XENOBIOTIC, BILE ACID, AND CHOLESTEROL TRANSPORTERS

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Table of Contents

1. Overview

Uptake and efflux transporters determine plasma and tissue concentrations of a broad variety of drugs, nutrients, and xenobiotics (1-3). The liver is the primary organ for the metabolic degradation of xenobiotics. Transmembrane transport proteins from the ATP binding cassette (ABC) and the solute carrier (SLC) families mediate the uptake and efflux of endogenous compounds, xenobiotics, and their metabolites in hepatocytes. The activity of these transporters is an important aspect of the pharmaco-kinetics and dynamics of drugs and is important for drug-drug and drug-nutrient interactions. Additionally, monogenetic diseases exist which can be explained by absence of function or dysfunction of specific hepatic transporters, such as progressive familial intrahepatic cholestasis. Functional impairment or inhibition of these transport systems may lead to liver injury. Several nuclear receptors, such as pregnane X receptor (PXR) and constitutive androstane receptor (CAR), coordinately regulate the levels of important enzymes like cytochrome P450 3A4 (CYP3A4) as well as drug transporters including ABCB1 (MDR-1). Understanding of the molecular mechanisms that affect enzyme and transporter activity may contribute to the predictability of drug-drug interactions and eventually help to develop safer therapeutic regimens.

2. Significance of Hepatic Transport Systems in Drug Disposition

Uni- or bi-directional basolateral transport systems translocate polar molecules from hepatic cytosol into blood, whereas active canalicular transport systems are responsible for the biliary excretion of drugs and metabolites (see Figure 1). There is widespread interest in the hepatic transport of drugs and metabolites among pharmaceutical scientists, including medicinal chemists, pharmacologists, and clinicians, for several reasons:

a. Drug Design (Drug Delivery)

Knowledge of structure-transport relationships for hepatic transport proteins would aid in the design of compounds with optimal transport properties. In some cases, extensive hepatic uptake or enhanced biliary excretion may be desirable characteristics for a potential drug candidate. In other situations, extensive hepatic uptake and biliary excretion may reduce systemic exposure and limit pharmacological activity, thus representing undesirable properties of the molecule.

b. Bioavailability

The liver is an important organ of first-pass elimination. Reduced or erratic systemic availability of drugs after oral administration may be related to dietary, disease, or drug-induced alterations in hepatic transport systems. For example, induction of a hepatic transport protein responsible for the hepatic uptake or biliary excretion of a drug could decrease systemic availability of that drug after oral administration. In addition to the liver, drug transport proteins that reside on the basolateral and apical membranes of the gastrointestinal epithelial cells are also crucial determinants of the bioavailability of many drug molecules.

c. Biliary Excretion

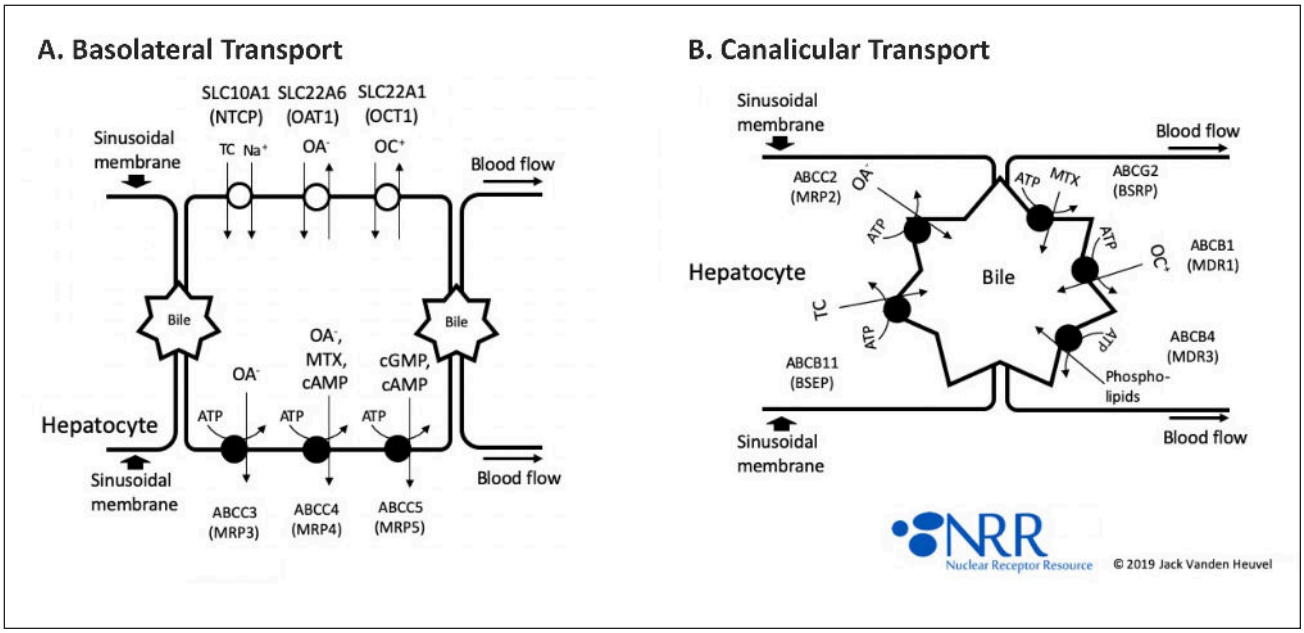
Compounds are excreted into bile by ATP-dependent canalicular transport proteins. The extent to which most drugs and metabolites undergo biliary excretion in humans is not readily appreciated due to the difficulties inherent in directly accessing bile drainage in healthy individuals. Many potentially useful therapeutic agents may be excluded in the early stages of drug development due to extensive biliary excretion that limits systemic exposure. Biliary excretion of drugs or metabolites may expose the intestinal epithelia to pharmacologically active or toxic species that can exert dose-limiting toxicities.

d. Interindividual Variability in Drug Pharmacokinetics and Pharmacodynamics

Disease-associated or genetic alterations in the expression and/or function of hepatic transport proteins may alter significantly the disposition of many endogenous and exogenous compounds, including drugs

and metabolites. Although this field of research is still in its infancy, hepatic transport systems are clearly responsible for important variations in the disposition, pharmacological activity, and toxicity of some drugs. Elucidating mechanisms of interpatient variability in hepatic drug transport systems is prerequisite to achieving desirable therapeutic outcomes in diverse patient populations.

Figure 1. Xenobiotic, bile acid, and cholesterol transporters in the liver. TC, taurocholine (bile salts); OA-, organic anions; OC+, organic cations; MTX, methotrexate; cAMP, adenosine 3'5'-cyclic monophosphate; cGMP, guanosine 3',5'-cyclic monophosphate.



e. Drug/Nutrient-Transport Interactions

Drugs and nutrients may interact with hepatic transport proteins resulting in enhanced or impaired transport activity. Such interactions may be direct or indirect in nature and may involve alterations in expression as well as function of the transport protein. Elucidation of the clinical importance of hepatic transport interactions and development of methods to predict these interactions offer many exciting opportunities for research in the upcoming decades.

3. Xenobiotic, Cholesterol and Bile Acid Transporters in Liver

A variety of uptake and efflux transporters are localized to the apical and basolateral membranes of hepatocytes (1). Molecules may be excreted from the hepatocyte across the basolateral membrane into sinusoidal blood with subsequent elimination by other organs (e.g., kidney) or across the canalicular membrane into bile, which is stored in the gallbladder and is released periodically into the upper small intestine (Figure 1, adapted from (4)). Hepatic transport proteins belong to either the superfamily of sodium-independent transport systems designated "solute carriers" with the root gene symbol designated "SLC" or to the ATP-binding cassette superfamily with the root gene symbol designated "ABC." Trivial names are still prevalently used and include, organic anion transport (OAT), organic cation transport (OCT), and multidrug resistance proteins (MRPs, MDR).

a. Drug Transport Proteins of the Hepatic Basolateral Membrane

The basolateral transport proteins, belonging to the gene superfamily of solute carriers (SLC), mediate the movement of compounds to and from the sinusoidal blood (some examples are shown in Figure 1, panel A). SLC10A1 (NTCP) is a bile acid uptake transporter that localizes to the basolateral membrane of hepatocytes. SLC22A6 (OAT1) and SLC22A1 (OCT1) are involved in uptake/export of organic anions (OA) and organic cations (OC), respectively. Removal of chemicals from the hepatocyte to the sinusoidal blood is accomplished by transporters on the basolateral membrane including ABCC3 (MRP3), ABCC4 (MRP4), and ABCC5 (MRP5). Glucuronidation is important for the detoxification and excretion of polar chemicals. Glucuronide conjugates and other organic anions are substrates of ABCC3. Several predominate transport proteins on the basolateral membrane and their substrates are summarized in Table 1.

Table 1. Human hepatic basolateral transport proteins

GENE SYMBOL	TRIVIAL NAMES	SUBSTRATES
SLC10A1	NTCP	Binds two sodium ions and one (conjugated) bile salt molecule, thereby providing a hepatic influx of bile salts. Other transported molecules include steroid hormones, thyroid hormones, and various xenobiotics.
SLC22A6	OAT1	Organic anion exchanger where uptake of one molecule of an organic anion is transported into a cell and one molecule of an endogenous dicarboxylic acid (such as glutarate, ketoglutarate, etc.) is simultaneously transported out of the cell.
SLC22A1	OCT1	Transport of organic cations (protonated molecules), such as tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium (MPP+), N1-methylnicotinamide (NMN), dopamine, and choline.
ABCC3	MRP3	Transports organic anions, such as bile acids and drug-glucuronide conjugates, including morphine-3-glucuronide and acetaminophen-glucuronide.
ABCC4	MRP4	Regulator of intracellular cyclic nucleotide levels and as a mediator of cAMP-dependent signal transduction to the nucleus. Transports prostaglandins, for example PGE2, out of the cell where they can bind receptors.
ABCC5	MRP5	Cellular export of its substrate, cyclic nucleotides as a potential elimination pathway. Provides resistance to thiopurine anticancer drugs, 6-mercatopurine and thioguanine.

b. Drug Transport Proteins of the Hepatic Canalicular Membrane

The canalicular transport system is comprised of ABC family members that are able to transport xenobiotics, phase 2 metabolites, and bile acids, against a concentration gradient into the bile duct. The bile salt export pump (BSEP, gene symbol ABCB11) is the primary transporter for the extrusion of bile salts into the bile canaliculi (3). Bile salts have detergent properties and may damage mitochondria, which leads to cytotoxicity and liver injury. In fact, the inhibition of BSEP is an important molecule initiating event in the adverse outcome pathway for cholestasis (4). Multidrug resistance protein 1 (MDR1, P-gp, ABCB1) is the most prominent xenobiotic transporter present in virtually all tissues with barrier function. This transporter was first discovered and extensively investigated in the context of resistance

of tumor cells against antineoplastic agents. The transporter mediates the elimination of a broad variety of xenobiotics from cells, and it shows wide overlap in substrate specificity with other outward-directed drug transporters. In Table 2, predominant canicular transport proteins and their substrates are depicted.

Table 2. Human hepatic canalicular transport proteins

GENE SYMBOL	TRIVIAL NAMES	SUBSTRATES
ABCC2	MRP2	Unidirectional efflux transporter that primarily transports organic anions, including drug conjugates and conjugated bilirubin.
ABCB11	BSEP	Unidirectional, ATP-dependent efflux transporter that plays an important role in the elimination of bile salts.
ABCG2	BSRP	Functions as a xenobiotic transporter which may play a role in multi-drug resistance to chemotherapeutic agents including mitoxantrone and camptothecin analogues.
ABCB1	MDR1, P-gp	ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs.
ABCB4	MDR3	Phosphatidylcholine is an important substrate ("flippase" activity) and transports phospholipids from liver hepatocytes into bile.

4. Regulation of Hepatic Transporters by Xenobiotic-Activated Transcription Factors

Understanding the basic regulation of hepatic transport proteins will facilitate predictions of how drugs and/or disease states alter hepatic transport of endogenous and exogenous compounds and potential impact toxicity. An important mechanism for regulation of membrane transport activity is changes in the number of transport molecules present in the membrane. Long-term modulation of transport protein expression can occur at several different levels: transcription, translation, and post-translation. Transcription factors play an important role in the regulation of transporter gene expression in hepatocytes, in particular members of the nuclear receptor superfamily.

Coordinated up-regulation of drug-metabolizing enzymes and transporters is mediated by a number of hepatic transcription factors (reviewed in (1; 3)). Transcription factor-mediated up-regulation of hepatobiliary transporters has been reported to be mediated by the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR, NR1I3), pregnane X receptor (PXR, NR1I2), peroxisome proliferator-activated receptor (PPAR, NR1C1), and farnesoid X receptor (FXR, NR1H4). These receptors (with the exception of AhR) function by heterodimerizing with the retinoid X receptor (RXR, NR2B1). Other transcription factors involved in transporter regulation include the oxidative stress sensor, nuclear factor erythroid 2-related factor 2 (Nrf2, NFE2L2) and the liver-specific regulators known as hepatocyte nuclear factors (HNF). The regulation of transporter expression is summarized in Figure 2.

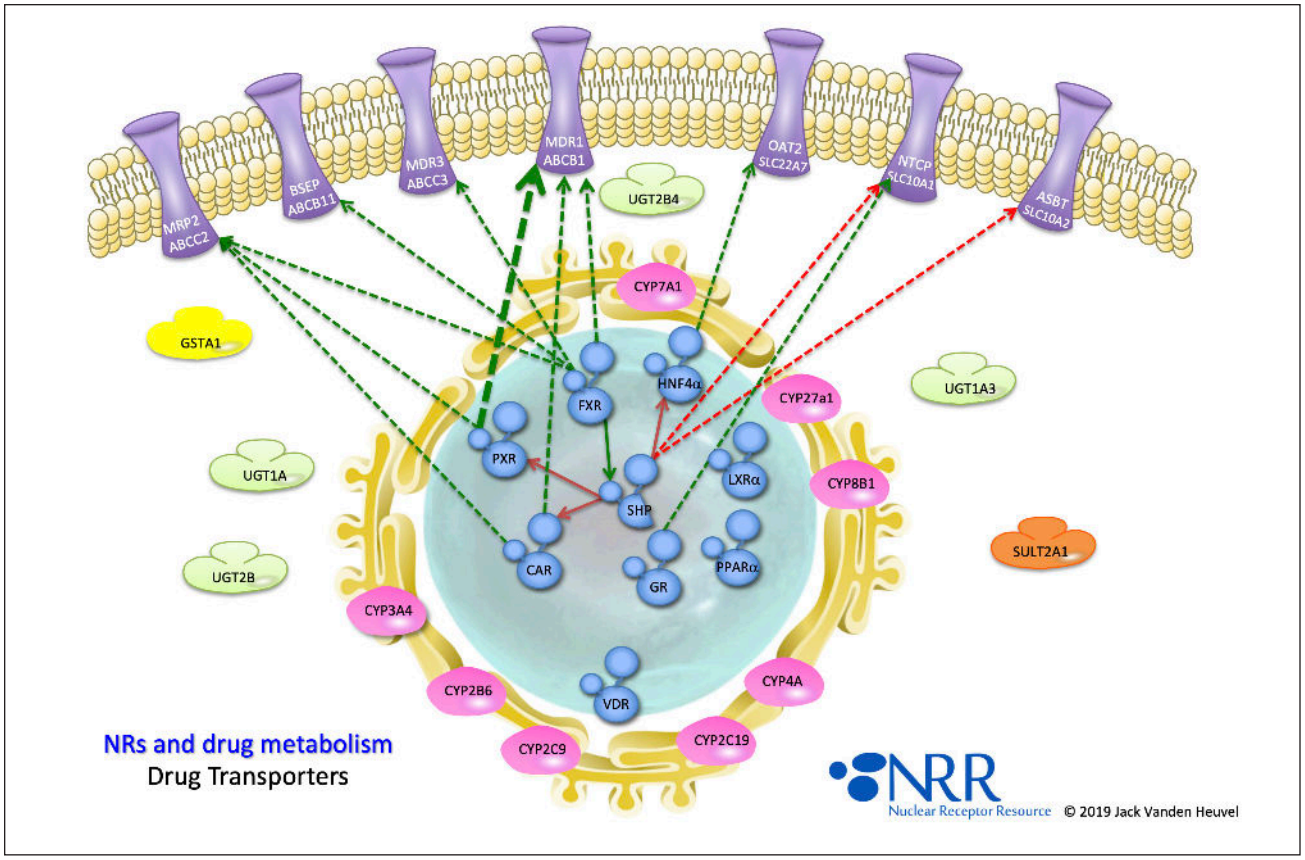


Figure 2. Regulation of drug transporters by nuclear receptors

5. Citations

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