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Minireview

Role and Regulation of Hepatobiliary ATP-Binding Cassette Transporters during Chemical-Induced Liver Injury

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ABSTRACT

Severity of drug-induced liver injury (DILI) ranges from mild, asymptomatic, and transient elevations in liver function tests to irreversible liver damage, often needing transplantation. Traditionally, DILI is classified mechanistically as high-frequency intrinsic DILI, commonly dose dependent or DILI that rarely occurs and is idiosyncratic in nature. This latter form is not dose dependent and has a pattern of histopathological manifestation that is not always uniform. Currently, a third type of DILI called indirect hepatotoxicity has been described that is associated with the pharmacological action of the drug. Historically, DILI was primarily linked to drug metabolism events; however, the impact of transporter-mediated rates of drug uptake and excretion has gained greater prominence in DILI research. This review provides a comprehensive view of the major findings from studies examining the contribution of hepatic ATP-binding cassette transporters as key contributors to DILI and how changes in their expression and function influence the development, severity, and overall toxicity outcome.

SIGNIFICANCE STATEMENT

Drug-induced liver injury (DILI) continues to be a focal point in drug development research. ATP-binding cassette (ABC) transporters have emerged as important determinants of drug detoxification, disposition, and safety. This review article provides a comprehensive analysis of the literature addressing: (a) the role of hepatic ABC transporters in DILI, (b) the influence of genetic mutations in ABC transporters on DILI, and (c) new areas of research emphasis, such as the influence of the gut microbiota and epigenetic regulation, on ABC transporters.

Introduction

Drug-induced liver injury (DILI) can be the outcome of use and/or misuse of prescription and nonprescription drugs, herbal products, and dietary supplements. Gradation of liver injury ranges from mild, asymptomatic elevations in liver function tests to complete acute liver failure (ALF). A large number of drugs have been implicated as potential causes of DILI. The Food and Drug Administration Liver Toxicity Knowledge Base lists over 1000 drugs divided into various categories based on their DILI-causing potential (Chen et al., 2011).

DILI can be historically classified by the presumed mechanism of toxicant action as intrinsic or idiosyncratic. The former, also called predictable, has a high frequency of occurrence and is dose-dependent. It usually occurs shortly after exposure to high therapeutic, supratherapeutic, or toxic doses. This toxicity can also be reproduced in various species of laboratory animals. Acetaminophen (APAP) is the prototype drug for this type of DILI, and misuse of APAP is responsible for approximately 50% of all cases of ALF in the United States (Ostapowicz et al., 2002; Lee, 2003; Larson et al., 2005; Lee, 2017). There is also a considerable number of other drugs that can cause intrinsic hepatic necrosis (i.e., niacin, aspirin, cocaine, amiodarone, methotrexate, cancer chemotherapy drugs).

The second class of DILI is idiosyncratic (IDILI) in nature or unpredictable and of rare occurrence. IDILI is often referred to as dose-independent, which is misleading because an adverse outcome cannot be entirely disassociated from dose. Classic drugs associated with IDILI include antibiotics such as amoxicillin–clavulanate, nitrofurantoin, minocycline, cephalosporins, fluoroquinolones, and isoniazid. In many developing countries, antituberculosis medications are the leading cause of IDILI (Ramachandran et al., 2013). This form of DILI seems to be the outcome of an interplay between drug exposure and environment

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ABBREVIATIONS: ABC, ATP-binding cassette; ALF, acute liver failure; AP, alkaline phosphatase; APAP, acetaminophen; APAP-Glu, APAP-glucuronide; APAP-Sulf, APAP-sulfate; ASCOM, cointegrator-2-containing complex; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; DIC, drug-induced cholestasis; DILI, drug-induced liver injury; IDILI, idiosyncratic drug-induced liver injury; FXR, farnesoid X receptor; GI, gastrointestinal; GSH, glutathione; HAX1, hematopoietic cell-specific Lyn substrate 1–associated protein X1; IL-1β, interleukin-1β; MDR, multidrug resistance protein; MH, monocyte-derived hepatocyte-like; MRP, multidrug resistance–associated protein; NAPQI, *N*-acetyl-*p*-benzobenzoquinoneimine; Nrf2, nuclear factor E2-related factor 2; PGP, P-glycoprotein; PXR, pregnane X receptor; SIRT1, sirtuin 1.

and/or host factors, such as adaptive immunity to damage-associated molecular pattern molecules (reviewed in Tujios and Fontana, 2011). IDILI usually occurs at a very low frequency, with drug treatment lasting several months to even years before toxicity manifests. These features make IDILI often difficult to identify, and its occurrence is often underestimated (Shapiro and Lewis, 2007). IDILI accounts for roughly 13% to 17% of all cases of ALF in the United States and Sweden (Ostapowicz et al., 2002; Wei et al., 2007).

A third type of DILI called indirect hepatotoxicity has been described. This classification include pharmaceuticals that can induce liver injury because of the drug's own pharmacological action, rather than through inherent hepatotoxic potential or immunogenicity. Examples of drugs associated with indirect DILI are certain antineoplastic agents, glucocorticoids, monoclonal antibodies, and tumor necrosis factor α, as well as inhibitors of checkpoint proteins and protein kinases (Hoofnagle and Björnsson, 2019). This third form is of much greater frequency than the idiosyncratic form and can have a distinct clinical manifestation. As yet, indirect hepatotoxicity is not a widely accepted classification distinct from intrinsic and idiosyncratic.

An important point to consider when defining mechanisms of DILI is that the concentration of a drug (or its metabolite) in hepatocytes is not only dependent upon drug metabolism but also interindividual variability in the rates of drug uptake and excretion. These transport processes significantly influence drug cellular concentration, tissue dosimetry, and ultimate DILI outcome. Given the interconnectivity of drug metabolizing enzymes and transporters, each drug can have distinct combinations of biotransformation and disposition pathway(s) that define the drug's pharmacokinetic and pharmacodynamic behavior as well as safety. Particularly in liver, there are two superfamilies of transporters, the solute carrier transporters and the ATP-binding cassette (ABC) transporters. The first one includes organic anion transporting polypeptides, organic anion transporters, organic cation transporters, and the sodium/taurocholate cotransporting polypeptide. These drug transporters are principally located in the plasma membrane of hepatocytes, mediating drug influx from sinusoidal blood into the cell. By contrast, the superfamily of ABC transporters is involved in drug efflux from hepatocytes into the bile, when the transporter is localized to the canalicular membrane, or into sinusoidal blood for transporters localized basolaterally. Intrinsic variability across individuals in the expression and function of drug transporters due to genetic polymorphisms and variable responses to environmental stressors and drugs is a well-recognized key contributor to the development, severity, and overall DILI outcome. This review focuses and summarizes the major findings from studies examining the role hepatic ABC transporters in the development or prevention of DILI.

Brief Historical Perspective

Studies on the mechanisms of DILI were initially centered on the role of phase I and II drug metabolism enzymes in the generation and net availability of cytotoxic reactive intermediates, as well as the ability of such reactive intermediates to covalently bind to cellular macromolecules, including proteins. Protein adduction by reactive intermediates may lead to loss of function, abnormal localization, or formation of immunogenic haptens. The potential consequences of such protein modifications are production of oxidative stress, mitochondrial dysfunction, and the triggering of an immune response against the liver (for a complete review, see Andrade et al., 2019).

The discovery of drug transporters and the increasing understanding of their role in cancer multidrug resistance in the 1980s and 1990s (Doring and Petzinger, 2014) opened the door for the study of their normal physiologic functions and pathophysiological consequences of altered expression, including DILI. The largest group of protein transport systems is the ABC class transporters. This transporter family is composed of 49 genes and 20 pseudogenes, divided into eight subfamilies, named from ABCA to ABCG. In the liver, all ABC proteins are efflux transporters with the capacity to transport a vast array of substances (for a complete review, see Kroll et al., 2021). Multidrug resistance–associated proteins (MRP) are members of the ABCC subfamily. Among them, MRP2 (ABCC2) is the only one located on the canalicular membrane of hepatocytes, while the remainder are located in the basolateral domain, with the most important and studied being MRP1 (ABCC1), MRP3 (ABCC3), and MRP4 (ABCC4). The only ABCA transporter localized to basolateral membranes of hepatocytes is ABCA1, which is involved in cholesterol efflux. The remainder of the ABC transporters present in the liver are located in the canalicular membrane, namely, the bile acid export pump (BSEP; ABCB11), multidrug resistant gene 3 (MDR3; ABCB4), MDR1 or P-glycoprotein (PGP; ABCB1), breast cancer resistance protein (BCRP; ABCG2), and ABCG5.

Key Recent Advances

In this review, key recent advances in the current understanding of the role of ABC transporters in drug liver damage are divided into intrinsic and idiosyncratic DILI.

Role of ABC Transporters in Intrinsic DILI

Intrinsic DILI is associated with ingestion of supratherapeutic or toxic doses of a compound. APAP is highlighted in this review as prototype compound, since it is extensively used in elucidating the role of ABC transporters in DILI.

The Role of ABC Transporters in APAP Metabolism. Ingestion of high doses of APAP saturates its normal detoxification routes of metabolism, sulfate, and glucuronide conjugation (Fig. 1) APAP undergoes bioactivation by cytochrome P450 enzymes to produce *N*-acetyl-*p*-benzo-quinoneimine (NAPQI). The conjugation of this reactive metabolite with glutathione (GSH) neutralizes its reactivity and produces nontoxic conjugates that are readily eliminated via bile and urine. However, upon depletion of the GSH intracellular pool, excess NAPQI is free to react with negatively charged macromolecules. This produces APAP–protein covalent adducts, mitochondrial dysfunction, and oxidative stress, which finally results in hepatocellular damage (for a review, see Ramachandran and Jaeschke, 2018).

The ABC transporters known to mediate APAP metabolites transport and directionality are shown in Fig. 1. APAP-glucuronide (APAP-Glu) is a known substrate for sinusoidal Mrp3 (Slitt et al., 2003; Manautou et al., 2005) and canalicular Mrp2 (Xiong et al., 2000; Chen et al., 2003; Silva et al., 2005). APAP-sulfate (APAP-Sulf) is primarily excreted into plasma, principally by Mrp4 and to a lesser extent by Mrp3 (Zamek-Gliszczynski, Nezasa, Tian, Bridges et al., 2006), while BCRP principally transports this metabolite into bile with some contribution by Mrp2 (Zamek-Gliszczynski et al., 2005; Zamek-Gliszczynski, Nezasa, Tian, Kalvass et al., 2006; Lee et al., 2009). Similarly, APAP-glutathione is transported into bile by Mrp2 and into plasma by Mrp1 and Mrp4 (Chen et al., 2003). The latter is known to also transport APAP-cysteine (Koenderink et al., 2020). The basal hepatic expression of MRP1 is very low, while MRP3 and MRP4 are expressed in liver, with interindividual variability in their expression and function (Borst and Elferink, 2002).

The Role of ABC Transporters in Modulation of APAP Toxicity and Autoprotection. The functional consequences of altered ABC transporters expression in DILI have been the subject of multiple investigations. Significant increments in the expression of the sinusoidal ABC transporters MRP1 and MRP4 at the mRNA level, with induction

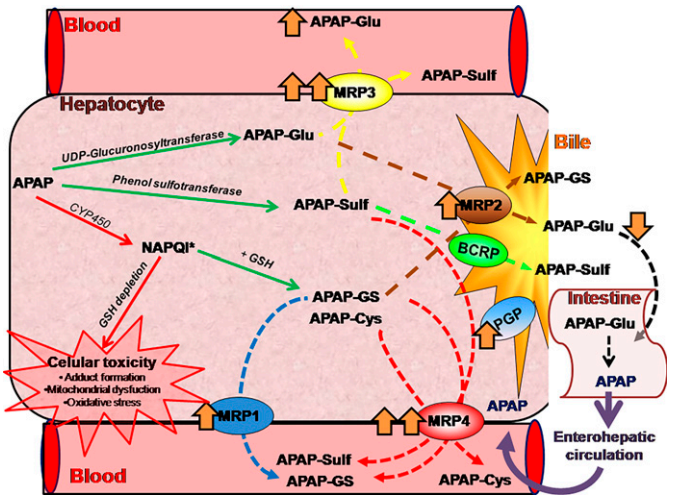

Fig. 1. ABC transporters involved in the most frequent form of intrinsic DILI: APAP intoxication. A hepatocyte is depicted with sinusoidal membranes in contact with blood flow and canalicular secretion, through bile ducts, arriving at the small intestinal lumen in the duodenum. Supratherapeutic doses of APAP enter freely into hepatocytes where it is transformed by saturable conjugative pathways to the nontoxic metabolites, APAP-Glu and APAP-Sulf, by UDP-glucuronosyltransferase and phenol sulfotransferase, respectively (depicted by green continuous lines). Excess APAP is oxidatively metabolized by CYP450 to form the reactive intermediate NAPQI. This toxication pathway is depicted by a red continuous line. NAPQI then undergoes conjugation with GSH to form APAP-glutathione that can then be transformed into APAP-cysteine. When the intracellular pool of GSH is depleted, unneutralized NAPQI can produce cellular toxicity by different mechanisms. APAP-Glu is excreted into bile by MRP2 (brown dotted line) or blood by MRP3 (yellow dotted line), while APAP-Sulf is excreted into sinusoidal blood, principally by MRP4 (red dotted line) and, to a lesser extent, by MRP3 (yellow dotted line). APAP-Sulf also undergoes biliary elimination, principally via BCRP (green dotted line), with some contribution by MRP2 (brown dotted line). APAP-glutathione is transported via bile by MRP2 (brown dotted line) and plasma by MRP1 (blue dotted line) and MRP4 (red dotted line). The latter transporter also translocates APAP-cysteine into plasma. Changes in ABC transporters (orange arrows) by APAP intoxication alter its disposition. Similarly, other chemicals and drugs can produce the same effect on transporters' expression and function. These changes consist of increased plasma efflux of APAP-Glu and urinary elimination, associated with a condominant decreased in biliary secretion of this metabolite. This vectorial change decreases the APAP-Glu biliary excretion and the amount of this conjugate that reaches the small intestine. Glucuronidases present in intestinal bacterial cleave APAP-Glu, releasing free APAP that is reabsorbed into the portal circulation, re-exposing the liver to APAP. A potential consequence of a shift in the vectorial transport of APAP-Glu from bile into urine due to changes in transporter expression is decreased enterohepatic circulation, less hepatic APAP exposure, and thus lower hepatotoxicity.

at the protein level for MRP4 and MRP5, are seen in patients with fulminant ALF produced by APAP (Barnes et al., 2007). The increased expression of the canalicular transporters BCRP and PGP is also observed in human liver specimens obtained from APAP poisoning cases during liver transplantation surgery. Additionally, this study demonstrates a strong association between increments in PGP expression and proliferating cell nuclear antigen–positive hepatocytes, suggesting the existence of a relationship between compensatory hepatocellular regeneration and increased efflux transporter expression (Barnes et al., 2007). What is unknown at this point is why is it important for hepatocytes undergoing active replication to enhance efflux transport processes while liver parenchyma is being restored.

At the canalicular membrane level, an increase in both the expression and activity of Mrp2 and Pgp occurs in rat liver (Ghanem et al., 2004), while in the mouse model of APAP-induced hepatotoxicity, Mrp2 mRNA (Aleksunes et al., 2005) and protein expression (Aleksunes et al., 2006) increase. Concomitantly, basolateral protein expression of Mrp3 is increased by toxic APAP treatment in rats (Ghanem et al., 2005) and mice (Aleksunes et al., 2005). Additionally, there is induction of Mrp4 mRNA expression and protein levels by APAP in both mice and humans (Aleksunes et al., 2006; Campion et al., 2008). Increments in Mrp3 and Mrp4 expression colocalize within regions of the hepatic lobule where cell proliferation is occurring in response to APAP toxicity, principally in hepatocytes surrounding the central vein (Aleksunes et al., 2006). The mechanism of the induction of both transporters is not totally understood, but there is evidence that induction is dependent on the paracrine actions of cytokines, such as tumor necrosis factor α and interleukin-1β (IL-1β), since depletion of Kupffer cells by liposomal clodronate treatment abolishes Mrp4 induction and sensitizes mice to APAP hepatotoxicity (Campion et al., 2008). APAP-mediated induction of Mrp3 and Mrp4 also involves activation of nuclear factor E2-related factor 2 (Nrf2), since mice with genetic ablation of Nrf2 do not show induction of Mrp3 and Mrp4 in response to APAP (Aleksunes, Slitt et al., 2008).

Mrp3 plays also plays a role in the hepatobiliary disposition of APAP and susceptibility to hepatotoxicity. Since Mrp3 preferentially transports glucuronide conjugates, it is not surprising that the intrahepatic accumulation of APAP-Glu is 20-fold higher in Mrp3 null mice, with a concomitant reduction in plasma APAP-Glu and greater biliary excretion (Manautou et al., 2005). Furthermore, Mrp3 null mice are more resistant to APAP-induced hepatotoxicity and experience a faster replenishment of hepatic GSH than wild-type mice. Thus, the basolateral excretion of APAP-Glu in mice is nearly completely dependent on Mrp3 activity, and altered disposition of APAP in the Mrp3 null mice is associated with lower hepatotoxicity.

There are species differences in basal expression of transporters and differential susceptibility to APAP dosing (Wang et al., 2015). For example, the expression of Mrp3 is very low in normal rats (Hirohashi et al., 1998) and much higher in mice (Zelcer et al., 2005). By contrast, Mrp4 is very lowly expressed in liver of mice (Aleksunes et al., 2005), rats (Chen and Klaassen, 2004), and humans (Barnes et al., 2007). In the normal human liver, high MRP3 mRNA expression has been reported. However, modest amounts of MRP3 protein are observed (Konig et al., 1999; Kool et al., 1999), with a high degree of variability among individuals (up to 80-fold) (Lang et al., 2004). This difference may be due to a single nucleotide polymorphism, 2211C>T, in the promoter region of MRP3, which lowers the binding affinity of nuclear transcription factors.

Induction of hepatic expression of Mrp3 (rats) and Mrp4 (mice) is associated with an increased resistance to subsequent toxic APAP treatment or re-exposure. This phenomenon is known as APAP autoprotection. It is observed with APAP (Aleksunes, Campion et al., 2008; Ghanem et al., 2009) as well as other drugs or nondrug xenobiotics that produce hepatotoxicity. In humans, evidence indicates that long-term abuse of opioid/APAP combination products or consumption of 3 times or more the daily recommended dose of APAP does not necessarily result in APAP hepatotoxicity, which is indicative of adaptation and development of tolerance (Tredger et al., 1995; Shayiq et al., 1999; Forootan et al., 2017). Interestingly, similar changes in hepatic expression of ABC transporters to those reported in mice and rats have also been reported in humans with toxic APAP exposure.

Although the precise mechanism of this adaptation remains unknown, the shift in APAP-Glu excretion from bile into urine following APAP pretreatment in rats (Ghanem et al., 2005) could be a contributing factor to the development of resistance to APAP DILI. This shift in the disposition pathway of APAP-Glu decreases its biliary excretion and therefore the amount of this conjugate that appears in intestinal tissue, where it is normally cleaved by bacterial glucuronidases, releasing free APAP that then undergoes intestinal reabsorption. The ultimate consequences are decreased enterohepatic circulation, less APAP hepatic exposure, and lower likelihood of hepatotoxicity (Watari et al., 1983; Ghanem

et al., 2009). It is worth noting that this observation obtained from rat studies is also reported in humans. Tredger et al. (1995) reports that the development of tolerance to APAP hepatotoxicity is associated with an increment in APAP-Glu and APAP-Sulf concentrations in serum and urine of patients, when compared with normal volunteers. Allegaert et al. (2005) reports an increase in urine APAP-Glu/APAP-Sulf ratio in newborns chronically treated with APAP.

The introduction of colchicine, an antimitotic agent, to the APAP autoprotection treatment regimen of mice not only blocked APAP-induced compensatory hepatocellular proliferation, but it also prevented the induction of Mrp4 and restored the normal susceptibility of mice to APAP hepatotoxicity (abolishment of autoprotection). These findings greatly reinforced the association between Mrp4 induction, compensatory hepatocellular proliferation, and susceptibility to APAP-induced hepatic damage (Aleksunes, Campion et al., 2008).

The basis of the hepatoprotective role of MRP3 induction by itself or in combination with MRP4 induction by APAP treatment is currently not known. It is known that induction of these two transporters leads to more efficient disposition of APAP metabolites, which should prevent inhibition of drug metabolizing enzymes (e.g., phase II enzymes) by their metabolic/detoxification products. Induction of these transporters can also dampen cellular injury by increasing efflux efficiency of endogenous molecules that are potential mediators of cytotoxic responses (Borst et al., 2007), such as bile salts (Bohan et al., 2003), acyl glucuronides (Iwamura et al., 2017) bilirubin, and other signaling molecules from hepatocytes into the bloodstream for subsequent renal excretion. These transporters also export multiple other endogenous molecules, such as folic acid (Zeng et al., 2001; Kitamura et al., 2010) and leukotriene C_4 (Zeng et al., 2000) for Mrp3 and cAMP (Chen et al., 2001), cyclic guanosine monophosphate (van Aubel et al., 2002), prostaglandin E_1 and E_2, (Reid et al., 2003), thromboxane B_2, and prostaglandin $F_{2\alpha}$ (Rius et al., 2005) for Mrp4. Therefore, their overexpression can also contribute to modulating signaling events occurring in adjacent hepatocytes and/or nonparenchymal cells that modify the hepatic microcirculation (Vollmar and Menger, 2009) and aid in recovery from injury (Alvarez and Lorenzetti, 2021).

Drugs and Pathologic Conditions That Modify the Expression of ABC Transporters and Their Impact on APAP Hepatotoxicity. Modulation of Mrp3 and Mrp4 gene expression in the liver results from several compounds, such as ciprofloxacin, clotrimazole, clofibrate, or various pathologic conditions, such as primary biliary cholangitis, hepatitis, and cholestasis (for a review, see (Ghanem and Manautou, 2019). Pretreatment with tanshinone IIA, an active component in the dried root of *Salvia miltiorrhiz*a commonly used in traditional Chinese medicine, protects mice against APAP hepatotoxicity in a Mrp4- and Mrp2-dependent manner (Zhang et al., 2020). This study also shows that induction of these two transporters is mediated by activation of Nrf2, a transcriptional regulator that binds to antioxidant responsive elements on the Mrp2 and Mrp4 gene promoters. Increased Mrp3 and Mrp4 expression is seen in animals models with hereditary deficiency in Mrp2, such as transport-deficient rats (Chen et al., 2005), or with experimental obstructive cholestasis (Hirohashi et al., 1998; Konig et al., 1999; Soroka et al., 2001; Keppler, 2011a). Induction is also seen in some forms of human cholestatic disease (Shoda et al., 2001). The severity of APAP toxicity is lower in the Mrp2-deficient, transport-deficient rats in comparison with wild-type rats (Silva et al., 2005) or after an obstructive cholestasis of 7 days in rats with bile duct ligation (Acevedo et al., 1995). Similarly, cholestasis induced by model organic anions, such as indocyanine green, protects from APAP hepatotoxicity in mice (Silva et al., 2006).

Role of ABC Transporters in IDILI

Cholestatic and mixed forms of hepatitis are the most frequent presentations of IDLI. The presence of jaundice, pruritus, and elevated levels of serum alkaline phosphatase (AP) are the main characteristics of drug-induced cholestasis (DIC) (Stapelbroek et al., 2010).

ABC Transporters Involved in Bile Formation. Bile formation is basically a mechano-osmotic process resulting from a concentrative process that brings primarily bile salts, GSH, and bicarbonate, among other substances, into the bile canaliculus. The canalicular translocation of these solutes is then followed by osmotic water diffusion primarily through aquaporin channels. It is known that ABC transporters play a crucial role in this highly concentrative process for these solute components of bile. Figure 2 summarizes the more important steps in bile formation and enterohepatic circulation. In addition, peristaltic actin contractions and paracellular fluid movement are also important for bile formation (for a review, see Boyer and Soroka, 2021). As the name implies, bile salt export pump (BSEP, ABCB11) is responsible for bile salt efflux at the canalicular level (Stieger et al., 1992; Gerloff et al., 1998) and is the rate-limiting factor for bile salt-dependent flow (see Fig. 2). Also, at the canalicular pole, Mrp2 is responsible for the efflux of a wide array of substances, such as GSH and GSH conjugates of bile salts, glucuronidated bile salts, and conjugated bilirubin (Chen et al., 2003). Mrp2 is the main transporter responsible for

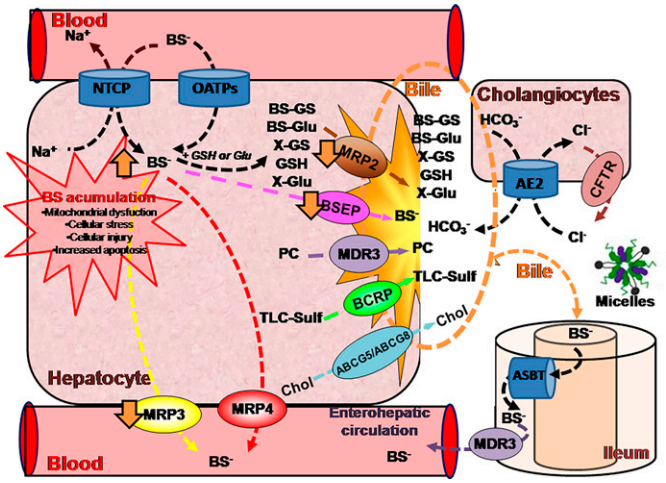

Fig. 2. ABC transporters involved in most frequent idiosyncratic form of DILI: drug-induced cholestasis. This figure shows the principal transporters involved in primary bile production by hepatocytes and the secondary modifications produced by cholangiocytes before mature bile arrives in the small intestine. Through the process of enterohepatic circulation, BS arrives in the liver via blood, entering hepatocytes via sodium/taurocholate cotransporting polypeptide and various organic anion transporting polypeptides, where they can be conjugated with glucuronide or GSH. At the apical side of hepatocytes, BSEP is the main transporter responsible for BS efflux (magenta dotted line) and the rate-limiting factor for bile salt independent flow generation. MRP2 is responsible for the canalicular efflux of GSH, GS-BS, and GS-X, as well as BS-Glu and X-Glu (e.g., bilirubin), with GSH being the main contributor to the establishment of the bile salt independent flow (brown dotted line). The osmotic concentration generated by these transport processes is then followed by passive inflow of water, principally through to aquaporin channels. This primary secretion then is modified in bile ducts by cholangiocytes. These biliary epithelial cells also contribute to the establishment of the bile salt independent flow through bicarbonate excretion via the chloride-bicarbonate anion exchanger and cystic fibrosis transmembrane conductance regulator. Bile acids self-aggregate into micelles, a process aided by phosphatidylcholine efflux into the bile canaliculus by MDR3 (violet dotted line) and cholesterol efflux by ABCG8/ABCG5 heterodimer (brown dotted line). BCRP collaborates with taurolithocholate-sulfate and probably other BS (green dotted lane). DIC is often the result of intrahepatic BS accumulation (orange arrows) produced by decreased activity of primarily BSEP and MRP2. Additionally, MRP3 and MRP4 mediates to basolateral efflux of BS (yellow and red dotted lines), so a decrease in MRP3 expression can also contribute to BS intrahepatic accumulation. Bil, bilirubin; CFT, cystic fibrosis transmembrane conductance regulator; TLC, taurolithocholate; X, xenobiotic.

establishing the bile salt–independent component of bile flow. MDR3 is a flippase, which exports phosphatidylcholine by its translocation from the inner to the outer membrane of the hepatocyte (Oude Elferink and Paulusma, 2007). In vitro studies have demonstrated that Bcrp is able to transport taurolithocholate sulfate (Suzuki et al., 2003); therefore, it might also contribute to the excretion of bile salts in vivo. After self-association due to their amphipathic properties, bile acids form micelles (Hofmann and Hagey, 2014) that are then charged with cholesterol and effluxed out of hepatocytes into bile via the ABCG8/ABCG5 heterodimer protein complex (Wittenburg and Carey, 2002). This primary secretion undergoes modifications in bile ducts by cholangiocytes. Micellar bile acids secretion is variable, and depending on the species, it can account for up to 40% of the total volume of daily bile output. The osmotic driving force provided by cholangiocytes is primarily generated by bicarbonate excretion via the chloride-bicarbonate anion exchanger, with chloride excreted by the cystic fibrosis transmembrane conductance regulator (ABCC7). However, regulation of the activity of these two apical proteins is very complex, involving not only intracellular levels of cAMP and calcium, but also luminal ATP that interacts with P2Y receptors to stimulate intracellular Ca^{2+} release (for a review, see Rodrigues et al., 2018). The exact contribution of MDR1 to bile formation has not been established yet, but it is well known that this is the main transporter of cationic drugs, and its contribution to the canalicular excretion of drugs and other xenobiotics into bile is well established (Pauli-Magnus and Meier, 2006).

DIC can be the result of the presence and/or generation of a toxic metabolite within the biliary system or from xenobiotic-mediated modulation in expression and/or inhibition of ABC transporters function. As a consequence, the intracellular accumulation of bile acids can produce hepatocellular injury due to their detergent properties that disrupt cell membranes (Hofmann and Hagey, 2014) and promotes generation of reactive oxygen species and mitochondrial damage (Krähenbühl et al., 1994). Furthermore, hydrophobic bile acids can also induce apoptosis by two different mechanisms. The first one is induction of the death signaling complex, which starts with the recruitment of Fas-associated death domain adaptor protein. The second one involves downregulation of the antiapoptotic Fas-associated death domain–like IL-1β–converting enzyme inhibitory protein, thus promoting caspase activation and apoptosis (for a review, see Engin, 2021). In summary, accumulation of bile acids contributes to hepatocyte necrosis and apoptosis, which can result in pathological tissue injury (Tujios and Fontana, 2011).

The functional activity of ABC transporters is dependent upon various factors, such as gene polymorphisms, transcriptional and post-translational modifications (e.g., glycosylation), allosteric modulators, membrane localization status, and changes in the transmembrane environment through alterations in lipid composition (Garzel et al., 2019).

Although an in-depth discussion of hepatic genetic diseases, such as progressive familial intrahepatic cholestasis, is beyond the scope of this article, it is important to mention that there is a heterogeneous group of autosomal recessive disorders related to gene mutations that affect bile production and bile acids secretion. Most of these mutations are on ABC transporters genes, and the phenotypic manifestations of these diseases are generally consistent with the function of these transporters (for a review, see Felzen and Verkade, 2021). These diseases are typically detected in early childhood, often followed by a severe course that can ultimately results in the need for liver transplantation either before or during adulthood. For some drug transporters, knockout mouse models develop a phenotype that is evidently independent of animal manipulation and/or treatment, while other transporters knockout animal models lack a phenotype unless challenged (Keppler, 2011b).

ABC Transporters Associated with DIC: Effect of Gene Mutations. The precise nature of the association between certain polymorphic forms of ABC transporters and DILI remains controversial, and more research is needed to bring clarity to this issue. It is well known that polymorphic forms of drug metabolic enzymes and certain human leukocyte antigen haplotypes are well associated with increments in susceptibility to DILI. However, genome-wide association studies have failed to identify similar associations between DILI and polymorphic forms of ABC transporters (Urban et al., 2012), at least on an individual basis. Despite the lack of a definite association between monogenetic variations in ABC transporters and DILI from genome-wide association studies, there are particular transporters polymorphisms that are more prevalent for certain drugs with a history of producing DILI (Daly, 2017). This is summarized in Table 1.

There are four highly conserved nonsynonymous mutations in genes of the ABC family of transporters, two in MDR3 and two in BSEP (Lang et al., 2007). In MDR3, there is a I764L polymorphism in the transmembrane domain and a L1082Q located close to the ATP-binding domain, both of them in evolutionarily conserved codons. The presence of these two heterozygous nonsynonymous mutations have been associated with the development of DILI in patients treated with risperidone and amoxicillin/clavulanic, respectively (Lang et al., 2007). This study also shows the presence of a common BSEP polymorphism in exon 13 (1331T>C-V444A) previously described in intrahepatic cholestasis of pregnancy (Keitel et al., 2006) and a newly reported one, D676Y. This latter polymorphic form of BSEP is more frequent in patients with DIC induced by fluvastatin. However, there are no differences in taurocholate transport when analyzed in vitro using an expression system for D676Y. In contrast, the V444A single nucleotide polymorphism, which is also more frequent in patients with DIC than in healthy controls or in patients with drug-induced disease, does exhibit lower transport activity when a construct of this polymorphic form of BSEP is expressed in vitro (Meier et al., 2006). Quantitative structure-activity relationship analysis shows that carriers of this mutation have an increased risk of developing hepatocellular DILI if treated with drugs containing a carbocyclic system with aromatic rings, such as some nonsteroidal anti-inflammatory drugs (Ulzurrun et al., 2013). It is important to note that this same mutation is associated with intrahepatic cholestasis of pregnancy (Pauli-Magnus et al., 2004).

The ABCC2 24C>T polymorphism (rs717620 T) is associated with increased risk of hyperbilirubinemia and mortality in a population of Japanese patients with DILI (Huang et al., 2021). This mutation is also more frequent in patients of European ethnic origin hospitalized due to diclofenac-induced DILI (Daly et al., 2007). A second well-characterized polymorphism for MRP2, –1774delG, shows a strong association with cholestatic or mixed-type hepatitis in the Korean population due to a decrease in MRP2 promoter activity and inducibility, principally by bile acids (Choi et al., 2007).

Mutations in BCRP are reported to alter transport activity and are also associated with DILI. The homozygous presence of the genetic polymorphism 421C>A (Q141K, rs2231142) in BCRP is associated with liver injury induced by the tyrosine kinase inhibitor sunitinib (Miura et al., 2014). This drug is a known substrate of BCRP, and the presence of this variant reduces its transport, leading to intracellular accumulation and drug toxicity. This polymorphism is approximately 3-fold higher in Asians than in Caucasians, and it is also associated with other adverse events, including thrombocytopenia (Low et al., 2016). Additionally, in vitro results from cells expressing the Q141K polymorphism showed decreased transport of mitoxantrone, a prototypical substrate for BCRP efflux (Morisaki et al., 2005). Finally, the MDR1 polymorphism 3435C>T has been associated with nevirapine-induced DILI in African (Haas et al., 2006) and US patients (Ritchie et al., 2006). Although the mechanistic role of this MDR1 mutation in DILI remains unknown, the presence of this variant alters PGP substrate

TABLE 1

Summary of different ABC transporters associated with DIC

ABC	Physiologic substrates	Drugs Associated with DILI (Wild Transporter or in the Presence of Polymorphism/s)		Reference(s)
		Name	Determined polymorphism	
BCRP (ABCG2)	Sulf-conjugates Anionic drug	Sunitinib (TKI)	421C>A Q141K, (rs2231142) Cholestatic (Reduction in transport activity)	(Miura et al., 2014)
BSEP (ABCB11)	BS (taurocholate and others cholate conjugates)	Bosentan; erythromycin; troglitazone; cyclosporine A; nefazodone; simvastatin; estolate; glibenclamide; sulindac; ethinylestradiol-17 -glucuronide (trans inhibition)	No association with wild-type transporter	(Stieger et al., 2000; Fattinger et al., 2001; Meier et al., 2006; Lang et al., 2007; Ulzurrun et al., 2013)
		Nonsteroidal anti-inflammatory drugs; drugs containing a carbocyclic system with aromatic rings	1331T>C V444A (rs2287622)	
		Heterozygous patient; fluvastatin	2026G>T D676Y	
		Heterozygous ethinylestradiol/gestodene	2568G>T G855R	
MDR3 (ABCB4)	Phosphatidylcholine	Oral contraceptives antifungal azoles (itraconazole)	No association with wild transporter	(Lang et al., 2007; Davit-Spraul et al., 2010; Yoshikado et al., 2011; Mahdi et al., 2016)
		Risperidone; oral anticonceptive	2290A>C I764L	
		Amoxicillin/clavulanic	3245T>A L1082Q	
MRP2 (ABCC2)	Bilirubin-Glu GSH Conjugates-GS Conjugates-gluc Organic anionic Drugs (−)	Ceftriaxone; diclofenac; synthetic estrogen	No association with wild transporter	(Choi et al., 2007; Daly et al., 2007; Braga et al., 2016; Huang et al., 2021)
		Homozygosis; deferasirox	−1774delG rs369192412	
		Diclofenac	−24 C>T polymorphism rs717620 T	
PGP (ABCB1)	Organic Cation Drugs (+)	Cyclosporine A; verapamil; erythromycin chlorpromazine; ivermectin	No association with wild-type transporter	(Haas et al., 2006; Ritchie et al., 2006)
		Nevirapine	3435C>T (rs1045642)	

specificity, which is of potential impact on the effectiveness of several drug treatments (Drain et al., 2010).

ABC Transporters Associated with DIC: Effect of Drugs. Perhaps the most relevant mechanism correlating ABC transporters function to DILI, particularly in the drug development area, is through chemical competition for transport and/or function inhibition (summarized in Table 1). Among all ABC transporters, inhibition of BSEP is considered to be a primary mechanism of DILI initiation that often escapes detection during preclinical studies (Kenna et al., 2018). The direct consequence of BSEP inhibition by drugs is the intrahepatic accumulation of cytotoxic bile acids. Although this mechanism of DILI has wide acceptance, our understanding of the relationship between BSEP inhibition of DILI is not complete. Given the multifactorial nature of DILI, BSEP inhibition should be considered in the context of other potential mechanisms when assessing the risk of a drug for producing DILI. A number of medications with a DILI liability produce Bsep inhibition via two different mechanisms: trans- or cis-inhibition (Kis et al., 2012). This distinction is important when selecting and interpreting in vitro Bsep inhibition/uptake screenings. Most Bsep inhibitors are direct-acting cis inhibitors (Morgan et al., 2010). Troglitazone and its metabolites produce cis-inhibition of Bsep, resulting in severe ALF. This, along with the mitochondrial damage and oxidative stress induced by troglitazone and its metabolites, led to the final withdrawal of this antidiabetic agent from the US market (Funk et al., 2001). There are various other compounds with DILI risks that produce cis-inhibition of BSEP, including cyclosporin A (Cadranel et al., 1992; Myara et al., 1996), rifampicin (Yang et al., 2021), bosentan (Fattinger et al., 2001), erythromycin (Mondragón-Navarro et al., 2016), and glibenclamide (Tholakanahalli et al., 1998; Vats et al., 2020). Concentration-dependent trans-inhibition of BSEP is reported for some steroid drugs, such as estrogen and progesterone metabolites, after their transport into the bile canaliculus by MRP2 (Stieger et al., 2000; Vallejo et al., 2006). Trans-inhibition of Bsep by estradiol-17β-glucuronide inhibits ATP-dependent taurocholate transport only in normal, but not Mrp2-deficient, canalicular liver plasma membrane vesicles, documenting the need for estradiol-17β-glucuronide to be secreted first into the bile canaliculus for inhibiting Bsep (Stieger et al., 2000). The endothelin receptor antagonist bosentan produces dose-dependent cholestasis (Fattinger et al., 2001). Co-administration with glyburide, another known BSEP inhibitor, potentiates the increment in serum levels of bile acids and AP produced by bosentan alone, while there is inhibition of taurocholate transport by bosentan and its metabolites in an in vitro study using canalicular rat liver plasma membrane and Bsep expressing Sf9-cell vesicles.

Dawson et al. (2012) examined the relationship between the potency of certain drugs for inhibiting BSEP and their propensity to produce clinically relevant DIC. This screening tested the potency of 85 pharmaceuticals to inhibit the ATP-dependent [3H]-taurocholate uptake into inverted plasma membrane vesicles from Sf21 insect cells expressing either human BSEP or its rat ortholog. The drugs were divided into three categories: cholestatic or mixed cholestatic/hepatocellular DILI ($n = 42$), hepatocellular DILI ($n = 22$), and no DILI drugs ($n = 21$). The results show that of the 42 tested compounds known to cause either DIC or DILI of mixed pathogenic manifestation, slightly over half (64%) of these drugs had higher BSEP inhibitory potencies than the drugs in the other two categories. Furthermore, the incidence of BSEP inhibition for the 22 compounds that are only associated with hepatocellular DILI was considerably lower than that for those compounds with documented mixed DILI risk. Similar results were obtained for compounds with no reported DILI risk. Of note, the study also showed differential inhibitory potencies for certain drugs between the human and rat forms of BSEP (Dawson et al., 2012). In a more recent article, Chan and Benet (2018) challenged this relationship between BSEP inhibitory potency by drugs and its implication in DILI pathogenesis and mechanism of action. This study concluded that there is no support for predicting DILI based solely on in vitro BSEP inhibition analysis (Chan and Benet, 2018).

Inhibition of other ABC transporters by drugs has been shown to produce DIC. A typical example is the antifungal azole family of drugs (Mahdi et al., 2016). In vitro studies demonstrate that itraconazole inhibits [^{14}C] phosphatidylcholine transport by MDR3 (Yoshikado et al., 2011). Similar results are seen in an in vitro transwell system with a monolayer of LLC-PK1 cells stably transfected with human MDR3 (Mahdi et al., 2016). Screening antifungal drugs in this system also shows inhibition of BSEP activity, thus indicating that the cholestatic actions of antifungals can involve more than one efflux transporter (Mahdi et al., 2016).

MRP3 and MRP4 are important basolateral efflux transporters for bile acids, especially during cholestasis (Keppler, 2011a). Köck et al. (2014) studied the inhibitory effect of 88 drugs on MRP3- and MRP4-mediated substrate transport using isolated membrane vesicles. The selected drugs for this screening are classified as 38 BSEP inhibitors (16 noncholestatic, 22 cholestatic) and 50 non-BSEP inhibitors (24 noncholestatic, 26 cholestatic). The results indicate that when MRP4 inhibition is detected, the risk of DIC is greater only with non-BSEP inhibitors. This strongly suggests that MRP4 inhibition does not provide any additional DILI risk among BSEP inhibitors. Furthermore, the same study also show that MRP3 inhibition does not increase the risk of DIC among the drugs screened (Köck et al., 2014), in contrast to troglitazone and its sulfated metabolite, which are able to inhibit not only BSEP transport but also MRP4 and MRP3 (Yang et al., 2014).

ABC Transporters Associated with DIC: Effect of Other Factors. In addition to drugs, changes in membrane microenvironment can modify the functionality of ABC transporters. For example, Bsep and Mrp2 are located in separate membrane microdomains and are both co-localized with ATP8B1 (Ismair et al., 2009). ATP8B1, a member of the P4 subfamily of the P-type ATPase superfamily, is a phosphatidylserine translocase that flips phospholipids from the outer to the inner leaflet of the plasma membrane (Ujhazy et al., 2001). ATP8B1 has a protective function by making the outer leaflet more resistant to the detergent action of bile salts in the canalicular lumen due to its richness in phosphatidylcholine, sphingomyelin, and cholesterol (Amigo et al., 1999). Decreased ATP8B1 activity is associated with a decrease in the activity of both Bsep and Mrp2 resulting from asymmetry in membrane microdomains characterized by an increment in phosphatidylserine outer membrane content, with these events leading to cholestasis (Paulusma et al., 2009).

A factor contributing to the development of DIC is the level of expression of drug transporters. In one study, 8 out of 12 patients with DIC show decreased expression of BSEP, MRP3, and MRP2 (Zollner et al., 2014). As with many other genes, ABC transporters expression is tightly regulated by transcriptional (for a review, see Rigalli et al., 2019) and post-transcriptional mechanisms (for a review, see Czuba et al., 2018).

Nuclear receptors are the principal modulators of transcriptional expression of ABC transporters (for a review, see Rigalli et al., 2019). Among them, and tightly correlated with drug-induced cholestasis, is the farnesoid X receptor (FXR, NR1H4). This transcription factor is the main regulator of BSEP expression and function (Plass et al., 2002). FXR also regulates MDR3 (Huang et al., 2003), MRP2 (Plass et al., 2002), and PGP (Jiang et al., 2013). When bile acids, such as chenodeoxycholic acid, deoxycholic acid, and cholic acid, bind to FXR, this interaction promotes FXR heterodimerization with the retinoid X receptor (Yu et al., 2002). This signaling-competent heterodimer binds to the

FXR response elements in the promoter region of target genes, inducing the expression of the aforementioned ABC transporters.

Interestingly, post-transcriptional modifications of FXR occurs under cholestatic conditions with an increased acetylation of lysine 217, which decreases its DNA binding and transactivation activity (Kemper et al., 2009; Kulkarni et al., 2016). FXR itself and its surrounding histones, when bound to DNA, can be deacetylated by sirtuin 1 (SIRT1), a class III nicotinamide adenine dinucleotide–dependent histone deacetylase; therefore, SIRT inactivation decreases DNA–FXR binding. SIRT1 lacks a DNA-binding domain, so it needs to be transactivated by FXR for recruitment into its own target genes promoters (Yang et al., 2017). Activation of the SIRT1/FXR pathway by small molecules produces FXR deacetylation, which helps reverse DIC (Qu et al., 2018; Zhao et al., 2019). However, continuous activation or overexpression results in ubiquitination and proteasomal degradation of FXR, which can revert back the cholestatic condition (Blokker et al., 2019).

Additional transcription factors can play a role in the regulation of hepatobiliary drug transporters. Hepatocyte-specific liver receptor homolog-1 (NR5A2) (Zwicker and Agellon, 2013) and the oxidative stress sensor Nrf2 regulate BSEP expression (Weerachayaphorn et al., 2009). Nrf2 is also involved in the induction of hepatic metabolic enzymes mediating phase I and II reactions in a coordinated fashion with the regulation of other ABC transporters, such as MRP3 and MRP4 (Taoka et al., 2016). Pregnane X receptor (PXR) is another ligand-dependent nuclear receptor that controls the inducible expression of drug metabolizing enzymes and drug transporters (Aleksunes and Klaassen, 2012). Transcriptional regulation of BSEP by PXR is particularly well-characterized. The recruitment of other components to the PXR/retinoid X receptor heterodimer to PXR responsive element complexes enhances BSEP expression. One of them is the cointegrator-2-containing complex (ASCOM), which normally interacts with FXR as the activating signal but is disrupted during cholestasis, decreasing BSEP expression (Ananthanarayanan et al., 2011). Other known PXR coregulators are coactivator-associated arginine methyltransferase 1 (Ananthanarayanan et al., 2004) and steroid receptor coactivator 2 (Chopra et al., 2011; for a review, see Sohail and Dönmez-Cakil, 2021).

Post-transcriptional targeting of Bsep, Mdr2 (the rat homolog of human MDR3), and Mrp2 to the canalicular membrane of hepatocytes is necessary to sustain bile formation functionality and biliary elimination of xenobiotics and endogenous compounds. (for a review, see Roma et al., 2008). Several ABC transporters, but more prominently Bsep, are constantly recycled between apical membranes and intracellular subapical endosomal compartments (Wakabayashi et al., 2004). When this recycling balance is disrupted by a shift toward greater endocytic internalization, reduced bile acid extraction from hepatocytes occurs, which can result in cholestasis (Crocenzi et al., 2012). In a model of intrahepatic cholestasis of pregnancy produced by estradiol-17β-D-glucuronide (Crocenzi et al., 2003) and in cholestasis produced by cyclosporin A (Román et al., 2003), an enhanced retrieval of Bsep into the subapical endosomal compartment has been demonstrated. This transporter recycling is a clathrin-dependent endocytosis process (for a review, see Miszczuk et al., 2018). Basically, this Ca2+-dependent internalization process during cholestasis induced by estradiol-17β-D-glucuronide is mediated by signaling events involving protein kinase C–p38 and phosphoinositide 3-kinase–extracellular signal-regulated kinase 1/2 (Crocenzi et al., 2008; Boaglio et al., 2010). Bsep internalization is decreased when its association with α- and μ2-adaptin (subunits of the AP2 adaptor complex) (Hayashi et al., 2012), and hematopoietic cell-specific Lyn substrate 1–associated protein X1 (HAX1) is disrupted (Ortiz et al., 2004). The same authors also demonstrated that HAX1 colocalizes with BSEP, MDR1, and MDR2 in the canalicular membrane. RNA interference that silences HAX1 leads to increases in BSEP protein levels in the apical membrane domain of Madin–Darby canine kidney cells in culture.

While individual factors can mechanistically modulate the expression and activity of ABC transporters, it is quite common for more than one mechanism to converge in the pathophysiology of DILI. The immunosuppressant drug cyclosporin A illustrates this concept (Tazuma, 2006). This drug, initially described as a competitive inhibitor of BSEP (Stone et al., 1987; Böhme et al., 1993), also decreases the targeting of Bsep to the canalicular membrane of hepatocytes (Román et al., 1990). Cyclosporin A also decreases Bsep activity by altering its microenvironment through a reduction in canalicular membrane fluidity (Yasumiba et al., 2001). The transport inhibitory function of cyclosporin A is not limited to Bsep; it also affects Mrps transporters and Pgp (Böhme et al., 1993). The coadministration of cyclosporin A and verapamil, another well-known inhibitor of Pgp, worsens the cholestasis produced by cyclosporin A alone in a rat model (Delle Monache et al., 1999). This interaction has also been demonstrated in humans (Padda et al., 2011).

Drugs that produce mitochondrial dysfunction and/or loss of mitochondrial integrity carry a risk of DILI (Dykens and Will, 2007; Labbe et al., 2008). This risk can be greater when the drug simultaneously interferes with ABC transporters function or by combining drugs that inhibit mitochondrial and transporter function separately. In this latter instance, the combined effect of a transporter inhibitor and a mitochondrial toxicant could lead to more severe forms of DILI (Porceddu et al., 2012). Illustrative of this, a high-throughput in vitro screening that used isolated mouse liver mitochondria was carried out with 124 chemicals (primarily drugs) to determine whether DILI can be predicted by the ability of these chemicals to impair mitochondrial function. Eighty-seven of the 124 chemicals had previously documented clinically relevant DILI, while the remaining 37 did not. Valproic acid, aspirin, tamoxifen, diclofenac, and tacrine are among the list of DILI compounds tested. This screening conclusively established a strong relationship between mitochondrial dysfunction (or toxicity) and DILI and had a high predictive value. Mitochondria targeting might explain the synergistic worsening of DILI when drugs that inhibit ABC transporter function are combined with drugs that interfere with mitochondrial function or drugs with both effects (Aleo et al., 2014). Another link between mitochondrial dysfunction and transporter activity is limitation in intracellular ATP availability, essential for driving ABC transporter function.

Current Challenges and Knowledge Gaps

Nowadays, polypharmacy is a very frequent practice that is primarily seen in elderly patients and can carry an increased risk of DILI (Chen et al., 2015). The current liver parameters recommendations, alanine aminotransferase, and AP values, generated by an international DILI Expert Working Group, allow for diagnosis and partial characterization of the DILI etiology (Aithal et al., 2011). However, these diagnostic endpoints cannot accurately predict the clinical course and ultimate DILI outcome, nor can they help identify the causative agent or the precise mechanism involved when more than one medication is used by the patient.

The current animal models available for studying intrinsic DILI are reproducible and mechanistically relevant to the human situation. The prototypical example is APAP, a classic hepatotoxicant studied for five decades. In contrast, for IDILI, there are relatively few animal models for its study, since its manifestation is dependent on the interaction of drug–host–environment (reviewed in Corsini et al., 2012). Despite the assumptions of similar modes of action/activation of the drug, there are limitations in traditional animal studies. A principal one is due to species differences resulting from the different genetic backgrounds that can influence drug–host interactions. A second limitation of animal

studies for IDILI is that only a limited number of drugs mimic the degree of injury produced in humans, and toxicity is observed in a small number of the animals treated. Lastly, animal studies often require prolonged drug exposure periods, increasing the study costs.

New biomarkers of DILI, such as high mobility group box protein 1, K18, and microRNA-122, are being studied in terms of increasing sensitivity, specificity, severity, and DILI predictability. The era of integrative "-omics" approaches and their application in investigative toxicology is expected not only to advance understanding of mechanisms of DILI but also to accurately identify drugs responsible for producing liver adverse events in humans (for a review see, Kullak-Ublick et al., 2017). Progress is further enhanced by the development of novel in vitro assays.

In the last decade, a biologic test called MetaHeps was recently introduced into the market. The assay is based on the use of monocyte-derived hepatocyte-like (MH) cells from the patient's own peripheral monocytes that are then cultured under a patented protocol (Benesic et al., 2012). A panel of drugs is then assayed for in vitro toxicity by lactate dehydrogenase release. MH cells have the same characteristics as primary human hepatocytes derived from the same donor, including drug-metabolizing enzymes capacity and ABC transporters function (Benesic et al., 2016). The MH cells assay was useful in identifying the culprit drug causing idiosyncratic DILI in a prospective study in patients consuming more than one medication (Benesic et al., 2016). Similarly, another prospective study demonstrated the value of this assay for studying hepatotoxicity of herbal and dietary supplements (Weber et al., 2021). This assay is useful to examine potential drug–drug interactions at the onset of DILI (Benesic et al., 2019). Adoption of the MH cell assay could be a tool to better define the origins of DILI reactions, particularly in patients on polypharmacy. Once the drug(s) responsible for DILI are identified, a systematic study of the drug metabolizing and transporters capacity can be performed on the same patient using the same MH cell tool. This investigative work could generate a much more robust comprehension of the interaction between drug therapy and host characteristics in a patient-by-patient manner.

Considering the current gaps in understanding of drug–host interactions, it is quite challenging to establish a comprehensive interplay between drug metabolism and transport networks activated and/or inhibited by drugs and its relationship to DILI. Future studies should be aimed at identifying factors that can modify risk, and research into the interactions of host–drugs is needed to deepen our knowledge of these interactions, allowing us to move toward a safer and more personalized medicine approach to treatment.

Perspective on Future Directions

The gastrointestinal (GI) microbiota and epigenetic regulation of drug-metabolizing enzymes and transporters are two research areas of emerging importance that can advance understanding of the pathogenesis of DILI and interindividual variability in DILI frequency and severity. The GI microbiota has the capacity of digesting food nutrients, producing endogenous metabolites, and biotransforming xenobiotics. The metabolic activity of intestinal microbes can produce dual and opposing effects, sometimes contributing to the maintenance of health, while in other instances promoting hepatopathological conditions, such as fatty liver disease (Dietert and Dietert, 2015; Sharpton et al., 2019). Illustrative of the importance of the gut–liver axis in liver disease is lithocholic acid, a secondary bile acid generated by the gut microbiota and a PXR antagonist that decreases BSEP expression and could produce cholestasis if abundantly generated (Yu et al., 2002). Glucuronidases in the intestinal microbiota cleave APAP-Glu or tacrine–glucuronide conjugates, releasing the parent compound that can then be reabsorbed and transported to the liver via enterohepatic circulation, re-exposing the liver again to the drug and increasing its potential for producing toxicity (Ghanem et al., 2009; Yip et al., 2018). Another area that deserves attention for its potential contribution to interindividual variability in DILI frequency and magnitude is circadian variations in the function of the GI microbiota (Gong et al., 2018).

The mechanistic link between the gut microbiota to the pathophysiology of DILI remains largely unknown and should receive greater attention. There is relatively recent new evidence indicating that the gut microbiota modulates several clusters of hepatic genes involved in xenobiotic metabolism through modulation of PXR binding to DNA (Selwyn et al., 2016). Selwyn et al. (2016) demonstrated that germ-free mice have a different pattern of Cyp450 gene expression. These mice showed a marked downregulation in the expression of Cyp3a gene isoforms, occurring concomitantly with an upregulation of Cyp4a isoforms. These changes correlated well with alterations in both PXR and peroxisome proliferator-activated receptor α DNA binding. Additionally, analysis of cholestasis induced by 5-day bile duct ligation using conventional and germ-free mice showed that in mice devoid of gut microbiota, hepatic bile infarcts are more pronounced, and there is greater susceptibility to hepatic inflammation. These findings are associated with microbiota-dependent alterations in the expression of genes involved in metabolism of fatty acids and amino acids and also due to increased gene expression of IL-1β and activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway. By contrast, the presence of gut bacteria enhances ductular reactions, cell proliferation, deposition of collagen 1, and cell autophagy (Juanola et al., 2021). Despite the dramatic surge in microbiome research in the last decade, much more needs to be known about the relationship between the gut microbiome and health and disease, including how changes in its composition and function influence liver diseases, including DILI (Forootan et al., 2017; Chan and Benet, 2018; Li et al., 2021).

Finally, epigenetic regulation of drug metabolizing enzymes (Jin et al., 2016) and transporters (Zappe and Cichna-Markl, 2020), such as DNA methylation, histone modification, and noncoding RNAs, has gained considerable attention recently. However, there are no systematic studies that associate epigenetic modifications with DILI susceptibility. There is evidence, however, that epigenetic mechanisms are involved in the pathogenesis of primary biliary cholangitis (Li et al., 2020) and intrahepatic cholestasis of pregnancy (Xiao et al., 2021). The H3K4me3 epigenetic mark is downregulated during cholestasis, decreasing the recruitment of the ASCOM complex to FXR binding elements in the BSEP and MRP2 gene promoters (Ananthanarayanan et al., 2011). It has been demonstrated that perturbations in the intrauterine environment during intrahepatic cholestasis of pregnancy produce an epigenetic imprinting during postnatal life that predisposes offspring to metabolic diseases in adulthood (Borges Manna et al., 2020).

This review highlights many recent advances in the study of drug transporters and DILI, yet more attention needs to be dedicated to addressing the knowledge in drug–host interactions, the role of GI microbiota, and the epigenetic regulation in the expression and activity of ABC transporters and their individual and combined contributions to DILIs.

Conclusion

Collectively, this review summarizes substantive evidence indicating that drug transporters, particularly ABC transporters, play a pivotal role in the development of DILI. The following statements capture the major points addressed in this review:

- ABC transporters are involved in the hepatobiliary disposition and hepatotoxicity of APAP, the most frequent drug causing intrinsic DILI.

- The pharmacokinetics and pharmacodynamics of multiple other drugs that are strongly associated with DIC and various forms of IDILI have well-documented interplays with drug transporters.
- There are different polymorphisms in hepatobiliary transporters identified, principally associated with BSEP, that modify protein expression and/or activity for this main efflux pump for bile acids. These polymorphisms are associated with DILI-causing drugs.
- Post-transcriptional modulation of transporters expression and/or function are considered to be causative of several types of DILI.
- The continuous development and increase in sophistication of multiomic platforms provide unique opportunities to deepen the mechanistic understanding of the role of ABC transporters in the various types of DILI.
- Greater knowledge is emerging from the fields of the gut microbiome and epigenetics. Although the GI microbiota status and composition is known to influence drug biotransformation, significantly less is known about its impact on drug transporters expression and function. Similarly, an increased understanding on the epigenetics of drug transporters can be instrumental in better defining mechanisms of DILI.

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