Comparison of Intestinal Permeabilities Determined in Multiple in Vitro and in Situ Models: Relationship to Absorption in Humans

Barbra H. Stewart,1,3 O. Helen Chan,1 Rosalind H. Lu,1 Eric L. Reyner,1 Heidi L. Schmid,1 Harriet W. Hamilton,2 Bruce A. Steinbaugh,2 and Michael D. Taylor2

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In vitro and in situ experimental models that are descriptive of drug absorption in vivo are valuable tools in the discovery of new chemical entities that are bioavailable after oral administration. The specific objective of the study was to compare the intestinal permeabilities obtained in the three absorption models for consistency, and to assess the utility of the models in predicting the fraction of dose absorbed in human studies. The intestinal absorption models that were compared are widely used: the rat in situ single-pass intestinal perfusion system, the rat everted intestinal ring method, and monolayers of human colon adenocarcinoma cell line (Caco-2). The models were compared using small molecular reference compounds, as well as a series of peptidomimetic (PM) analogs. Each model had strong potential for estimating the fraction absorbed. For small organic molecules, excellent correlation was observed when permeabilities from Caco-2 cells and perfusions, or everted rings and perfusions, were compared. Weaker correlation was observed between everted rings and Caco-2 cells. Permeabilities for the set of reference compounds and PMs were positively correlated between any two of the three systems. Variance between correlations for reference compounds and PMs are likely due to structural features and physicochemical properties that are unique to the latter class of compounds. The results support caution in extrapolating correlations based on findings with small organic molecules to the behavior of complex peptidomimetics. Corroboration of permeabilities with two methods of determination is a useful cross-validation of experimental systems, as well as producing a reliable permeability assessment. Caco-2 cell monolayers and rat single-pass intestinal perfusion combine the highest correlation between systems, most defined relationship with fraction absorbed in humans, and experimental logistics in-line with discovery candidates.

KEY WORDS: absorption of peptidomimetics; Caco-2 cell model; intestinal absorption models; fraction absorbed in humans; rat everted intestinal ring model; rat intestinal perfusion model.

INTRODUCTION

In vitro and in situ experimental models that are descriptive of drug absorption in vivo are valuable tools in estimating the biological transport properties of new chemical enti-

1 Department of Pharmacokinetics/Drug Metabolism, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.
2 Department of Chemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.
3 To whom correspondence should be addressed.

ties after oral administration. These experimental models are designed to isolate the barrier or process of interest so as to permit relatively rapid and mechanistic evaluation of drug candidates. In vivo studies permit determination of absolute or relative bioavailability, but are also more complex in terms of plasma assay development and assessing where rate-limiting processes occur. Meaningful feedback to the drug discovery effort generally requires a balance between the higher throughput of in vitro or in situ studies, and the more clinically relevant picture obtained in selected in vivo studies. In vitro or in situ models are of particular utility if they project when 1) absorption is rate-limiting to systemic availability, and 2) permeability is rate-limiting to absorption.

The specific objectives of this study were to compare the intestinal permeabilities obtained in three absorption models for consistency, as well as to use the fraction absorbed data in humans, available in the literature, to assess whether the models were predictive. The models were compared using reference compounds, as well as a series of peptidomimetic (PM) analogs (see Figure 1). The reference compounds were defined as small (<400 Da) organic molecules that were relatively well characterized in terms of transport and metabolism, either in our hands or in the literature. These compounds ranged from those poorly absorbed in humans, such as D-mannitol, to those well absorbed in humans, such as phenytoin, and included compounds that were absorbed by carrier-mediated and passive processes. The PMs originated in a renin inhibitor discovery program (1). Oral delivery of renin inhibitors and other PM-based therapeutic agents (2) has been a formidable obstacle that has resulted in close examination of each aspect of the delivery process. These compounds were selected to represent a wide range of physicochemical properties including lipophilicity, molecular weight and hydrogen-bonding capacity.

Three widely-used intestinal absorption models possessing individual strengths and weaknesses were compared. Each absorption model relied on a different means of determining membrane transport. The rat in situ single-pass intestinal perfusion system based permeability calculations on steady-state disappearance of the compound from the intestinal lumen. The rat everted intestinal ring method used tissue accumulation of compound in vitro to determine drug uptake rate. The human colon adenocarcinoma cell line, Caco-2, was grown on membrane filters, mounted in diffusion chambers, and the rate of compound appearing in the receiver compartment was the basis of the permeability measurement. This work extends that of other researchers (3-7) by examining how well permeabilities from multiple in vitro or in situ systems are correlated for a diverse set of molecules, as well as the relationship of intestinal permeability to fraction absorbed in vivo.

There are other factors that weigh in for each of these models in selecting in vitro/in situ methods for evaluation of discovery candidates. These factors are summarized below for each of the experimental systems used in this report. Considerations include ease and cost of preparation and maintenance, control of experimental conditions, reproducibility, uptake versus transcellular measurements, and ease of drug analysis.
Everted rat intestinal rings offer a relatively quick and inexpensive technique for measuring uptake of drug into tissue. These considerations are important in comparing the methods, as the everted ring technique does not entail the considerable time and expense of start-up and maintenance of the CACO-2 cells, nor does it require the greater number of animals, with associated husbandry costs, needed for statistical power with the perfusion method. There is a statistical advantage in that several conditions can be studied in replicate with controls obtained from the same animal. Rings can be prepared from virtually any segment of intestine, permitting study of axial differences in uptake. This technique has been widely used in mechanistic studies of amino acid and peptide transport and has a wide database in the literature (8). Critics of this method take issue with viability of the tissue over the time course of the experiment, although ring incubations require a small fraction of the time used for everted intestinal sac or excised tissue experiments. The technique is limited to uptake of drug by the tissue, unlike excised tissue preparations where transmucosal flux can be monitored (9). One of the most important considerations in whether to use everted rings is if the compound of interest is available in radiolabelled form; analysis of nonradiolabelled drug in intestinal tissue is relatively labor-intensive, although necessary when one is interested in obtaining metabolic information (10).

Rat single-pass intestinal perfusion is an in situ technique wherein the blood supply, innervation and clearance capabilities of the animal remain intact. Input of drug can be closely controlled in terms of concentration, pH, osmolality, composition, intestinal region and flow rate (11). The absorption model, per se, is physiologically and pharmacologically responsive which may account, in part, for the higher variability observed in some of these experiments. The drug is measured in buffer or perfusate, thus facilitating assay of drug by specific chromatographic means. This technique has been used extensively in establishing a database of permeabilities with correlation to human absorption data, as well as to elucidate absorption mechanism (12,13).

CACO-2 cell monolayers have evolved to a widely used model for intestinal absorption with a burgeoning database in many laboratories. This system offers the convenience of a continuously cultured cell line to model the small intestinal epithelium, despite the origin of CACO-2 from human colon adenocarcinoma. The human origin of the cells is desirable; however, the transformed nature of the cells may result in unpredictable differentiation markers (14). In addition, several reports have associated transepithelial electrical resistance, enzyme expression and some transport properties of the CACO-2 cells more closely with colon than small intestine (6,15). Transport can be measured by either transcellular flux or uptake directly into the cell monolayers, thus isolating apical and basolateral membrane processes. Measurements of drug are made either in buffer or from lysed and precipitated cells, thus minimizing assay difficulty.

MATERIALS AND METHODS

MES buffer was prepared from MES, NaCl and KCl (Sigma Chemical Co., St. Louis MO). Gabapentin (1-(amino-methyl)cyclohexanecatic acid, CI-945, lot XH370889) and [14C]-gabapentin (lot NO286-2701, radiochemical purity >98%) were used for studies. Reference compounds and PEG-4000 were also purchased from Sigma Chemical Co. Radiochemical reference compounds were purchased from either New England Nuclear/Dupont (Boston MA: [3H]-D-mannitol, [3H]-acetaminophen, [3H]-L-phenylalanine, [3H]-hydrocortisone, [14C]-phenytoin, [3H]- and [14C]-PEG-4000) or Amersham (Arlington Heights IL; [3H]-prednisolone). Tritated PMs were prepared using a common tritiated cyclohexyl precursor (Figure 1; 16,17). Dulbecco’s Modified Eagle’s Medium (DMEM) was purchased from Sigma (St. Louis MO). Solune and Hionic-Fluor were purchased from Packard Instrument Co. (Meriden CT). Neutralizing solution (PGM) was prepared from a saturated solution of sodium pyruvate in methanol, glacial acetic acid and methanol in the ratio of 4:3:1 by volume.

Single-Pass Intestinal Perfusion in Rats. Male white Wistar rats (250 to 470 g) were fasted overnight with water ad libitum. Rats were anesthetized with a cocktail of ketamine, xylazine and pentobarbital; animals were sacrificed at the end of the experiment before recovering from the anesthesia. Laparatomy was performed after onset of deep anesthesia and the upper jejunum was identified. Proximal and distal ends of a 3 to 15 cm segment of intestine were cannulated with glass tubing. Perfusion solutions of drug and PEG-4000 were prepared with radiolabelled tracer plus cold material if necessary to achieve desired concentrations in iso-osmotic buffer (10 mM MES, 135 mM NaCl, 5 mM KCl; pH 6.5). Specific activity of the tritium label was 1 to 3 × 105 dpm/mL, while the carbon-14 label was 1 to 3 × 104 dpm/mL. Drug solution containing the nonabsorbable water marker, PEG-4000 (0.01% w/v), was perfused into the proximal intestine at a constant concentration, C_in, and constant flow rate, Q (0.125, 0.20 or 0.25 mL/min), using a Harvard Apparatus Infusion Pump (Model 4200, South Natick MA). Exiting perfusate was collected from the distal cannula at concentration, C_out, over 10-min intervals for 90 min. Drug and