Establishment of the Relay Method for Low Turnover Compounds in Hepatocyte Stability Assays

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Abstract

In vitro assays using liver subcellular fractions or suspended hepatocytes for characterizing the metabolism of drug candidates play an important role in the lead optimization stages. However, conventional *in vitro* assays have limitations in their ability to predict clearance for low turnover (slowly metabolized) drug molecules[1-2]. In this study, two *in vitro* methods, one using suspended cryopreserved hepatocytes (routine method) and a relay of sequential incubations with suspended cryopreserved hepatocytes (relay method) have been developed. We validated the relay method with 7 commercial compounds [3-4]. The data were in good agreement with those reported in the literature. The *in vitro in vivo* correlation (IVIVC) analysis of low turnover compounds showed that almost all the compounds were within 3-fold of unity.

Objectives

The objective of the study was to explore a methodology for determining the intrinsic clearance of low turnover compounds that can be applied in preclinical drug development.

Methods

1. Pooled cryopreserved human and animal hepatocytes were purchased from BioIVT.
2. For the routine method, test compounds (1 μM) were incubated with a suspension of hepatocytes (0.5 million cells/mL). At seven time points (0, 0.25, 0.5, 1, 1.5, 2, 4 hours), aliquots were removed and quenched by acetonitrile.
3. For the relay method, test compounds (1 μM) were incubated with a suspension of hepatocytes, the incubation mixture at the end of a 4-hour incubation period was centrifuged and the supernatant was subsequently drawn off and frozen until the next incubation period. This supernatant was thawed and introduced into a freshly prepared cryopreserved hepatocyte suspension to continue the incubation period. The incubation time was prolonged to 20 hours (5 days of 4-hour incubation).

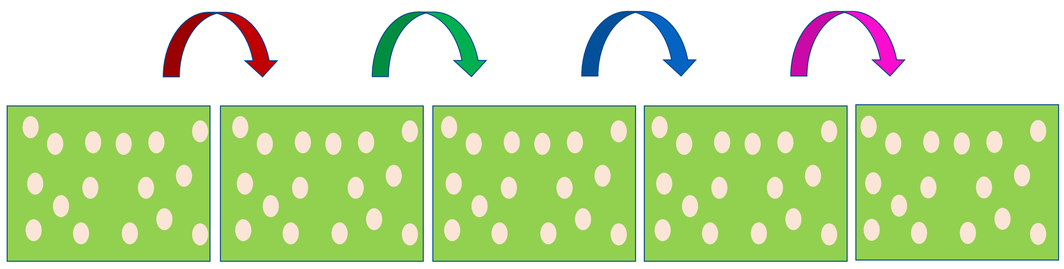

Figure 1. The process of relay method (continuous transfer of supernatant)

Results

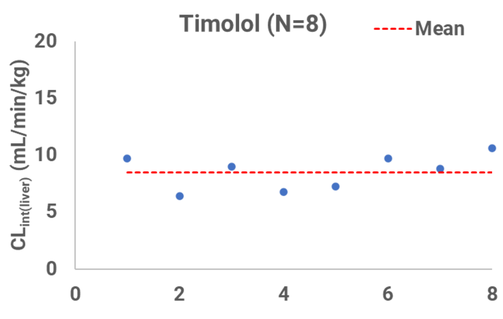

Figure 2. The intrinsic clearance value of Timolol obtained from 8 different runs by relay method

Table 1. Comparison of *In vitro* $T_{1/2}$ and $CL_{int(liver)}$ values in human hepatocyte using relay method between in-house and literature data

Compound ID	In house		Literature[3]
	$T_{1/2}$ (hour)	$CL_{int\ (liver)}$ (mL/min/kg)	$CL_{int\ (liver)}$ (mL/min/kg)
Tolbutamide	8.2	7.80	7.4
Warfarin	26.0	2.47	4.2
Timolol	6.6	9.72	14.0
Zolmitriptan	24.8	2.59	3.5
Theophylline	23.8	2.70	2.8
Atorvastatin	4.4	14.72	NA
Mephenytoin	5.2	12.26	NA

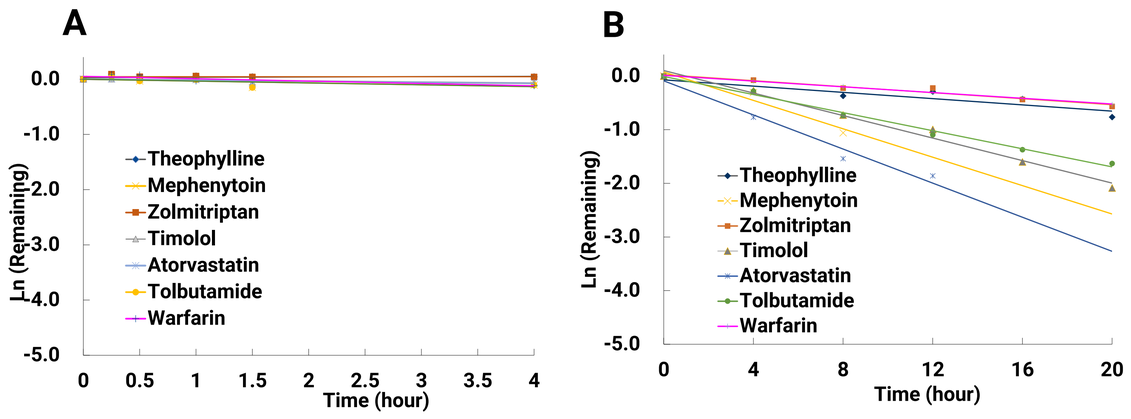

Figure 3. The metabolic stability profile of 7 tested compounds in routine method (A) and relay method (B)

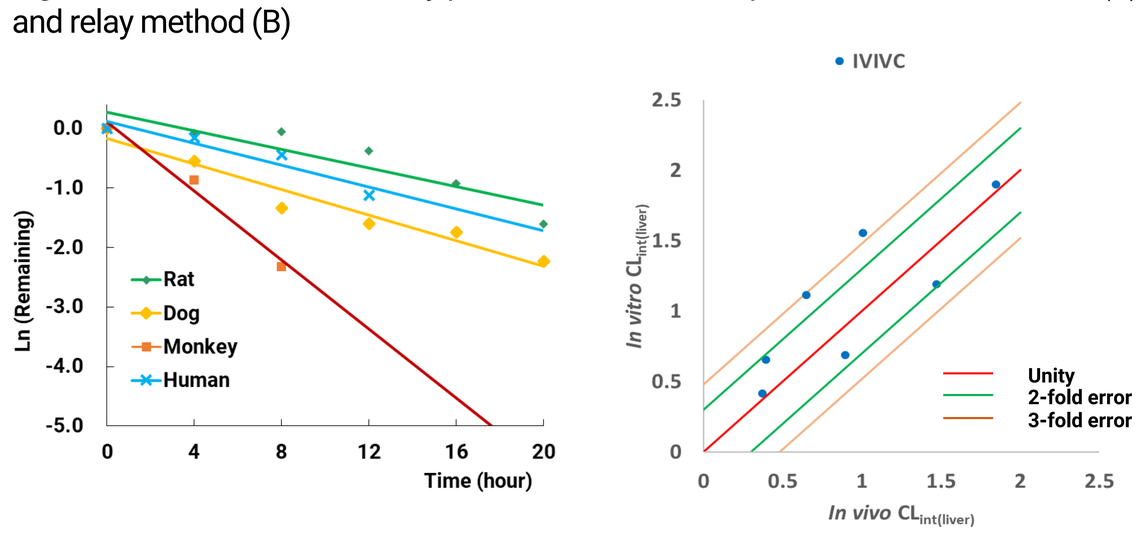

Figure 4. The metabolic stability of Mephenytoin in rat, dog, monkey and human hepatocytes

Figure 5. The IVIVC analysis of 7 low turnover compounds in human hepatocyte

- The relay method showed good reproducibility. The CV% of 8 runs in different days was 18 (Figure 2).
- Of the seven compounds tested, the clearance data of five compounds were comparable to values reported in literature[5] with the fold of difference less than 1.7 (Table 1).
- If compounds do not show significant turnover (>25%) in routine method, it will be difficult to determine the metabolic rate. For these low turnover compounds, the relay method showed significant turnover after incubation for 20 h (Figure 3).
- The intrinsic clearance values (*in vivo* $CL_{int(liver)}$) were calculated from reported human intravenous clearance data[6-9] based on the well stirred model. The *in vitro* intrinsic clearance (*in vitro* $CL_{int(liver)}$) was measured using the relay method. Six out of seven compounds were within 3-fold of unity (Figure 5).

Conclusion

1. Data from the relay method showed good correlation with *in vivo* human clearance data for low turnover compounds.
2. The relay method can be applied in hepatocytes from species other than rat, dog, cyno monkey, and human in our laboratory.
3. The method presents a solution to address low-clearance related issues in drug discovery and development.

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