Simulation Modelling of Human Intestinal Absorption Using Caco-2 Permeability and Kinetic Solubility Data for Early Drug Discovery

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ABSTRACT: Measurement of permeation across a monolayer of the human adenocarcinoma cell line, Caco-2, is a popular surrogate for a compound's permeation across the human intestinal epithelium. Taken alone, however, Caco-2 permeability has certain limitations in the prediction of the extent of absorption of an orally-administered compound, because it does not take into account confounding factors such as solubility and dissolution in the gastrointestinal (GI) tract fluids. A simulation model is described that uses Caco-2 permeability measured in the apical to basolateral direction plus kinetic solubility in buffered solution (both measured at pH 7.4) to predict human intestinal absorption. The model features novel treatment of time-varying fluid volume in the GI tract, as a consequence of secretions into, and absorption of fluid from, the upper part of the GI tract. The model has been trained and cross-validated with data for 120 combinations of compound and dose. It has superior predictive power to recently published simulation and quantitative structure property relationship models, and is suitable for high-throughput screening during lead identification and lead optimisation in drug discovery. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:4557–4574, 2008

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INTRODUCTION

One of the major challenges during the lead identification (LI) and lead optimisation (LO) phases of drug discovery is to identify, from amongst the known efficacious compounds, those that are predicted to have appropriate PK properties in man. In the case of a drug to be administered orally this includes having appropriate human intestinal absorption (HIA). At this stage, the focus is usually on intestinal permeability as the major determinant of HIA. A common approach to is to measure, *in vitro*, the permeation of test compounds across a barrier (such as a monolayer of Caco-2 cells) that represents the *in vivo* permeability barrier of the intestinal epithelium, and to use the permeability data to make a prediction of HIA. The prediction of HIA can potentially be improved by

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also taking account of solubility and physiological parameters: absorption of an orally-dosed compound is a multistep process involving dispersion and dissolution of solid forms, dynamic interconversion between dissolved and precipitated states in the lumen of the gastrointestinal (GI) tract and, finally, absorption across the GI epithelium. Depending on the compound any of these can potentially limit the amount absorbed and, amongst other factors, can be influenced by formulation and dose. This potential for dose limitation of absorption to occur requires that HIA be considered in terms of the dose to be administered—HIA has little meaning unless the amount dosed is known.

A means of measuring solubility has been developed[1] that is amenable to high throughput, requires considerably less compound than equilibrium measurement, and is therefore suitable for routine use in LI and LO where compound availability can be limiting. This is a kinetic assay, in which compound stock solution in solvent (e.g. DMSO) is diluted to various degrees and then a small volume further diluted into water or buffer, and the solution assayed for precipation after a set period of time, for example by measuring light absorbance or scattering due to turbidity. The method imitates the procedure that typically occurs in assays for ADME properties, such as clearance and plasma protein binding, in which the assay is initiated by just such a process of dilution. It has been developed to be used as a primary screen to determine whether compounds possess the minimum solubility required so that ADME screening data can be reliably used. Thus, if a Caco-2 permeability assay is to be run with a substrate concentration of 10 μM, then a minimum solubility of 10 μM is required for any compound to be measured using the assay.

The joint effects of chemistry and physiology on intestinal absorption can be investigated by means of simulation modelling. With the availability of Caco-2 and solubility screens as described such modelling can be applied in early drug discovery. A study investigating this approach is described in this paper. A simulation model has been developed that incorporates transit through the GI tract, solubility-dependent dissolution of solid compound and absorption of dissolved compound from the lumen of the GI tract. The model features novel treatment of time-varying fluid volume in the GI tract, as a consequence of secretions into, and absorption of fluid from, the upper part of the GI tract. The method also features the novel use of solubility data determined in the kinetic assay as an input. Caco-2 permeability (P_{Caco}) and kinetic solubility in buffer have been generated for 80 compounds. The model has been trained and cross-validated with data for 120 combinations of compound and dose. The results are compared to those obtained by performing the same model development process with thermodynamic solubility data in water, and with two recently described *in silico* methods for the prediction of HIA.[2,3] Although the expression of transport proteins differs between Caco-2 cells and the human intestinal epithelium,[4–7] the use of Caco-2 permeability to predict intestinal permeability at least takes some account of carrier-mediated processes in a manner that is not possible when using physicohemical properties alone.

MATERIALS AND METHODS

Materials

Talinolol was supplied by AWD pharma (Radebeul, Germany). All other control compounds, test compounds, and chemicals were commercially available.

Cell Culture

Caco-2 cells obtained from the American Type Cell Culture Collection (Manassas, VA), at passage number 27 were used. Cells (passage number 40–60) were seeded on to Multiscreen® Caco-2 plates (Millipore Ltd, Watford, UK) at 1×10^5 cells/cm^2. The cells were cultured for 20 days in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1% (v/v) nonessential amino acids, penicillin (44 IU/mL) and streptomycin (44 IU/mL) at 37°C in an atmosphere of 5% CO_2 with a relative humidity of 95% to allow the formation of confluent monolayers. On day 20 the Caco-2 permeability assay was performed.

Caco-2 Permeability Assay

The Caco-2 permeability assay was adapted from the method described by Burton et al.[8] The specific details of the method used are described in Supplementary Material.