Scaling factors for intestinal metabolism in dogs: Examining interindividual and regional variability, and correlations to hepatic scaling factors

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Abstract

- The lack of validated microsomal intestinal scaling factors currently limits *in vitro-in vivo* extrapolation (IVIVE) of first-pass metabolism for orally administered xenobiotics [1,2].
- The main focus of this project was to characterise intestinal metabolism in the Beagle dog, in order to aid prediction of *in vivo* intestinal extraction (E_G) and its contribution to oral bioavailability [3].
- The impact of regional intestinal metabolism is important to reflect the differing metabolic capacities between segments. Therefore, generation of zonal microsomal protein per gram mucosa (MPPGM) scaling factors is required for IVIVE within physiologically-based pharmacokinetic (PBPK) models, to aid prediction of oral clearance (**Fig. 1**).

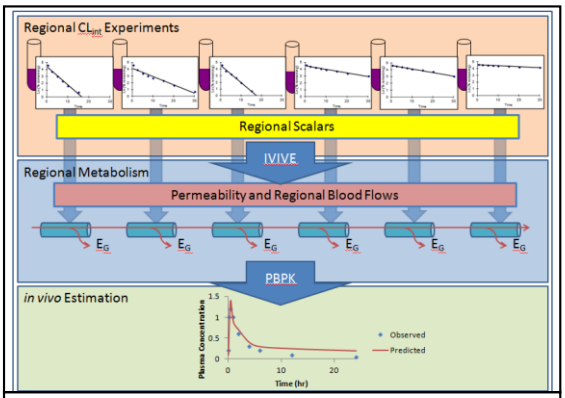

Figure 1| Relevance of regional scaling factors (along with permeability and blood flow) for IVIVE of intestinal first-pass in vivo using PBPK modelling approach.

Aims

- Characterise intestinal scaling factors in the Beagle dog as preclinical species
- Obtain regional intestinal scalars and assess differences in activity using CYP and UGT probes.
- Comparison of intestinal and hepatic scalars

Methods

- Intestinal microsomes from beagle dogs (n=3 per sex/segment) were prepared using the enterocyte elution method evaluated and optimized previously in the rat [4].
- The entire small intestine of the dog was obtained and split into *circa* six 60cm segments. Liver and kidneys were also collected from the same dogs.
- To correct for enzyme losses during microsome preparation, markers were measured in each sample, including cytochrome P450 (CYP) content, CYP3A activity (testosterone 6β-hydroxylation), and UGT activity (4-nitrophenol-glucuronide formation)

Results

- Microsomal recovery was higher in the dog liver *vs.* intestine (61±8% *vs.* 20±4%), but showed low coefficient of variation (CV 8% and 18%, respectively), highlighting the reproducibility of the method.
- MPPGM from dog proximal intestine were 39±12 and 23±11 mg protein/ g mucosa for male and female dogs (p<0.05).Values for the corresponding hepatic scalars were 43±8 and 37±8 mg protein/g liver in male and female dogs, respectively. Values of hepatic and intestinal scalars were strongly correlated (**Fig. 2**).

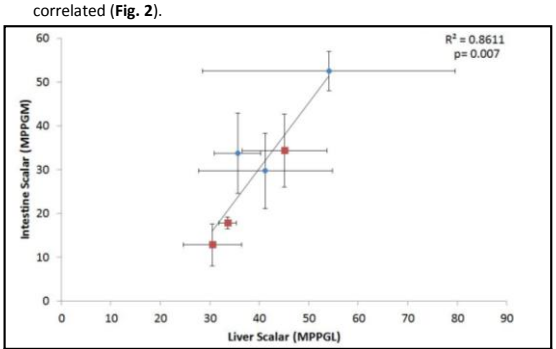

Figure 2| Correlation of liver and proximal intestinal scalars in male (■) and female (◆) dogs. MPPGM: microsomal protein per gram mucosa, MPPGL: microsomal protein per gram liver

Results continued

- Normalisation of $CL_{int,u}$ for a broad range of compounds by reported CYP3A12 content [6] indicated a good correlation between observed liver and intestinal activity for CYP3A substrates, but was 10 fold higher in the intestine (**Fig. 3**).

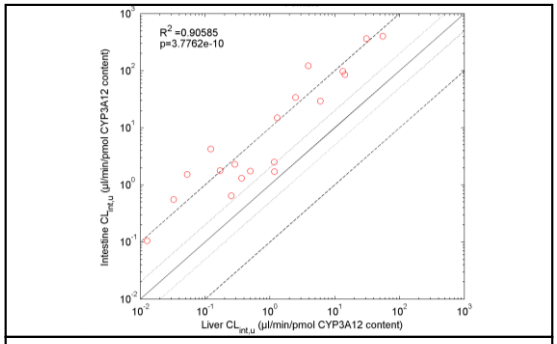

Figure 3| Depletion of a broad range of compounds (n=24) in female dog intestinal and hepatic microsomes, normalised for testosterone 6β hydroxylation activity. Testosterone Incubations performed with 100µM testosterone at 1mg/ml protein concentration. n=3. CL_{int} and fu_{inc} determinations performed at 1mg/ml and 1µM compound. Solid line: line of unity, dashed lines: 2 fold (grey) and 10 fold (black).

- Regional scaling factors in female dogs increased steadily to the third segment (10.5±1.1). Distal microsomal scalars decreased to levels comparable with the proximal tissue (5.24±1.0) (**Fig. 4**).

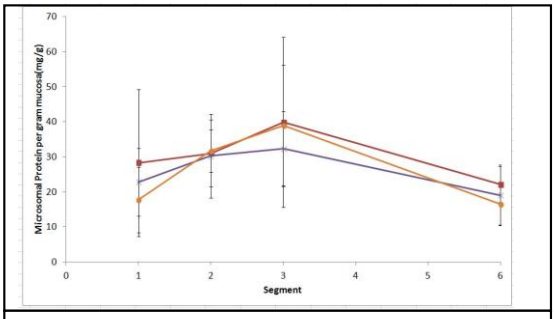

Figure 4| Marker corrected female dog regional intestinal microsomal protein per gram mucosa scalars. Data represent mean ± sd of n=3 per segment. Correction for losses marker key: CYP (■). 6β testosterone hydroxylation (×), 4-nitrophenol glucuronidation (●)

- Regional differences in dog intestinal activity were observed. Testosterone 6β-hydroxylation (CYP3A) was highest in the proximal segment 1 and segment 3 (0.8 nmol/min/mg). Formation was lowest in the segment 6 (0.15 nmol/min/mg).
- 4-NP-glucuronide formation in was significantly lower in distal compared to proximal segment 1 ($p<0.05$). Mean maximal 4-NP glucuronide formation declined distally from 1.3 nmol/min/mg (segment 1) to 0.4 nmol/min/mg (segment 6).

Conclusions and On-going Work

- This is the largest known comparison of matched liver and proximal intestinal prepared microsomes in beagle dogs. Strong correlations were observed between intestinal scalars as well as CYP3A substrate activities normalised for reported CYP3A expression in each organ. A mean 10 fold higher activity was observed in the intestine, but this is based on limited data from 2 dogs [6]. Further work is required to assess these dog liver and intestinal relationships.
- Lower recoveries in the dog intestine *vs.* liver, probably reflects the mucus content and its effect on microsomal preparation [5].
- MPPGI showed initial increase in the proximal dog intestine, and were the lowest in the final segment, in agreement with recent reports [5]. Regional differences in activity showed distally decreasing UGT activity along the course of the intestine. CYP activity peaked in segments 1 and 3.
- On-going work is focused on IVIVE of measured CL_{int} for selected compounds in dog intestinal and liver intestinal microsomes using the derived scaling factors in order to make predictions of E_G and oral bioavailability

References

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