Prediction of Caco-2 pH-Dependent Permeability based on High-Quality in vitro Training Set

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ABSTRACT

The aim of the study was to predict the pH-dependent permeability profiles of drugs in a Caco-2 assay, using an *in combo* procedure based on measured Double-Sink™ PAMPA permeability values and the calculated Abraham solvation descriptors (α, β, π, R, V_x).

INTRODUCTION

Cellular (e.g., Caco-2)[1] and low-cost parallel artificial membrane permeability assays (PAMPA)[2] are used to predict intestinal absorption properties of compounds in drug discovery projects. However, prior to doing these assays, reliable *in silico* prediction of the permeability of test compounds can be very useful. For example, knowledge of the predicted property can help the medicinal chemist to design additional structural features into the molecule to improve the prospects of bioavailability. The predicted effects of intestinal pH could be similarly useful (Fig. 1). Furthermore, the predicted permeability value can improve assay design for poorly soluble compounds. Unwittingly, investigators sometimes report "zero" permeability for compounds that are likely to be highly permeable, because of limitations due to extremely low solubility. In such instances, a "contrary" *in silico* permeability alerts the chemist to critically examine the assay design.

The first successful quantitative *in silico* procedure to establish a correlation between PAMPA and Caco-2 was based on fluoroquinolones from three congeneric series.[3] Several areas of inconsistencies in data treatment in cellular studies were critically assessed, to further improve the effectiveness of the *in silico* methods.[4,5]

Out of these studies, a computer program, *p*CEL-X (*p*ION), was developed, which can be used to model the various factors controlling transport of drug-like molecules across artificial or cell-based membranes in permeability measurements, such as: (a) passive (uncharged), (b) passive (ionic), (c) paracellular (Renkin sieving function; electrostatic potential model), (d) aqueous boundary layer (ABL), and (e) carrier-mediated model for acids.

MATERIALS AND METHODS

The method applied to correlating the Caco-2 permeability coefficients of 18 weak-base drugs to Double-Sink™ PAMPA permeability data augmented by calculated Abraham descriptors, producing $r^2 = 0.98$,[5] (Fig. 2) was extended to a larger training set consisting of 53 high-quality Caco-2 measurements (taken at two or more different pH) collected from the literature, and pre-processed to extract the passive permeability components (Fig. 3). Acids, bases, neutrals, and zwitterions were included in the extended training set of compounds.

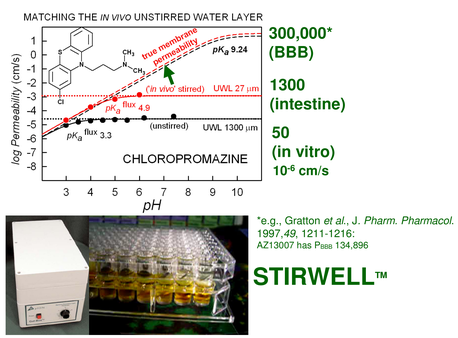

Fig. 1. The permeability of ionizable compounds depends on pH. But this dependency can be masked by poor stirring efficiency in the *in vitro* assay (both Caco-2 and PAMPA). An environmental chamber has been designed to overcome such limitations, to produce *in vivo* mimetic stirring conditions.

The Abraham[6] linear free energy (LFER) descriptor calculation and the computational model testing used the Algorithm Builder V1.8 and ADME Boxes V4.1 computer programs[7] from Pharma Algorithms, Toronto (www.ap-algorithms.com). The prediction of the pH-dependent Caco-2 permeability profile took into account, transcellular passive (neutral and charged species), paracellular, aqueous boundary layer (ABL), and filter-limited permeability.

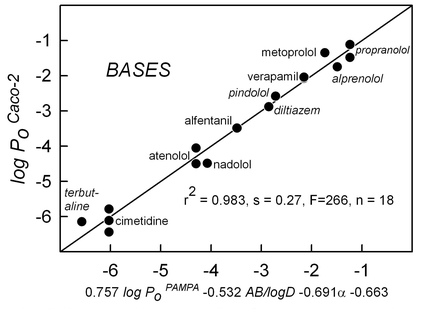

Fig. 2. Prediction of the *intrinsic* Caco-2 permeability, using *intrinsic* Double-Sink PAMPA permeability, calculated apparent partition coefficients (AB/logD, pH 7.4), and Abraham's H-bond acidity, α, for a series of bases.[5]

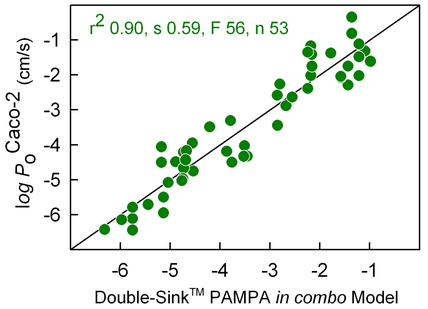

Fig. 3. Prediction of the *intrinsic* Caco-2 permeability, using *intrinsic* PAMPA permeability Abraham's H-bond descriptors for a 53-compound series of acids, bases, neutrals, and zwitterions (this study).

RESULTS AND DISCUSSION

The extended training set of 53 Caco-2 measurements was correlated in the *in combo* model to the extent of $r^2 = 0.90$, as shown in Figure 3.

For example, as a test of the procedure, the pH-dependent Caco-2 apparent permeability (in units of 10^{-6} cm/s) at 500 RPM stirring rate for itraconazole: $P_{app} = 100$ at pH 5.0 (factors controlling transport: 68% passive, 17% ABL, 15% filter), $P_{app} = 192$ at pH 6.8 (39% passive, 33% ABL, 28% filter).

Below is a case study of indomethacin, illustrating the steps in calculating the pH-dependent Caco-2 permeability profile.

CASE STUDY: Caco-2 PERMEABILITY OF INDOMETHACIN (donor pH 5 – 8, receiver pH 7.4; 450 RPM)

The simulation program can calculate Caco-2 permeability from just a 2-D structure of the molecule, provided as a mol file. The simulation first predicts the pK_a, the octanol-water partition coefficient, log P_{oct}, and the intrinsic (neutral species) Double-Sink PAMPA permeability, log P_o^{DS}, along with the five Abraham LFER descriptors. However, the simulation can be improved if *measured* pK_a, log P_{oct}, $logP_o^{DS}$ values are provided as input (Fig. 4).

In a separate dialog box (Fig. 5), the aqueous boundary layer permeability is calculated, which is a function of diffusivity, stirring speed, and temperature.

Finally, the paracellular and filter permeability values are calculated (Fig. 6) from the size of the molecule, the junction pore size, the junction potential gradient, and the porosity of the supporting filter.

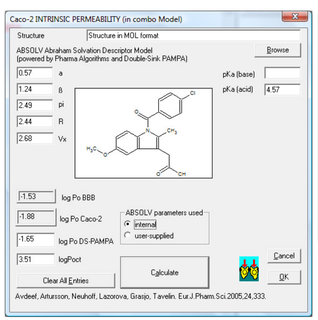

Fig. 4 Prediction of the intrinsic permeability for PAMPA., Caco-2, and blood-brain barrier (BBB) models.

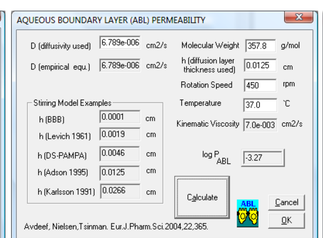

Fig. 5 Prediction of the aqueous boundary layer permeability (P_{ABL}).

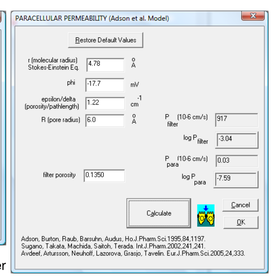

Fig. 6 Prediction of the paracellular and filter permeability (P_{ABL}).

The simulated model (Figs. 4-6) was compared to measured data reported b Neuhoff et al.[8] The solid curves in Figure 7 are based on the simulated model. Th green points from the Caco-2 data are close to the simulated curves. Refinement c the simulated parameters further improves the fit (Figs. 8-9). The factors controllin transport are shown in the pie charts (Fig. 10).

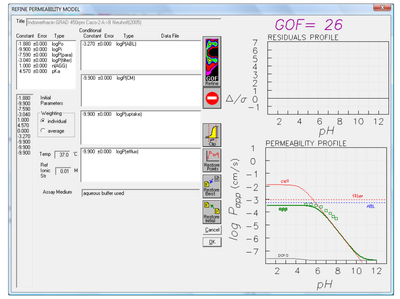

Fig. 7 Simulation (solid curves) tested against actual pH-dependent Caco-2 permeability reported for indomethacin by Neuhoff et al. [8] (green points).

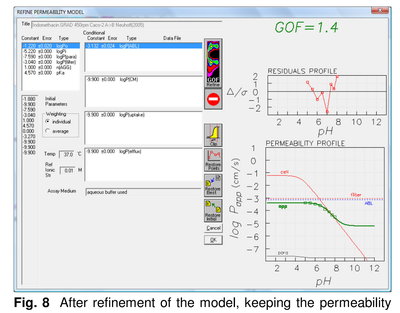

Fig. 8 After refinement of the model, keeping the permeability of the anionic indomethacin species four log units below the refined intrinsic permeability.

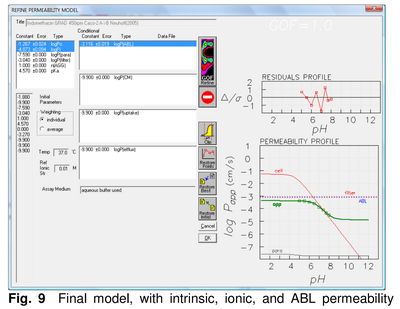

Fig. 9 Final model, with intrinsic, ionic, and ABL permeability coefficients refined, and the paracellular permeability included as a fixed contribution.

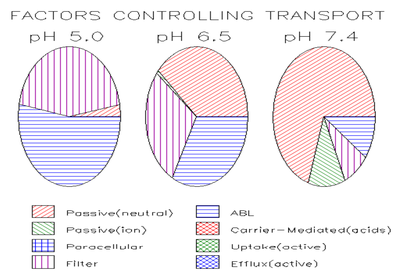

Fig. 10 Factors controlling tranaport. At pH 7.4, the main contribution comes from passive diffusion (70%), with ionic , filter, and ABL accounting for about 10% each.

CONCLUSION

The ability to predict with confidence how Caco-2 permeability will depend on the physicochemical properties of a drug is important in both planning measurements of practically-insoluble molecules and interpreting the results of difficult measurements. The method we developed appears to be useful in saving cost, by avoiding poorly-designed assay protocols, and in some cases, altogether avoiding the need for the costly measurements.

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