In Vitro Species Comparison Using Long-Term Hepatocyte Co-Cultures Model and Highly Sensitive UHPLC-QTOF-MS with SWATH Analysis

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OVERVIEW

Purpose
To develop a reliable, quicker, and cost-saving in vitro method to accurately predict major human metabolite profile in vivo and to de-risk disproportional or unique human metabolites before a drug candidate nomination

Method
Using long-term animal and human hepatocyte co-cultures coupled with non-targeted MS/MS\textsuperscript{ALL} with SWATH acquisition by a UHPLC-QTOF system to generate metabolite profile information

Results
Metabolites of the tested compounds identified in human hepatocyte co-cultures were also found in those of rat and/or monkey and the major human circulating and excreta metabolites of these compounds were also found in human and/or animal hepatocyte co-cultures. The proposed approach appears to be reliable.

INTRODUCTION

One of the main goals of in vitro species comparison studies is to assess whether there is adequate coverage from the preclinical species to humans with respect to disproportional and/or unique human metabolites. Also important is to accurately predict major human metabolite profile in vivo. Traditionally, this is performed with subcellular fractions and/or suspended hepatocytes; however, these short-term in vitro systems do not usually provide multi-generation metabolites.\textsuperscript{[1, 2, 3, 4]}

In this study, we incubated selected compounds of diverse chemical structures (linezolid, ziprasidone, and diclofenac, Figure 1) that were subjected to a wide range of biotransformation pathways with long-term hepatocyte co-cultures model over an extended time period, and mined the metabolite information from the mass spectra generated by an UHPLC-QTOF-MS system through non-targeted MS/MS\textsuperscript{ALL} with SWATH acquisition to compare the metabolite profiles across species and to the major metabolite profile found in humans in vivo.

METHODS

Sample Preparation
Linezolid, ziprasidone (ZIP), and diclofenac (\texttextit{@} 50 \textmu M) were incubated with rat, monkey, or human HepatoPa\textsuperscript{TM} co-cultures at 37°C in a 24-well format. Incubations with stromal cells served as the negative control. The plates were placed inside a humidified incubator over 168 hours. The enzymatic reactions were terminated by adding 400 \textmu L of ice-cold acetonitrile solution directly to the well at 0, 4, 48, and 168 h. The mixture was vortex-mixed, centrifuged, and the supernatants were analyzed by UHPLC-MS/MS.

RESULTS

Incubation of ZIP with Hepatocyte Co-Culture
1. Three major human circulating and excreta metabolites S-methyl-dihydro-ZIP, ZIP sulfone, and N-dealkyl ZIP sulfone,\textsuperscript{[3, 4]} were identified in both monkey and human hepatocyte co-cultures (Table 3). S-Methyl-dihydro-ZIP and ZIP sulfone were also found in rat.
2. S-Methyl-dihydro-ZIP and S-Methyl-dihydro-ZIP-SO were the major metabolites in rat, monkey, and human hepatocyte co-cultures (Figure 2).
3. Metabolites identified in human were also found in animals.

Incubation of Linezolid with Hepatocyte Co-Culture
1. Two major human circulating and excreta metabolites, PNU-142586 and PNU-142300,\textsuperscript{[5]} were identified in both animal and human hepatocyte co-cultures as major or significant (Table 3).
2. Metabolite profiles were qualitatively similar across all species tested, with three morpholine ring-opened products PNU-142300, PNU-142586, and PNU-143010 as the major metabolites in human. PNU-142586, PNU-142300, and PNU-143131 were the major metabolites in monkey, while PNU-142300 and PNU-142618 were major metabolites in rat (Figure 3).
3. Metabolites identified in human were also found in animals.

CONCLUSIONS

1. Major human circulating and excreta metabolites, four acyl glucuronides, 4'-hydroxyl and 5-hydroxyl diclofenac,\textsuperscript{[5, 11]} were identified in both animal and human hepatocyte co-cultures as major or significant (Table 3).
2. Four diclofenac acyl glucuronides were the major metabolites in all species at 4 h. At 48 h, acyl glucuronides were the major metabolites in rat and monkey, while acyl glucuronides and 4'-hydroxyl and 5-hydroxyl diclofenac were major metabolites in human.
3. In addition, multiple hydroxydiclofenac glucuronides and a dehydrogenated diclofenac (detected in negative mode) were also identified in animal and/or human.
4. Metabolites identified in human were also found in animals.

REFERENCE

2. Slatter JG et al., Pharmacokinetics, metabolism, and excretion of linezolid following an oral dose of \texttextit{[14C]}linezolid to healthy human subjects. DMD, 2001; 29(8):1136-1145.
5. Stierlin H et al., Biotransformation of diclofenac sodium (Votaren) in animals and in man, I: Quantitative determination of the major metabolites in all species at 4 h. At 48 h, acyl glucuronides were the major metabolites in monkey and human, while acyl glucuronides and 4'-hydroxyl and 5-hydroxyl diclofenac were major metabolites in rat.