Improving the prediction of oral bioavailability using fresh human intestinal tissue

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Abstract

Here we present data obtained using fresh human small intestinal mucosa tissue mounted in Ussing chambers to showcase its utility as a predictive model of drug intestinal absorption and metabolism. When comparing the permeability data sets, a sigmoidal relationship was observed between *in vitro* permeability coefficients (Papp) and the reported clinical Fa values for the same test compounds. Both metabolic (phase 1 and phase 2 enzyme systems) and transporter activities were also shown to be preserved in the test system. Ussing chambers and fresh human gastrointestinal tissue therefore offer the opportunity to model human absorption whilst taking into account physiologically relevant intestinal metabolism and transporter effects. Ussing chambers also allow the opportunity to directly compare and understand regional and/or preclinical species differences in intestinal absorption and metabolism.

Introduction

Orally administered drugs continue to be the most common route of drug therapy. It is also increasingly recognised that intestinal biology not only influences absorption but is also an important site of metabolism that influences oral bioavailability. Before a drug progresses to the clinic an estimation of the fraction reaching the systemic circulation is required to optimise the first in man dose. At present, these predictions routinely rely on inputs from animal *in vivo* or cell based assay models. While the implementation and utilisation of models such as Caco-2 have greatly increased prediction performance over the years, the predictions are recognised to be superior for IV profiles over the more relevant PO profiles for the same set of drugs[1]. This suggests a need to better model the intestinal absorption and metabolism of oral drugs in humans. Here we present data obtained using fresh human small intestinal mucosa tissue mounted in Ussing Chambers.

Methods

Fresh human intestinal tissue, residual from resection surgery, was obtained from the ReproCELL clinical network. On arrival, the mucosa was dissected free from surrounding tissue and mounted in Ussing chambers.

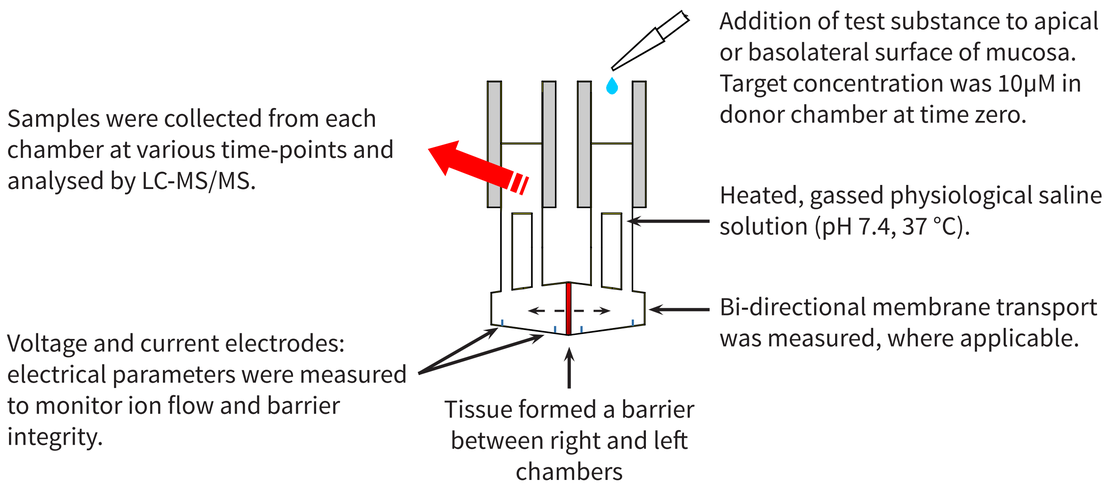
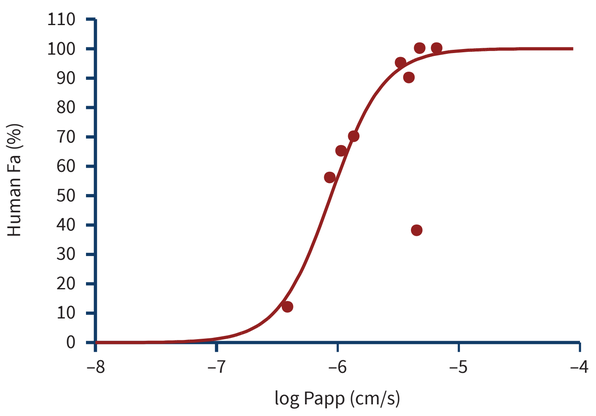


Figure 1: Diagrammatic representation of the Ussing Chamber bath set-up used. Multiple baths were set-up from each donor tissue.

Results



Compounds	Human Fraction Absorbed (Fa, %)	Log Papp (log cm/sec)
Warfarin	95	–5.47
Methotrexate	70	–5.86
Clonidine	90	–5.41
Digoxin	65	–5.46
Verapamil	100	–5.18
Mannitol	38	–5.34
Atenolol	56	–6.06
Antipyrine	100	–5.32
Sulfasalazine	12	–6.41

Figure 2: Graph and associated table showing a steep sigmoidal relationship (Hill slope = 2) between *in vitro* derived duodenum Papp and corresponding human Fa. Although steep, there was a good window between the highest (6.66 x 10^{-6} cm/s) and lowest (0.39 x 10^{-6} cm/s) permeability values observed *in vitro*. On the graph, each dot represents an individual test compound and data was fitted using a 4 parameter logistic model curve fit. The Papp value shown for each test compound is presented as a mean of the calculated Papp values for n = 3 individual donors.

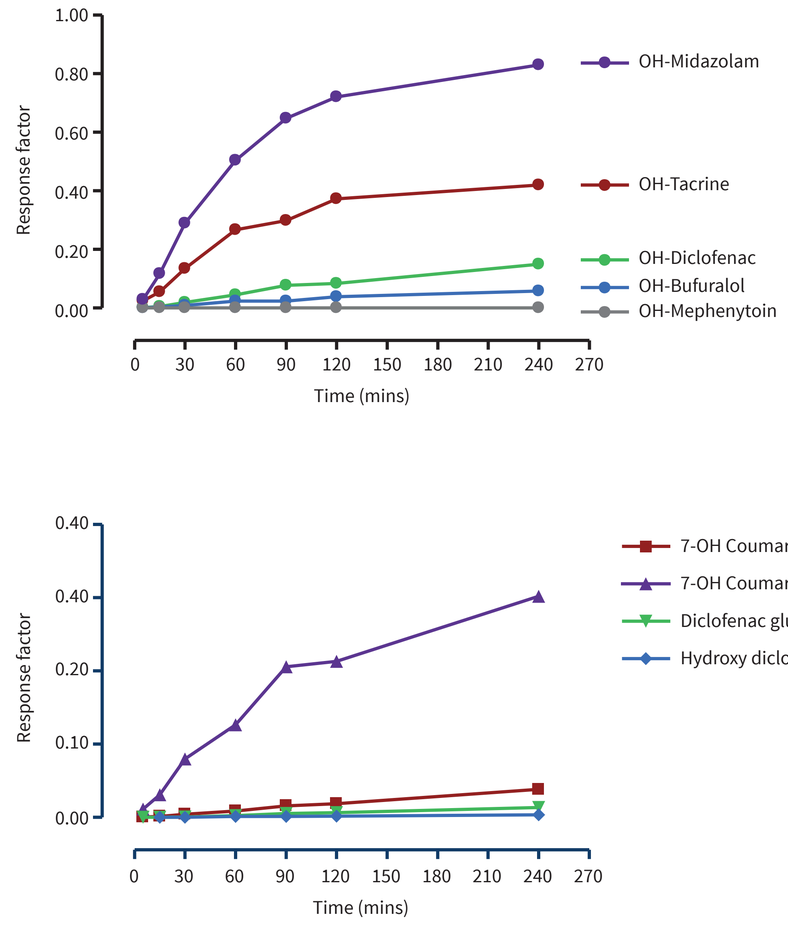


Figure 3: **A** – Graph showing Phase 1 metabolic activity in human duodenum mucosa mounted in Ussing chamber apparatus. Metabolic activity was assessed using model cytochrome p450 (CYP) substrates midazolam (CYP3A4), tacrine (CYP1A2), bufuralol (CYP2D6), diclofenac (CYP2CP) and mephenytoin (CYP2C19). Hydroxy metabolite formation was measured in both apical and basolateral baths (apical bath data shown above). Data presented as the mean of n = 2 individual donors. **B** – Graph showing Phase 2 metabolic activity in human duodenum mucosa mounted in Ussing chamber apparatus. Metabolic activity was assessed using model phase 2 enzyme substrates coumarin and diclofenac. Glucuronide and sulphate metabolite formation was measured in both apical and basolateral baths (apical bath data shown above). Data presented as the mean of n = 2 individual donors.

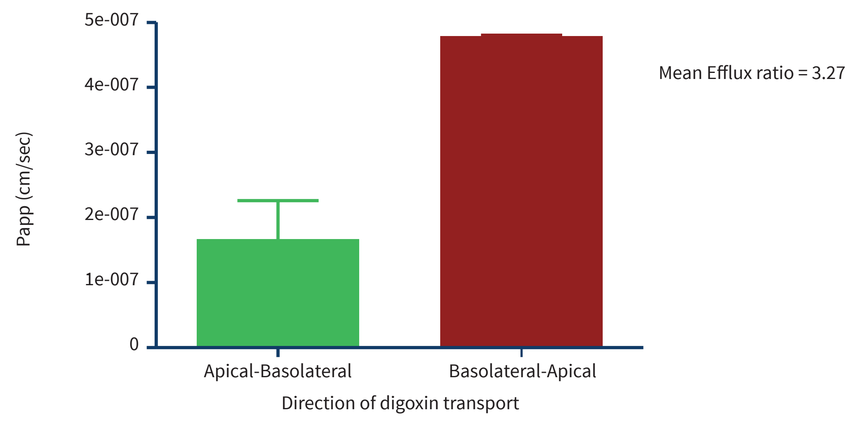


Figure 4: Graph showing Pgp activity in human jejunum tissue mounted in Ussing chamber apparatus. Pgp activity was assessed using digoxin as a model Pgp substrate and measuring its permeability (Papp) in both apical-basolateral and basolateral-apical directions. Data presented as the mean ± SEM of n = 2 individual donors.

Conclusions

- **Ussing chambers and fresh human gastrointestinal tissue provide the opportunity to model human intestinal absorption whilst taking into account physiologically relevant metabolic and transporter effects.**
- **Ussing chambers also provide the opportunity to directly compare and understand regional and/or preclinical species differences in intestinal absorption and metabolism.**
- **The data presented here supports and expands upon the findings of other researchers[2,3].**

References

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