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Cellular Pharmacokinetic Model-Based Analysis of Genistein, Glyceollin, and MK-571 Effects on 5 (and 6)-Carboxy-2′,7′-Dichloroflourescein Disposition in Caco-2 Cells

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ABSTRACT

Pharmacokinetic modeling was used to describe 5 (and 6)-carboxy-2′,7′-dichloroflourescein (CDF) disposition in Caco-2 cells following CDF or CDFDA (CDF diacetate) dosing. CDF transcellular flux was modeled by simple passive diffusion. CDFDA dosing models were based on simultaneous fitting of CDF levels in apical, basolateral, and intracellular compartments. Predicted CDF efflux was 50% higher across the apical versus the basolateral membrane. This difference was similar following apical and basolateral CDFDA dosing, despite intracellular levels being 3-fold higher following basolateral dosing, thus supporting nonsaturable CDF efflux kinetics. A 3-compartment catenary model with intracellular CDFDA hydrolysis described CDF disposition. This model predicted that apical CDF efflux was not altered in the presence of MK-571, and that basolateral membrane clearance was enhanced to account for reduced intracellular CDF in the presence of this multidrug resistance-associated protein (MRP) inhibitor. Similar effects were predicted for glyceollin, while genistein exposure had no predicted effects on CDF efflux. These modulator effects are discussed in the context of model predicted intracellular CDF concentrations relative to reports of CDF affinity (measured by K_m) for MRP2 and MRP3. This model-based analysis confirms the complexity of efflux kinetics and suggests that other transporters may have contributed to CDF efflux.

Introduction

Polyphenols derived from plants are a subset of phytochemicals that continue with high interest to be investigated for their health-promoting effects.[1] Curcumin, genistein, and resveratrol are 3 polyphenols that have received the most attention regarding their therapeutic potential in cancer, diabetes, and in cardiovascular and neurodegenerative diseases.[2-5] Evaluation of their pharmacokinetic properties has revealed they have poor oral bioavailability due to efficient enterocyte extraction via glucuronide and sulfate conjugate formation and transporter-mediated efflux of both untransformed compound and conjugate metabolites to the intestinal lumen.[6] The important role that intestinal transporters play in determining the systemic availability of polyphenols has led to numerous studies to identify the transporters involved, and, through *in vitro* and *in vivo* work, has led to a fuller appreciation of the role transporters play not only in the absorption of these chemicals but also in their downstream pharmacological effects and systemic elimination.[7] Given that they are substrates for several transporters also important in the disposition of many pharmaceuticals, their potential to alter transporter-mediated drug disposition, especially given that they are available without a prescription, is high and merits increased attention on both research and regulatory fronts.[8]

Glyceollin is a relatively new polyphenol to emerge with interest in its health-promoting and disease-sparing effects.[9] Like genistein, it is produced from soy beans, but is referred to as a phytoalexin, being produced only under stressed conditions of plant growth. Preliminary studies conducted in rats demonstrated the production of glucuronide and sulfate conjugates following oral administration.[10] A sulfate conjugate was also identified on exposure to Caco-2

Abbreviations used: ABCC2, ATP binding cassette sub-family C, member 2; ABCC3, ATP binding cassette sub-family C, member 3; AB, apical-to-basolateral; BA, basolateral-to-apical; BCRP, breast cancer resistance protein; CDF, 5 (and 6)-Carboxy-2′,7′-Dichloroflourescein; CDFDA, CDF diacetate; Cl, clearance; MRP, multidrug resistance-associated protein.

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cells[11]; thus, like other polyphenols, the possibility that conjugate metabolites would be exported to the lumen via breast cancer resistance protein (BCRP) and multidrug resistance-associated protein (MRP)2, much like genistein[12] and resveratrol,[13] is reasonable. Likewise, the potential that these conjugates could alter the efflux of another compound at the apical membrane was also evaluated by co-exposing Caco-2 cells to glyceollin and genistein[14]; these studies demonstrated reduced genistein conjugate efflux in the presence of glyceollin. Companion studies conducted with substrates of BCRP (BODIPY-prazosin)[15] and MRP2 (CDF)[16] demonstrated that glyceollin reduced the transport of these substrates, suggesting that this polyphenol may inhibit BCRP and MRP2 function.

Increasingly, pharmacokinetic models describing drug and metabolite concentrations in whole-cell *in vitro* models are recognized as useful tools to advance our understanding of the role that carrier-mediated transport plays in drug disposition and in the assessment of drug safety and efficacy.[17-19] Their usefulness in predicting alteration of intracellular concentrations of victim drugs and their metabolites due to perpetrator drug alteration of transporter-mediated disposition has been demonstrated.[20,21] A recent application of modeling to decipher the various transporters involved in resveratrol disposition in HeLa cells demonstrated the usefulness of this approach to quantify MRP4-mediated contributions to overall glucuronide excretion.[22] A surprising finding in the aforementioned studies with glyceollin in Caco-2 cells was a decrease in intracellular levels of CDF following exposure to MK-571, a well-recognized inhibitor of MRP2 and MRP3.[23] Both transporters have been demonstrated to be responsible for CDF efflux, respectively, across the canalicular and serosal membranes of hepatocytes.[16,24,25] Based on mRNA analysis, MRP2 and MRP3 were expressed in Caco-2 cells[26,27]; however, a recent proteomic analysis of Caco-2 cells grown on filters for 2-4 weeks, demonstrated high expression of MRP2, but no detectable expression of MRP3.[28] Observation of increased retention of compounds in the presence of MK-571 has been taken to implicate that they are MRP substrates; such has been the case for Caco-2.[22,27] Thus, the purpose of the present study was to apply a retrospective modeling analysis of CDF transport data that had been obtained from several experiments conducted in Caco-2 cells grown on filters to determine if such an approach could provide insight regarding the effects of MK-571 (i.e., normalization of basolateral-to-apical (BA) dosing CDF flux ratios from approximately 3-0.8, but in the presence of reduced intracellular CDF for both dosing conditions). Population models that described the time course of CDF amount transported over 2 h in the receiver compartment, and terminal donor compartment and intracellular CDF amounts, following apical and basolateral CDFDA dosing were developed to quantify CDF efflux across both apical and basolateral membranes.

Materials and Methods

Conduct of In Vitro *Transport Evaluations*

Details of the materials used in the conduct of the CDF and CDFDA transport evaluations in the absence versus presence of MK-571, glyceollin, and genistein have been described.[14,29] Likewise, conduct of the transport studies has also been described in these same 2 publications and will be summarized in abbreviated fashion herein.

Caco-2 cells were seeded onto collagen-coated 0.4-μm-PTFE Transwell-Col® permeable supports (12 mm/12-well/1.12 cm^2) with 1.2×10^5 cells/mL and cultured for 18-24 days before use in a transport evaluation. On the day of a transport experiment, both sides of each Transwell insert were washed twice with 37°C pH 7.4 transport buffer and incubated on both sides for 30 min in a CO_2 incubator with either transport buffer containing only the cosolvent system used to prepare MK-571, glyceollin, or genistein stocks (final concentration 0.1% dimethyl sulfoxide, 0.1% ethanol) for controls, or transport buffer with 10, 30, or 100 μM of 1 of the 3 agents. Following this preincubation, a 50-μM stock solution of CDF or CDFDA prepared in 1% dimethyl sulfoxide, 1% ethanol in transport buffer was added to achieve a final concentration of 5-μM CDF or CDFDA on either the apical side or basolateral side, and the 12-well plate placed on an orbital shaker (150 rpm) maintained at 37°C. The volume on the apical side was 0.5 mL, whereas on the basolateral side, it was 1.5 mL. During an experiment, these volumes were held constant by taking a 0.4 mL sample for analysis of CDF concentration from the receiver side (basolateral compartment in an apical dosing experiment, and apical compartment in a basolateral dosing experiment) with immediate equivalent volume replacement. Receiver-side samples were taken every 30 min for 120 min (4 samples). At the end of an incubation, a donor-side sample was taken. Also at 120 min, cells on monolayers were washed 3 times with ice-cold transport buffer (0.5 mL on apical side, and 1.5 mL on basolateral side), and then exposed on the apical side to 0.2 mL of methanol for 15 min at room temperature. Subsequently, the methanol was collected, centrifuged for 5 min at $2000 \times g$, and saved for analysis of CDF concentration in Caco-2 cells at 120 min.

CDF concentrations in receiver, initial and final donor, and in cells were determined by comparison to CDF standards prepared by serial dilution in transport buffer over the range 7.8-1000 nM (separate standards were prepared for controls and a given agent [MK-571, glyceollin or genistein] at the final concentration of that agent used in a transport experiment). CDF measurements were made with a 96-well plate reader set to 485 nm/530 nm for excitation and emission wavelengths, respectively. Rate of CDF appearance (dCDF/dt, pmol/min) into the receiver compartment was determined by linear regression analysis of the amount of CDF transported versus time between 30 and 120 min, thus zero-order, steady-state kinetics with no back diffusion of CDF were applied. In experiments in which CDF was dosed, CDF clearance across the cells was determined using the following equation, where Cl refers to clearance (mL/min), dCDF/dt is the rate of CDF transport across the monolayer (pmol/min), and C_{donor} (pmoles/mL) is the average of the initial and final donor CDF concentrations.

$$Cl = \frac{dCDF}{dt} \Big/ C_{donor} \quad (1)$$

Modeling and Simulation of CDF Transport

There were 5 occasions in which CDF was dosed, and 16 occasions in which CDFDA was dosed. The data used to model CDF transport following CDF dosing have not been previously published. The data used to model CDF transport following CDFDA dosing have been published previously.[14,29] Each occasion consisted of 12 filters: 6 filters for apical dosing: 3 without (control) versus 3 with a defined concentration of an agent, and 6 filters for basolateral dosing: 3 without versus 3 with an agent. The range of Caco-2 passages used in these experiments was from 32 to 58.

Mass transport kinetic analyses were conducted using Phoenix® with NLME™ 7.0 (Pharsight®, Certara, L.P., Princeton, NJ). A population approach was used to model CDF transport following either CDF or CDFDA dosing experiments in the apical-to-basolateral (AB) and BA directions. Population parameter estimates (fixed effects) and between-occasion and between-filter variability within an occasion (random effects) were estimated using the method of quasi-random parametric expectation maximization (QRPEM),