Evaluation of an Integrated Human Multi-Organ Culture Plate For Predicting Systemic Toxicity

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ABSTRACT

The framework put forth in the report entitled "Toxicity Testing in the 21st Century" (2007) focused on the need for the development of alternative methods to animal testing. Emphasis was placed on the use of human cells combined with adverse outcome pathways for determining adverse effects. Predicting systemic toxicity *in vitro* requires a platform that incorporates multiple organs interconnected via a fluidics network. The aim of this study was to evaluate a new Dynamic Multi-Organ Plate (DMOP) that provides the ability to use human tissues or cells, which are in communication via fluidics and dialysis. A simple transwell-based, two organ compartment model containing Caco-2 cells and rat primary hepatocytes was used initially. A standard 6-well plate was fitted with a fluidics/dialysis network. The dialysis membrane allows for exchange of test article and metabolites while maintaining each cell type under optimized media conditions. Caco-2 cells were grown to confluency on a transwell insert and then transferred to the DMOP. To mimic oral dosing, Acetaminophen (APAP) (200 µM) was added to the upper chamber of transwells containing confluent monolayers of Caco-2 cells. Dialysis samples from the lower chamber were collected at a flow rate of 1 µL/min. Integrated time samples were collected at 1, 2, 3, and 6 hr. APAP was first detected in the 1-2 hr dialysate with a relative recovery of approximately 70%. APAP was added to the second compartment containing a monolayer of primary rat hepatocytes and dialysis samples collected over 2 hr. APAP, as well as its glucuronide and sulfate conjugates, were detected in the perfusate. Relative recovery of APAP in the hepatocyte dialysate was >95%. These data demonstrate that a meso-scale multi-organ culture platform linked by fluidics/dialysis can provide a means of evaluating the movement of chemicals and their metabolites between organs in order to predict systemic effects *in vitro*.

INTRODUCTION

The development of new technologies that mimic human organ structure and circulation is important for evaluating the effects of drugs and chemicals on systemic toxicity without the use of animals. With the advent of new tissue models for skin, lung, liver, and heart, the opportunity to link these systems together has never been better. 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 this study was to design and validate a new larger-scale multiple organ plate with a fluid system modeled after to the human vascular system. In this model, the fluidics system uses a dialysis membrane that is present only in the organ compartment. Wells and tissues are linked by non-permeable tubing. This design keeps each organ isolated from the other organ wells. The only exchange between wells is the movement of small molecules that across the dialysis membrane. Perfusion fluid is circulated through the fluidic system using a micro syringe pump, thereby maintaining a diffusion gradient until steady-state kinetics are achieved. The administration of a drug or chemical to the apical side of the intestine well (Caco-2) can be followed as it moves into the basolateral compartment. The analyte then diffuses into the circulating perfusion medium where it is carried to the liver compartment. In the liver compartment, the analyte is delivered to the culture medium until the chamber reaches equilibrium. By collecting medium directly from each organ compartment, the total analyte concentration can be determined. The relationship between analyte concentration and distribution mimics a two-compartment oral-absorption model.

METHODS

Preparation of Plates
Initially, standard 6-well plates were used and the fluidic system installed manually. The fluidic system consisted of microdialysis loop probes manufactured by Bioanalytical Systems Inc. (BASi)(Lafayette, IN). The loop probes (3 cm) and their tubing were custom fit into the plate such that only the dialysis membrane was in contact with each organ compartment. A perfusion rate of 1 µl/min was used in each experiment.

Cell Culture
Intestine Compartment
Intestinal absorption was simulated by using Caco-2 cells cultured under standard conditions on transwell inserts (Snapwells, Corning®) in 6-well plates. The cells were checked for use by transepithelial electrical resistance (TEER). A reading of >250 ohms indicated that the cells had formed tight junctions and were ready to be placed into the dynamic multi-organ plate (DMOP). The cells were cultured in Eagles Modified Essential Medium (MEM) with 20% FBS.
Liver Compartment
The liver compartment was simulated with the human hepatoma cell line HepG2. Cells were cultured in MEM containing 10% FBS. The cells were added to the 6-well plate in culture media at a density of 500,000 cells/well and incubated at 37 degrees C, 5% CO_2 for 48 hr prior to beginning the experiments.

Single Bolus Dose Experiment
A diagrammatic view of the plate design is shown in Figure 1. A single 500 µL aliquot of culture medium containing 200 µM acetaminophen was added to the apical side of the Caco-2 chamber. The perfusion pump was started at a constant flow rate of 1 µL/min. The perfusion solution was phosphate buffered saline, pH 7.0. Fluidics tubing connected the basolateral portion of the Caco-2 chamber with the chamber containing HepG2 cells. Perfusate and media samples from each compartment were collected every hour. A control plate with no cells was also included to determine the effect of cells on APAP movement from the apical to basolateral chamber, from the basolateral chamber into the perfusate, and from the perfusate into the delivery well (liver well).

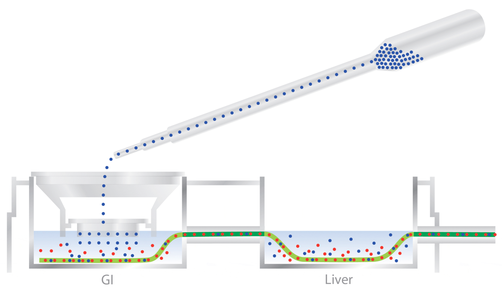
Analytical Procedures
A LC-MS/MS method was developed by verifying suitable transition ions for acetaminophen (APAP), and acetaminophen-glucuronide (APAP-Gluc) by infusion of compounds into the mass spectrometer and manually optimizing cone voltage and collision energy. APAP standard curve and QC samples were prepared in PBS and compared to standard curves and QC samples in media with and without serum.

METHODS

Sample analysis was performed using an Agilent 1100 HPLC in-line with a Waters Quattro Micro mass spectrometer outfitted with a Waters X Bridge Shield C-18 2.1x50 mm column. The Mobile Phase used was Solvent A (MPA): Ultrapure water with 0.1% formic acid, and Solvent B (MPB): methanol.
Column temperature was set to 40 degrees Celsius and a flow rate of 0.5 mL/min. MPB was 0% initial, 60% MPB at 2.8 minutes, 100% MPB at 3.3 minutes and back to 0% MPB at 3.8 minutes. APAP and APAP-Gluc were monitored by LC-MS/MS via MRM specific for each compound as shown in the table below. The results were acquired with the Mass Lynx data system and quantified via the Quan Lynx application.

FIGURE 1

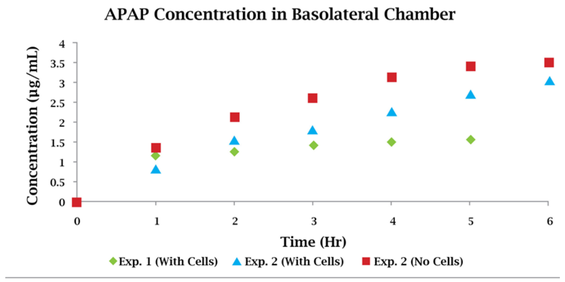
Diagrammatic Representation of a Two Chamber Plate System with Fluidics



A simple two-compartment organ model was tested by adding a single bolus concentration (200 µM) of Acetaminophen (APAP) to the apical side of the Caco-2 chamber, then periodically measuring APAP concentrations throughout the system. Each simulated-organ compartment was essentially isolated from the other compartments. Movement of drug between organ chambers was solely by the fluidics system.

FIGURE 2

Time-Dependent Accumulation of APAP in the Basolateral Chamber With and Without Cells

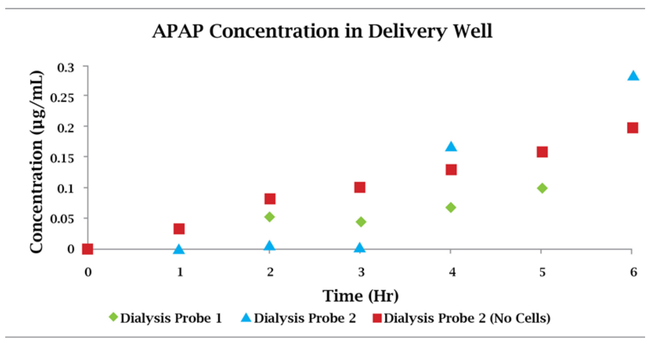


APAP kinetics were measured with (green and blue symbols) and without (red symbol) Caco-2 cells. APAP movement from the apical chamber to the basolateral chamber was slightly slower in the presence of cells.

RESULTS

FIGURE 3

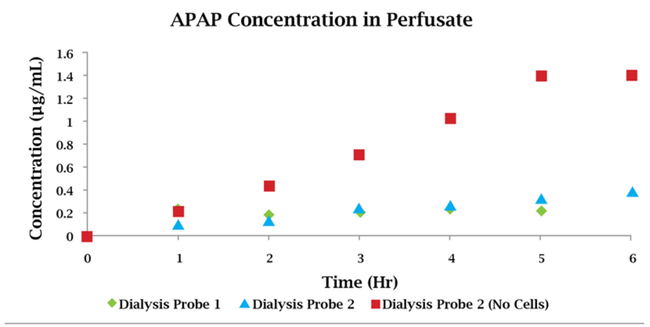
Time-Dependent Movement of APAP From The Intestine Chamber to The Liver (Delivery) Chamber



The simulated-intestine chamber was essentially isolated from the simulated- liver well. The only connection between the two organs was the fluidics system. Uptake and delivery of APAP occurred via passive diffusion across the dialysis membrane in the intestine basolateral chamber and in the delivery chamber (HepG2 cells). In the delivery well, the detection of APAP was later in the presence of cells (green and blue symbols) than without cells (red symbols). Diffusion from the fluidics tubing was dependent on the dialysis membrane, cellular uptake, concentration in the basolateral Caco-2 chamber, and concentration in the delivery chamber.

FIGURE 4

Time-Dependent Changes in APAP Concentration in the Fluidics Tubing With and Without Cells

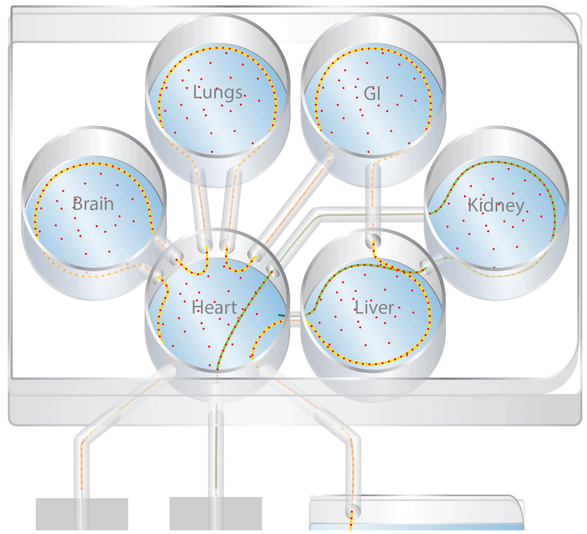


APAP concentration in the fluidics system (perfusate) reached its maximum concentration by 5 hr in the absence of cells (red symbols). In the presence of cells, concentrations in the perfusate were significantly lower (green and blue symbols). This delay in the increase in APAP concentration was most likely due to rapid cellular (HepG2) uptake. Concentrations at each time point were determined from perfusate samples collected over the sample interval.

RESULTS

FIGURE 5

Complete DMOP Plate Design Showing Six Organs



US and EU Patent Pending

SUMMARY

The results of this first study confirm that the incorporation of microdialysis into a multiple-organ tissue culture plate provides a mechanism that allows inter-tissue communication while maintaining the unique culture conditions required for each tissue or cell type.

The kinetics of acetaminophen (APAP) could be followed after a single bolus-dose administration to the apical compartment of the Caco-2 intestinal model.

By monitoring the total amount of APAP in each compartment as well as in the perfusate, a full kinetic picture was possible.

Delivery of APAP to the liver compartment was via the release of drug through the dialysis membrane. Thus, APAP was picked up in the basolateral chamber of the intestinal model and delivered to the liver chamber.

As more compartments are connected, the system should be capable of handling up to six different organ models.

ACKNOWLEDGEMENTS

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