# Determining regional scaling factors and activity for intestinal metabolism: A crucial step to understand interspecies differences in drug bioavailability

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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 within preclinical models and ultimately in human, in order to aid prediction of *in vivo* intestinal extraction ($E_G$) and its contribution to oral bioavailability in various species and give insight to interspecies differences in 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 intestine (MPPGI) scaling factors is required for IVIVE within physiologically-based pharmacokinetic (PBPK) models , to aid prediction of oral clearance (Figure 1).

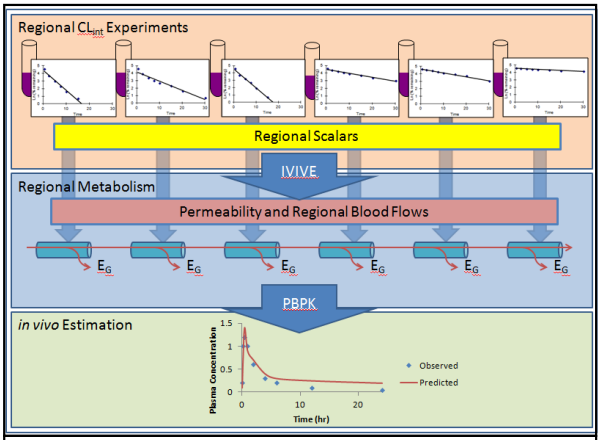


**Figure 1|** Relevance of regional scaling factors (along with permeability and blood flow) for IVIVE of intestinal first-pass in vivo using PBPK modelling approach. Key: $E_G$: intestinal extraction

## Aims

- Characterise intestinal scaling factors in preclinical species (rat and dog) and man.
- Obtain regional intestinal scalars and assess differences in activity using various probes.

## Methods

- Intestinal microsomes from Han Wistar rats (3 pools of n=9 rats per pool), beagle dogs (n=3 per sex/segment) and human tissue (1 pool of n=3 jejunum and n=2 ileum donors) were prepared using the enterocyte elution method evaluated and optimized previously in the rat [4,5].
- Pooled proximal tissue was obtained for the rat. Human intestinal segments were collected from gastric bypass (jejunum) or colon cancer (ileum) surgery patients. 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

- Proximal scalars for individual species are shown in figure 2.

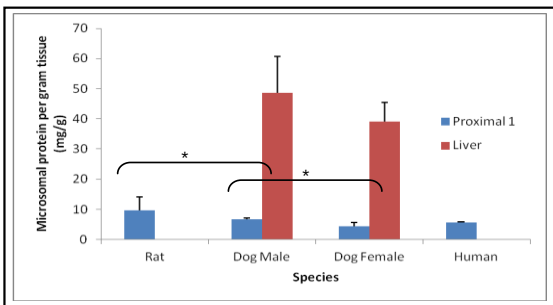


**Figure 2|** Inter-species comparison of proximal MPPGI scalars. Data represent mean ± sd of n=3 per segment. Key: * : p<0.05.

## Results continued

- 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) (Figure 3).

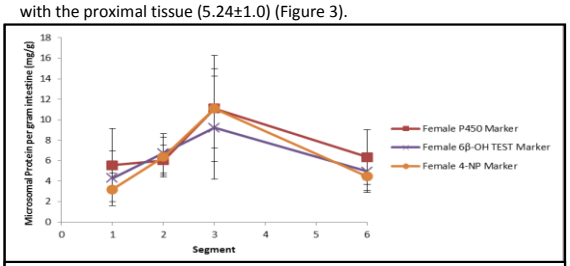


**Figure 3|** Dog regional intestinal microsomal protein per gram intestine scalars. Data represent mean ± sd of n=3 per segment. Key: 6β-OH TEST: 6β testosterone hydroxylation, 4-NP: 4-nitrophenol

- 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. Mean intestinal recovery was higher for rat (32±10%) and human tissue (41±6%).
- Testosterone 6β-hydroxylation (CYP3A) was highest in the human tissue, and lowest in the rat, unlike dog and human, testosterone 16β-hydroxylation (CYP2B) was a major enzyme pathway in the wistar rat (Table 1).
- Mean maximal 4-NP glucuronide formation was 84.7±28.7, 0.7±0.4, 1.3±0.2 1.4±0.2 nmol/mg in proximal rat, male dog, female dog and human intestine respectively.
- Regional differences in dog intestinal activity were observed. 4-NP-glucuronide formation was significantly lower in distal compared to proximal segment 1 ($p<0.05$). Testosterone 6β-hydroxylation was highest in proximal segments and lowest in the distal segment (Table 1).

| | | | Microsomes Rate of OH-Testosterone $V_{MAX}$(nmol/min/mg) | |
|---|---|---|---|---|
| **Species** | **Metabolite** | **P450 Isoform** | **Proximal** | **Distal** |
| **Rat** | 16β-OH-TEST | 2B1 | 0.06 ±0.01 | |
| | 6β-OH-TEST | 85% specific to 3A1 (1A1, 1A2, 3A1) | 0.06±0.01 | |
| | 16α-OH-TEST | 2B1 ( 2B2 2C13, 2C11) | 0.05 ±0.01 | |
| **Dog Male** | 6β-OH-TEST | 3A12 | 0.3±0.07 | |
| **Dog Female** | 6β-OH-TEST | 3A12 | 0.82 ±0.49 | 0.15 ±0.05 |
| **Human** | 6β-OH-TEST | 3A4 | 1.37 ± 0.2 | 0.37 ± 0.06 |

**Table 1| Rate of Formation of major testosterone metabolites for rat dog and human intestinal microsomes.** Incubations performed with 100µM testosterone at 1mg/ml protein concentration. n=3, OH-TEST:

## Conclusions and On-going Work

- Highest scaling factors were observed in proximal rat tissue. Lower recoveries in the dog probably reflect mucus content and its effect on microsomal preparation[6].
- MPPGI showed initial increase in the proximal dog intestine, and were the lowest in the distal segment, in agreement with recent reports[7]. Regional differences in activity showed decreasing phase I and phase II activity along the course of the intestine.
- Species differences in intestinal activity highlighted the importance of CYP2B in wistar rat metabolism consistent with reported protein expressional data[8]. Female dog 6β-hydroxylation was within 2-3 fold of human. Phase II metabolism of 4-NP was 60-100 fold lower in rat *vs.* dog and human highlighting the importance of this metabolism pathway in the rat.
- On-going work is focused on IVIVE of measured $CL_{int}$ for selected compounds in rat, dog and human intestinal microsomes using the derived scaling factors.

## References

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