Kinetic Analysis of Human and Canine P-Glycoprotein-Mediated Drug Transport in MDR1–MDCK Cell Model: Approaches to Reduce False-Negative Substrate Classification

JIBIN LI, YING WANG, ISMAEL J. HIDALGO

Absorption Systems LP, Exton, Pennsylvania

Received 21 December 2012; revised 5 March 2013; accepted 5 March 2013

Published online 5 April 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23523

ABSTRACT: Madin–Darby canine kidney (MDCK) cells transfected with the multidrug resistance 1 (*MDR1*) gene, MDR1–MDCK, are widely used as an *in vitro* model to classify compounds as human P-glycoprotein (hPgp) substrates or nonsubstrates. Because MDCK cells express endogenous canine Pgp (cPgp), which is prone to downregulation after transfection with hPgp, this situation could lead to false-negative classification of hPgp substrates. The aim of this study was to investigate factors that influence hPgp substrate classification in MDR1–MDCK model and to seek ways to reduce false classification. Three-compartment models were used to derive flux equations describing the drug transport processes; factors influencing hPgp substrate classification were evaluated by simulations. Pgp functionality was assessed by determining the bidirectional permeability of a series of test compounds. Expressions of hPgp and cPgp were measured by quantitative polymerase chain reaction (qPCR). Kinetic model analysis revealed that the current net flux ratio calculation for hPgp substrate classification is influenced by endogenous cPgp expression as well as hPgp–cPgp expression ratio; the effect was more pronounced in low hPgp–cPgp region and diminished in high ratio region. On the basis of kinetic considerations, this study provides a rational experimental approach and appropriate mathematical corrections to minimize the potential occurrence of false-negative classification of new molecular entities. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3436–3446, 2013

Keywords: ABC transporters; transporters; P-glycoprotein; MDCK cells; mathematical model; membrane transporter; *in vitro* model; drug transport; drug interactions

INTRODUCTION

P-glycoprotein (Pgp) is a member of the ATP-binding cassette family of transporters. Encoded by the multidrug resistance gene 1, *mdr1* (in humans, *MDR1*), Pgp is found in the intestine, liver, kidney, and brain, and has been shown to transport compounds exhibiting a wide range of chemical diversity.[1,2] As a result of its broad substrate affinity and ubiquitous distribution, Pgp can be involved in numerous drug–drug interactions (DDIs).[3,4] Because the identification of drugs that are substrates and/or inhibitors of Pgp, and the prediction of their potential involvement in DDIs in humans, would help reduce the drug-related toxicity, several *in vitro* assays are used for classifying compounds as Pgp substrates or inhibitors.[4–6] Bidirectional transport assays across polarized cell monolayers are often the preferred method to study drug interactions with Pgp because they measure Pgp-mediated drug efflux in a direct manner. MDR1-transfected Madin–Darby canine kidney (MDR1–MDCK) cells have the added advantage that they develop polarized monolayers in a few days as opposed to Caco-2 cells that may take up to 3 weeks.[7,8] In spite of the canine kidney origin of MDCK cells, the high expression of human Pgp (hPgp) and short culturing time make MDR1–MDCK cells an attractive model to study drug interactions with

Abbreviations used: A→B, apical to basolateral; B→A, basolateral to apical; ER, efflux ratio; NFR, net flux ratio; HEPES, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]; MDCK, Madin–Darby canine kidney; MDR1, human multidrug resistance 1; Pgp, P-glycoprotein; hPgp, human Pgp; cPgp, canine Pgp; PS, permeability–surface area product; P_{app}, apparent permeability coefficient; qPCR, quantitative polymerase chain reaction; wt, wild type.

Correspondence to: Ismael J. Hidalgo (Telephone: +610-280-7300; Fax: +610-280-3779; E-mail: ihidalgo@absorption.com)

Journal of Pharmaceutical Sciences, Vol. 102, 3436–3446 (2013)

hPgp and to classify drugs as hPgp substrates and/or inhibitors. Because MDR1–MDCK cells express both hPgp and canine Pgp (cPgp), in the absence of evidence to the contrary, the possibility that assay results obtained with these models are influenced by endogenous cPgp cannot be ignored. In fact, two recent studies reported a decrease in endogenous cPgp expression in MDCK cells transfected with the human *MDR1* gene.[9,10] According to the US FDA Draft Guidance for Industry on Drug–Drug Interaction Studies, for a test compound to be classified as an hPgp substrate, its net flux ratio (NFR), efflux ratio (ER) in MDR1–MDCK cells divided by the ER in parental (nontransfected) MDCK cells (ER′), must be at least 2.[11] If the endogenous cPgp expression in MDR1–MDCK cells is attenuated compared with that in MDCK cells, the cPgp-mediated transport is lower in MDR1–MDCK than in MDCK cells and the ensuing NFR would be underestimated, which, in turn, could lead to false-negative classification of hPgp substrates. In addition, other factors, such as culture conditions, that can alter endogenous cPgp expression, and thus influence assay results, may lead to disparities among different laboratories in the classification of hPgp substrates.

However, to date, there are no definitive reports evaluating the contribution of the cPgp (endogenous) and hPgp (transfected) in the assays performed to classify hPgp substrates using MDR1–MDCK cell lines. The objectives of this study were (1) to determine the factors that influence the NFR calculation and (2) to seek rational approaches to minimize false classification of hPgp substrates. Three-compartment (apical, cellular, and basolateral) models representing MDR1–MDCK and wild type (wt) MDCK cell monolayers were used to derive flux equations describing drug transport in these cells. The relationship between the NFR and endogenous cPgp expression, hPgp- and cPgp-mediated drug transport, and passive diffusion was solved mathematically. Simulations were used to illustrate the factors that influence hPgp substrate classification using the NFR calculations, and thus, shed light on approaches that can help minimize the effects of these factors. To verify findings from the kinetic analysis, the expressions of hPgp and cPgp in MDR1–MDCK and wt MDCK cells were quantified using quantitative polymerase chain reaction (qPCR), and the bidirectional transports of a set of test compounds were determined in these cells. By calibrating the MDR1–MDCK cell system, taking into account the extent of downregulation of endogenous cPgp, it is possible to eliminate, or at least minimize, the occurrence of false-negative classification of Pgp substrates in this model.

THEORETICAL CONSIDERATIONS

Transport of Pgp substrates across MDCK and MDR1–MDCK cell monolayers can be described using three-compartment kinetic models (Fig. 1). To

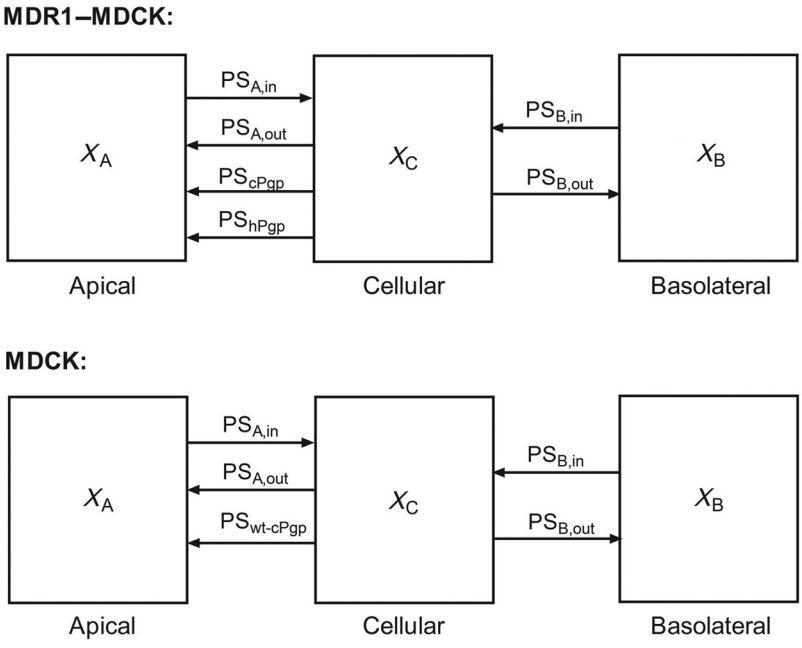

Figure 1. Three-compartment models describing drug transports in MDR1–MDCK (top) and MDCK (bottom) cells. PS_A (assuming $PS_A = PS_{A,in} = PS_{A,out}$) and PS_B (assuming $PS_B = PS_{B,in} = PS_{B,out}$) represent the permeability-surface area product of passive diffusion across the apical and basolateral membrane, respectively; PS_{hPgp} represents the PS resulted from recombinant hPgp efflux in MDR1–MDCK cells; PS_{cPgp} and PS_{wtcPgp} represent the PS resulted from the endogenous canine efflux in MDR1–MDCK and MDCK cells, respectively.