Comparative Metabolism of Eight Model Pharmaceutical Compounds in Rat and Human Liver Microsomes, Suspension Hepatocytes and Micropatterned Co-cultures of Primary Hepatocytes

Hepregen

Enoru JO[1], DeMaio W[1], Watanyar A[1], Draper K[1], Moore A[2] & Ukairo O[2]

1. Ricerca Biosciences LLC, 7528 Auburn Rd, Concord, OH 44077
2. Hepregen Corporation, 200 Boston Ave, Medford, MA 02155

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ABSTRACT

In vitro metabolism evaluations for compounds in early development are typically conducted using liver microsomes and suspension hepatocytes. While these systems have been serving the drug development community, they have their limitations, with examples of "metabolite surprises" - where metabolites, which were not predicted or observed *in vitro* or *in vivo* during pre-clinical studies, were 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 *in vitro* systems that can more accurately predict human *in vivo* metabolism.

In this study, the metabolite profile of eight model pharmaceutical compounds with various biotransformation pathways was investigated using a functionally stable model of primary hepatocytes [micropatterned co-cultures (MPCCs)] (HepatoPac™) in parallel with the traditional liver microsomal and suspension hepatocyte systems. 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 MPCCs. Metabolites were identified by LC/MS employing a narrow range of chromatographic conditions, representative of drug metabolism screening in an early development setting.

Preliminary results show that MPCCs are more metabolically active than the traditional platforms, based on greater disappearance of test article and formation of metabolites. Formation of metabolites from non-P450 mediated reactions, especially UGTs, and secondary metabolites appear to be more predominant in MPCCs.

The results from this investigation support the usefulness of MPCCs for metabolite profiling studies with the potential of eliminating "metabolite surprises." MPCCs also provide the advantage that the cells maintain metabolic activity over an extended period in culture, which probably explains why secondary metabolites were more abundantly generated 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

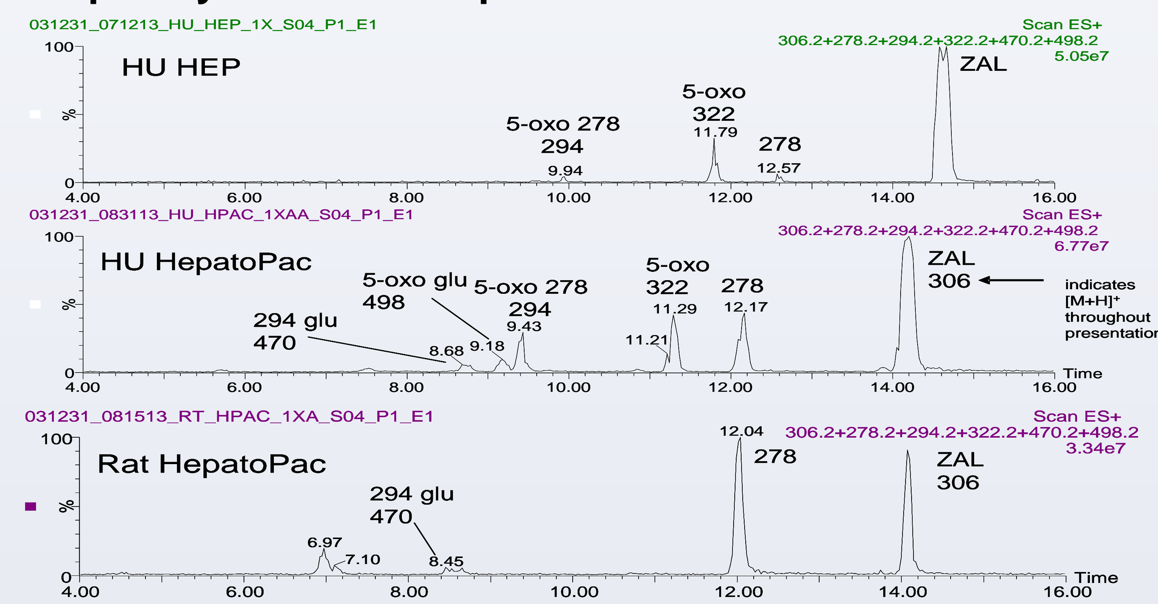
- Liver microsomes fortified with cofactors and cytosol- limited non-P450 enzymes
- Suspension hepatocytes - Loses phenotype in culture
- Liver S9 fortified with cofactors - Limited amount of phase II enzymes
- Recombinant enzymes - "More artificial" than other *in vitro* systems

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

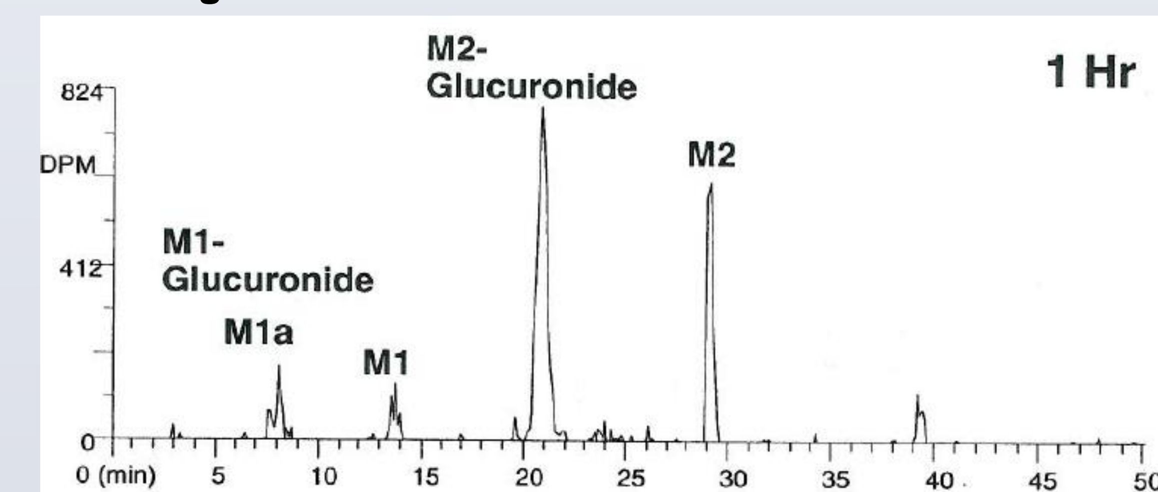
Figure 1: Zaleplon – Metabolite Profiles in Human LM, Hepatocytes and Micropatterned Platform



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

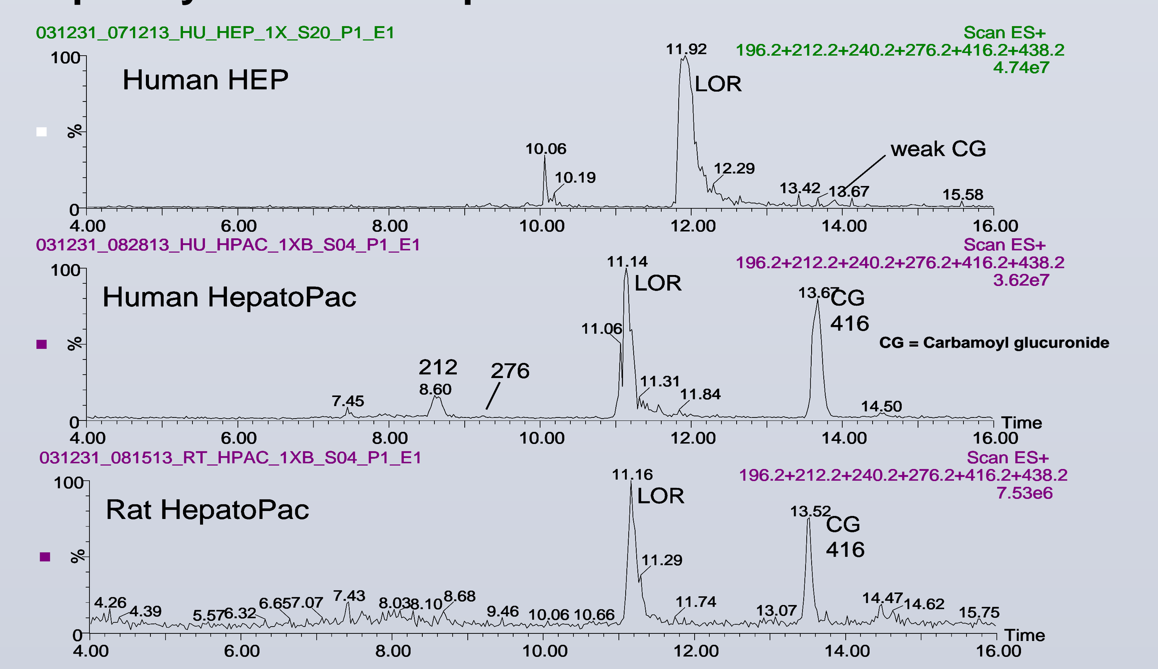
Figure 2: Zaleplon – Metabolite Profiles in Human Plasma 1 hr after 20 mg dose

(Reference: DeMaio et al., 1994.)



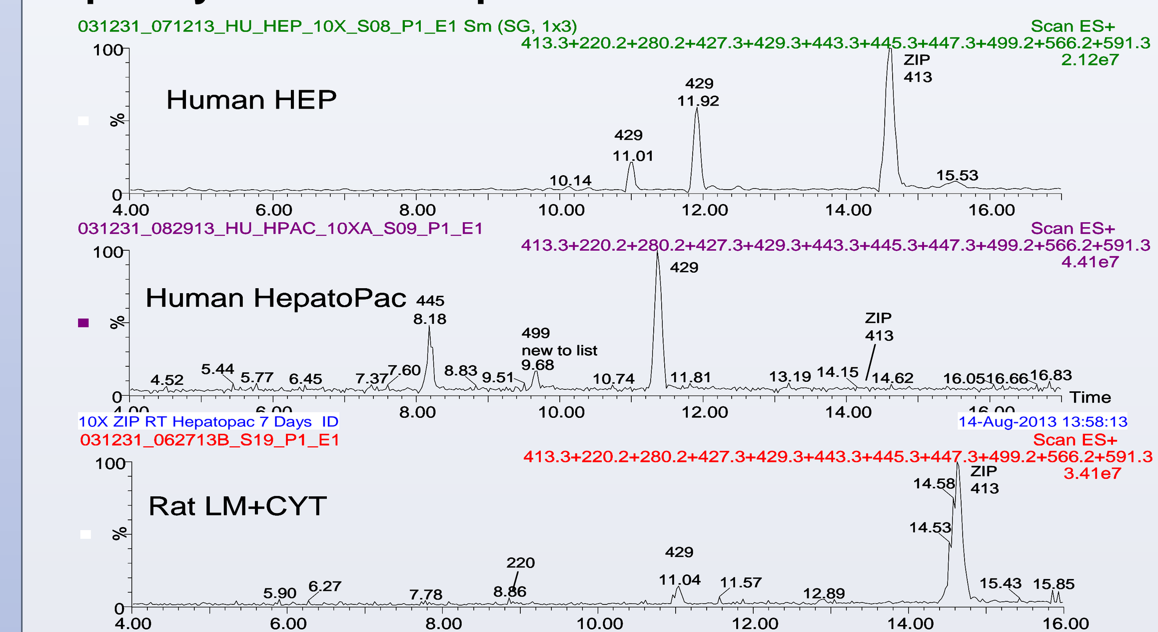
HepatoPac consistent with human plasma radiochromatogram, except for 278, which was present as an additional metabolite. 5-oxo ZAL, a minor metabolite also observed in rat bile and urine, was not observed with rat HepatoPac. Desethyl ZAL metabolite (278) was also the major metabolite in dog plasma.

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



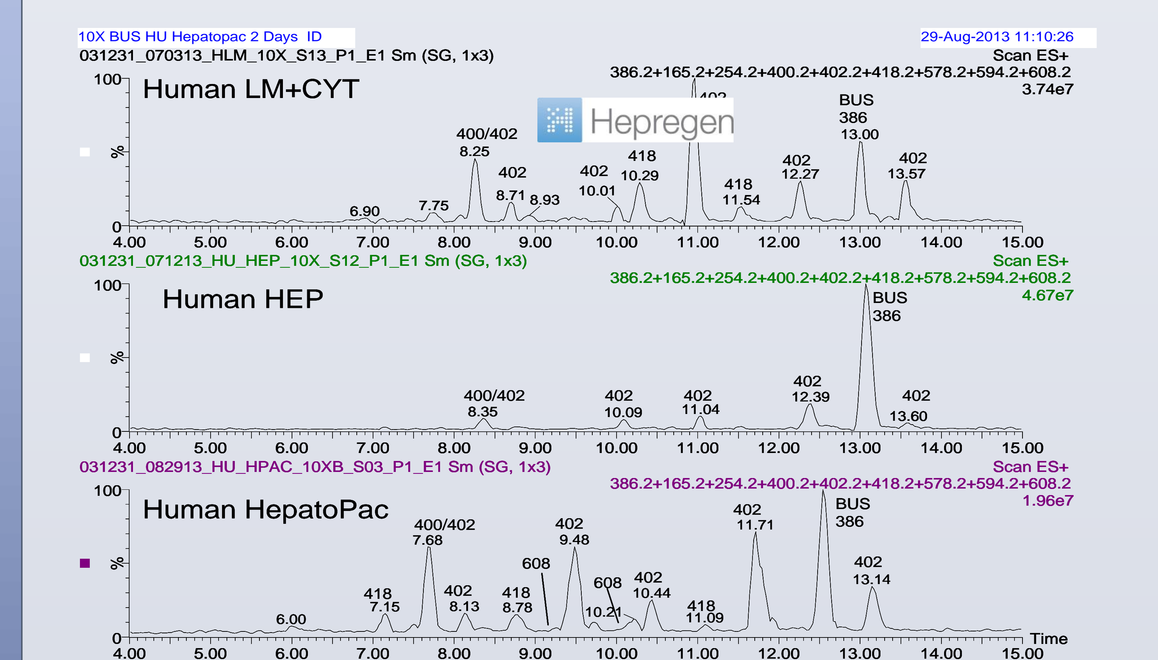
A major human metabolite carbamoyl glucuronide (CG) is generated by HepatoPac™ but not predicted by LM and hepatocytes.

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



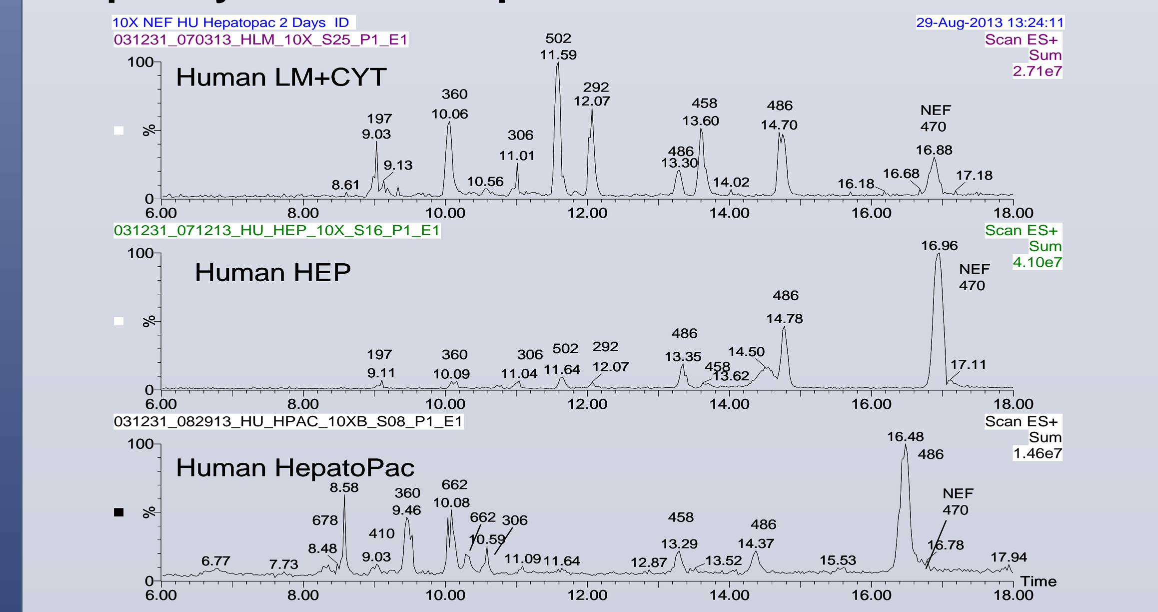
HepatoPac™ and hepatocytes confirmed aldehyde oxidase and *S*-methyl transferase metabolism that was not observed in Liver MIC+CYT

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



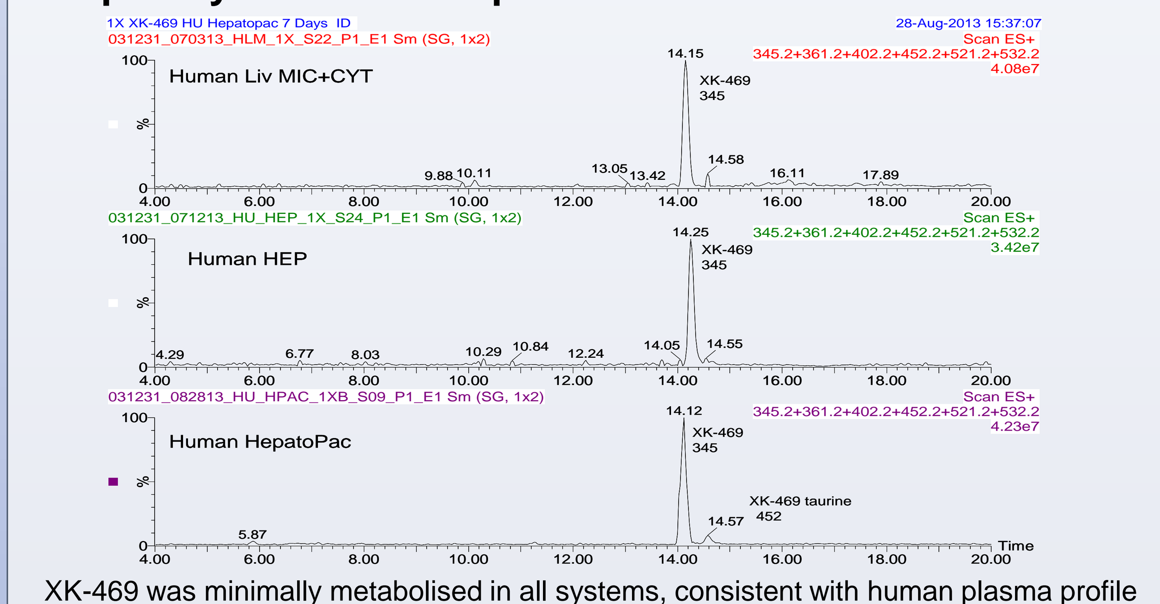
All systems generated numerous oxidation products. HepatoPac™ metabolism was more extensive than other systems.

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



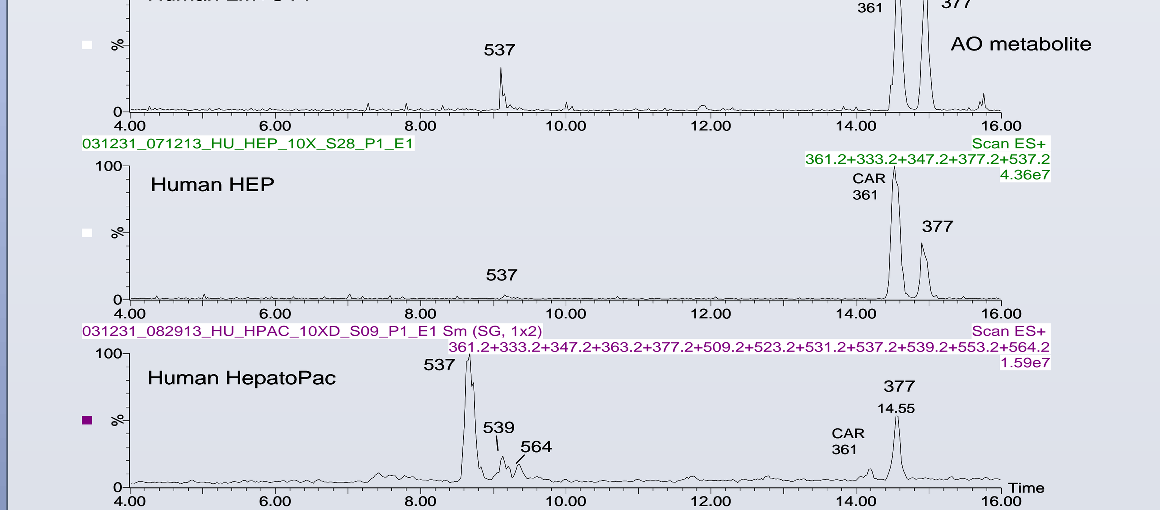
Human HepatoPac™ closest to human plasma profile than the traditional in vitro platforms

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



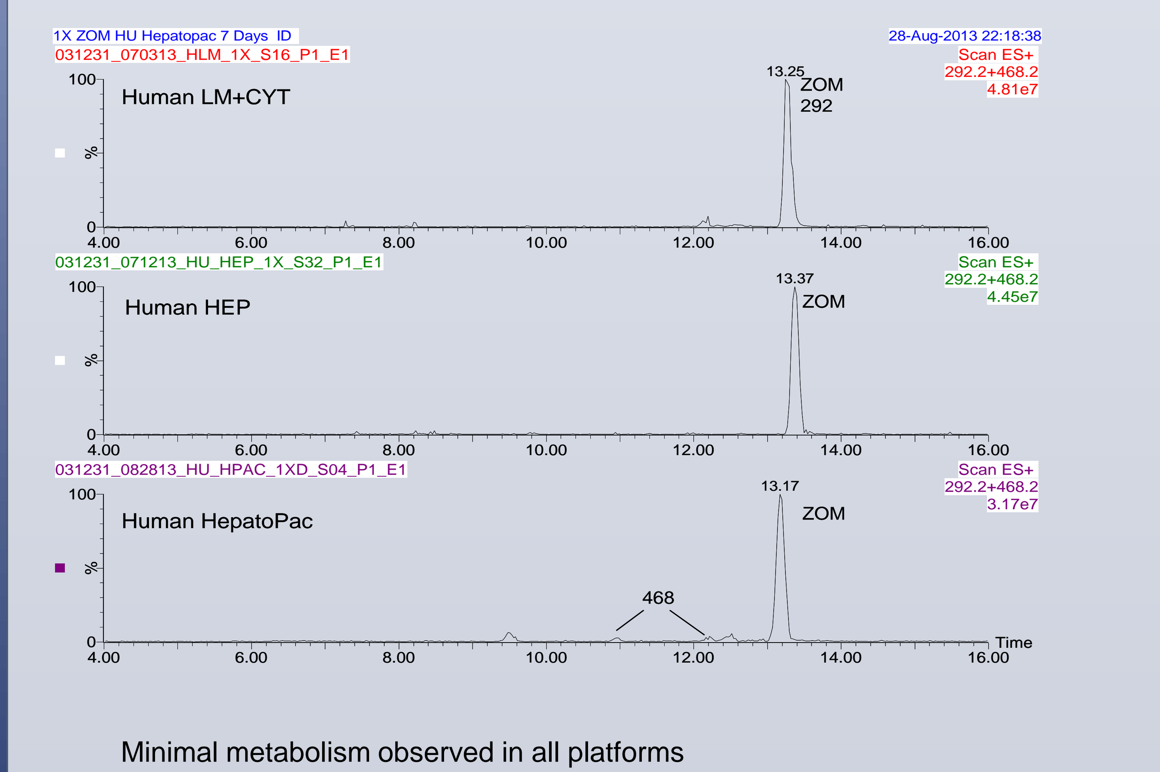
XK-469 was minimally metabolised in all systems, consistent with human plasma profile

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

1. Z. Tong, A. Chandrasekaran, W. DeMaio, R. Espina, W. Lu, R. Jordan, and J. Scatina. 2010. Metabolism of Varbicaserin in Mice, Rats, Dogs, Monkeys and Humans. *Drug Metab Dispos*, 38 (12), 2266-2277.
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