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Usefulness of A Model-Based Approach for Estimating *In Vitro* P-Glycoprotein Inhibition Potency in a Transcellular Transport Assay

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ABSTRACT

In vitro half-maximal inhibitory concentration (IC_{50}) is a key parameter for accurately predicting the potential risk for P-glycoprotein (P-gp)–mediated drug–drug interactions. We aimed to compare the IC_{50} values estimated by different approaches and determine the usefulness of model-based approaches. Transcellular transport of digoxin across Caco-2 monolayer was investigated using various concentrations of P-gp inhibitors, quinidine, verapamil, and zosuquidar. To calculate IC_{50} values, 3 traditional parameters were used: apical-to-basal (AtoB) and basal-to-apical (BtoA) clearance (CL) with inhibitors ($CL_{AtoB,i}$ and $CL_{BtoA,i}$) and the difference between the efflux ratios (ERs) with P-gp inhibitors (ER_i) and those under complete P-gp inhibition [$ER_{(-P\text{-}gp)}$]. Furthermore, a new model-based approach was applied that uses the difference between the reciprocals of CL_{AtoB} with P-gp inhibitors ($1/CL_{AtoB,i}$) and those under complete P-gp inhibition [$1/CL_{AtoB(-P\text{-}gp)}$] as parameters. IC_{50} values obtained from 2 model-based approaches [$ER_i - ER_{(-P\text{-}gp)}$ and $1/CL_{AtoB,i} - 1/CL_{AtoB(-P\text{-}gp)}$] were comparable, whereas 2.6- to 6.6-fold larger IC_{50} values were estimated from empirical approaches ($CL_{AtoB,i}$ and $CL_{BtoA,i}$). The reason for such difference in IC_{50} values is that indicators for model-based approaches, but not empirical approaches, directly reflect the P-gp function. Our new approach [$1/CL_{AtoB,i} - 1/CL_{AtoB(-P\text{-}gp)}$] based on only AtoB transcellular transport could substitute for current estimation methods using ER.

Introduction

P-glycoprotein (P-gp; encoded by the *MDR1* or *ABCB1* gene in humans) is expressed in various tissues and involved in the efflux of substrates from the cells. Numerous studies have clearly indicated that P-gp expressed in the gastrointestinal tract is important for suppressing the intestinal absorption of various compounds, including clinically used drugs.[1,2] The number of P-gp–mediated drug–drug interaction (DDI) cases in humans has been gradually increasing, thereby making it necessary to predict potential DDI signals during drug development. Under such circumstances, the Food and Drug Administration, European Medicines Agency, and Ministry of Health, Labour and Welfare recently published a draft or final guidance/guideline in which P-gp is nominated as an important transporter to be investigated when evaluating DDI risks.[3-5] In these regulations, if the $[I]_1/IC_{50}$ value (where $[I]_1$ = total inhibitor concentration in plasma) is more than 0.1, or the $[I]_2/IC_{50}$ value ($[I]_2$ = dose/250 mL) is more than 10, clinical DDI studies are recommended to further investigate the impact of potential P-gp inhibition on the pharmacokinetics of P-gp substrate drugs in humans. Thus, IC_{50} is a critical parameter for judging the potential risk of P-gp–mediated DDIs.

Among the *in vitro* experimental tools available for investigating the P-gp function, monolayers of P-gp–expressing polarized cells cultivated on culture inserts are widely used. However, considerable interlaboratory variations in IC_{50} values for the same inhibitors estimated from transcellular transport experiments[6] have recently become a great concern when considering the accurate determination of potential DDI risks. Several reasons, such as differences in

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cell lines, probe substrates, concentration used, transport buffer pH, and IC_{50} calculation methods, are proposed as contributing to these variations.[6-9] In terms of IC_{50} estimation, various methods are mixed up in the previous reports with the use of unidirectional permeability coefficient (or clearance [CL]) [$Papp_{BtoA}$ (or CL_{BtoA}) and $Papp_{AtoB}$ (or CL_{AtoB})], a concentration-dependent net secretory flux [differences of apparent permeability coefficient (Papp) between BtoA and AtoB; $Papp_{BtoA}$ to $Papp_{AtoB}$], and efflux ratio (ER) [ratio of $Papp_{BtoA}$ to $Papp_{AtoB}$; ER ($Papp_{BtoA}/Papp_{AtoB}$)]. The difference between an ER and a unidirectional transport for IC_{50} calculation is that an ER is proportional to the permeability–surface area product for P-gp–mediated efflux ($PS_{P\text{-}gp}$) across the cell monolayers,[10] whereas a unidirectional transport is not proportional to $PS_{P\text{-}gp}$. Thus, it is understandable that different IC_{50} values for a certain inhibitor drug are estimated using these different methods, and it is desirable that IC_{50} values are rationally estimated with a theoretical background.

In the present study, the impact of different estimation approaches (3 traditional approaches using CL_{BtoA}, CL_{AtoB}, and $ER_i - ER_{(-P\text{-}gp)}$ and our novel approach [$1/CL_{AtoB,i} - 1/CL_{AtoB(-P\text{-}gp)}$]) on the IC_{50} values for 3 prototypical P-gp inhibitors was demonstrated using digoxin as an *in vitro* probe substrate which is the most commonly used for P-gp.

Materials and methods

Chemicals

[^{3}H]Digoxin was purchased from PerkinElmer (Waltham, MA). Zosuquidar was synthesized at Boehringer Ingelheim Pharma GmbH & Co. KG (Biberach, Germany). Digoxin, quinidine, verapamil, and bovine serum albumin were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM) with 3.7 g/L sodium bicarbonate was obtained from Bio-Chrom AG (Berlin, Germany). Fetal bovine serum, nonessential amino acids, penicillin–streptomycin mixture, and L-glutamine were purchased from Invitrogen (Carlsbad, CA). Collagen R solution was obtained from Serva (Heidelberg, Germany). All other chemicals were of the highest reagent grade available from commercial sources.

Cell Line and Cell Culture

Caco-2 cells were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Caco-2 cells were maintained at 37°C, 8% CO_2, and 90% relative humidity in 75-cm^2 cell culture flasks supplemented with DMEM culture medium containing heat-inactivated fetal bovine serum, nonessential amino acids, L-glutamine, and penicillin–streptomycin mixture. Transwell® filter inserts with a polycarbonate membrane (#3396; Corning, NY) were coated with collagen R solution. At 1-2 days after coating, Caco-2 cells were seeded at 0.625×10^5 cells/filter. Cells were cultured to confluence (15-16 days) at 37°C, 8% CO_2, and 90% relative humidity in DMEM culture medium. The culture medium was replaced every 2-3 days. All other details are described in Kishimoto et al.[9]

Transcellular Transport Assay

Each transport assay was composed of triplicate incubations using different filter inserts for both AtoB and BtoA directions. Experiments were performed at 37°C and involved using a robotic workstation (Freedom EVOware, Tecan, Switzerland). Monolayers with transepithelial electrical resistance values of >500 $\Omega \cdot cm^2$ were used. Cells were equilibrated in the transport buffer for 30 min. For AtoB or BtoA transport assay, the apical or basal side of the cell monolayer (donor compartment), respectively, was filled with donor solution (transport buffer containing the radiolabeled compound). The opposite chamber (receiver compartment) was filled with receiver solution (transport buffer supplemented with 2% bovine serum albumin). The inhibitor was added to both donor and receiver solutions. After preincubation for 30 min, the transport assay was initiated. Samples were taken at 0, 30, 60, and 90 min from the receiver compartment to measure the amounts of compound that had passed through the monolayer. Samples were then transferred into a LUMA plate (PerkinElmer), dried, and radioactivity was determined using a 10 min measurement in a TopCount NXT (PerkinElmer) microscintillation counter. All other details are described in Kishimoto et al.[9]

Transport CL and Efflux Ratio

The AtoB or BtoA CL (CL_{AtoB} or CL_{BtoA}, respectively) is described by the following Equation 1:

$$CL_{AtoB} \text{ or } CL_{BtoA} = Papp_{AtoB} \text{ or } Papp_{BtoA} \times A = \frac{dQ}{dt} \times \frac{1}{C_{t0}} \quad (1)$$

where Q is the amount of compound transported over time t of the experiment, and therefore, dQ/dt is the amount of drug transported within a given period (nmol/sec). C_{t0} is the initial concentration of the test compound added to the donor compartment (μM). A is the membrane surface area of transwell insert used in Caco-2 transcellular transport assay (1 cm^2). CL_{AtoB} and CL_{BtoA} can be determined from multiplying A by the apparent permeability coefficient ($Papp_{AtoB}$ or $Papp_{BtoA}$).

The ER is described by the following Equation 2:

$$ER = \frac{CL_{BtoA}}{CL_{AtoB}} \quad (2)$$

Empirical Approaches

When CL_{AtoB} and CL_{BtoA} are used as indicators of P-gp–mediated transport function to calculate IC_{50} values, the following Equations 3 and 4 were applied:

$$CL_{AtoB,\,i} = CL_{AtoB,\,I=\infty} - \left(CL_{AtoB,\,I=\infty} - CL_{AtoB,\,I=0}\right) \times \frac{1}{1 + \frac{I}{IC_{50}}} \quad (3)$$

$$CL_{BtoA,\,i} = CL_{BtoA,\,I=\infty} + \left(CL_{BtoA,\,I=0} - CL_{BtoA,\,I=\infty}\right) \times \frac{1}{1 + \frac{I}{IC_{50}}} \quad (4)$$

where $CL_{AtoB,i}$ and $CL_{BtoA,i}$ represent the observed CL_{AtoB} and CL_{BtoA} in the presence of inhibitor, $CL_{AtoB,I=0}$ and $CL_{BtoA,I=0}$ represent the CL_{AtoB} and CL_{BtoA} in the absence of inhibitor, $CL_{AtoB,I=\infty}$ and $CL_{BtoA,I=\infty}$ represent the CL_{AtoB} and CL_{BtoA} under the complete inhibition of P-gp function, and I and IC_{50} represent the initial inhibitor concentration in the donor compartment and the inhibitor concentration that elicits 50% inhibition, respectively.