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In Vitro P-glycoprotein Assays to Predict the in Vivo Interactions of P-glycoprotein with Drugs in the Central Nervous System

Bo Feng, Jessica B. Mills, Ralph E. Davidson, Rouchelle J. Mireles, John S. Janiszewski, Matthew D. Troutman, and Sonia M. de Morais

Pharmacokinetics, Dynamics, and Metabolism Department, Pfizer Global Research and Development, Groton, Connecticut

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ABSTRACT:

Thirty-one structurally diverse marketed central nervous system (CNS)-active drugs, one active metabolite, and seven non-CNS-active compounds were tested in three P-glycoprotein (P-gp) in vitro assays: transwell assays using MDCK, human MDR1-MDCK, and mouse Mdr1a-MDCK cells, ATPase, and calcein AM inhibition. Additionally, the permeability for these compounds was measured in two in vitro models: parallel artificial membrane permeation assay and apical-to-basolateral apparent permeability in MDCK. The exposure of the same set of compounds in brain and plasma was measured in P-gp knockout (KO) and wild-type (WT) mice after subcutaneous administration. One drug and its metabolite, risperidone and 9-hydroxyrisperidone, of the 32 CNS compounds, and 6 of the 7 non-CNS drugs were determined to have positive efflux using ratio of ratios in MDR1-MDCK versus MDCK transwell assays. Data from transwell studies correlated well with the brain-to-plasma area under the curve ratios between P-gp KO and WT mice for the 32 CNS compounds. In addition, 3300 Pfizer compounds were tested in MDR1-MDCK and Mdr1a-MDCK transwell assays, with a good correlation (R^2 = 0.92) between the efflux ratios in human MDR1-MDCK and mouse Mdr1a-MDCK cells. Permeability data showed that the majority of the 32 CNS compounds have moderate to high passive permeability. This work has demonstrated that in vitro transporter assays help in understanding the role of P-gp-mediated efflux activity in determining the disposition of CNS drugs in vivo, and the transwell assay is a valuable in vitro assay to evaluate human P-gp interaction with compounds for assessing brain penetration of new chemical entities to treat CNS disorders.

Human P-glycoprotein (P-gp, MDR1) is known to be a determinant of drug absorption, distribution, and excretion of a number of clinically important drugs (Ambudkar et al., 1999; Fromm, 2000). P-gp is widely expressed in major organs, and, more specifically, P-gp is highly expressed in the capillaries of the blood brain barrier (BBB) and poses a barrier to brain penetration of its substrates (Schinkel, 1999). Given that P-gp efflux liability can be a major hurdle for CNS therapeutic drugs to cross the BBB and reach the target, the interactions of CNS compounds with P-gp may lead to the lack of CNS activity as a result of the decreased brain penetration. Thus, the prediction and understanding of the relevance of P-gp-mediated efflux transport have become important activities in the discovery and development of CNS drugs. In attempts to predict the effects of P-gp in vivo, a variety of in vitro P-gp assays have been developed to classify compounds as P-gp substrates. For instance, transwell-based assays using polarized cell lines such as the Madin-Darby canine kidney (MDCK) cell line. The MDCK cell line can be stably transfected with human MDR1 or mouse Mdr1a (MDR1-MDCK or Mdr1a-MDCK, respectively). Comparison of the efflux ratios between MDR1-MDCK and MDCK transwell assays can provide a measure of the specific human P-gp-mediated efflux activity (Polli et al., 2001). Another widely used P-gp in vitro assay is the P-gp ATPase assay for assessing drugs with P-gp interactions as substrates (Sarkadi et al., 1992; Ramachandra et al., 1998). The principal reagent of the ATPase assay is a membrane preparation from insect cells highly expressing human P-gp, and functional human P-gp will transport P-gp substrates across the membrane resulting in the release of inorganic phosphates (Scarborough, 1995; Litman et al., 1997). Finally, the in vitro calcein AM P-gp inhibition assay can be used to detect compounds that inhibit P-gp-mediated efflux of the fluorescent P-gp substrate, calcein. This assay can differentiate P-gp inhibitors from noninhibitors by measuring the fluorescence of calcein (Liminga et al., 1994; Tiberghien and Loor, 1996). However, it is important to note that the calcein AM assay is a P-gp inhibition assay and not a substrate assay and that P-gp substrates do not necessarily correlate with P-gp inhibitors.

In addition to P-gp in vitro assays, animal models have been used to assess the impact of P-gp on substrate pharmacokinetics in humans. Comparison of the brain/plasma (B/P) exposure ratios in *Mdr1a/Mdr1b* KO mice versus WT mice has become a standard experimental approach to determine whether P-gp-mediated efflux poses a potential threat to the activity of CNS agents in vivo (Schinkel et al., 1996).

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ABBREVIATIONS: P-gp, P-glycoprotein; MDR1/Mdr1a, multidrug resistance protein; BBB, blood-brain barrier; CNS, central nervous system; MDCK, Madin-Darby canine kidney; MDR1-MDCK, MDR1-transfected MDCK; Mdr1a-MDCK, Mdr1a-transfected MDCK; B/P, brain/plasma ratio; KO, knockout; WT, wild type; PAMPA, parallel artificial membrane permeation assay; DMSO, dimethylsulfoxide; A, apical; B, basolateral; MES, 4-morpholineethanesulfonic acid; MRM, multiple reaction monitoring; PBS, phosphate-buffered saline; ER, efflux ratio ($P_{appB>A}/P_{appA>B}$); RR, ratio of ratios; AUC, area under the curve.