Metabolite Profiling Using Human Hepatocyte Co-cultures and UHPLC-Q-TOF-MS with Data Independent MS/MS

Ronghua Wang; Ragu Ramanathan; Cornelia Smith; Caroline Lee; Helen Shen; Zamas Lam

Introduction

The identification of human derived metabolites is an imperative step in drug discovery and development process. Typical studies are conducted in human *in vitro* systems and metabolites are identified utilizing HPLC-MS/MS. Human hepatocyte co-culture is a new *in vitro* system, which is comprised of a mixture of hepatocytes and fibroblast cells, capable of remaining viable and highly functional for up to 4 weeks. In this presentation, the application of high resolution mass spectrometry (HRMS), ultrahigh-pressure liquid chromatography (UHPLC), full scan acquisition, and Sequential Window acquisition of All THeoretical fragment ion spectra (SWATH™) was used to profile and characterize metabolites of diclofenac, linezolid, and ziprasidone following incubations in human hepatocyte co-cultures.

Methods

Diclofenac, linezolid, ziprasidone, buspirone as IS, and 96-well human hepatocyte co-culture plates were purchased commercially. Each compound (@10 µM) was incubated at 0, 4, 48, and 168 hours in human hepatocyte co-culture. After incubation, 400 µL termination solution (acetonitrile containing IS) was added directly to the well and samples were collected. The metabolite profiling method was developed on a TripleTOF® 5600 system coupled with a Shimadzu Nexera UHPLC. Data were acquired with high resolution TOF-MS survey scan and SWATH MS/MS, and processed with MetabolitePilot software which utilized mass defect filtering (MDF), isotope pattern filtering (IPF), and background subtraction

Preliminary Data

4'- and 5-hydroxydiclofenac (m/z 310 in negative ionization) were the predominant metabolites of diclofenac (m/z 294) after 168 h incubation in human hepatocyte co-culture. Minor metabolites included diclofenac glucuronide (m/z 470) and diclofenac-quinone-imine (m/z 308). Four new metabolites were identified as hydroxydiclofenac glucuronides (m/z 486).

For linezolid (m/z 338 in positive ionization), a total of 15 metabolites were identified. All the metabolites were products of linezolid phase I metabolism, which included hydrolysis of amide bond and oxidations on the different positions of morpholine ring. In a human clinical study reported by Slatter *et al*. (1), 12 metabolites were identified and the morpholine ring opening metabolite (M4, m/z 370) was the predominant metabolite. In addition to the 12 metabolites reported in the human AME study, three new oxidation metabolites of linezolid (m/z 354) were identified in hepatocyte co-culture incubations.

In the ziprasidone (m/z 413 in positive ionization) incubations, all 13 metabolites were products of ziprasidone phase I metabolism, which included *S*-oxidation, *S*-methylation with the N-S bond cleavage in the benzisothiazole ring, and *N*-dealkylation on both nitrogens from piperazine moiety followed by oxidations. In a human clinical study reported by Prakash *et al.* (2), 12 metabolites were identified with 11 phase I metabolites, one glucuronide, and *N*-dealkylation on the nitrogen bearing the ethylene group from piperazine moiety followed by oxidations was the major metabolic pathway. In addition to the 11 phase I metabolites reported in the human AME study, two new di-oxidation metabolites of ziprasidone (m/z 445) were identified in human hepatocyte co-culture incubations.

The preliminary results from this study demonstrated that analysis of human hepatocyte co-cultures incubation samples using UHPLC-Q-TOF-MS with data independent MS/MS generated metabolite profiles similar to those in human AME studies in a higher throughput workflow.

Novel Aspect

Identification of new metabolites in human hepatocyte co-culture using Sequential Window acquisition of All THeoretical fragment ion spectra.