Development of a New Permeability Assay Using Low-Efflux MDCKII Cells

LI DI,[1] CARRIE WHITNEY-PICKETT,[1] JOHN P. UMLAND,[1] HUI ZHANG,[1] XUN ZHANG,[2] DAVID F. GEBHARD,[3] YURONG LAI,[1] JAMES J. FEDERICO III,[1] RALPH E. DAVIDSON,[1] RUSS SMITH,[4] ERIC L. REYNER,[5] CAROLINE LEE,[5] BO FENG,[1] CHARLES ROTTER,[1] MANTHENA V. VARMA,[1] SARAH KEMPSHALL,[6] KATHERINE FENNER,[6] AYMAN F. EL-KATTAN,[1] THEODORE E. LISTON,[1] MATTHEW D. TROUTMAN[1]

[1]Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., Groton, Connecticut

[2]Compound Safety Prediction Group, WWMC, Pfizer Inc., Groton, Connecticut

[3]Primary Pharmacology Group, Pfizer Inc., Groton, Connecticut

[4]Current address: Vertex Pharmaceuticals, Cambridge, Massachusetts

[5]Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., La Jolla, California

[6]Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., Sandwich, UK

Received 4 March 2011; revised 13 April 2011; accepted 3 June 2011

Published online 15 July 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22674

ABSTRACT: Permeability is an important property of drug candidates. The Madin–Darby canine kidney cell line (MDCK) permeability assay is widely used and the primary concern of using MDCK cells is the presence of endogenous transporters of nonhuman origin. The canine P-glycoprotein (Pgp) can interfere with permeability and transporter studies, leading to less reliable data. A new cell line, MDCKII-LE (low efflux), has been developed by selecting a subpopulation of low-efflux cells from MDCKII-WT using an iterative fluorescence-activated cell sorting technique with calcein-AM as a Pgp and efflux substrate. MDCKII-LE cells are a subpopulation of MDCKII cells with over 200-fold lower canine Pgp mRNA level and fivefold lower protein level than MDCKII-WT. MDCKII-LE cells showed less functional efflux activity than MDCKII-WT based on efflux ratios. Notably, MDCKII–MDR1 showed about 1.5-fold decreased expression of endogenous canine Pgp, suggesting that using the net flux ratio might not completely cancel out the background endogenous transporter activities. MDCKII-LE cells offer clear advantages over the MDCKII-WT by providing less efflux transporter background signals and minimizing interference from canine Pgp. The MDCKII-LE apparent permeability values well differentiates compounds from high to medium/low human intestinal absorption and can be used for Biopharmaceutical Classification System. The MDCKII-LE permeability assay (4-in-1 cassette dosing) is high throughput with good precision, reproducibility, robustness, and cost-effective. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:4974–4985, 2011

Keywords: absorption; P-glycoprotein; ADME; drug transport; *in vitro* models; bioavailability; LC–MS; Biopharmaceutics Classification System (BCS); cell culture; MDCK cells; transporters

Abbreviations used: ADME, absorption, distribution, metabolism, and excretion; Bcrp, canine breast cancer resistant protein; BCS, Biopharmaceutical Classification System; Caco-2, human colonic adenocarcinoma cells; DDI, drug–drug interaction; FACS, fluorescence-activated cell sorting; HIA, human intestinal absorption; HTS, high-throughput screening; IVIVC, *in vitro–in vivo* correlation; MDCK, Madin–Darby canine kidney cell line; MDCKII-LE, MDCKII-Low Efflux cells are a subpopulation of MDCKII cells with low expression of endogenous canine Pgp transporter [it is also named RRCK (Ralph Russ Canine Kidney) for Pfizer internal use]; MDR1, human multidrug resistance gene that codes for Pgp; mRNA, messenger RNA; MRM, multireaction monitoring; Mrp, canine multidrug resistant proteins; PAMPA, parallel artificial membrane permeability assay; P_{app}, apparent permeability; PCR, polymerase chain reaction; Pgp, P-glycoprotein; SAR, structural activity relationship.

Correspondence to: Li Di (Telephone: +860-715-6172; Fax: +860-441-6402; E-mail: li.di@pfizer.com)

Journal of Pharmaceutical Sciences, Vol. 100, 4974–4985 (2011)
© 2011 Wiley-Liss, Inc. and the American Pharmacists Association

INTRODUCTION

A successful drug discovery and development program involves selecting the right disease target, identifying the best chemical matter, and executing a flawless development program. High-throughput absorption, distribution, metabolism, excretion and toxicity (ADME/TOX) screening is an integrated part of the drug discovery process to enhance the success of drug candidates in the clinic.[1–4]

The ADME/TOX assay panels are most typically comprised solubility, permeability, lipophilicity, metabolic stability, drug transporters, drug–drug interaction (DDI) mediated by cytochrome P450 enzyme inhibition, and hERG (human ether-a-go-go gene) channel blockage.[1,2,5] Among the various ADME properties, permeability has been widely recognized as an important property of drug candidates. It plays a critical role in oral absorption, blood–brain barrier permeation, cell-membrane penetration for intracellular targets, skin absorption of transdermal products, and others.[3,4] Permeability also has significant impact on metabolism and transporter effects, drug disposition and pharmacokinetics–pharmacodynamics relationships.[6–8] Permeability, along with solubility, are key determinants for Biopharmaceutical Classification System (BCS),[9,10] which is widely used in the pharmaceutical industry and by regulatory agencies, notably US Food and Drug Administration (FDA), for benchmarking *in vitro–in vivo* correlation (IVIVC) and biowaiver studies.[10–12]

Various permeability assays have been developed and successfully applied in drug discovery and development, including *in silico*, *in vitro*, *in situ*, and *in vivo* approaches.[3,13–16] Among the most common *in vitro* permeability assays are human colonic adenocarcinoma cell (Caco-2),[17,18] Madin–Darby canine kidney cell line (MDCK),[19,20] and parallel artificial membrane permeability assay (PAMPA).[21] The selection and implementation of a particular permeability assay for a research organization is dependent on the scientific rationale as well as personal preference, experience, and cultural and historical prospective of the organization. The MDCK permeability assay has been widely used in many companies because of its many favorable characteristics. These characteristics include spontaneous differentiation into a polarized epithelial tissue in a short culture time (∼3–5 days), which minimizes maintenance and reduces the chance for contamination, low expression of metabolizing enzymes and transporters, tight paracellular junctions, high-monolayer integrity, and morphologic homogeneity, which contributes to good interlaboratory reproducibility. Additionally, the MDCK cells are easily transfected to express specific transporters and produce robust assay results in a 96-well high-throughput screening (HTS) format.[22]

Because of the nonhuman origin of MDCK cells, the endogenous canine transporters[23] can have different activity and selectivity against various substrates and interfere with passive permeability measurement in the MDCK-WT assay and can alter efflux ratios (ERs) in transfected cell assays such as MDCK–MDR1 (human multidrug resistance 1 gene). The apparent permeability (P_{app}) values can appear to be artificially lower when measuring passive permeability and the ERs can be falsely higher due to the contributions of endogenous canine efflux transport. The most important endogenous transporter in MDCK cells is the canine P-glycoprotein (Pgp) because of its relatively high abundance, wide substrate specificity, and significance *in vivo*. Compounds can be substrates for canine Pgp, but are not necessarily substrates for human Pgp and vice versa, leading to false positives in efflux transport assays.[24] For these reasons, FDA Draft Guidance for Industry on Drug Interaction Studies[25] (study design, data analysis, and implications for dosing and labeling) recommends using the net flux ratio (MDCK–MDR1 ER divided by the MDCK-WT ER) to cancel out the endogenous canine transporter effect. However, this method suffers from false negatives, particularly when a given compound such as erythromycin shows a high-ER in the wild-type cells; indeed, the ER and effect that Pgp may play in transport is not entirely linearly related to expression and this consequently leads the net flux ratio approach to produce erroneous results regarding substrate classification under certain circumstances.

The area of measuring *in vitro* permeability is well understood and a mature one; however, there are aspects that could be improved. More specifically, although MDCK cells are desirable over Caco-2 and PAMPA for enabling robust high throughput or time-critical recurring profiling, reproducibility, ease of use of data by nonexperts, and potential for more closely measuring passive cellular (epithelial) permeability, undesirable aspects in these MDCK cells remain, notably the presence of functionally active canine Pgp. The goal of the studies described in this paper was to identify a subpopulation of MDCK cells that expressed low levels of endogenous canine Pgp to minimize the interference of canine Pgp on passive permeability measurement. Additionally, studies were performed to confirm or refute the use of this cell model to provide high-quality data to predict oral absorption of drug candidates and for BCS classification. The successful isolation of MDCK cells with very-low-canine Pgp expression and function raises the potential of their use as a parental cell line to transfect with various human transporters for transporter and DDI studies without the added interferences from endogenous canine Pgp. This represents an elegant improvement to the current state in one study, rather than two, that can be used to more accurately

Download English Version:

https://daneshyari.com/en/article/2486196

Download Persian Version:

https://daneshyari.com/article/2486196

Daneshyari.com