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Special Section on Transporters in Toxicity and Disease—Commentary

The Role of Transporters in Toxicity and Disease

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ABSTRACT

The significance of transporters in the disposition, metabolism, and elimination of drugs is well recognized. One gap in our knowledge is a comprehensive understanding of how drug transporters change functionality (their amount and activity) in response to disease and how disease and its inevitable pathology change transporter expression. In this issue of *Drug Metabolism and Disposition* a series of review and primary research articles are presented to highlight the importance of transporters in toxicity and disease. Because of the central role of the liver in drug metabolism, many of the articles in this theme issue focus on transporters in the liver and how pathology or alterations in physiology affects transporter expression. The contributing authors have also considered the role of transporters in drug interactions as well as drug-induced liver injury. Noninvasive approaches to assessing transporter function in vivo are also described. Several articles highlight important issues in oncology where toxicity must be balanced against efficacy. In total, this theme issue will provide a stepping-stone to future studies that will establish a more comprehensive understanding of transporters in disease.

Introduction

Transporters facilitate the movement of compounds across biologic membranes which, in turn, regulate the intracellular concentration of a transported substance. These transported compounds can be either drugs (xenobiotics) or endogenous compounds (endobiotics). On a rudimentary level the types of transporters can be classified into uptake and efflux carriers. In vitro model systems, under rigorously defined conditions, have provided a wealth of information about individual transporter substrate selectivity, affinity, and driving forces. From these studies, we know that many "drug" transporters are multispecific and feature substrate redundancy, resulting in more than one transporter interacting with a particular substrate (Giacomini et al., 2010). One possible explanation for the broad substrate specificity is the presence of a large substrate binding pocket, as is found in P-glycoprotein (P-gp, aka MDR1 and ABCB1) (Aller et al., 2009). Ultimately, the combination of in vitro model systems and structural analysis will provide mechanistic insights into individual transporters. However, determining the contribution of a single transporter to a drug's tissue concentration is complicated by multiple factors, including the expression of the same transporter in more than one tissue, the redundancy of transporter function, and the fact that many tissues express both uptake and efflux transporters in the same or opposing membranes. For instance, for hepatocytes, we need to consider the possibility that a drug might interact with both canalicular efflux and basolateral uptake and efflux transporters in the hepatocyte.

Key findings such as digoxin toxicity (Ho and Kim, 2005) and myopathy associated with high statin plasma levels (Giacomini et al., 2013) have been linked to alterations in transporter function. These events were either a function of drug-drug interactions (DDI), such as inhibition of P-gp for digoxin (Fenner et al., 2009) and inhibition of organic anion transporting polypeptides (OATPs) by statins (Link et al., 2008) or reduced transporter activity for statins with allelic variants of OATPs (Giacomini et al., 2013). The role of transporters in various diseases (Ho and Kim, 2005) and in response to drugs and various toxicants (Ho and Kim, 2005; Sweet, 2005) has been considered. Just as drug metabolizing enzymes reduce the exposure to xenobiotics through direct clearance and increased excretion, transporters can also function to modulate the intracellular concentrations of compounds (McBride et al., 2009; Chu et al., 2013), which would ultimately alter cellular physiology with toxicologic sequelae. Deconvoluting the specific role(s) of transporters in tissue and plasma exposure will come from modeling (Shitara et al., 2006; Poirier et al., 2009; Menochet et al., 2012) and the application of new approaches to assess in vivo function, such as positron emission tomography (Sasongko et al., 2005) and mass spectrometry (Deo et al., 2012). In comparison with drug metabolizing enzymes there are a limited

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ABBREVIATIONS: ABC, ATP binding cassette; ABCB11, bile salt export pump, BSEP; ABCC2, multidrug resistance protein 2, MRP2; DDI, drug-drug interaction; DILI, drug-induced liver injury; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; MRP, multidrug resistance protein; OATP, organic anion transporting polypeptide; 6β-OHF, 6β-hydroxycortisol; PCFT, proton-coupled folate transporter; P-gp, P-glycoprotein; RFC, reduced folate carrier; SBE, steroid-binding element.

number of specific substrates, and inhibitors for many transporters and alternative approaches to overcome this significant challenge to experimental paradigms are needed (Brouwer et al., 2013).

Clearly, transporter affinity, spatial distribution, and membrane localization influence the degree to which individual transporters impact a drug or endobiotic concentration. These are important issues which are not specifically addressed in this theme issue, but have been reviewed elsewhere (VanWert et al., 2010). Instead, we have attempted to extend this foundation by presenting both primary and review articles that highlight the complexity of understanding how transporters impact disease and are, in turn, affected by disease state.

Liver Transporters in Health and Disease

Bile acids are synthesized from cholesterol in the liver. After conjugation, they require transporters, ABCB11 (aka bile salt export pump, BSEP) and ABCC2 (aka multidrug resistance protein 2, MRP2), to cross the bile canalicular membranes. These secreted bile acids are efficiently (>95%) reabsorbed from the intestinal lumen, mostly by the apical sodium-dependent bile acid transporter (ASBT, SLC10A2) in the terminal ileum (Dawson et al., 2003). The reabsorbed bile acids return to the liver via the portal circulation and are taken up by the basolateral uptake transporters of the hepatocyte (enterohepatic recirculation). The sodium/taurocholate cotransporting polypeptide (NTCP, SLC10A1) transports the majority (~90%) of these bile acids, but OATPs (SLCO1B) are also involved. Because transporters play a crucial role in modulating the uptake and export, hence the amount of liver bile acid, it is important to have a comprehensive overview of the transporters and major factors (e.g., nuclear receptors) that are crucial to bile formation and bile acid homeostasis. Integrating this knowledge is important to provide insight into hepatic inflammation, fibrosis, and cirrhosis. To address the role of transporters in the etiology of liver disease, in this issue, Cuperus et al. (2014) provide an overview of the major canalicular ATP binding cassette (ABC) transporters and their regulation by nuclear receptors and function in cholestasis.

Elevation in bilirubin is a biomarker for liver damage, but one caveat is that significant liver damage is required to raise serum bilirubin levels (Kaplowitz, 2005). The importance of transporters to bilirubin homeostasis is exemplified by the inclusion of ABCC2 and ABCB11, transporters involved in bilirubin and bile acid transport, in the recently issued DDI guidances from the European Medicines Agency (EMA) (CHMP, 2012) and the U.S. Food and Drug Administration (FDA) (CDER, 2012). In this context, it is important to understand which transporters have affinity for and transport bilirubin. Variant *ABCC2* alleles can cause Dubin-Johnson syndrome (Dubin and Johnson, 1954), which is characterized by a mild conjugated hyperbilirubinemia. However, the exact importance of ABCC2 in hyperbilirubinemia remains difficult to assign. For example, patients with Dubin-Johnson syndrome have hyperbilirubinemia due to loss of MRP2 function and MRP3 upregulation, which increases bilirubin efflux into the systemic circulation (Konig et al., 1999). As outlined by Keppler (2014), besides ABCC2, OATP (SLC01B) and other uptake transporters contributes to bilirubin uptake. Bilirubin is also metabolized in humans exclusively by uridine-diphosphoglucuronate glucuronosyltransferase (UGT1A1) in the liver to form bilirubin glucuronides (Kadakol et al., 2000). Alteration of any, or all, of these proteins can contribute to increases in bilirubin levels. It is possible that expression of human transporters in mice and rats will provide insight into the relative role of human transporters in DDI and in various disease states (van de Steeg et al., 2013).

The FDA recommends studies with ABCB11 to help provide a mechanistic understanding of cholestasis, and the EMA guidance suggests a more proactive role in assessing the interaction of a compound with this transporter. For example, the causal link relating inhibition of ABCB11 to cholestasis is not always clear. In this issue, Rodrigues et al. (2014) consider the interaction with ABCB11 and the proposed association with cholestasis in a more holistic manner, discussing bile acid enterohepatic recirculation, bile acid clearance, and the interplay of additional transporters (MRPs, ABCC subfamily) and metabolic enzymes (sulfotransferases). The regulation of these transporters and enzymes by nuclear hormone receptors as part of a feedback loop in response to elevations in bile acids is also presented. Rodrigues et al. (2014) provide interesting perspectives on the possible contributions of a number of processes. In addition to the well-appreciated aspects of bile homeostasis, they consider the possible role of kinases involved in intracellular trafficking of proteins and also enterobacterial metabolism of bile acids. As the authors note, the complexity of the overall process can make it challenging to discern the primary mechanism(s) by which a drug elevates bile acids either by directly impacting bile acid homeostasis or as a secondary effect as a consequence of hepatotoxicity.

ABCG2 (breast cancer resistance protein, BCRP) is ubiquitously expressed and capable of exporting a wide array of endobiotic and xenobiotic compounds. Studies in knockout mice indicate that Abcg2 (Bcrp) does not have a protective role in adaptation to acute cholestasis (Mennone et al., 2010), suggesting it does not interact with bile acids. However, findings from in vitro studies are not as clear-cut and have not ruled out a role for Abcg2 in bile acid transport. A potential explanation is that ABCG2 activity is influenced by a combination of the membrane microenvironment and the inherent properties of ABCG2 (Hegedus et al., 2009b). For example, recent studies have demonstrated that the activity of some ABC transporters is regulated by membrane cholesterol levels (Paulusma et al., 2009). Telbisz et al. (2014) extend this by reporting in this issue that human ABCG2 contains a domain referred to as a steroid-binding element (SBE). ABCG2 apparently requires this domain because mutations in the SBE abrogate cholesterol stimulation of ABCG2 ATPase. These studies are extended to show that ATPase activity in ABCG2 harboring mutations in the SBE are refractory to bile acid inhibition, unlike wild-type ABCG2. These studies suggest that bile acids, while not being substrates for ABCG2, may impact its function.

A better understanding of the impact of disease on the expression of transporters is key not only to developing treatments for specific conditions but also to understanding the increased or decreased potential for DDIs in the patient population. Animal models that more closely recapitulate human diseases can provide needed insight into how disease progression changes transporter function. Two primary articles presented in this theme issue address this topic. First, Canet et al. (2014) apply a model of nonalcoholic steatohepatitis (NASH) as a surrogate of the human disease to characterize the alterations in transporter function. In these studies, an interesting outcome of this disease model, in comparison with healthy animals, was both an up-regulation of efflux transporters and a repression of uptake transporters. The second article by Merrell et al. (2014) uses an animal model that mimics human infection with an enteropathogenic *Escherichia coli.* They use this model system to elucidate how the toll-like receptor (TLR) pathway and certain cytokines modulate expression of the transporters and the metabolizing enzymes regulating liver bile acid concentrations.

An example of pathogen-induced transporter regulation is also provided in this issue. In Sub-Saharan Africa, an estimated 25% of all pregnancies are complicated by placental malaria infection (Dellicour et al., 2010). The article by Cressman et al. (2014) in this issue takes a unique perspective on the complex interplay of transporter and enzyme expression during the pregnancy of malaria-infected female mice. They find that malaria-induced alterations in the expression of transporters and drug-metabolizing enzymes in maternal and fetal tissues may alter drug disposition, potentially impacting maternal and

fetal outcomes. This work is especially relevant in light of the drugs currently used for malaria treatment (e.g., quinine, chloroquine, halofantrine, artemisinin, etc.), many of which are also substrates for CYP3A4 and efflux transporters such as P-gp and MRPs.

Transporters and Oncology

The impact of transporters in cancer chemotherapy can be viewed from two perspectives: decreasing host toxicity and increasing tumoricidal activity. To mitigate and understand the factors causing the untoward toxicities of cancer chemotherapy, a comprehensive knowledge of the transporters in these tissues is needed. This will be accomplished with systematic studies investigating the drug specificity of transporters in susceptible host tissues. Such knowledge can improve therapeutic efficacy. In this theme issue, Sprowl and Sparreboom (2014) provide important illustrations of how this can be realized. They tackle the known uptake carriers and highlight how unexplained drug-induced pathologies can be explained by tissue-specific expression of transporters, and they highlight some potential strategies to minimize clinical toxicity due to transporters. Knowledge of these transporters can account for the unique side effects of some chemotherapeutic agents.

P-gp was discovered in the mid-1970s in rodent cells, which acquired reduced sensitivity to anticancer drugs (Juliano and Ling, 1976). In the past 20 plus years, other ABC transporters have been discovered that also confer resistance to chemotherapeutic drugs (Gottesman et al., 2002). However, much effort has been directed toward developing inhibitors of P-gp as a strategy to overcome tumor drug resistance. This focus on P-gp is probably not misplaced, considering the number of compounds that interact with it. In addition, many of the new "targeted therapies" (e.g., tyrosine kinase inhibitors) are substrates for P-gp (Hegedus et al., 2009a). The strategy to increase antitumor efficacy by inhibiting P-gp has been effective in vitro but has not successfully translated in vivo. In this issue, Callaghan et al. (2014) account for this lack of success. In addition, they propose a paradigm shift beyond the simple P-gp inhibition model and suggest approaches such as modulating P-gp expression by affecting signaling pathways as an approach to increasing antitumor efficacy.

Folates are essential for cell growth and tissue development and must be obtained from exogenous sources because mammals cannot synthesize these derivatives de novo. Folates take part in essential biosynthetic pathways that produce the building blocks for DNA and RNA as well as certain amino acids. Folates are anions at physiologic pH and do not cross biologic membranes by diffusion alone. The major folate transporters are the reduced folate carrier (RFC) (SLC19A1) and the proton-coupled folate transporter (PCFT) (SLC46A1) (Zhao et al., 2009). RFC is the major membrane transporter of circulating folates, and all substrates are anions. Antifolates such as methotrexate are administered intravenously for cancer treatment. Methotrexate is also used for the treatment of autoimmune/inflammatory diseases, but administered orally. Methotrexate and other antifolates are good substrates for RFC and PCFT. Matherly et al. (2014) provide a comprehensive review of the biology and role of RFC and PCFT in cancer chemotherapy.

Transporters and Drug Interactions

Updates to the recent DDI guidances from both the FDA (draft, CDER, 2012) and the EMA (CHMP, 2012) include a consideration of the possible contributions of drug metabolites to DDI and toxicity. It is usually very challenging to discriminate the relative contribution of parent drug versus metabolite(s) in DDI. One significant complicating factor is that metabolites (a result of phase I or phase II metabolism) are likely to interact with transporters. Zamek-Gliszczynski et al. (2014) in this issue discuss the possible role of metabolites in affecting transporter function, thus altering the efficacy and safety of drugs. The definition of a major metabolite according to the metabolites discussion in the International Harmonization Committee's Safety Testing document (ICH, 2010) is 10% of the total drug-related material in systemic circulation. Whether this level of metabolite, or the 25% of parent proposed in the DDI regulatory guidance, is sufficient to contribute to the overall drug related effects has been questioned (Yu and Tweedie, 2013). Zamek-Gliszczynski et al. (2014) also propose that excretory metabolites in modifying transporter function may also need to be taken into consideration. An important case could be made for drugs and endobiotics that undergo enterohepatic recirculation, particularly as levels of noncirculating metabolites can be significant (Li et al., 2014). A trend in the pharmaceutical industry toward producing more metabolically stable drug candidates to reduce clearance may in fact, as the authors propose, lead to greater interactions with drug transporters. A number of case examples are presented to illustrate their perspective.

Drugs that inhibit multiple transporters have the capability of increasing the retention of both drug substrates and endobiotics (e.g., bile acids), which may increase the probability of tissue damage. Indeed, impaired bile-acid export could contribute to the development of cholestatic drug-induced liver injury (DILI) due to the increased concentrations of liver bile acids producing hepatocellular apoptosis and/or necrotic death. ABCB11 is the primary bile acid exporter located in the canalicular membrane of the hepatocyte. Loss-of-function mutations in ABCB11 cause the disease progressive familial-intrahepatic cholestasis type 2 (PFIC 2) (Strautnieks et al., 1998). A number of studies have highlighted the potential contribution of ABCB11 inhibition to DILI; thus, ABCB11 inhibition is considered a risk factor in DILI. However, the drugs that interact with ABCB11 in vitro do not always produce cholestasis. Notably, the basolateral ABC transporters ABCC3 and ABCC4 also transport bile acids (Zelcer et al., 2003a,b). In this issue, Kock et al., (2014) hypothesize that interaction with the basolateral exporters (ABCC3 and ABCC4) might further increase the likelihood of identifying drugs that produce cholestasis. This is because ABCC3 and ABCC4 have the capability of exporting bile acids (Zelcer et al., 2003a) but also because ABCC4 is up-regulated in cholestasis in mouse and humans (Schuetz et al., 2001; Keitel et al., 2005). Moreover, Abcc4 also protects against acute cholestatic injury (Mennone et al., 2006). Thus, predicting drug and transporter interactions contributing to cholestasis might be improved by knowing which drugs interact with ABCB11 and the basolateral efflux transporters ABCC4 and ABCC3. Using a combination of in vitro transport assays and an inventory of drugs associated with cholestasis, the authors determine that drug interactions with both ABCB11 and ABCC4 might be important parameters to consider when assessing a drug's cholestatic potential. At the very least, this study may facilitate the development of additional preclinical models to enhance more reliable prediction of drug-induced hepatotoxicity.

In Vivo Probes to Dissect Transporter Interaction

An emerging area is to noninvasively investigate the functional capability of the uptake and efflux transporters in the liver using both contrast and fluorescent dyes. The review by Pastor et al. (2014) highlights how MRI can provide a noninvasive approach to determining how hepatic concentrations are impacted by influx and efflux membrane transport systems. Currently, these dyes exploit the OATPs and MRP2

transporters in the liver. This approach can also reveal variation in transporter function in both normal and injured hepatocytes. However, there is also a practical, clinically important reason to know which transporters use the contrast agents and fluorescent dyes. These dyes are used to assess liver function, and it important to discriminate between altered transporter activity versus reduced liver function. Furthermore, a refined knowledge of transporters involved in transporting contrast dyes will facilitate assessment and identification of potential DDI in vivo.

Another potential DDI concern is renal tubular secretion. In their article, Imamura et al. (2014) evaluate 6β-hydroxycortisol (6β-OHF) as a novel endogenous probe substrate of human OAT3 (SLC22A8) and multidrug and toxin extrusion protein (MATE, SLC47A1), which also may be a novel tool to evaluate renal DDI. Typically, 6β-OHF and 4β-cholesterol are used to evaluate hepatic CYP3A4 activity, but the renal excretion of 6β-OHF can be affected significantly by inhibition of OAT3. In an elegant series of experiments using specific transporter inhibitors, they demonstrate that OAT3, but not MATE, plays a significant role in the urinary clearance of 6β-OF, thus revealing a barometer of in vivo OAT3 function.

Conclusions

The past 20 plus years have seen an expansion in the number of transporters of therapeutic and disease relevance. Transporters have well-known importance in impacting therapeutic outcome (e.g., RFC is crucial to antifolate therapy for cancer) or affecting oral drug absorption and brain penetration (e.g., P-gp). We have attempted to highlight some important and emerging areas where transporters are critical to drug response, such as how pathology or changes in physiology affect transporter expression and function. These articles underscore the need to better understand the whole body distribution of drugs. This can be accomplished by more clearly elucidating the transport processes responsible for distribution into various organs and also how disease and/or other xenobiotics modulate transporters. Such studies suggest the need for further development of human relevant preclinical models that incorporate not only expression data but also functional analysis in particular. The latter determinations of in vivo transporter activity can be achieved through the use and development of additional noninvasive probes. The use of in vivo probes of transporter function will also aid in the prediction of DDIs or the identification of metabolites that interfere with transporter function. However, although in vivo approaches will be a boon to our knowledge, in vitro studies are also essential. In vitro transporter studies can further inform us by judiciously using databases that catalog drug side effects. In combination, these approaches will allow us to fill in the gaps in our understanding of how individual and combinations of transporters affect tissue concentrations.

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Wrote or contributed to the writing of the manuscript: Schuetz, Swaan, Tweedie.

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