The Rat as a Model for Screening Intestinal Metabolism Potential

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Abstract

- The contribution of the intestine to first-pass metabolism may be significant for some orally administered drugs, potentially resulting in low bioavailability [1, 2] (Figure 1). Determining the fraction of dose escaping intestinal extraction (F_G) is limited *in vivo* due to the difficulties in separating contributions of the liver and intestine following oral dosing [2].

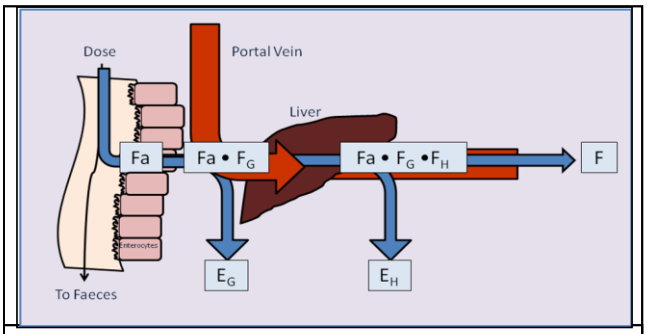

Figure 1| First-pass metabolism. Oral bioavailability (F) is determined by the extent of absorption from the intestinal lumen (Fa), fraction escaping intestinal metabolism (F_G) and the fraction escaping hepatic metabolism (F_H). E_G: intestinal extraction, E_H: hepatic extraction.

- *In vitro* tools (e.g. microsomes) provide greater mechanistic understanding of first-pass clearance processes in pre-clinical models and humans. However, unlike in the liver, established *in vitro* systems, *in vitro* to *in vivo* scaling factors and well characterized mathematical models do not exist for the intestine.
- In addition, there are large inconsistencies in the methodologies used for preparation of microsomes (e.g. scraping/ elution techniques, varied sample pool sizes, different buffer constituents) compromising estimates of *in vitro* clearance and data comparison [1,2,3]. A systematically optimised method for preparing intestinal microsomes was presented previously [3,4].
- Furthermore, the lack of validated subcellular intestinal scaling factors currently limits *in vitro-in vivo* extrapolation (IVIVE) and prediction of first-pass metabolism and bioavailability of orally administered xenobiotics.

Objectives

- Characterise 'in house' prepared rat intestinal microsomes in terms of method reproducibility and through the use of enzyme substrate probes, and a diverse range of metabolically cleared drug compounds.
- Explore any limitations in cocktailing phase I and II cofactors, and relative enzyme activities in commercial *vs.* 'in house' prepared microsomes.

Methods

- Two pools of Han-Wistar rat intestinal microsomes were prepared using the enterocyte elution method evaluated and optimized previously [3,4].
- 24 phase I and phase II substrate literature compounds were incubated at 1µM at either 1mg/ml or 0.5mg/ml microsomal protein concentration, with corresponding co-factors to generate values of intestinal intrinsic clearance ($CL_{int,gut}$) via the substrate depletion approach.
- Rates of metabolite formation for phase I and phase II probe substrates (testosterone hydroxylation for a range of CYP enzymes and 4-nitrophenol for glucuronidation) were obtained to characterize the prepared microsomes.
- Using commercially available Han-Wistar rat intestinal microsomes, 8 compounds were used to generate comparative measures of $CL_{int,gut}$ in the presence of either phase I or phase II cofactors, or cocktailed together [5]
- *In vivo* measures of F_G were determined indirectly through low dose oral and IV administration.

Results

- Mean coefficient of variation between rat intestinal pools was 31% (±28%), and indicated no significant trends in terms of route of metabolism (Figure 2).
- $CL_{Int,gut}$ ranged from <5 (e.g. pirenzepine) to >300 µl/min/mg in the case of 7-hydroxycoumarin and nicardipine.
- The highest $CL_{Int,gut}$ were obtained for UGT, CYP2B and CYP3A substrates.

Results cont

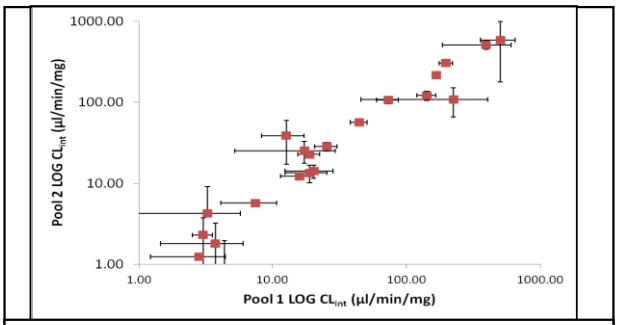

Figure 2| Correlation of measured CL_{int} between two pools of rat intestinal microsomes using an identical set of 24 drugs, at 1 or 0.5mg/ml protein concentration and 1µM probe concentration. Data represent mean ± sd of n=3.

- In commercial rat microsomes, observed $CL_{int,gut}$ was on average 2 fold lower than 'in house' prepared microsomes.
- No significant difference was observed in $CL_{int,gut}$ using phase I and phase II cofactors either combined or in isolation.
- In rat microsomes, 16α-, 16β- and 6β-hydroxytestosterone metabolites were predominant phase I metabolites formed, corresponding to CYP2B and CYP3A enzyme activities, consistent with reported intestinal enzyme protein expression [6] (Table 1). Rate of metabolite formation was higher than reported previously [7]. No significant difference in metabolite formation was shown between pools; however, the value was reduced 30% over one freeze-thaw cycle for 6β-hydroxytestosterone formation.

Metabolite	Enzyme Isoform	Rate of Metabolite formation (nmol/min/mg)
16β-OH-T	2B1	0.06 ±0.02
6β-OH-T	85% specific to 3A1 (1A1, 1A2, 3A1)	0.11±0.03
16α-OH-T	2B1, 2B2, 2C13 low amounts, 2C11	0.05 ±0.01
4-NP Gluc	UGT	58 ±21

Table 1| Rate of Formation of major testosterone and 4-nitrophenol metabolites for 'in house' rat intestinal microsomes. Incubations performed with 100µM testosterone or 4-nitrophenol at 1mg/ml protein concentration. n=2 pools rat, n=3 incubations, OH-T: hydroxytestosterone 4-NP Gluc: 4-Nitrophenol glucuronide

Conclusions and Ongoing Work

- A reproducible method of intestinal microsome preparation was demonstrated, with high levels of enzyme activity. Characterisation indicates likely metabolism routes of elimination, important when considering species differences in metabolic pathways between species.
- No significant differences in $CL_{int,gut}$ was observed either through using separation of cofactors or using a cocktailed approach, implicating a greater utilisation potential, beneficial considering the low intestinal microsome yields.
- Assessing the predictability of *in vivo* F_G in rat (using scaling factors incorporating correction for losses [4,5] and an appropriate mathematical model, such as 'Q_{GUT}'[8]), and application to dog and human intestinal tissue is ongoing.

References

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