Combination of *in vitro* Caco-2 and aqueous solubility screens with *in silico* physiological modelling for the prediction of human intestinal absorption in early drug discovery

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INTRODUCTION

Commercial drug discovery is a process of design, optimization and selection of candidate compounds with both appropriate pharmacological activity and favourable absorption, distribution, metabolism and elimination (ADME) properties. With the increasing application of high-throughput assays for the determination of the *in vitro* ADME properties of compounds in lead identification and optimization, there is a growing need for efficient and cost-effective methods for interpreting the resulting data to enable well-informed selection to be made for compound progression.

Physiologically-based pharmacokinetic (PBPK) modelling can provide such a means of integrating the large quantity of *in vitro* data routinely generated in order to predict the human pharmacokinetic (PK) properties of compounds. Full-body PBPK models are mathematical descriptions of the flow of blood throughout the body, developed for the simulation of xenobiotic absorption, distribution and elimination. However, less extensive physiologically-based models, describing the movement of compounds through defined body sub-systems, can also be derived. We have developed an approach that utilizes aqueous solubility and Caco-2 permeability data, in conjunction with physiological modelling, to predict human intestinal absorption (HIA). These data are routinely obtained during early drug discovery from primary ADME screens. An estimate of solubility is essential for assessing the reliability of data generated by *in vitro* ADME assays, as poorly soluble compounds may precipitate during the course of an assay leading to unreliable results. Caco-2 permeability (P_{app}) in the apical-to-basolateral (A-B) direction is frequently used as an indicator of the likely *in vivo* intestinal absorption.

Our approach provides not only a quantitative estimate of HIA, but also highlights possible dose-dependence. Furthermore, because the clinical exposure that is required for any compound depends on its efficacy, the approach enables very early integration of pharmacokinetic and *in vitro* efficacy data for better-informed compound selection than could be made by consideration of Caco-2 P_{app} data alone.

METHODS

Experimental

The following experimental data were determined at Cyprotex, using our standard Cloe® Screen (Cyprotex Discovery Ltd., 2006) screening methods:

- Aqueous solubility at pH 7.4, measured using a high-throughput turbidimetric assay; this is a rapid, low-cost screen that returns solubility as an estimated range (i.e., lower bound and upper bound) and a calculated mid-range value.
- Caco-2 apparent A-B permeability coefficient (P_{app}) at pH 7.4.

Modelling

The model we have used here for the prediction of intestinal absorption is a modified version of the Advanced Compartmental Absorption and Transit (ACAT) model (Rose *et al*., 1998), which is itself an extension of the CAT model originally proposed by Yu and co-workers. (Yu *et al*., 1996; Yu, 1999). The lumen of the gastrointestinal (GI) tract is conceptually subdivided into nine compartments representing the lumens of the stomach, duodenum, jejunum, ileum and colon.

The entire oral dose of a compound enters the stomach lumen compartment at zero time. Dissolution, transfer to successive compartments and absorption via both passive transcellular and paracellular diffusion are modelled.

Within each compartment, dissolved and undissolved compound are represented; only the dissolved fraction is available for absorption. The amount absorbed from each individual compartment over the time course of the simulation is determined, and thereby the total intestinal absorption can be predicted.

A schematic representation of flow through the intestinal absorption model is given in Figure 1.

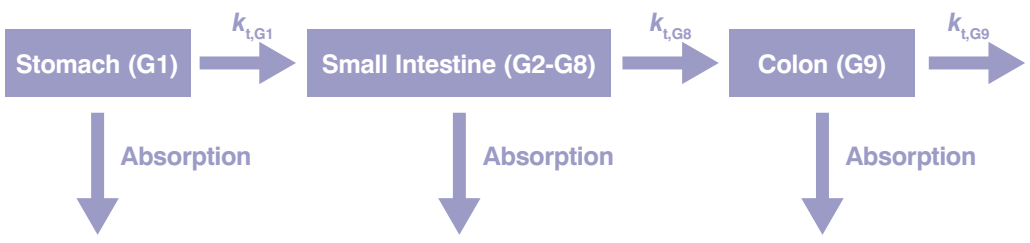

FIGURE 1. Schematic diagram of the intestinal absorption model.
G1 and G9 represent the stomach lumen and colon lumen, respectively, whilst compartments G2-G8 collectively represent the small intestinal lumen. Transit between compartments G1 and G2, between G8 and G9, and from G9 are governed respectively by the transit rate constants, $k_{t,G1}$ and $k_{t,G8}$ and $k_{t,G9}$. Transit between the individual compartments representing the small intestinal lumen forms part of the model but is not shown above.

The only required inputs to the model are effective intestinal permeability (P_{eff}) and Cloe® Screen aqueous solubility at pH 7.4. There are no pH-dependent components to the model, and hence pKa is not required. P_{eff} is derived from Cloe® Screen Caco-2 P_{app} through a correlation model. This model was developed using a training set of *in vivo* HIA data and validated using a separate test dataset. The training and test datasets were compiled from data presented in a single source (Zhao *et al*., 2001), in which reported HIA data for a large set of drugs have been collated and their reliability assessed.

The composition of the training and test sets, in terms of observed HIA and Caco-2 P_{app}, is illustrated by Figure 2. A high proportion of drugs are extensively absorbed, but this is not necessarily the case for discovery compounds. Hence, in order to minimise any resulting bias, drugs with a reported HIA of 100% were excluded from the training set.

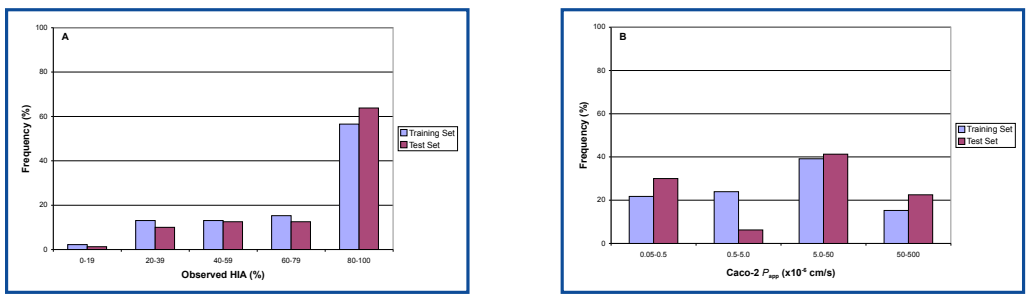

FIGURE 2. Composition of the training and test sets, in terms of (A) observed HIA and (B) Caco-2 P_{app}.

RESULTS

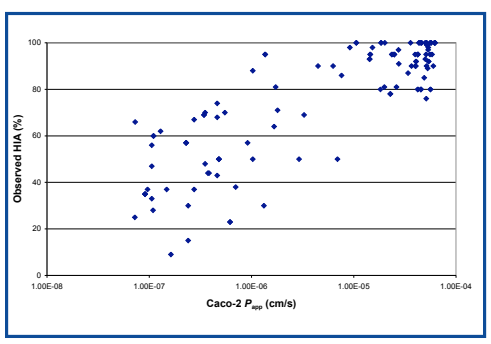

FIGURE 3.
HIA plotted against P_{app} for a diverse set of drugs.

A plot of HIA against A-B P_{app} for a diverse set of drugs demonstrates that P_{app} is reasonably predictive of HIA (Figure 3). Complete, or almost complete absorption (HIA ≥ 80%), can generally be expected for compounds with P_{app} greater than approximately 5×10^{-6} cm/s. A lower P_{app} value indicates that incomplete absorption is likely, the extent of which shows some correlation with P_{app}. However, it is difficult to predict dose dependency using Caco-2 data alone.
Compounds that are not completely absorbed *in vivo* tend to be those that have a high molecular weight, subject to significant active efflux in Caco-2, and/or poorly soluble. These properties are often associated with discovery compounds and isolated Caco-2 P_{app} data for such compounds must be interpreted with caution; solubility should also be taken into account in order to generate a reliable estimate of the extent of absorption in man.

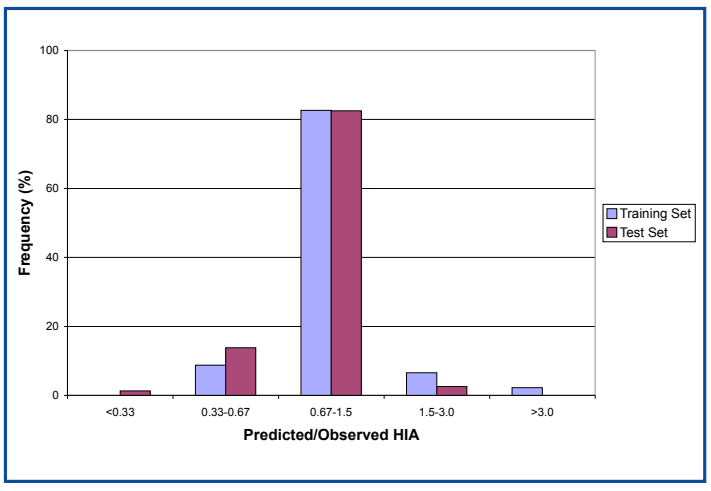

FIGURE 4. Frequency distributions of the predicted/observed ratios of HIA for the training and test sets.

The approach we have developed provides a quantitative prediction of HIA from both Caco-2 P_{app} and solubility data. The vast majority (greater than 80%) of the predicted HIA values are within a factor of two of the observed HIA (Figure 4). Furthermore, the most notable underprediction (predicted/observed ratio < 0.33) is for a high dose (10 mg/kg) of methotrexate, a test set compound that is subject to active uptake. At low dose (0.1 mg/kg), prediction is more accurate (predicted/observed $= 0.63$). A second compound (gabapentin) that undergoes active uptake *in vivo* is also underpredicted but to a lesser degree, with predicted/observed ~ 0.4 for 300 and 900 mg doses.

The method presented here can also provide an indication of likely dose-dependent absorption and the potential limitations of solubility on HIA. Whilst HIA may be somewhat underestimated by the model in some cases, the observed reduction in HIA with increasing dose is generally well-predicted, and a probable variation in HIA with solubility is indicated (Figure 5).

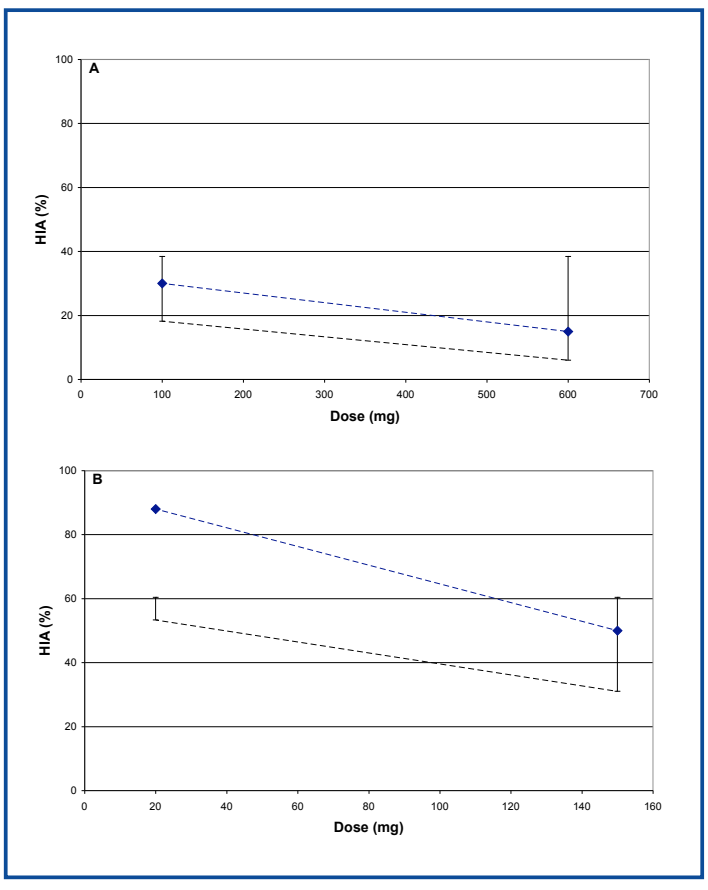

FIGURE 5. Predicted and observed change in actual HIA with dose, and predicted variation with solubility for: (A), acyclovir; (B), ranitidine.
The filled symbols represent the observed HIA, whilst the bars indicate the range of predicted HIA values over a range of solubilities (from an experimentally-determined minimum of 100 μM to an upper limit of 100 mM, above which no increase in HIA has been predicted for any compound).

CONCLUSIONS

Quantitative prediction of HIA is not straightforward. However, we have developed a methodology that combines data generated by primary *in vitro* ADME screens with physiologically-based modelling of oral absorption to yield not only estimates of HIA that are adequate for informing decision-making in early drug discovery, but also provides an indication of how HIA is likely to vary with dose and solubility. An alternative to the physiologically-based simulation approach is provided by quantitative structure-pharmacokinetic relationship (QSPR) modelling, as exemplified by the model of Zhao and co-workers (Zhao *et al*., 2001), which utilizes Abraham descriptors as a basis for estimating HIA. However, such methods are not capable of providing information on dose-dependence, and tend to be less robust when applied to compounds that fall outside the descriptor space of the training data for the model.

In the approach presented here, the prediction of HIA is made on the basis of two readily-determined *in vitro* ADME properties: unidirectional Caco-2 P_{app}, which is commonly used as an indicator of intestinal permeability, and aqueous solubility. This approach avoids the use of bidirectional Caco-2 data, which are more costly to obtain and pKa, which is not generally available in the early stages of drug discovery.

By utilizing data that are routinely available early in the drug discovery process in conjunction with physiological modelling, we have developed a rapid and cost-effective means of not only estimating HIA, but also determining whether there is any likely dose-dependence or solubility limitation, thereby maximising the information that can be derived from two relatively simple *in vitro* assays. Furthermore, by providing information on dose-dependence, this combined approach provides a means of linking *in vitro* ADME data with a consideration of efficacy to better inform compound selection.

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