ABSTRACT

Using the Human Dynamic Multi-Organ Plate (Hu-DMOP™) to Evaluate Cholestasis In Vitro.

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BACKGROUND

In vitro integrated organ models are of interest in preclinical drug and chemical safety assessment as they provide important information on toxicokinetics, bidirectional transport, metabolism, and other in vivo physiological processes. While the Human Dynamic Multi-Organ Plate (Hu-DMOP™) allows each tissue type to be culturally independently in its own optimized media, communication between tissues is allowed through a common blood flow system, allowing exchange of key artefacts, allowing inter tissue communication. Here we assessed liver response to Troglitizone in the presence of Cyclosporin + CDCA. This system mimics the human primary hepatocyte in umbilical cord (HC11). Intestinal tissue was exposed to the liver out-put (30 µM stock Cyclosporin A, Cyclo, and the 30 µM stock CDCA + troglitazone, Trog). The CDCA and troglitazone were combined separately and infused into the perfusate to assess whether this affects the expression, and a reduction in CYP3A1 expression. These gene changes were evident, but minimal (30% decrease) and 50% less CDCA reducetion in response to Cyclo + Trog. Unfortunately limited (LCH) increase of 10% was only observed with Cyclo, but no significant difference was observed with the hu-DMOP™ system as an evidence it was only integrated organ model to assessing increasing.

INTRODUCTION

Cholestasis and mixed cholestasis-hepatic injury represent 50% of all drug induced liver failures, and are one of the most common reasons for drug failure in the market. Therapies aimed at reducing injury results from biliary in situ damage, toxicity, or signaling pathways that inhibit primary bile acid transport, resulting in accumulation of bile acids (BA) within the liver and extrahepatic organs. (1) In the liver, BA accumulation increases the risk of liver injury is believed to be due to toxic metabolites, which is correlated to chronic cholestasis. (2) This is the leading cause of liver failure in the United States. Other hepatotoxic BA homeostasis is tightly regulated in part by enterohepatic circulation by the secretory axis for excreted bile acids (4). This is common, and this axis has been previously implicated in the development of hepatic and cholestasis-related hepatic disease. The ability to assess cholestasis-related drug candidates prove to be valuable to understanding potential drug liabilities. In addition, the ability to screen for these liabilities enables pharmaceuticals to decrease potential hepatotoxic compounds substantially and in the absence of conventional methods. In order to assess cholestasis-related potential, the system was expected to be the biliath excretor junction (Figure 3) and the bile acid export pump (Figure 4). For human cells, cholestasis is due to cholestasis, and Troglitazone is frequently associated with cholestasis. While cholestasis is the least that is to the BA metabolism involved both BSEP and FXR activation, thereby blocking the BA axis and the activation of the adaptive response. The results of this study reveal that the bile acid concentration, and the bile acid export pump is impacted under cholestasis conditions. It is the adaptive response in the liver that is to be assessed for cholestasis in the hu-DMOP™.

METHODS

Preparation of Plates

hu-DMOP™ custom designed plates (Figure 1) were used and equipped with a simulated blood flow system consisting of tubing connected to a peristaltic pump. The system was then set to run at 1.0 mL/min and the tubing was attached to the plates, such that only the arterial connections was in contact with each organ compartment. A perfusion rate of 1.0 mL/min was used.

Cell Culture

Caco-2 cells (ATCC CCL-243) were cultured in DMEM/F12 containing 2% fetal bovine serum (FBS; Sigma-Aldrich) and 1% Pen-Strep (Lonza). Cells were passaged every 4-6 days. Caco-2 cells were cultured to confluence in 24-well plates (Corning) for 8-10 days before transferring them to the hu-DMOP™. Cultures were maintained in DMEM/F12 containing 10% FBS and 1% Pen-Strep. Cells were maintained between 35% and 40% confluent. Caco-2 cells were passaged every 4-6 days.

Analytical Procedures

Caco-2 and Trop were measured by LC/MSMS (AB Sciex, Framingham, MA). A Caco-2 and a Trop, standards were prepared in H1 and compared against the standard curves and QC standards in media.

RESULTS

Figure 1. qPCR Indicating Basal Expression of ASBT

Figure 2. Diagram (top) of the two compartment setup used here showing the parent compound (blue dots) and metabolites (red dots) as well as the simulated blood flow (green). This allows compounds and metabolites to be exchanged between compartments. Each cell or tissue type remains in its own optimal media, so the media is not circulated between compartments. Photo of a three compartment setup that is routinely used at IONTIX (intestine – liver – kidney) complete with pump.

Figure 3. The kinetics of Cyclo and Trog distribution in the basolateral compartment of the hu-DMOP™. The data were determined at the indicated time points post-exposure by LC-MS/MS. Mass balance was performed and the permeability kinetics of both Cyclo and Trog closely matched known human bioavailability.

Figure 4. qPCR data show that the ASTB gene, which is necessary for enterohepatic circulation of bile acids, is present in the Epithelial™ tissue. Although present, there was no meaningful change in ASTB or Fxr related genes in Epithelial™ tissue after exposure to Cyclo, Trog, Cyclo+CDCA and Trog+CDCA in 20 µM Stock CDCA and 30 µM Stock Cyclo. The black dotted line represents a two-fold induction in gene expression, which is considered to be biologically relevant.

Figure 5. The kinetics of Cyclo and Trog distribution in the media of the primary human hepatocytes in culture system were determined at the indicated time points post-exposure by LC-MS/MS. Mass balance was performed and the permeability kinetics of both Cyclo and Trog closely matched known human bioavailability.

REFERENCES