In vitro to in vivo extrapolation (IVIVE) for low intrinsic clearance compounds using platable Hepatocytes system

NOVARTIS

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Abstract

In vitro systems have been long used to determined metabolic clearance of compounds. Liver subcellular fractions such as microsomes, S9, cytosol and suspended hepatocytes have the ability to screen for metabolic instability of new chemical entities (NCE) and help optimization and development activities. However, these assays often fail to provide an accurate metabolic response to predict in vivo metabolic fate of low turnover compounds. In vitro incubation with suspended hepatocytes could only be performed for few hours, to avoid loss in cell viability and activity of drug metabolizing enzymes. (Griffin and Houston, 2005) Unlike suspended hepatocytes, primary plated hepatocytes have been shown to have sustained enzyme activity and cell viability in prolonged incubation. (Ma et al., 2017)

The purpose of our study is to validate the pooled plated hepatocytes system and use the system to capture slow rate of metabolism of selected low clearance compounds. The obtained intrinsic clearance results will be used to predict in vivo metabolic and to determine the in vitro-in vivo extrapolation (IVIVE) accuracy. In the current study, we have established and validated pooled primary plated rat and human hepatocytes assay by determining intrinsic clearance (CLint), through substrate depletion approach at low concentrations.

Experiment Design

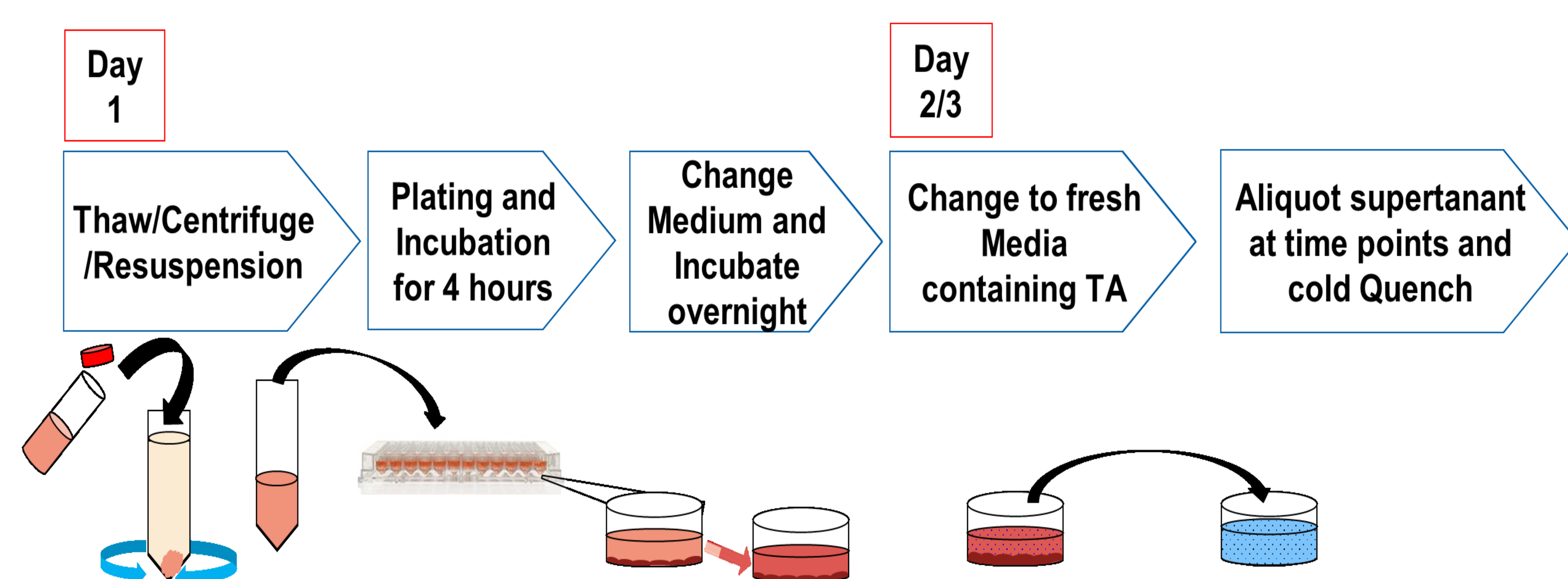

Figure 1. Plated hepatocytes System Workflow. Post substrate incubation, a 50 ul aliquot was taken at six different time points over 30 hours period and mixed with cold Quench solution (80 % Acetonitrile) with internal standard. LC/MS was used to analyze the aliquots at different time points to quantify the parent compound disappearance.

Results

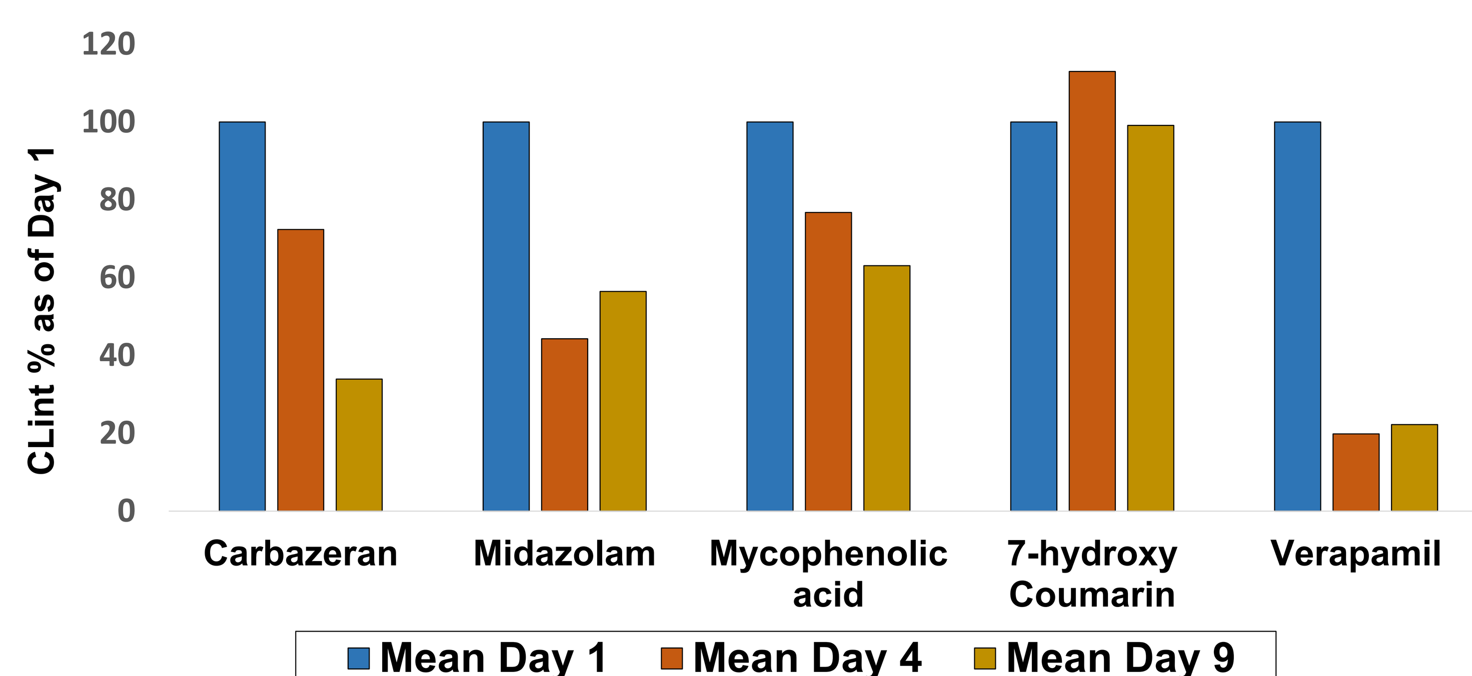

Figure 2. CLint of Enzyme Specific Substrates over nine day period. Day 1 was found to be the most suitable for the initiation of compound incubation, as it showed the highest enzyme activity. From these results, we designed the Incubation assay with six time points over the period of 30 hours.

Eznyme Substrates	Rat Plated Hepatocytes $CL_{int, in vitro}$ (ul/min/million cells)	Human Plated Hepatocytes $CL_{int, in vitro}$ (ul/min/million cells)
Ramipril (CES)	13.5	22.5
Carbezaran (AO)	12	4.2
Midazolam (CYP 3A)	13.5	7.5
Mycophenolic Acid (UGT)	2.55	4.04
7-hydroxycoumarin (UGT, SULT)	35.5	25.08

Table 1. CLint value of Enzyme Specific Substrates in Rat and Human Plated Hepatocytes. In both rat and human plated system, we observed parent disappearance kinetics confirming sustainable enzymatic activity over the period of assay.

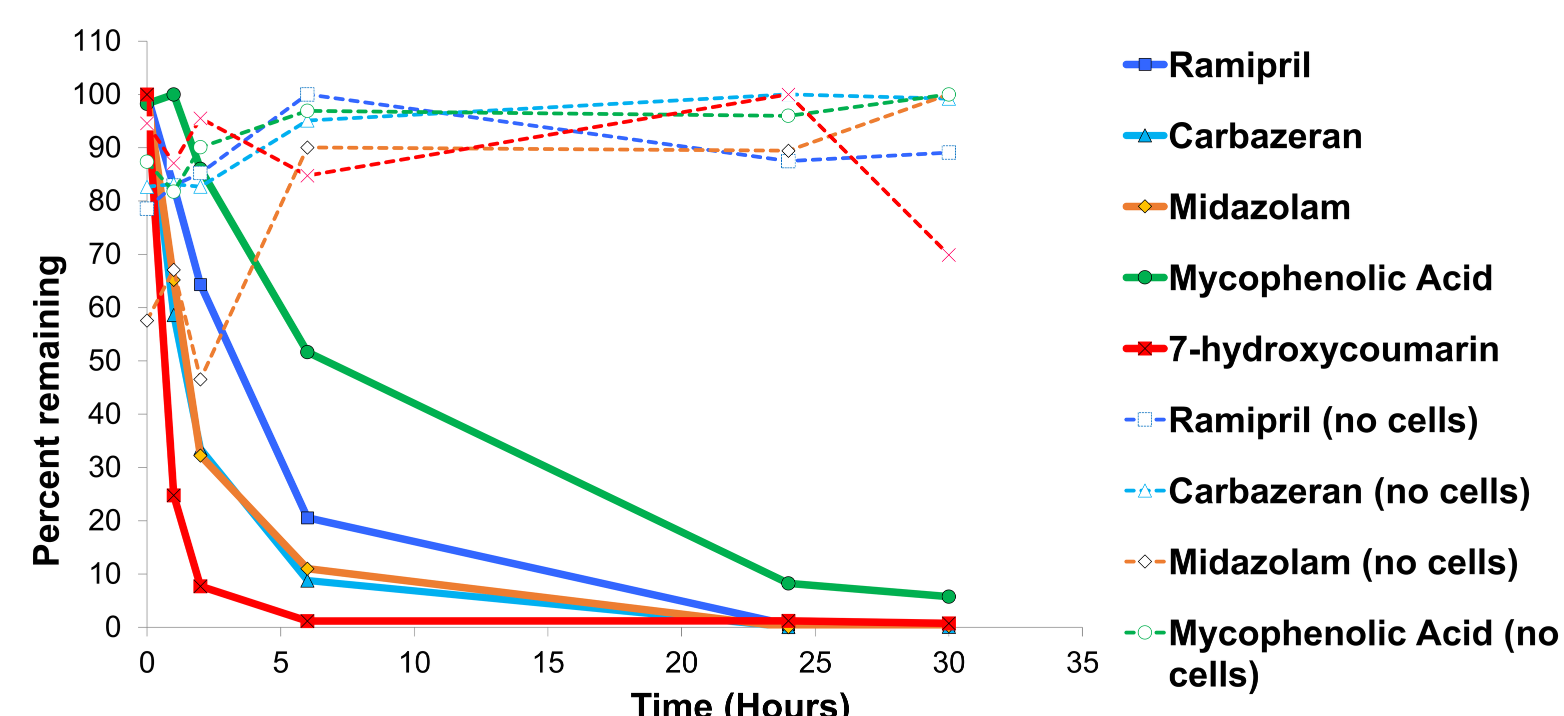

Figure 3. Metabolic Stability of Enzyme Specific Substrates in Rat Platable Hepatocytes. We found good resolution for parent disappearances curve over 30 hours period in rat plated hepatocytes assay. The enzymatic activity driven parent disappearance was further confirmed by control set, incubated without cells. The developed assay protocol was then used to measure intrinsic clearance in human plated hepatocytes.

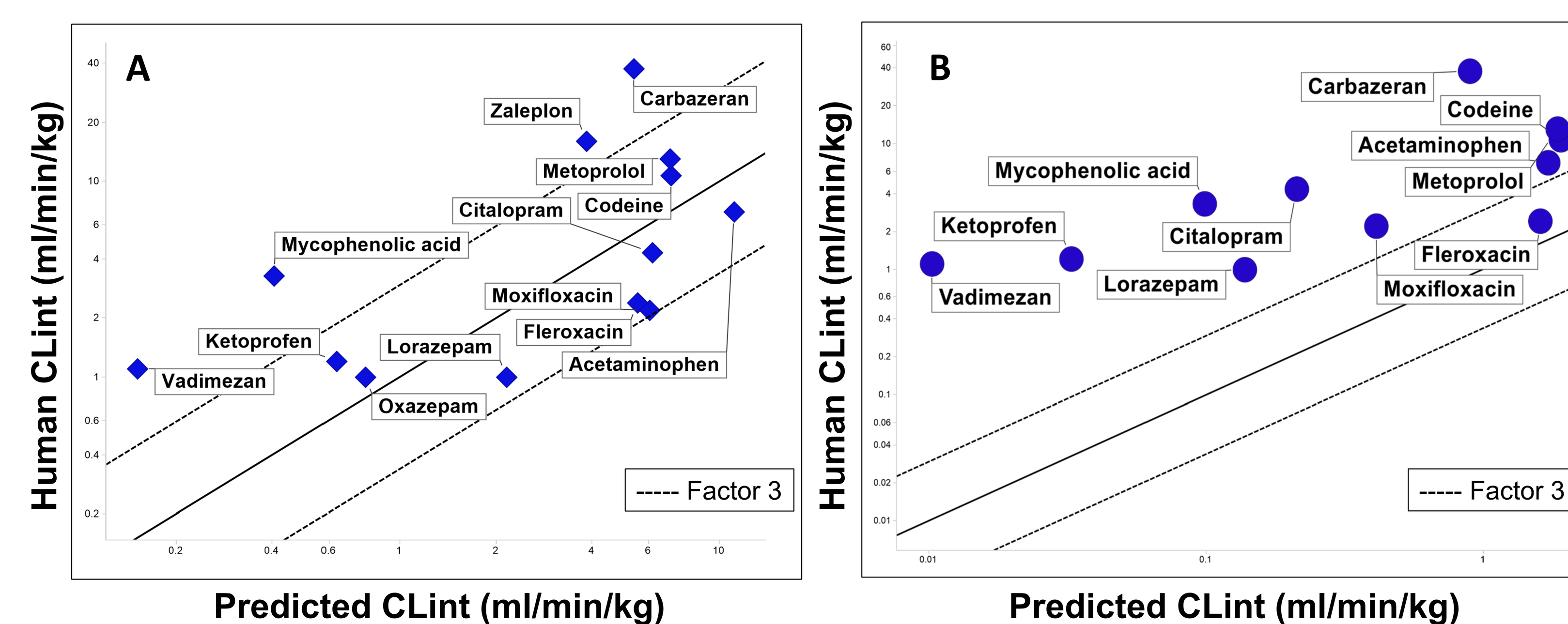

Figure 4: IVIVC using plated human hepatocytes for a test set. All compounds were "low CLint" in human liver microsomes (<25ul/min/mg). IVIVE is performed using the well-stirred model either excluding or including fraction unbound in plasma and fraction unbound in hepatocytes, plot A and B respectively. Correlation is significantly improved with binding is not incorporated suggesting binding in the incubation.

Conclusions and Future Work

Conclusions

- Enzymatic activity were observed in both rat and human primary hepatocytes platable system, confirmed by enzyme driven compound disappearance.
- Based on the data and its reproducibility, the assay protocol for the rat and human platable hepatocytes have been established.
- IVIVE for low CLint compounds in human plated hepatocytes shows reasonable IVIVC when fraction unbound in plasma is assumed to be equal to fraction unbound in incubation.

Next Steps

- Expand low CLint dataset and test in human and rat plated hepatocytes under the established experimental conditions.
- Look into quantitative approach for measuring cell viability over the experiment period, current approach is visual inspection.
- Automation (VIAFLO) development for liquid aliquot and dispensing.

References

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