Intraluminal Drug and Formulation Behavior and Integration in *In Vitro* Permeability Estimation: A Case Study with Amprenavir

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Received 8 August 2005; revised 25 October 2005; accepted 26 October 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20553

ABSTRACT: The purpose of this study was to assess the effect of biorelevant apical conditions on intestinal permeability estimation in the Caco-2 system for amprenavir, a poorly water-soluble substrate of the efflux carrier P-glycoprotein (P-gp). To establish biorelevant conditions, human intestinal fluids (HIF) were aspirated from the duodenum and jejunum in fasted subjects, before and during 4 h after the intake of a standard formulation of amprenavir (Agenerase®). The HIF samples were characterized with respect to the concentrations of phospholipids, individual bile salts, amprenavir, and the excipient d-α-tocopheryl polyethyleneglycol 1000 succinate (TPGS); subsequently, the use of these samples in the Caco-2 system during permeability estimation for amprenavir was compared to standard conditions (amprenavir 10 μM dissolved in HBSS-based transport medium). The presence of the solubilizing excipient TPGS resulted in high intraluminal amprenavir concentrations (mM-range) and affected the permeability in a concentration-dependent way. At the observed intraluminal TPGS concentrations (mM-range), TPGS appeared to completely inhibit the interaction between amprenavir and P-gp, suggesting that the effect of P-gp on transepithelial transport of amprenavir in a clinical setting is probably negligible. This study illustrates the importance of the evaluation of intraluminal conditions after drug intake and their integration in permeability estimation *in vitro*. J Pharm Sci 95:372–383, 2006

Keywords: Caco-2 cells; intestinal absorption; amprenavir; TPGS; P-glycoprotein; biorelevance; human intestinal fluid

INTRODUCTION

Cell-based model systems of the human intestinal mucosa are commonly used to estimate permeability for drugs or drug candidates and to gain insight into the mechanisms of transepithelial transport. As these model systems are expected to be as representative as possible for the *in vivo* situation, their physiological relevance is an important issue. However, in the human Caco-2 cell culture system, drug transport is typically studied using plain aqueous buffers, enriched with glucose, at a fixed pH of 7.4. It may be questioned whether this buffered salt solution as standard apical medium is representative for the *in vivo* luminal environment in the gastrointestinal tract after oral drug intake. *In vivo* conditions are more complex and more variable, affected by gastrointestinal secretions, motility and hydro-

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Journal of Pharmaceutical Sciences, Vol. 95, 372–383 (2006)

dynamics, food effects, and formulation factors. Some of these complicating factors may directly influence permeability by interacting with the transcellular or paracellular permeation pathway or by modulating the activity of intestinal transport carriers or enzymes. Furthermore, the *in vivo* luminal environment may affect drug release, dissolution, and solubility, especially of poorly water-soluble compounds[1,2]; together with gastrointestinal transit, hydrodynamics, and absorption, these factors determine aqueous intraluminal drug concentrations after oral drug intake. Selecting relevant drug concentrations for *in vitro* permeability estimation may be critical for the biorelevance of the assay, especially when concentration-dependent processes are involved in transepithelial transport.[3]

To improve the physiological relevance of the apical medium used in the Caco-2 system, some options have already been explored.[4] First, as the reported luminal pH of the upper small intestine under fasted conditions is generally lower than the standard apical pH in the Caco-2 system (7.4), adjustment of the apical pH to 6.5 (creating a pH gradient over the monolayer) has become a common approach.[5] This influences ionization and thus partitioning of drugs as well as the activity of pH-dependent transport carriers. However, because it has recently been reported that the use of a differential pH may create a false efflux component for weakly basic drugs,[6] careful interpretation is necessary. Another option to increase the biorelevance of Caco-2 transport studies is the inclusion of bile salts in the apical compartment. Apart from being a physiologically relevant approach to increase the solubility of poorly water-soluble compounds,[7] including bile salts may affect membrane fluidization or the activity of transport carriers. An inhibitory effect of taurocholate has indeed been reported on the efflux carriers P-glycoprotein (P-gp)[8] and MRP3.[9] Also fasted state simulated intestinal fluid (FaSSIF), containing sodium taurocholate and phosphatidylcholine (lecithin) at pH 6.5, has been investigated as potential biorelevant medium for permeability estimation.[8,10] Finally, inclusion of solubilizing excipients such as polyethyleneglycol, polysorbate, Cremophor, and d-α-tocopheryl polyethyleneglycol 1000 succinate (TPGS) in the apical medium may be relevant for the *in vivo* condition in specific cases, regarding their use in several oral formulations of poorly water-soluble drugs.[11] In addition to their effect on solubility—and thus on luminal drug concentrations—they may exert a variety of concentration-dependent effects on membrane permeability for drugs through micellar encapsulation and modulation of membrane fluidization and/or the activity of transport carriers.[12–14]

Although several of these studies suggest that the use of more physiologically relevant apical media affect drug transport across Caco-2 monolayers, certain aspects remain to be further investigated. The impact of biorelevant conditions on the predictive value of *in vitro* permeability estimation is sometimes unclear, as well as the precise mechanism behind the observed effects. Also with respect to the precise *in vivo* luminal conditions—being the reference for biorelevant media—the existing literature remains incomplete. For instance, the knowledge about intraluminal concentrations of drugs and excipients after intake of an oral dosage form is very limited. Recently, we have evaluated a method to sample human intestinal fluid (HIF) from duodenum and jejunum after administration of an oral formulation.[15] Characterization of these fluids may increase insight into drug and formulation behavior in the gastrointestinal tract and may assist in the selection of relevant media, drug and excipient concentrations during *in vitro* permeability estimation.

In the present paper, we characterized intraluminal conditions after intake of an oral dosage form of the HIV protease inhibitor, amprenavir. Subsequently, the impact of these physiologically relevant conditions on *in vitro* permeability estimation for amprenavir was investigated. As amprenavir has a low intrinsic aqueous solubility (0.08 mM at pH 7 and 37°C), it is typically administered as soft gelatin capsules containing the solubilizing excipient TPGS in order to achieve sufficient oral bioavailability.[16] With respect to permeability, both *in vitro* (Caco-2)[16] and *in vivo* (*Mdr1a/1b* knockout mice)[17] studies have revealed an interaction between amprenavir and the apical efflux carrier, P-gp. In this study, luminal samples were aspirated from two sites along the gastrointestinal tract (i.e., duodenum and jejunum) before and after oral administration of the soft gelatin formulation of amprenavir; these HIF samples were characterized with respect to pH, individual bile salts and the concentrations of lecithin, TPGS, and amprenavir. Next, the effect of apical media representative for these intraluminal conditions on *in vitro* permeability estimation for amprenavir was assessed in the Caco-2 system.