (2.8-fold and 2.7-fold, respectively) were observed in the intestinal tissue. These results suggest no substantial irritation response to PE or PAA which fits the known toxicological profile of PE at the concentrations assessed. Additionally, the PE Tmax obtained here (1-4 hr) matches known *in vivo* results (1 hr). The observed PE Cmax, while dose dependent, appeared to correlate with known *in vivo* results as well. These data indicate that the HuDMOP™ model may be an effective platform to properly model and predict PK and PD in humans.

INTRODUCTION

Repeat low dose systemic toxicity testing is a key component of chemical safety assessment. These studies are currently performed in animals and provide information on target organ toxicity, dose-response for each endpoint, identification of critical endpoints, and the development of NOAELs and NOELs which are required for quantitative risk assessment (QRA). Currently, there are no *in vitro* alternatives for this important phase of safety assessment. Nevertheless, significant efforts are being made to develop alternative methods. To achieve this goal, it is generally agreed that the *in vitro* models must provide an integrated organ approach and use human tissue models. In addition, the system must incorporate a fluidics system (simulated blood) that closely mimics the *in vivo* environment. The system should be affordable and should be on a scale that allows the use of multiple tissue types (organs), and allow for several endpoints to be monitored. Standard pharmacokinetic models should describe the movement of test agents in the *in vitro* system and therefore enable scaling of the *in vitro* data to *in vivo* effects. One promising platform is the patented HuDMOP™ model that is a dynamic multiple-organ system where the individual organ models of choice are connected with a fluid system modeled after the human vascular system. The system is on a meso-scale and uses a unique dialysis component to the fluidics system that prevents complete media exchange between compartments and allows for chemical uptake and delivery in a manner that mimics *in vivo* systems. HuDMOP™ allows for toxicokinetic modelling and measurement of toxicodynamic effects *in vitro*.

2-Phenoxyethanol (PE) is widely used as a preservative and germicidal compound in the cosmetic and personal care industry. The metabolism and toxicological effects of PE are well understood. At very high concentrations, PE has been shown to cause hepatotoxicity, renal toxicity, and hemolysis at dosages ≥ 400 mg/kg/day in numerous species.[1,2] Upon ingestion, PE rapidly converted to 2-phenoxyacetic acid (PAA), the primary metabolite of PA, via alcohol dehydrogenase and aldehyde dehydrogenase.[3,4] This, as well as the low LogP (~1.16) and low toxicity, make PE an excellent molecule to assess the repeat-dose predictive capabilities of the HuDMOP™ platform.

In order to assess repeat-dose predictive potential of the HuDMOP™ system, EpiIntestinal™ tissues were linked, via a simulated blood flow, to Transporter Certified™ human primary hepatocytes in sandwich culture and then to primary human renal proximal tubule epithelial cells (HRPTEC). The EpiIntestinal™ tissues were exposed to numerous concentrations of PE (0.5, 1, 5, 50 mM) for 24 hours. The absorption and distribution of PE to the liver and kidney compartments, and subsequent metabolism to PAA, was assessed by LC-MS/MS. Viability was assessed in each tissue by determining LDH release in each tissues compartment and changes in expression of the following genes was determined in each tissue as well; Interleukin 8 (IL-8), tumor necrosis factor alpha (TNFα), Cytochrome P450 1A1 (CYP1A1) and Cytochrome P450 3A4 (CYP3A4).

METHODS

Cell Culture
Transporter Certified™ human primary hepatocytes (BioIVT, Durham, NC) in sandwich culture and the primary renal proximal tubule epithelial cells (HRPTEC; Cell Applications, San Diego, CA) were both seeded at 1.1 x10^6 cells/well using the provided culture media in proprietary 6-well cups. EpiIntestinal™ tissues (MatTek Corp., Ashland, MD) were placed in the proprietary 6-well cup containing the provided EpiIntestinal™ media (SMI-100-MM). The cells and tissues were maintained at 37°C, 5% CO_2.

Dosing Solutions
PE was purchased from Sigma-Aldrich and was ≥99.5% pure. Dosing solutions were prepared under sterile conditions on the day of dosing. The PE dosing solutions were prepared in Phosphate Buffered Saline (PBS) at 0.5, 1, 5, and 50 mM concentrations. The EpiIntestinal™ tissues were exposed to 50 µL of 0.5, 1, 5 and 50 mM PE (3.5, 7, 35 and 350 µg total dose of PE, respectively). A single dose was administered at T0.

Lactate Dehydrogenase Cytotoxicity Assay
Cytotoxicity was determined as LDH leakage. LDH activity was measured with Promega's CytoTox-ONE™ Homogenous Membrane Integrity Assay. The assay was performed according to the manufacturer's instructions.

METHODS

qPCR
RNA was isolated using the Qiagen RNeasy Mini Kit (QIAGEN, Valencia, California, USA). Briefly, Buffer RLT was added to each well to cover the primary hepatocytes, HRPTECs and the EpiIntestinal™ tissues, and the plates were placed at -80°C.

After thawing, the RNA was isolated according to the manufacturer's instructions. RNA was diluted to 10 ng/µL and reverse transcription was performed using the High Capacity cDNA Reverse Transcription kit from Applied Biosystems (Foster City, CA). The assay was performed according to the manufacturer's instructions.

qPCR was performed using the TaqMan Master Mix 2 with UNG along with the TaqMan Gene Expression Primer Assays from Applied Biosystems (Foster City, CA). The genes assessed were GAPDH (Hs_99999905_m1), as a housekeeping gene, and Interleukin 8 (IL-8 Hs_00174103_m1), tumor necrosis factor alpha (TNFα; Hs01113624_g1), Cytochrome P450 1A1 (CYP1A1; Hs01054797_g1) and Cytochrome P450 3A4 (CYP3A4; Hs00430021_m1) as target genes. The assay was set up in an optically clear qPCR plate. The plates were placed in a QuantStudio 5 qPCR machine () and run at the following settings: 1 cycle at 50°C for 2 minutes, one cycle at 95°C for 10 minutes. This was followed by followed by 40 cycles of the following: 15 seconds at 95°C, 60 seconds at 60°C.

LC-MS/MS Analysis
At the indicated time points (1, 2, 4, 6 and 24 hr) media samples were removed from the intestinal, liver, kidney, and perfusate (simulated blood). LC-MS/MS was performed on media/perfusate samples according to standard LC-MS/MS methods.

Figure 1. The three compartment HuDMOP™ system routinely used at IONTOX (intestine – liver – kidney) complete with pump. Each cell or tissue type remains in its own optimal media, so the media is not circulated between compartments.

RESULTS

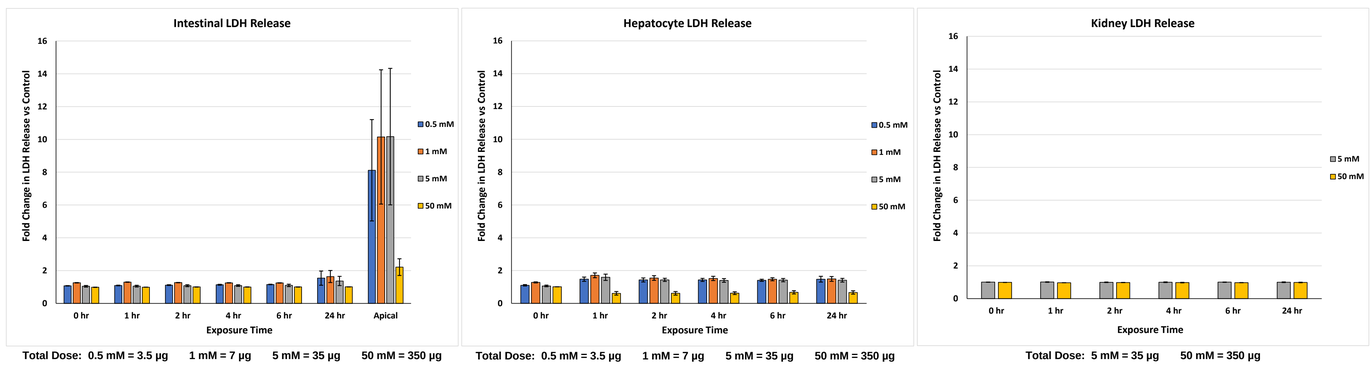

Figure 2. Intestinal, hepatic, and renal toxicity was assessed by lactate dehydrogenase (LDH) activity. The data are presented as a % increase in LDH compared to the PBS or media exposed control. The results are means of duplicate runs(n = 2 to 6) with the error bars representing the standard error of the mean (SEM) of all samples (three samples analyzed per run for a total of 6 samples taken for LDH).

RESULTS

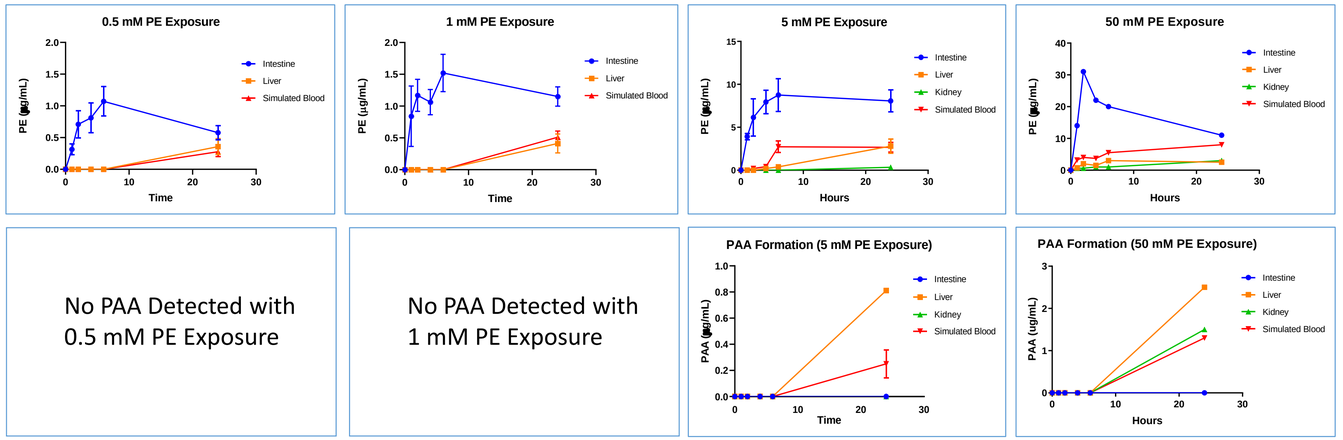

Figure 3. Distribution of PE and PAA over time in the basolateral media of the intestinal tissue, the hepatocyte media, the renal media, and in the simulated blood flow (perfusate). The data is presented as µg/mL PE and PAA. Values represent the mean of duplicate runs (n = 2 to 6) with the error bars representing the standard error of the mean (SEM). Total PE Dose: 0.5 mM = 3.5 µg, 1 mM = 7 µg, 5 mM = 35 µg and 50 mM = 350 µg.

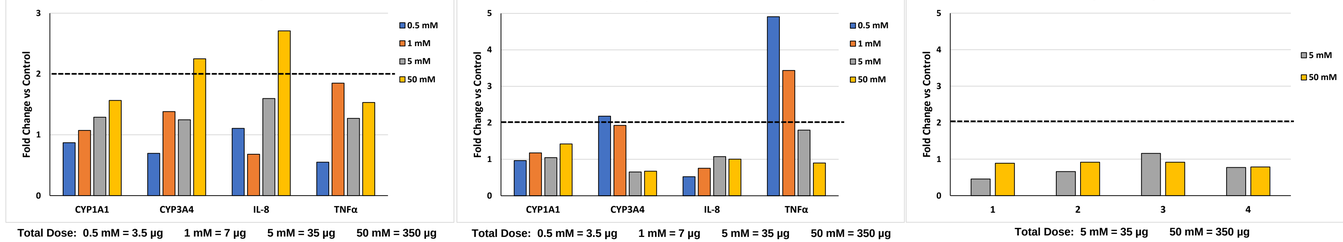

Figure 4. Intestinal, hepatic, and renal expression of CYP1A1, CYP3A4, IL-8, and TNFα genes after 24 hours exposure. The black dotted line represents a 2-folds induction which is considered biologically relevant. The data shows the only observed induction is with IL-8 (both 5 and 50 mM) and CYP3A4 (50 mM) in the intestine. No induction over 2-fold was observed in the kidney or the liver. The data is presented as fold induction vs PBS exposed control. Values represent the means of duplicate runs (n = 2 to 6).

Conclusions

PE exposure concentration up to 50 mM (350 µg total dose) induced no observable toxicity in any tissue model as determined by LDH release.

Analysis of gene expression changes showed a slight induction above 2-fold for CYP3A4 and IL-8 in the intestinal tissue in response to 50 mM PE (350 µg total dose). No changes in expression were observed in the kidney.

The HuDMOP™ system appeared to properly model PE distribution to the liver and kidneys, as well as properly model PE and metabolism to PAA.

Similar to *in vivo* results, the max concentration in the simulated blood and the basolateral media (interstitial space) was both dose dependent and time dependent. The higher the initial exposure concentration, the higher the concentration in the basolateral intestinal media and simulated blood, as well as the faster the Cmax is reached (Tmax or Time to Cmax) and the higher the Cmax. Future work includes determining AUC after multiple doses over 48 hr and evaluation of transporters.

The HuDMOP™ model may be a highly predictive *in vitro* platform to properly model and predict PK and PD in humans.

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