Metabolite Profile Comparison Using a HμREL Co-culture Hepatocyte Model versus a Conventional Hepatocyte Suspension for Low Turnover Drugs

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Overview

- Investigated four commercially available drugs known to exhibit low metabolic turnover using a conventional hepatocyte suspension and a HμREL co-culture model.
- Metabolite identification conducted using accurate mass data acquired from an LTQ-Orbitrap XL mass spectrometer.
- Mass spectrometric (MS) data searched for predicted and previously reported [1-4] in vivo human metabolites.
- Tandem MS data (MS/MS) probed for metabolite-related fragment ions.
- Human in vitro incubations for meloxicam, timolol, linezolid, and XK-469 produced little metabolic turnover using conventional hepatocyte incubations.
- Incubations employing the HμREL co-culture model generated much higher levels of metabolic turnover, yielding all major metabolites observed for all four drugs in previous human clinical studies.

Introduction

- In vitro incubations are commonly used in drug discovery in order to provide an early prediction of in vivo human metabolism.
- Hepatocytes (liver cells) are frequently utilized as they contain a full complement of Phase I/Phase II enzymes, thus providing a generally good prediction of metabolism for the corresponding in vivo system.
- Conventional systems employ hepatocyte suspensions, which are typically viable out to only 4 hours. For low turnover compounds, this may be insufficient to provide an adequate assessment of human metabolism.
- For this study, four commercially available drugs (meloxicam, timolol, linezolid, and XK-469) were investigated using a conventional hepatocyte suspension, as well as the HμREL co-culture model. These compounds have been reported to exhibit low metabolic turnover using conventional systems.
- The primary goal of this study was to determine whether the HμREL model could produce metabolites consistent with those observed in previous human clinical studies.

Methods

Pooled Hepatocyte Suspension

- The conventional in vitro model employed for this comparison was a pooled (10 donor) human hepatocyte suspension (0.5 x 10^6 hepatocyte/mL). Test substrates (10 μM) were incubated in 0.5 mL for 4 hours.
- Following incubation, samples were quenched with an equal volume of acetonitrile and then centrifuged and prepared for analysis.

HμREL Hepatocyte Suspension

- The hepatocyte co-culture 24-well plates were generated at HμREL using a plateable pool (5 donor) of cryopreserved hepatocytes (188,000 hepatocytes/well), plated with stromal cells.
- HμREL plates were received, media changed, and incubations were initiated 4 hours later by addition of test substrates (10 μM).
- Test substrates were incubated for 4 hours, 24 hours, 3 days, and 7 days in a 37 °C incubator with moderate shaking (no media change).
- Aliquots collected at time points were quenched with an equal volume of acetonitrile and then centrifuged and prepared for analysis.

Metabolite Identification

- Accurate mass MS and MS/MS data were obtained using a Thermo Fisher LTQ-Orbitrap XL coupled to a Shimadzu 10AD VP HPLC.
- The MS data were searched for predicted biotransformations as well as metabolites previously reported in human in vivo studies.
- Accurate mass MS/MS data were acquired using data-independent acquisition (DIA).
- The MS/MS data were searched for parent drug fragment ions, as well as predicted metabolite fragment ions.
- Metabolite target lists were generated from the MS (predicted metabolite hits) and MS/MS (DIA – predicted metabolite fragment hits) data.
- Data dependent acquisition (DDA) was utilized to obtain more definitive metabolite structural information.
- Metabolite structures were correlated with literature data obtained from human clinical studies.

Metabolite Identification Regulatory Considerations

- Guidance expectations around metabolite profiling (FDA/EMA/Japan):
  - Are there "major" circulating human metabolites, and are they covered in the toxicity species – MIST (Metabolites in Safety Testing);
  - Are there "major" circulating human metabolites that may elicit clinically relevant drug interactions – DDI (drug-drug interactions);
  - What are the primary metabolic clearance pathways, including the enzymes responsible – DDI.
- Prior to Phase I dosing, in vitro systems are relied upon to predict human metabolites:
  - To support selection of toxicity species;
  - To predict the potential for victim-based DDIs and/or PK variability in the clinic;
  - For moderate/high clearance drugs, traditional in vitro systems are used for these assessments.

Note: For low clearance drugs, human liver microsomes (HLM) or suspended hepatocytes are often inadequate at investigating metabolic pathways.

Results

- For meloxicam, the primary metabolic pathway (oxidation of the methyl substituent of the methylthiazole moiety) had been reported as nearly 70% of the dose in human subjects. Both the hydroxymethyl and subsequent carboxylic acid (major in vivo metabolite) of meloxicam were readily generated using the HμREL hepatocyte co-culture model.
- With timolol, in vivo human data had revealed that 22% of the dose represents oxidative opening of the morpholine ring, a pathway not observed with conventional hepatocyte suspensions. The HμREL model generated strong turnover, and the morpholine ring-opening metabolites were detected.
- For linezolid, oxidative opening of the morpholine ring to yield two carboxylic acid metabolites had been reported as 56% of the dose in human subjects. While these metabolites were observed at low levels in conventional suspensions, their formation was significantly more robust using the HμREL model.
- In the case of XK-469, the primary metabolic pathway involves aldehyde oxidase-mediated mono-oxidation of the quinoxaline ring, which had accounted for 54% of the total urinary excretion in human subjects. While this metabolite was not observed in conventional suspensions, the HμREL model produced the metabolite starting at approximately 24 hours, and by Day 7 it was abundant, at over 50% of parent. In addition, low-level taurine and glycine conjugates were identified.

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Figure 1. Meloxicam

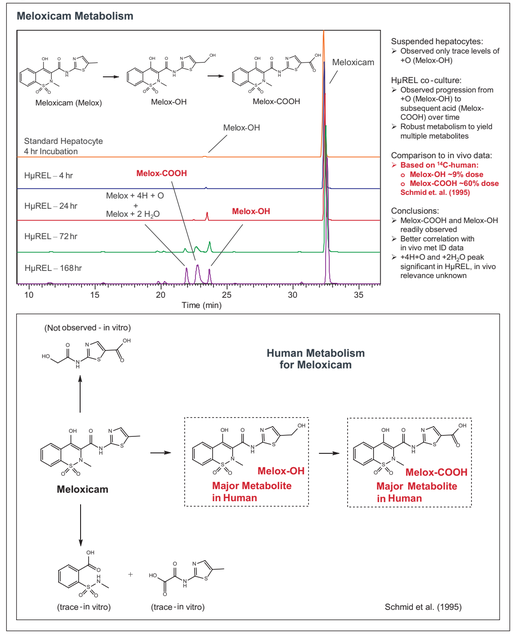


Figure 2. Timolol

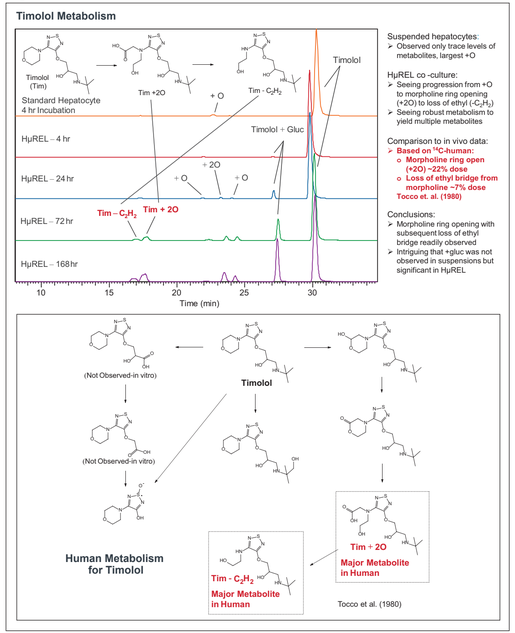


Figure 3. Linezolid

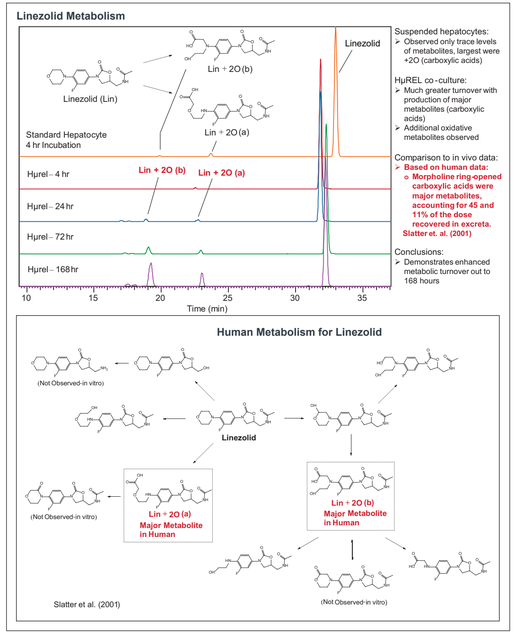
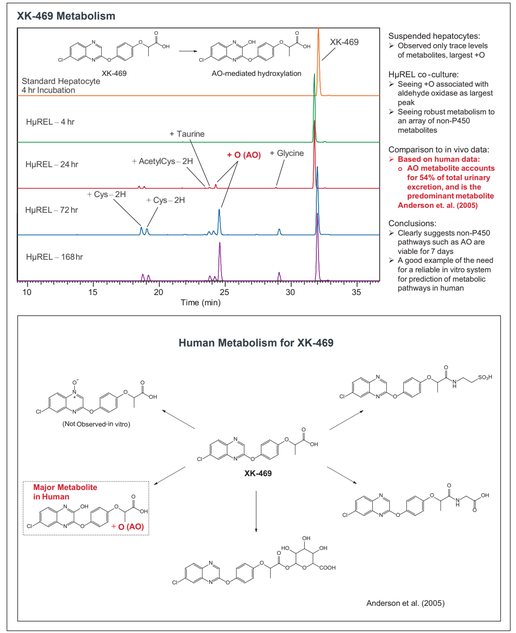


Figure 4. XK-469



Conclusions

- Human in vitro incubations for meloxicam, timolol, linezolid, and XK-469 were first conducted using hepatocytes in suspension. For all of these drugs, minimal to no metabolic turnover was observed, in agreement with literature findings, which characterize these compounds as low turnover.
- Incubations employing the HμREL co-culture model greatly enhanced overall levels of metabolic turnover for all four compounds, with time-dependent generation of metabolites consistent with that reported in human in vivo studies.
- All previously reported major in vivo human metabolites were observed for meloxicam, timolol, linezolid, and XK-469 using the HμREL co-culture model.
- For each of the four drugs examined, continuous metabolic turnover was observed out to 7 days of incubation in the HμREL model, covering a range of metabolic mechanisms, including cytochrome P450, aldehyde oxidase, and numerous Phase II pathways.
- Additional metabolites were generated using the HμREL model that were not previously reported for the four drugs. The relevance of these metabolites remains in question.

References

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