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A combined cell based approach to identify P-glycoprotein substrates and inhibitors in a single assay

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Abstract

The objective of this project was to develop a cell based in vitro experimental procedure that can differentiate P-glycoprotein (P-gp) substrates from inhibitors in a single assay. Caco-2 cells grown to confluency on 12-well Transwell® were used for this study. The efflux permeability (B to A) of P-gp specific probe (viz., digoxin) in the presence of test compounds (e.g. substrates, inhibitors and non-substrates of P-gp) was monitored, and the influx permeability (A to B) of test compounds was evaluated after complete P-gp blockade. Radiolabelled digoxin was added on the basolateral side with buffer on the apical side. The digoxin concentration appearing on the apical side represents digoxin efflux permeability during the control phase (0–1 h period). After 1 h, a test compound (10 uM) was added on the apical side. The reduced efflux permeability of digoxin suggests that the added test compound is an inhibitor. The influx permeability of test compound is also determined during the 1–2 h study period by measuring the concentration of the test compound in the basolateral side. At the end of 2 h, a potent P-gp inhibitor (GF120918) was added. The increased influx permeability of test compound during the 2–3 h incubation period indicates that the added test compound is a substrate. Samples were taken from both sides at the end of 1–3 h and the concentrations of the test compounds and digoxin were quantitated. Digoxin efflux permeability remained unchanged when incubated with P-gp substrates (e.g., etoposide, rhodamine123, taxol). However, when a P-gp inhibitor was added to the apical side, the digoxin efflux (B to A permeability) was significantly reduced (ketoconazole = 51% reduction) as expected. The influx permeability of substrates increased significantly (rhodamine123 = 70%, taxol = 220%, digoxin = 290%) after the P-gp inhibitor (GF120918) was introduced, whereas the influx permeability of P-gp inhibitor and non-substrates was not affected by GF120918. Thus, this combined assay provides an efficient cell based in vitro screening tool to simultaneously distinguish compounds that are P-gp substrates from P-gp inhibitors.

Keywords: Permeability; Efflux; Caco-2 cell; High-throughput; P-glycoprotein; Inhibitor; Substrate

Abbreviations: A to B, apical to basolateral; ADME, absorption, distribution, metabolism, elimination; B to A, basolateral to apical; BCRP, breast cancer resistance protein; HBSS, Hank's balanced salt solution; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; IAM, immobilized artificial membrane; LSC, liquid scintillation counter; LRP, lung cancer resistance protein; MRP, multi-drug resistance protein; NCE, new chemical entity; PAMPA, parallel artificial membrane permeability assay; P-gp, P-glycoprotein; P_c, permeability co-efficient; TEER, trans-epithelial electrical resistance

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1. Introduction

Despite tremendous innovations in the drug delivery methods in the last few decades, the oral route still remains as the most preferred route of administration for most New Chemical Entities (NCE). The oral route is preferred by virtue of its convenience, low cost and high patient compliance compared to alternate routes. However, compounds intended for oral administration must have appropriate physico-chemical properties (i.e. adequate intestinal permeability without much efflux component) in order to achieve therapeutic concentrations. With the explosive growth in the field of genomics and combinatorial chemistry in the last few years, synthesizing a large number of potential drug candidates is no longer a bottleneck in the drug discovery process. Instead, the task of screening compounds simultaneously for biological activity and biopharmaceutical properties (e.g., solubility, permeability/absorption, stability, etc.) has become the major challenge. This has provided a great impetus within the pharmaceutical industry to implement appropriate screening models that are high capacity, cost-effective and highly predictive of in vivo permeability and absorption (Balimane et al., 2000; Hidalgo, 2001; Hillgren et al., 1995; Kerns, 2001; Lipinski et al., 2001).

Amongst the biopharmaceutical properties that need to be considered in early discovery, permeability assessment and P-glycoprotein (P-gp) interaction studies are critical in determining the fate of a compound. Transport of drug substances across the intestinal membrane is a complex and dynamic process. It includes the passage of compounds across various functional pathways in parallel. Passive transport occurs through the cell membrane of enterocytes (transcellular) or via the tight junctions between the enterocytes (paracellular). Various influx and efflux mechanisms (via carriers and transporters) are also functional. Drug efflux transporters such as P-gp may be a major determinant of absorption, distribution and elimination of a wide variety of drugs (Benet et al., 2003; Lin, 2003; Lin and Yamazaki, 2003; Malingre et al., 2001; Matheny et al., 2001; Polli et al., 1999; Sababi et al., 2001). P-gp is known to limit the oral absorption of drugs such as docetaxel and taxol; it can limit entry of drugs such as HIV protease inhibitors into brain and CNS; and it can actively facilitate excretion of drugs via biliary and urinary routes. Since P-gp interactions of a drug can play such a pivotal role in dictating their pharmacokinetics, increasing efforts are being made in early discovery and development to identify compounds that can potentially interact with P-gp.

Drug discovery scientists in evaluating permeability/absorption of drug candidates during the drug candidate selection process currently employ various techniques. The most pervasive pre-clinical methodologies currently used throughout the industry are: in vitro methods (ussing chamber, membrane vesicles, cell based Caco-2 cells, MDCK, etc., artificial lipid based PAMPA or IAM), in situ methods (single pass perfusion), in vivo methods (whole animal studies), and even in silico methods. These models provide information on permeability characteristics of test compounds but provide no information on their potential to interact with P-gp. An entirely different set of models is needed to gauge the potential of a test compound to interact with P-gp either as a substrate or as an inhibitor. There are literature reports of various in vitro and in vivo models that are used for assessing P-gp interactions with test compounds (Adachi et al., 2001; Balimane et al., 2004; Perloff et al., 2003; Polli et al., 2001; Yamazaki et al., 2001). In vitro assays such as ATPase assay, rhoadmine-123 uptake assay, calcein AM uptake assay, cell based bi-directional assay, radio-ligand binding assay along with in vivo models such as transgenic (knockout mice) and mutant animal models are most commonly used. However, all these models have a major drawback that they provide information regarding only one aspect of P-gp interaction: whether the test compound is a substrate or inhibitor of P-gp. In other words, two separate assays have to be performed, one for substrate and other for inhibitor identification. Keeping in mind the utility of these models as screening tools (i.e. early identification of the drug's potential to interact with P-gp) running these assays twice can lead to a significant loss of time and effort which can be highly counter-productive. In addition to the inefficiency, performing two assays lead to more compound requirement which can be a severe bottleneck in early discovery stage. Therefore, an ideal P-gp screening model would be the one that is efficient, cost-effective, predictive and provide two critical answers in one assay: (1) whether or not the compound is a P-gp substrate and (2) whether or not the compound is a P-gp inhibitor.