Pharmacokinetic Interplay of Phase II Metabolism and Transport: A Theoretical Study

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ABSTRACT: Understanding of the interdependence of cytochrome P450 enzymes and P-glycoprotein in disposition of drugs (also termed "transport–metabolism interplay") has been significantly advanced in recent years. However, whether such "interplay" exists between phase II metabolic enzymes and efflux transporters remains largely unknown. The objective of this article is to explore the role of efflux transporters (acting on the phase II metabolites) in disposition of the parent drug in Caco-2 cells, liver, and intestine via simulations utilizing a catenary model (for Caco-2 system) and physiologically based pharmacokinetic (PBPK) models (for the liver and intestine). In all three models, "transport–metabolism interplay" (i.e., inhibition of metabolite efflux decreases the metabolism) can be observed only when futile recycling (or deconjugation) occurred. Futile recycling appeared to bridge the two processes (i.e., metabolite formation and excretion) and enable the interplay thereof. Without futile recycling, metabolite formation was independent on its downstream process excretion, thus impact of metabolite excretion on its formation was impossible. Moreover, in liver PBPK model with futile recycling, impact of biliary metabolite excretion on the exposure of parent drug [(systemic (reservoir) area under the concentration–time curve (AUC_{R1})] was limited; a complete inhibition of efflux resulted in AUC_{R1} increases of less than 1-fold only. In intestine PBPK model with futile recycling, even though a complete inhibition of efflux could result in large elevations (e.g., 3.5–6.0-fold) in AUC_{R1}, an incomplete inhibition of efflux (e.g., with a residual activity of $\geq$20% metabolic clearance) saw negligible increases ($<$0.9-fold) in AUC_{R1}. In conclusion, this study presented mechanistic observations of pharmacokinetic interplay between phase II enzymes and efflux transporters. Those studying such "interplay" are encouraged to adequately consider potential consequences of inhibition of efflux transporters in humans. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:381–393, 2012

Keywords: phase II metabolism; interplay; efflux transporters; Caco-2 cells; PBPK; UGTs; glucuronidation; CYP enzymes

INTRODUCTION

Drug elimination is a highly complex process that is governed by multiple individual and interacting components (e.g., metabolism, influx, and efflux). Among the intricate relationships within and between metabolic and transport pathways, the interdependence of transport and metabolism (also called "transport–metabolism interplay") has received considerable attention, particularly with cytochrome P450 enzymes (CYPs) and P-glycoprotein (P-gp) as the players.[1–6] The underlying mechanism of transporter–enzyme interplay is becoming clear after extensive studies and discussion.[6–8] It is now generally agreed that kinetic "interplay" of CYPs and P-gp [i.e., a reciprocal relationship between their respective clearances (CLs) or a seesaw phenomenon] is resulted from their competition for the same drug substrate within the cells.[6]

Abbreviations used: PBPK, physiologically based pharmacokinetic modeling; CYPs, cytochrome P450 enzymes; P-gp, P-glycoprotein; UGTs, UDP-glucuronosyltransferases; SULTs, sulfotransferases; BCRP, breast cancer resistance protein; MRPs, multidrug resistance proteins; f_{met}, the fraction of metabolized; ER, extraction ratio; CL, clearance; CL_d, diffusional clearance; CL_{met}, conjugation intrinsic clearance; CL'_{met}, deconjugation intrinsic clearance; $CL_{app,met}$, apparent metabolic clearance; $CL_{app,biliary}$, apparent biliary clearance; AUC, area under the concentration–time curve; AUC_R, systemic (reservoir) area under the concentration–time curve; AUC_L, hepatic area under the concentration–time curve; AUC_I, intestinal area under the concentration–time curve; *f*, fraction of unbound; *V*, volume of distribution; *Q*, blood flow.

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Drug elimination via phase II metabolic pathways (or the conjugative metabolism in contrast to CYP-catalyzed phase I metabolism) such as glucuronidation and sulfation involves (at least) two distinct and sequential processes, namely, metabolite (or conjugate) formation and excretion.[9–11] The metabolite formation is mediated by phase II metabolizing enzymes such as UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs), and metabolite excretion by efflux transporters [e.g., breast cancer resistance protein (BCRP) and multidrug resistance proteins (MRPs)]. Instead of sharing the same substrate such as CYP/P-gp, the phase II enzymes and efflux proteins act on different substrates (i.e., the parent drug for the former and the metabolite for the latter). The involvement of efflux transporters in elimination of phase II metabolites is necessitated by the fact that these metabolites usually are too polar to passively diffuse out of the cells.[11–13] Interestingly, most phase II enzymes and efflux transporters coexist in both human liver and intestine (two most important metabolizing organs), although the expression level is organ and isoform specific.[9,14–17]

The current literature provides strong lines of evidence indicating that efflux transporters are responsible for clearance of phase II metabolites such as glucuronide and sulfate, thus facilitating the metabolic pathways. For example, organ perfusion studies show that BCRP and/or MRP2 contribute significantly to the intestinal and/or biliary excretion of glucuronides and/or sulfates of various compounds such as harmol,[18] acetaminophen,[19,20] and dietary flavonoids.[21] The recognition of cooperation between phase II enzymes and efflux transporters in elimination of phase II metabolites leads to the hypothesis that phase II metabolism is dependent on the transporter-mediated clearance of metabolites (or "interplay" of metabolism and efflux).[11–13,22] Most likely, inhibition of efflux transporters would decrease the metabolism or vice versa.[23] However, at present, the evidence of such interplay is rare probably due to experimental difficulties, one of which refers to the lack of specific inhibitors for the phase II enzymes and/or efflux transporters.

The objective of this study therefore is to enhance our understanding of possible interplay of phase II metabolism and efflux transporters in Caco-2 cells and major metabolic organs (i.e., the liver and intestine), and its effects on disposition of the parent drug from a pharmacokinetic standpoint. Toward this end, a catenary model for the Caco-2 system and physiologically based pharmacokinetic (PBPK) models for the liver and intestine were employed because these models have established solid theoretical and experimental bases.[6,24,25] It is noteworthy that interplay might involve changes in the expression of enzymes via regulation of nuclear receptors, which is not the focus of this paper.[1] To contrast the difference between phase II metabolism highlighted here and CYP-catalyzed phase I metabolism, aglycone/conjugate was preferably used in the following paragraphs instead of the general term drug/metabolite.

MATERIALS AND METHODS

The Catenary Model

The Caco-2 system in a catenary model was used to describe transport and metabolic processes within the apical, cellular, and basolateral compartments (Fig. 1).[8,24–26] For simplicity, it was assumed that transport of aglycone (D) across the cell membrane was mainly driven by passive diffusion, which is true for most UGT/SULT substrates.[11,27] Diffusional clearances CL_{d1} and CL_{d2} denote passive transport CLs on the apical membrane, whereas CL_{d3} and CL_{d4} denote passive transport CLs on the basolateral membrane. Unless specified, it was assumed that the CLs were equal for both influx and efflux at both apical and basolateral membranes ($CL_{d1} = CL_{d2} = CL_{d3} = CL_{d4} = CL_d$). Conversion of aglycone to its conjugate (M) followed Michaelis–Menten (saturable) kinetics described by V_{max} and K_m, whereas the futile recycling (i.e., deconjugation or a backward conversion) used the deconjugation intrinsic clearance CL'_{met}.[8] The formed conjugate inside the cells was effluxed to the apical and basolateral compartments using the intrinsic CLs CL_{sec} and CL_{ef}, respectively.

The fraction of metabolized (f_{met}) was calculated as described previously,[8] which is a more appropriate index to reflex the extent of metabolism in transport–metabolism interplay than others such as extraction ratio (ER).

$$\text{Fraction of metabolized}\,(f_{met}) = \frac{\sum \text{Conjugates}}{\sum \text{Conjugates} + P_{receiver} + P_{cell} + P_{donor}}$$

where, Σ Conjugates is the summed total amount of conjugate formed. $P_{receiver}$, P_{cell}, and P_{donor} are the amounts of aglycone in the receiver, cellular, and donor compartments, respectively.

PBPK Modeling of the Liver

The liver PBPK model, consisting of four compartments [the reservoir (R, or blood compartment), liver blood (LB), liver tissue (L), and bile compartment (bile)], was well developed and used in this study (Fig. 2).[6,7,25,28] In this model, aglycone (D) was administered to the reservoir as a bolus. The blood flow (Q) rate and protein binding were denoted by Q_L for the liver blood, f_{LB} for the unbound fraction in the blood, and f_L for the unbound fraction in the liver,

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