METABOLISM OF EIGHT MODEL PHARMACEUTICAL COMPOUNDS IN RAT- and HUMAN- HEPATOPAC® VERSUS LIVER MICROSOMES AND SUSPENSION HEPATOCYTE PLATFORMS

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ABSTRACT

Metabolism evaluations for compounds in early development are typically conducted *in vitro* using liver microsomes and suspension hepatocytes. While these systems have served the drug development community for many years, they have limitations and result in infrequent "metabolite surprises", a term used to describe the observation of human metabolites that were previously undetected in preclinical studies. Such surprises can have a significant impact on the timeline of a drug development program and has triggered investigations to develop *in vitro* systems that more accurately predict human *in vivo* metabolism.

In this study, the metabolite profiles of eight model pharmaceutical compounds with various biotransformation reactions were investigated in parallel using a functionally stable model of primary hepatocytes [micropatterned co-cultures (MPCCs), referred to as Hepatopac™] and the traditional liver microsomal and suspension hepatocyte platforms (Khetani & Bhatia, 2006). Incubations were performed for 0 and 60 min in liver microsomes; 0 and 2 hr in suspension hepatocytes; and 0, 4 hr, 2 days and 7 days in Hepatopac™. Metabolites were identified by LC/MS employing a narrow range of chromatographic conditions, which were representative of drug metabolism screening parameters used in an early drug development setting.

Our results show that Hepatopac™ are metabolically more active than the traditional platforms, as judged from levels of test article disappearance and formation of metabolites. Formation of metabolites from non-P450 mediated reactions, especially UGTs, and secondary metabolites appear to be more predominant in Hepatopac™.

Results from this investigation also suggest that use of Hepatopac™ for metabolite profiling studies will be broadly useful in minimizing "metabolite surprises". Use of Hepatopac™ platform also provides cells that maintain metabolic activity over an extended period in culture; a difference that likely explains the increased abundance of secondary metabolites in this system.

INTRODUCTION

Regulatory guidance specifies safety testing of new human metabolites which represent greater than 10% of the parent drug-related components in circulation. Early *in vitro* metabolite profiling studies can provide a clue regarding any potential differences in metabolite profiles between humans and animals. Such *in vitro* evaluations are typically conducted using human liver microsomes and suspension hepatocytes. These systems however have their limitations, sometimes resulting in "metabolite surprises" - where metabolites which were not predicted or observed *in vitro* or *in vivo* during pre-clinical studies, are observed in the clinic. Such surprises can have a significant impact on the timeline of a drug development program and has prompted investigations aimed at developing new *in vitro* systems and models that can more accurately predict human *in vivo* metabolism.

This poster presents our comparative metabolite profiling analysis of eight model pharmaceutical compounds with various biotransformation reactions using a functionally stable model of primary hepatocytes [micropatterned co-cultures (MPCCs)] in parallel with the traditional liver microsomal and suspension hepatocyte systems.

Current In Vitro Platforms and Limitations

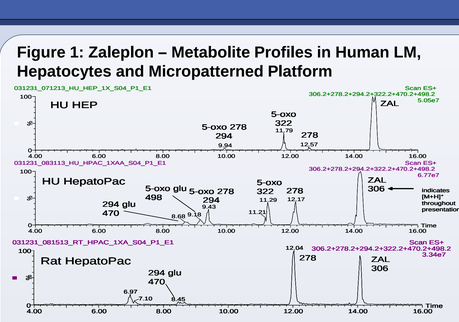
- Liver microsomes fortified with cofactors and cytosol- limited non-P450 enzymes
- Suspension hepatocytes - Loses phenotype in culture
- Liver S9 fortified with cofactors - Limited amount of phase II enzymes
- Recombinant enzymes - "More artificial" than other *in vitro* systems

Note: All systems show a decrease in P450 enzyme activity with time in culture

Micropatterned Platform

- Micropatterned co-cultures of hepatocytes and other cells
- 3-D context
- Cell-cell interactions
- Cell-Matrix interactions
- Maintains metabolic activity over extended periods in culture

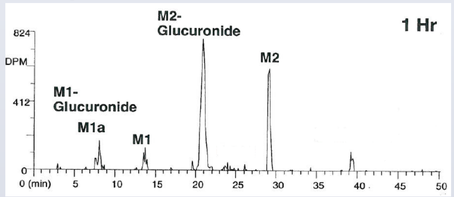
Figure 1: Zaleplon – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



HepatoPac™ and cytosol fortified LM predicted actual aldehyde oxidase (AO) species difference between humans and rats as expected

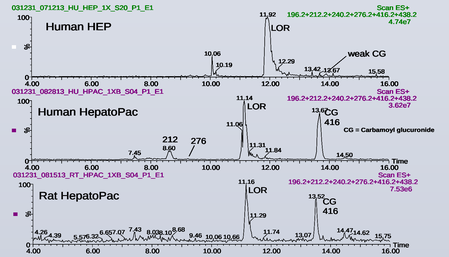
Figure 2: Zaleplon – Metabolite Profiles in Human Plasma 1 hr after 20 mg dose

(Reference: DeMaio et al., 1994.)



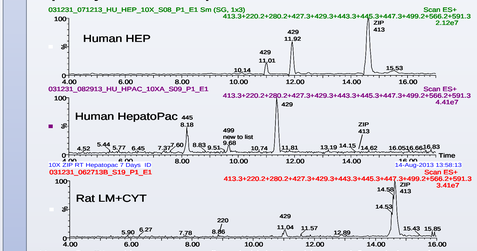
HepatoPac consistent with human plasma radiochromatogram, except for 278, which was present as an additional metabolite. 5-oxo ZAL, a minor metabolite also observed in rat bile and urine, was not observed with rat HepatoPac. Desethyl ZAL metabolite (278) was also the major metabolite in dog plasma.

Figure 3: Lorcaserin – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



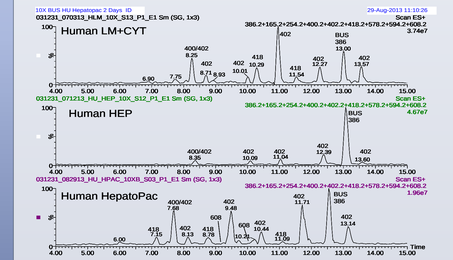
A major human metabolite carbamoyl glucuronide (CG) is generated by HepatoPac™ but not predicted by LM and hepatocytes.

Figure 4: Ziprasidone – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



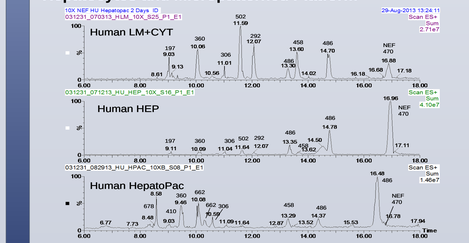
HepatoPac™ and hepatocytes confirmed aldehyde oxidase and *S*-methyl transferase metabolism that was not observed in Liver MIC+CYT

Figure 5: Buspirone – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



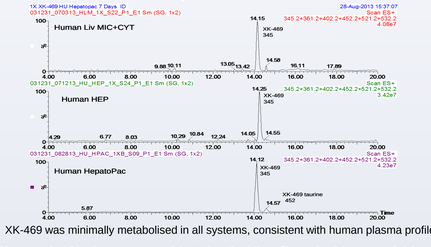
All systems generated numerous oxidation products. HepatoPac™ metabolism was more extensive than other systems.

Figure 6: Nefazodone – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



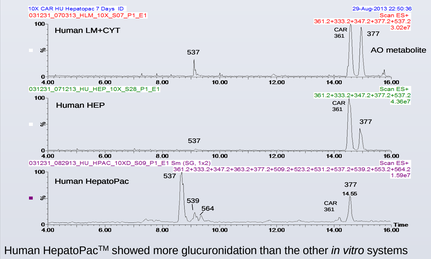
Human HepatoPac™ closest to human plasma profile than the traditional in vitro platforms

Figure 7: XK-469 – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



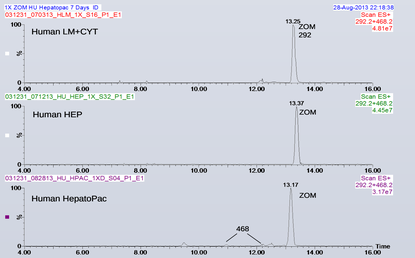
XK-469 was minimally metabolised in all systems, consistent with human plasma profile

Figure 8: Carbazeran – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



Human HepatoPac™ showed more glucuronidation than the other *in vitro* systems

Figure 9: Zomepirac – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



Minimal metabolism observed in all platforms

MATERIALS and METHODS

Model Compounds

-Zaleplon (ZAL)
-Ziprasidone (ZIP)
-Buspirone (BUS)
-Nefazodone (NEF)
-Lorcaserin (LOR)
-XK-469
-Carbazeran (CAR)
-Zomepirac (ZOM)

Experimental Design:

-Liver Microsomes (LM) with Cyt - 1 hr incubation in presence of NADPH and UDPGA
-Suspension Hepatocytes - Incubation time: 2 hr
-HepatoPac™ - Incubation time: 4 hr, 2 days, 7 days
-Compound concentration = 10 μM
-Note: Suspension hepatocytes and HepatoPac™ were derived from the same lot of primary hepatocytes.

LC/MS Analysis:

Use same HPLC column and mobile phase for all compounds
Determine DP (Q1MS scan) and CE (MS/MS) for each parent drug
Determine mobile phase gradient to get parent drug retention time in 10 to 15 min range, starting gradient at 5% organic.
Phenomenex Luna C18(2) 100 x 2.00 mm, 3 micron
Mobile Phase A = 10 mM NH4 acetate in water, pH 4.5
Mobile Phase B = Acetonitrile
Run time = 30 min
LC/MS (+) ESI mode AB Sciex API4000, Q1 scan, undiluted and 10x-dilution
LC/MS/MS (+)ESI mode, product ion scans for selected metabolites

SUMMARY OF RESULTS

- Zaleplon - Actual AO species difference between humans and rats predicted by HepatoPac™ and cytosol fortified liver microsomes as expected
- Lorcaserin - A major human metabolite (carbamoyl glucuronide) is generated by HepatoPac™ that was not predicted by LM or HEP
- Ziprasodone - Aldehyde oxidase and S-methyl transferase metabolism confirmed in hepatocytes and HepatoPac™ that was not observed in Liver MIC+CYT
- Buspirone - Numerous oxidation products observed in all systems as expected. HepatoPac™ metabolism more extensive than other systems
- Nefazodone - Human HepatoPac™ closest to human plasma profile than the traditional in vitro platforms
- XK-469 - Minimal metabolism in all systems consistent with minimal metabolism indicated by human plasma profile
- Carbazeran - More glucuronidation observed in human HepatoPac™

CONCLUSION

-HepatoPac™ maintains enzyme activity for longer periods.
-HepatoPac™ provides more extensive metabolism than other platforms and therefore may serve as a useful system for predictive toxicology evaluations.
-Metabolic stability in HepatoPac™ appears more predictive of actual human *in vivo* metabolic stability than obtainable with suspension hepatocytes.
-UGT activity higher with HepatoPac™ than traditional platforms
  - *N*-carbamoyl glucuronidation activity high
  - Acyl glucuronidation activity low.
-Aldehyde oxidase (AO) species differences readily apparent (ZAL and CAR)
-Metabolite profiling with HepatoPac™ is a service that would be valuable to our drug development clients.

REFERENCES

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HepatoPac™ is a trademark of Hepregen Corporation

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