Human Drug Absorption and Metabolism

Predictive Drug Discovery Services

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The increasing use of human intestine in Ussing chambers: improving the prediction of human oral bioavailability

Introduction

Orally administered drugs continue to be the most common route of drug therapy. As development costs increase, models which can more effectively predict human bioavailability at an early stage in development have increasingly come to the fore.

It is also increasingly recognised that the intestine not only influences absorption but is also an important site of first-pass metabolism that influences oral bioavailability. Common approaches do not accurately reflect the biology of the human small intestine — the site of absorption of most drugs.

Benefits of Human Intestinal Tissue

- Data is generated in the actual site of drug absorption
- Accurate metabolic and transporter profile
- Avoids species differences
- Adds commercial value during preclinical development
- GI metabolism, binding, transport and permeability in one experiment
- Reduces the risk of clinical failure

The only system reflecting interplay between f_a and f_g

The main uses of the Ussing chamber in drug discovery and development are to offer a system where both permeability and metabolism can be considered in a single experiment.

Systems for the prediction of compound absorption have been in use for some time, e.g. the colon cancer cell line, Caco-2, is useful for the prediction of f_a for passively absorbed compounds, but the low level expression of metabolic enzymes mean that intestinal permeability (f_a x f_g) cannot be predicted.

Similarly, predictions of metabolic instability can be provided by the use of subcellular fractions or slices of intestine (i.e. prediction of f_g) but permeability cannot be predicted due to the lack of an intact cell membrane (for fractions) or intact barrier (for intestinal slices).

Use of high-throughput approaches mean that the in vivo situation is therefore seldom accurately predicted[1].

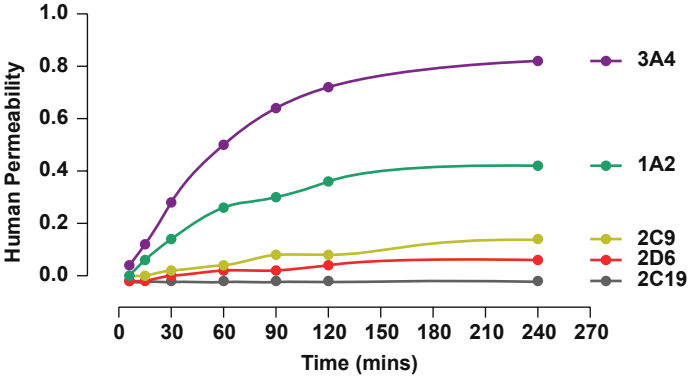


Figure 1. Human duodenal mucosa mounted in the Ussing chamber shows time-dependent Phase 1 and Phase 2 metabolism of model enzyme substrates (data on phase 1 metabolites is shown relative to specific cytochrome P450 enzymes).

Are Animal Tissues Predictive?

In many instances, animal tissues from rats, dogs and non-human primates are used experimentally; but do they predict human bioavailability?

Rats: there is a good correlation between rat and human permeability; however, there is no correlation with oral bioavailability[2].

Dogs: there is a poor correlation between dog and human f_a[3].

Non-human primates: there is a good correlation between monkey and human permeability; however, there is a poor correlation with oral bioavailability as the expression of CYP enzymes is much higher in the GI tract of monkeys[4].

Permeability and f_a

Permeability and the free drug concentration within the gastrointestinal tract are two key variables controlling the fraction of drug that is absorbed (f_a)[2].

The apparent permeability, 'P_{app}' can be calculated in vitro using human intestinal mucosa.

The P_{app} value reflects permeability regardless of transport mechanism.

Intestinal Metabolism and f_g

Once the drug has moved into the enterocyte, it may undergo Phase I and Phase II metabolism.

Phase I metabolism is controlled by CYP enzymes, in particular CYP3A4, which is expressed in the small intestine, but not the large intestine.

Both Phase I and II metabolism can be measured in the Ussing chamber using human intestinal tissues.

Moreover, by combining permeability and metabolism, regional differences along the length of the gastrointestinal tract can be investigated.

Ussing chamber studies show good correlation with clinical data

Compound	Human F_a (%)	P_{app} (cm/sec)	Log P_{app} (cm/sec)
Warfarin	95	3.35231E-06	–5.475
Methotrexate	70	1.38046E-06	–5.860
Clonidine	90	3.93057E-06	–5.406
Digoxin	65	1.08418E-06	–5.965
Verapamil	100	6.66E-06	–5.177
Mannitol	38	4.55E-06	–5.342
Atenolol	56	8.71472E-07	–6.060
Antipyrine	100	4.83289E-06	–5.316
Sulfasalazine	12	3.90602E-07	–6.408

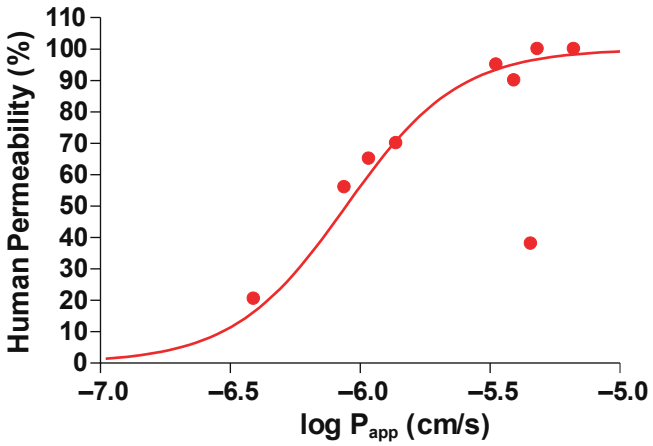
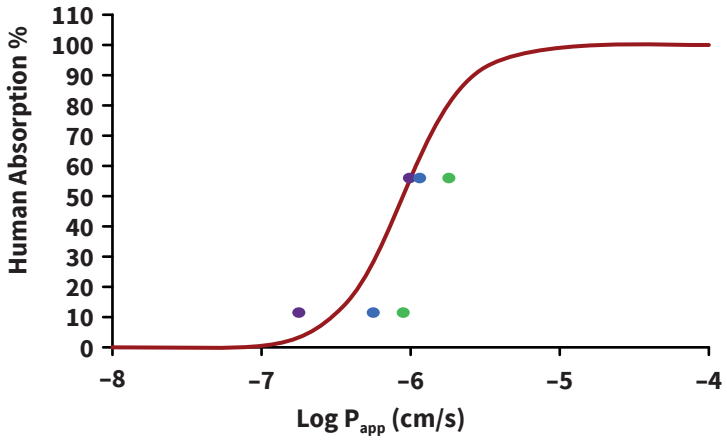


Figure 2: Relationship between drug permeability (P_{app}) in healthy intestine and clinical drug permeability in humans. The P_{app} and clinical permeability values for nine drugs (Warfarin, Methotrexate, Clonidine, Digoxin, Verapamil, Mannitol, Atenolol, Antipyrine, Sulfasalazine) were measured and then plotted on the above graph, showing good correlation between the two values.

Advantages of human Ussing experiments over other experimental models:

- Caco-2 cells express very low levels of CYP3A4 , thus passive permeability may be well predicted but overall bioavailability is not (because metabolism may not occur)[2]
- Inter-laboratory variability is high for Caco-2 and is reported to be lower for human intestinal Ussing[2]
- Poor interspecies correlations in bioavailability[5]

Estimation of oral bioavailability in healthy and IBD tissues



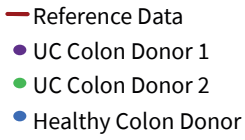


Figure 3. Comparison of the absorption profile for two Ulcerative Colitis (UC) donors and one healthy donor. P_{app} (calculated permeability in cm per second) values were calculated as a mean of the four baths in each donor and then plotted against Biopta (REPROCELL) reference data. The results showed that the drug absorption varied for the IBD donors compared to the healthy donor and reference data.

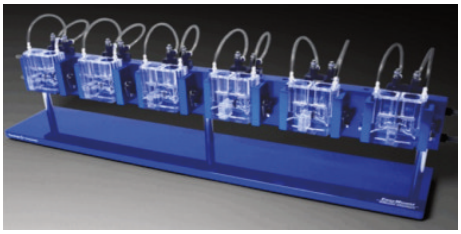
Experimental set up for estimating oral bioavailability

The Ussing chamber technique consists of two chambers separated only by the intact tissue, which may be an intact mucosal layer dissected free from the stomach, small intestine or large intestine.

Clearly, high quality fresh tissue is of great importance and viability is typically assessed before and during an experiment by measurement of electrical resistance ("transepithelial electrical resistance", TEER). Functional tissues also have a measurable potential difference (reflecting the voltage gradient across the tissue) and basal short circuit current (Isc), reflecting the ionic fluxes across the tissue.

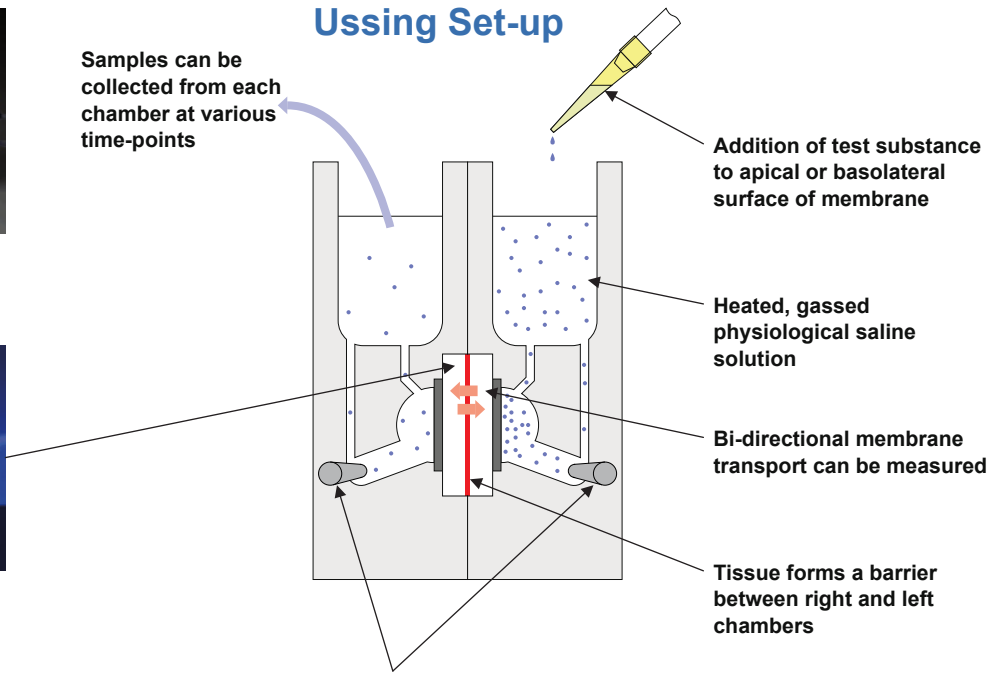
Tissues are ethically donated as residual to surgical procedures or from organ donors and are transported to the laboratory in a physiological saline solution. Once mounted in the chamber, tissues are allowed to equilibrate at 37°C and the solution is gassed with 95% O_2 / 5% CO_2.

Electrical parameters are recorded via voltage and current electrodes within each chamber; TEER values are collected every 2-5 minutes during the protocol by measuring the current injection required to briefly clamp the voltage at 1 mV. From Ohm's Law (V = IR), TEER can be calculated.



Multiple tissues per donor can be studied

Holder for intact mucosa



References

1, Sjoberg, A et al. Eur J Pharm Sci (2013) 166-180; 2, Lennernas, H. Adv Drug Del Rev (2007) 59, 1103-1120; 3, Chiou, W.L. et al. Pharm Rev (2000) 17, 135-140; 4, Chiou, W.L. et al. Pharmaceutical Res (2002) 19, 868-874; 5, Gotoh, Y et al. J Biomol Screening (2005) 10 (5) 517-523