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Refining Transporter DDI Studies with Endogenous Biomarkers

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BACKGROUND

ATP-binding cassette (ABC) and solute carrier (SLC) membrane transporters play an integral role in the absorption, distribution, metabolism and excretion (ADME) of small molecule drugs. Alterations to transporter function due to interfering drug reactions, genetic polymorphisms, or disease can influence a drug's pharmacokinetic (PK) properties which may lead to reduced efficacy or safety concerns. Therefore, it is important to understand whether investigational products in development are transported by specific drug transporters and, more importantly, whether they are a potential transporter perpetrator.

ROLE OF ENDOGENOUS TRANSPORTERS IN CLINICAL STUDIES

Out of the nearly 450 transporters identified responsible for shuttling proteins, nutrients, hormones, neurotransmitters, antioxidants and signaling molecules, only 8 are clinically relevant for drug-drug interaction (DDI) studies due to their role in drug and metabolite transport (reviewed in [1]). Results from preclinical analysis and/or ADME studies can guide which drug transporters to interrogate during a drug-drug interaction (DDI) study (**Table 1**). Since there are no clinical index drugs for transporters (drugs that predictability affect a metabolic pathway), the choice of substrate or perpetrator drug for a DDI transporter study typically stems from the likelihood of concomitant administration of both drugs [2, 3]. Also, many of the inhibitors in **Table 1** can affect other transporters or are recognized as CYP enzyme inhibitors, and consequentially interpretation of results can be challenging. To this end, endogenous biomarkers of drug transporters have emerged as a powerful tool to enrich PK findings. They can help elucidate the mechanism of complex DDI results when multiple transporters or CYPs may be involved. For example, changes in an endogenous biomarker could differentiate the relative contribution of a given transporter during a DDI assessment. Furthermore, endogenous transporter biomarkers can be examined in early phases of drug development. This includes during multiple ascending dose (MAD) studies to guide future transporter DDI study designs as well as provide a mechanistic understanding of the drug's metabolism. In later stage studies, endogenous biomarkers could serve as a signal to detect transporter inhibition. For example, modest increases in serum creatinine observed during a clinical trial with tucatinib, a tyrosine kinase inhibitor (TKI), were subsequently confirmed to be the result of organic cation transporter (OCT)2 and multidrug and toxin extrusion protein (MATE)1/2K inhibition rather than renal dysfunction [4].

Endogenous transporter biomarkers must display good selectivity, specificity and sensitivity as well as have limited intrinsic and dietary confounding factors that can influence their concentrations. In addition, comprehensive understanding of the biosynthesis of biomarkers and their physiological role as well as their influence on a disease state should be known. Owing to this stringent set of criteria, only a handful of endogenous transporter biomarkers have been identified (**Table 1**).

Endogenous transporter biomarkers are gradually gaining acceptance from drug developers [6] and regulators [1]. The most established biomarkers with extensive clinical experience are coproporhyrins and N1-methylnicotinamide for organic anion transporting polypeptide 1B (OATP1B) and OCT2/MATE1/2-K transporters, respectively. A brief summary of the utility of these biomarkers is described below.

COPROPORPHYRIN (CP)

CP-I and CP-III are heme metabolites taken up by OATP1B1 and B3. Their plasma concentrations increase during treatment with OATP1B inhibiting drugs or in conditions of reduced OATP1B activity such a genetic polymorphism [6]. The *OATP1B1*15* allele is typically associated with Japanese populations and is characterized by decreased OATP1B transporter activity that coincides with elevated CP-I levels (reviewed in [7]). CP-I and CP-III are ideal biomarkers for displaying minimal diurnal fluctuations. They can be used to understand the complex DDI involvement for drugs with potentially multiple elimination pathways. For example, preclinical data suggested that fenebrutinib, a TKI, potentially inhibited BCRP and/or OATP1B1 [8]. Therefore, a DDI study with rosuvastatin (a dual substrate for both BCRP and OATP1B1) was conducted, during which CP-I and CP-III were measured to determine OATP1B1 contribution. As expected, rosuvastatin plasma exposure was increased more than 2.5-fold with fenebrutinib co-administration compared to rosuvastatin treatment alone. However, CP-I and CP-III plasma concentrations were

similar in both conditions, suggesting that fenebrutinib inhibits BCRP activity, yet not OATP1B1 [8]. These results were corroborated with PBPK prediction and informed con-med recommendations in later phase studies [9]. Another example of a complex DDI study where CP-I and CP-III clarified the contribution of transporter inhibition is the case of itraconazole and ipatasertib. Ipatasertib is a putative substrate for CYP3A4 and P-glycoprotein (P-gp) and inhibitor of P-gp and OATP1B1 [10]. Itraconazole is a strong inhibitor of CYP3A and P-gp but also inhibits other transporters to some degree [11]. In a DDI study with ipatasertib, CP-I and CP-III did not change suggesting a low likelihood of OATP1B involvement [10].

N1-METHYLNICOTINAMIDE (NMN)

NMN is derived from tryptophan and vitamin B3 metabolism, and its synthesis and renal excretion are well characterized by diurnal processes. Plasma and urine NMN levels are elevated in the morning (shortly after waking) and gradually decrease over the course of a day to slowly rise again in the overnight hours [13]. NMN is actively transported by OCT2 and MATEs into urine, and unchanged NMN renal clearance accounts for ~35% of total clearance [12]. The utility of NMN as an endogenous substrate for MATE transporters was evaluated in a DDI study where metformin, a reference probe drug, was administered to healthy volunteers with or without increasing doses of pyrimethamine, a MATE inhibitor [14]. In this study, a dose-dependent decrease in renal clearance (CLr) of metformin and NMN was observed over 24 hours with increasing doses of pyrimethamine, and the CLr of metformin positively correlated with that of NMN [14]. Another application of NMN is to rule out the involvement of OCT2 or MATE1/2K. This strategy was used for abrocitinib, an oral Janus kinase 1 (JAK1) inhibitor. In vitro studies suggested that abrocitinib is a potential MATE1/2K inhibitor and therefore, a DDI study was conducted with metformin. Results demonstrated that both plasma exposure and CLr of metformin and NMN were unaffected by abcroitinib administration [15]. Overall, in both of these examples the NMN data complemented and matched the metformin results, supporting the role of this endogenous biomarker to assess MATE transporter activity.

CONCLUSION

Endogenous transporter biomarkers can clarify complex DDI study results by providing insight into drug metabolism and excretion pathways. When applied in a MAD study, changes in these biomarkers could indicate the need for a dedicated DDI study. Drug sponsors are increasingly including endogenous biomarkers in their trials to obtain greater understanding of their drugs' disposition and to optimize their drug development programs.

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Table 1. When to Perform a Clinical DDI study for New Drugs that are Likely Transporter Substrates or Inhibitors

Transporter (Gene)	Clinical Substrates [a]	Clinical Inhibitors [a]	Endogenous Biomarkers [b]
Intestinal absorption, biliary excretion or renal active secretion is likely to be a major cause of the variability in a drug's PK and response			
• P-gp (ABCB1)	• Dabigatran etexilate • Digoxin • Edoxaban • Fexofenadine	• Amiodarone • Clarithromycin • Cobicistat • Cyclosporine • Dronedarone • Erythromycin • Itraconazole • Ketoconazole • Lapatinib • Lopinavir and Ritonavir • Quinidine • Ranolazine • Saquinavir and Ritonavir • Verapamil	None
• BCRP (ABCG2)	• Rosuvastatin • Sulfasalazine	• Curcumin • Cyclosporine A • Darolutamide • Eltrombopag • Febuxostat • Fostamatinib • Folapitant (PO only) • Teriflunomide	None
Hepatic/biliary elimination is a significant clearance pathway requiring active uptake of drug into liver			
• OATP1B1 (SLCO1B1) • OATP1B3 (SLCO1B3)	• Atorvastatin • Bosentan • Docetaxel • Elagolix • Fexofenadine • Glecaprevir • Glyburide • Grazoprevir • Letermovir • Paclitaxel • Pitavastatin • Pravastatin • Repaglinide • Rosuvastatin • Simvastatin	• Atazanavir and Ritonavir • Clarithromycin • Cyclosporine • Gemfibrozil • Lopinavir and Ritonavir • Rifampin (single dose)	• Bile acids • Bilirubin (conjugated / unconjuated) • **CP-I** • **CP-III** • HDA • TDA
Drug undergoes significant active renal secretion or there are concerns over renal toxicity			
• OAT1 (SLC22A6)	• Adefovir • Baricitinib • Bumetanide • Cefaclor • Ceftizoxime • Ciprofloxacin • Famotidine • Furosemide • Methotrexate • Oseltamivir • Carboxylate • Benzylpenicillin • Tenofovir	• Probenecid • Teriflunomide	• HVA • Pyridoxic Acid • Taurine
• OAT3 (SLC22A8)			• 6βHC • GCDCA-S • Pyridoxic Acid
• OCT2 (SLC22A2)	• Metformin	• Cimetidine • Dolutegravir • Isavuconazole • Pyrimethamine • Ranolazine • Trilaciclib Vandetanib	• Creatinine • **NMN** • Thiamine • Tryptophan
• MATE1 (SLC47A1) • MATE2-K (SLC47A2)			• Creatinine • Dopamine • **NMN** • Thiamine

Adapted from FDA Guidance for Industry [2]; a. Per FDA Table 5-1 and Table 5-2 [3]; b. Adapted from Li et al. [5]. Prototypical substrates or inhibitors are underlined and key biomarkers of interest are in bold. ***6βHC****, 6β-hydroxycrotisol;* ***ABC****, ATP-binding cassette;* ***BCRP****, breast cancer resistance protein;* ***CP****, coproporphyrin;* ***GCDCA-S****, glycochenodeoxycholate-3-sulfate;* ***NMN****, N1-methylnicotinamide;* ***HAD****, hexadecanedioate;* ***HVA****, homovanillic acid;* ***MATE****, multidrug and toxin extrusion protein;* ***OAT****, organic cation transporter 2;* ***OATP1B****, organic anion transporting polypeptide B1;* ***OCT****, organic cation transporter;* ***P-gp****, P-glycoprotein;* ***SLC****, solute carrier;* ***TDA****, tetradecanedioate.*