HIGH-THROUGHPUT APPROACH FOR METABOLIC STABILITY SCREENING IN HEPATOCYTES

Milić A, Jurmanović S, Marković A, Marjanović N, Padovan J

Fidelta Ltd., Zagreb, Croatia

Introduction

Metabolic stability plays a major role in drug clearance, with the liver being the primary site for drug biotransformation via two major enzymatic reactions:

- Phase I (modifications to the molecular structure itself) and
- Phase II reactions (conjugation reactions)

The metabolic stability of compounds is commonly investigated early on in the drug discovery process using various *in vitro* test systems, such as liver microsomes, S9 fraction and hepatocytes.

Among these *in vitro* test systems hepatocytes are considered to be the golden standard for investigating metabolic stability as they represent an independent *in vitro* cellular test system, containing both Phase I and Phase II enzymes, as well as all cofactors and drug transporters.

However, the use of hepatocytes can be quite laborious, and both time and money consuming.

Objectives

The aim was to develop a quick and high-throughput approach for metabolic stability screening in hepatocytes.

Materials and Methods

A total of 600 compounds (200 compounds per species) were tested in human, rat and mouse hepatocytes.

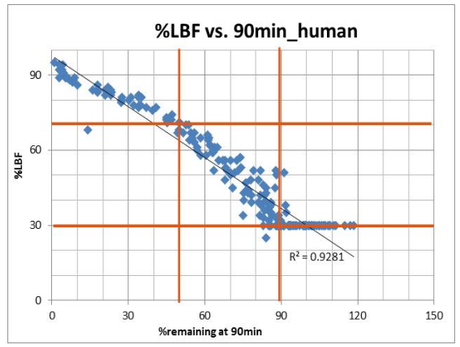
All tested compounds were incubated for 3h at 37°C in the hepatocytes resuspended at a density of $0.5x10^6$ cells/mL in modified Krebs–Henseleit buffer. Aliquots were taken at several different time points (0, 30, 60, 90,120, 150 and 180 min) and reaction was terminated by addition of acetonitrile/methanol (2:1) mixture, containing internal standard. After centrifugation (at 4000 rpm, at 4C, for 10 min) of collected aliquots, the resulting supernatants were subjected to LC-MS/MS analysis.
The *in vitro* half-life (t1/2) was in GraphPadPrism software from % remaining vs. time regression using non-linear regression fit. The hepatic *in vitro* intrinsic clearance (mL/min/g liver) was determined from the *in vitro* half-life (t1/2), normalized to the cell concentration in the incubation mixture and assuming hepatocellularity number of $120x10^6$ cells/g of liver.
This value was further scaled to predicted *in vivo* hepatic clearance and % of liver blood flow, enabling classification of all compounds as either low, moderate or highly stable drugs.

A correlation analysis of the percent remaining at 3 different time-points (45, 90 and 120min) was conducted, in order to evaluate the time-point with best predictivity of *in vitro* clearance and predicted *in vivo* hepatic clearance.

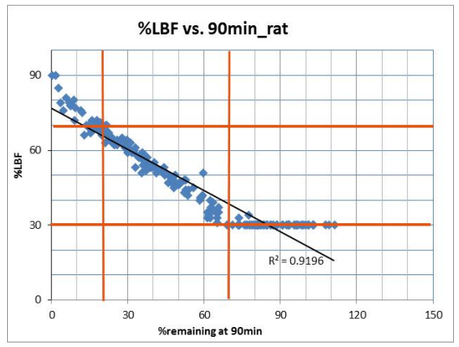
Results

Among the three different time-points investigated, our results suggest that the 90 min time-point gives the best predictivity (R^2>0.9) in all three tested species, i.e. the most accurate ranking of tested compounds.
Very similar results were obtained for the 120 min time-point, while overlaps were seen at the 45 min time-point.
Due to species differences, a different range of % remaining values was determined for each species at the 90min time-point, corresponding to the low, moderate and high stability classification group.

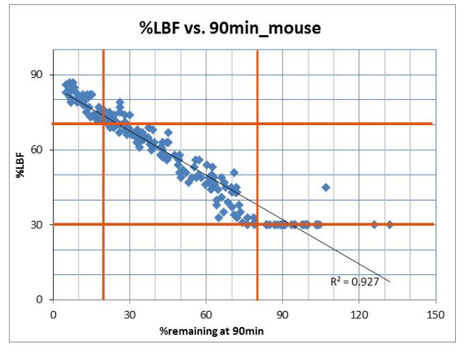
Human hepatocytes



Rat hepatocytes



Mouse hepatocytes



Species	%remaining at 90min	Classification
HUMAN	<50	LOW
	50-90	MODERATE
	>90	HIGH
RAT	<20	LOW
	20-70	MODERATE
	>70	HIGH
MOUSE	<20	LOW
	20-80	MODERATE
	>80	HIGH

Conclusion

A good correlation found for the 90 minute time point and an accurate ranking of the majority of tested compounds favors the routine use of the two time-point approach for high-throughput ADME screening of hepatic stability of new compounds.

In addition, the high-throughput approach presented here is both time- and cost effective as the following improvements have been achieved:

- a significantly higher number of compounds can be tested per vial of hepatocytes
- the analytical time per compound is shortened
- an overall decrease of assay cost per compound

Acknowledgement

We thank Maja Koša Prtenjača and Mira Šelendić for technical assistance.

References

1) Chao P et al, Expert Opin Drug Metab Toxicol, 2010, 6:189-198
2) Riley RJ and Girme K, Drug Discovery Today: Technologies, 2004, 1:365-372

Poster available online at:
www.fidelta.eu