

Drug-Induced Cholestasis

Manmeet S. Padda,¹ Mayra Sanchez,² Abbasi J. Akhtar,³ and James L. Boyer²

Recent progress in understanding the molecular mechanisms of bile formation and cholestasis have led to new insights into the pathogenesis of drug-induced cholestasis. This review summarizes their variable clinical presentations, examines the role of transport proteins in hepatic drug clearance and toxicity, and addresses the increasing importance of genetic determinants, as well as practical aspects of diagnosis and management. (HEPATOLOGY 2011;53:1377-1387)

The liver is the central organ responsible for the selective uptake, metabolism, and excretion of drugs, xenobiotics, and environmental toxins. This essential function predisposes the liver to drug toxicity and is the primary reason for the failure of pharmaceutical agents during drug development. Hepatic drug toxicity is the most common cause of acute fulminant hepatic failure, accounting for more than 50% of cases.¹ More than a thousand drugs and herbal remedies have been reported to cause a variety of different liver disorders. However, specific diagnostic markers for drug-induced liver injury (DILI) are lacking, and convincing cause-and-effect evidence exists for few cases.² Indeed, establishing causality has been a major hindrance in the understanding of DILI.^{3,4}

Cholestatic & mixed cholestatic and hepatocellular injury are two of the most severe manifestations of drug-induced liver disease (DILD),⁵ and account for close to half of all hepatic drug toxicity in some epide-

miologic reports.⁶ There is increasing evidence that drugs that are excreted by the liver into bile are prime candidates for producing cholestatic liver disease in the susceptible patient.⁷

Several forms of cholestatic liver injury can be produced by drugs, and these can present acutely or in the form of chronic liver disease. Drug-induced cholestasis may mimic other intrahepatic and extrahepatic cholestatic diseases. Not recognizing a drug as a triggering factor for cholestasis prolongs exposure to the toxic agent, which may lead to worse liver injury and unnecessary diagnostic and therapeutic interventions.

Acute and chronic cholestatic liver injury results from dysfunction of the mechanisms of bile formation. However, drug-induced cholestasis can present with asymptomatic disease where the only clinical manifestation is an elevation in alkaline phosphatase (AP). Moreover, the target of injury can vary from a mixed hepatocellular cholestatic injury, to impairment of canalicular bile flow resulting in pure intrahepatic cholestasis, or to an "obstructive" drug-induced cholangiopathy where the initial site of injury is located at various levels of the bile duct epithelium.⁸⁻¹⁰

The incidence and associated health care costs secondary to drug-induced cholestasis are not available, in part because most drugs commonly cause asymptomatic cholestasis associated with mild abnormalities in the serum liver profile. A Danish study of 110 cases of DILI from 1978 to 1987 reported a 17% prevalence of acute cholestatic injury.¹¹ In the United States, the prevalence of drug-induced cholestasis was reported to be 20% in the elderly population. However, on examination, not all of these cases included patients with cholestasis.¹² Approximately 2%-5% of hospitalized cases with jaundice are caused by drugs but cholestasis is expressed in only some of these patients.¹³⁻¹⁶ A Swedish adverse drug reactions advisory committee reviewed 784 reported cases of DILI between 1970

Abbreviations: ABC, ATP-binding cassette; ALT, alanine aminotransferase; ANIT, α -naphthylisothiocyanate; AP, alkaline phosphatase; AST, aspartate aminotransferase; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CYP, cytochrome P450; DILD, drug-induced liver disease; DILI, drug-induced liver injury; GGT, gamma glutamyl transferase; MDR1, multidrug resistance-1 protein; MRP, multidrug resistance protein; NTCP, sodium-dependent taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; PXR, pregnane X receptor; UDCA, ursodeoxycholic acid; VBDS, vanishing bile duct syndrome.

From the ¹Division of Gastroenterology, Centennial Hills Hospital Medical Center, Las Vegas, NV; ²Department of Internal Medicine, Digestive Disease Section and Liver Center, Yale University School of Medicine, New Haven, CT; and ³Division of Gastroenterology, Charles Drew University of Medicine and Science, Los Angeles, CA.

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Address reprint requests to: James L. Boyer, M.D., Department of Internal Medicine, Digestive Disease Section and Liver Center, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208019, New Haven, CT 06520-8019. E-mail: James.Boyer@yale.edu; fax: 203-785-7273.

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and 2004, almost half of which had either cholestatic or mixed cholestatic hepatic toxicity.⁶ Nevertheless, a wide variety of commonly used drugs can induce cholestatic liver injury including nonsteroidal anti-inflammatory drugs, antihypertensives, antidiabetics, anticonvulsants, lipid-lowering agents, and psychotropic drugs.¹¹⁻¹⁷ Many drugs target the biliary epithelium and result in drug-induced cholangiopathy and vanishing bile duct syndrome (VBDS). Terms such as “drug-induced bile duct injury” and “disappearing intrahepatic bile ducts” are also used to refer to this type of drug-induced injury that can mimic primary biliary cirrhosis or small duct primary sclerosing cholangitis (PSC).⁸ A few rare agents such as 2-fluoro 2'-deoxyuridine can also produce injury to the larger bile ducts; in these cases, injury to the hepatic artery must be excluded as ischemia to the biliary epithelium may result in a similar complication.

Clinical Features

Individual drugs that induce drug-induced cholestasis tend to have a characteristic signature, which is composed of a clinical and pathological pattern, but a single drug can exhibit more than one specific signature. Cholestatic reactions tend to be prolonged after the discontinuation of the causative agent, presumably because cholangiocyte repair and regeneration is slower than that of the hepatocyte, and because bile secretory function may be slower to recover than other hepatocyte functions. In some cases, persistence of a self-propagating immune response may play a role in prolonging drug-induced cholestasis.

Drug-induced cholestasis may present as an acute illness that promptly subsides with the withdrawal of the offending agent. It may present with or without jaundice. However, parenchymal liver injury may elicit nonspecific symptoms such as nausea, malaise, anorexia, and fatigue. Abdominal pain or discomfort may be present in drug-induced cholestasis, especially that caused by amoxicillin-clavulanate or erythromycin.¹⁸ Symptoms may occur weeks or months after beginning treatment. Chronic drug-induced cholestasis can result in development of xanthomas, pruritus, and melano-derma.¹⁹ Pruritus can be the major reason that patients seek medical care. Chronic drug-induced cholestasis often will resolve following withdrawal of the offending agent, but in some cases, if there is significant loss of the interlobular bile ducts (as in VBDS), drug-induced cholestasis can lead to chronic liver disease that may progress to liver failure.²⁰ Rarely, drugs can induce cholelithiasis or may mimic large duct scleros-

Table 1. Classification of Drug-Induced Cholestasis Syndromes

I. Intrahepatic Drug-Induced Cholestasis
a) <i>Acute</i>
i. Cholestasis without hepatitis (pure, simple, canalicular, or bland cholestasis)
ii. Cholestasis with hepatitis (hepatocanalicular hepatitis or mixed cholestatic hepatitis)
iii. Cholestasis with bile duct injury (ductular, cholangiolar, or cholangiolytic cholestasis)
b) <i>Chronic (Cholangiopathies)</i>
i. Mild, nonspecific bile duct injury
ii. VBDS
iii. PSC-like
II. Extrahepatic Drug-Induced Cholestasis
i. Cholelithiasis
ii. PSC-like

ing cholangitis, resulting in extrahepatic obstruction.²¹ Occasionally, extrahepatic manifestation of drug toxicity may provide clues to the diagnosis. Amoxicillin-clavulanate can cause acute interstitial nephritis and acute lacrimal gland inflammation along with hepatic injury.²² Similarly, contaminated rapeseed oil poisoning can cause both pulmonary toxicity and drug-induced cholestasis concomitantly.²³

Drug-induced cholestasis can be categorized into several groups (Tables 1 and 2):

Acute Drug-Induced Cholestasis Without Hepatitis. These drug-induced cholestatic disorders are rare and cause minimal or no hepatic parenchymal involvement. This form of drug-induced cholestasis manifests itself histologically by pure canalicular cholestasis, typically produced by estrogen or anabolic steroids.

Acute Drug-Induced Cholestasis With Hepatitis. Cholestasis associated with hepatitis is characterized by portal inflammation and varying degrees of hepatocyte injury and necrosis.

Acute Drug-Induced Cholestasis With Bile Duct Injury. These forms of drug-induced cholestasis exhibit bile duct injury associated with minimal involvement of parenchymal liver cell injury.

Chronic Drug-Induced Cholangiopathies. These drug-induced cholestatic disorders vary from asymptomatic patients with isolated elevations in AP or gamma glutamyl transferase (GGT) and liver histology showing only mild bile duct disarray or “ductopenia”, to progressive forms of the VBDS.²⁴ Although some reports of asymptomatic idiopathic adulthood ductopenia fail to identify a causative agent,²⁵ others suggest that these cases may originate from overlooked drug-induced bile duct injury.^{26,27} The common drugs known to cause the various drug-induced cholestasis syndromes are listed in Table 3.

Table 2. Clinical Syndromes of Drug-Induced Cholestasis

Syndrome	Pathology	Clinical Features	Biochemical Features
Intrahepatic			
<i>ACUTE</i>			
Cholestasis without hepatitis	Dilated canaliculi filled with few bile casts, especially in centrilobular area (acinar zone 3). Minimal or no inflammation or necrosis.	Influenza-like prodrome (nausea, anorexia, malaise)	Hyperbilirubinemia, <3 times elevation of AP, 1-8 times elevation of AST/ALT
Cholestasis with hepatitis	Early portal inflammation-with or without eosinophils, hepatocyte necrosis.	Influenza-like prodrome, hypersensitivity symptoms, right upper quadrant pain (can mimic acute cholangitis or cholecystitis)	Hyperbilirubinemia, >3 times elevation of AP, 2-10 times elevation of AST/ALT
Cholestasis with bile duct injury	Biliary ductules filled with numerous bile casts, scattered steatosis, and minimal or no hepatocellular damage	Eosinophilia, renal failure, Stevens-Johnson syndrome, prolonged jaundice (>6 months), may progress to VBDS	Hyperbilirubinemia, >3 times elevation of AP, elevated GGT, 2-10 times elevation of AST/ALT
<i>CHRONIC</i>			
Mild nonspecific bile duct injury	Minimal bile duct epithelial disarray	asymptomatic	Mild elevation in AP or GGT Occasional inflammatory cells in or around biliary epithelia in portal triad
VBDS	Loss of interlobular or septal bile ducts in ≥50% of portal tracts or complete disappearance, portal tract inflammation, fibrosis, hepatocellular necrosis, marked ductal destruction	Hepatosplenomegaly, hyperlipidemia, malabsorption, xanthelasmas, xanthomas, leads to cirrhosis	Hyperbilirubinemia, antimitochondrial antibody absent, >3 times elevation of AP, 2-10 times elevation of AST/ALT, initial elevation of GGT, hypercholesterolemia
PSC-like	Nonspecific findings that may resemble PSC, marked ductal destruction, hepatocellular necrosis	Jaundice develops within 3-6 months of drug administration.	Hyperbilirubinemia, >3 times elevation of AP, 2-10 times elevation of AST/ALT, hypercholesterolemia.
Extrahepatic			
Cholelithiasis		Biliary colic, gallstone pancreatitis, common bile duct dilatation	Hyperbilirubinemia, elevated AP
PSC-like	PSC-like pathology of extrahepatic ducts	Jaundice develops within 3-6 months of the drug administration,	Hyperbilirubinemia, >3 times elevation of AP, 2-10 times elevation of AST/ALT, hypercholesterolemia

Pathophysiology of Drug-Induced Cholestasis

Hepatocytes are highly polarized cells with distinct sinusoidal, lateral, and apical membrane domains. Lipid-soluble drugs with molecular weights ~500 daltons or greater are selectively removed by the liver across the sinusoidal domain. Although some drugs diffuse across the cellular membrane, most require active or facilitated transporters (phase 0).^{5,28,29} Cellular uptake and binding to cytosolic proteins is followed by phase 1 and phase 2 biotransformation resulting in more water-soluble metabolites. Phase 1 reactions involve oxidation, hydroxylation, and other reactions mediated by the cytochrome P450 (CYP) system, particularly CYP3A4. The activity of the CYP system varies greatly among individuals and their transcription is highly regulated by xenobiotic sensing nuclear receptors such as the pregnane X receptor (PXR) and the constitutive androstane receptor. Phase 2 reactions involve esterification reactions that form conjugates with sulfate, glucuronic acid, amino acids, or glutathione molecules. Generally, this results in increased water solubility and decreased pharmacologic activity, which

enhances detoxification of the compounds. However, this same process can also lead to the production of toxic intermediates.³⁰ Drug-induced cholestasis may occur particularly under conditions of increased drug concentrations, genetic alterations in expression of enzymes or transporters, and/or reduced hepatic concentrations of antioxidants such as glutathione. Drug-induced cholestasis can be caused by direct toxic effects of drugs or their metabolites on different cell types of the liver or through an immune-mediated process.³¹

Role of Hepatic Transport Proteins in Drug Clearance and Toxicity

The molecular identification of transport proteins that mediate the sinusoidal uptake and biliary secretion of bile acids and other organic solutes, many of which are drugs, has greatly expanded the understanding of the cellular mechanism for bile formation and its dysregulation in cholestatic conditions, including drug-induced cholestasis.^{5,28,29} A list of these membrane transporters, their gene nomenclature, and their function in transporting drug substrates is given in Table 4 and illustrated in Fig. 1. Drug substrates that are

Table 3. Common Drugs Causing Various Drug-Induced Cholestatic Syndromes**Cholestasis without hepatitis**

Anabolic steroids, estrogens, tamoxifen, azathioprine, cyclosporine, nevirapine, glimepiride, metolazone, infliximab, cetirizine

Cholestasis with hepatitis

Isoniazid, halothane, methyl dopa, macrolide antibiotics, tricyclic antidepressants, amoxicillin-clavulanate, azathioprine, oxypenicillins, NSAIDs, chlorpromazine, troglitazone, celecoxib, carbamazepine, repaglinide, terbinafine, cephalixin, fenofibrate, hydrochlorothiazides, ticlopidine, pyritinol, methimazole, metformin, gemcitabine, orlistat, celecoxib, gabapentin, propafenone, acitretin, isoflurane, bupropion, captopril, resperidone, propafenone, chlorambucil, risperidone, glimepiride, proplthiouracil, itraconazole, dextromethorphan, atorvastatin, *Senna*, *Cascara sagrada*, *Lycopodium serratum*

Cholestasis with bile duct injury

Carmustine, toxins (paraquat, methylenedianiline), flucoxacinil, dextropropoxyphene, tenoxicam, gold therapy, pioglitazone, amoxicillin-clavulanate

Vanishing Bile Duct Syndrome (Ductopenia)

Aceprometazine, ajmaline, amineptine, amitriptyline, amoxicillin-clavulanic acid, ampicillin, azathioprine, barbiturates, carbamazepine, carbutamide, chlorothiazide, chlorpromazine, cimetidine, ciprofloxacin, clindamycin, co-trimoxazole, cromolyn sodium, cyamemazine, cyclohexyl propionate, cyproheptadine, D-penicillamine, diazepam, erythromycin, estradiol, flucloxacillin, glibenclamide, glycyrrhizin, haloperidol, ibuprofen, imipramine, methyltestosterone, norandrostenedione, phenylbutazone, phenytoin, prochlorperazine, terbinafine, tetracyclines, thiabendazole, tiopronin, trifluoperazine, tolbutamide, trimethoprim-sulfamethoxazole, troleandomycin, xenalazine

Sclerosing cholangitis-like cholestasis

Flouxuridine, intralesional agents (hypertonic saline, iodine solution, formaldehyde, absolute alcohol, silver nitrate)

known to induce cholestasis are listed in italics in Table 4. The rate-limiting step in the systemic clearance of lipophilic drugs and their metabolites is their excretion into bile. This process is regulated by adenosine triphosphate (ATP)-dependent canalicular transporters, including the bile salt export pump (BSEP, ATP-binding cassette B11 [ABCB11]), the major determinant of bile salt-dependent canalicular bile secretion,³² and the multidrug resistance protein-2 (MRP2, ABCG2) that determines bile salt-independent bile flow by excretion of glutathione.²⁸ MRP2 also transports drug conjugates and divalent bile salt conjugates into bile. Other ATP-dependent transporters include the multidrug resistance-1 protein (MDR1, ABCB1), which transports organic cations (often tertiary or quaternary amines), the breast cancer resistance protein (BCRP, ABCG2), which transports organic anions (including drug conjugates), and the multidrug resistance protein 3 (MDR3, ABCB4), a phospholipid oipase. A number of drug substrates for these transporters are known to produce drug-induced cholestasis (Table 4).

Insight into specific mechanisms of drug-induced cholestasis in patients has been gained mostly from animal models of cholestasis. These studies carried out in

isolated membrane vesicles, hepatocyte cultures, and in bile duct-cannulated rodent models indicate that cholestatic drugs can inhibit bile secretion and bile acid transport at many levels, including uptake and efflux across the sinusoidal membrane, as well as canalicular efflux. For example, rifampicin, cyclosporine A, rifamycin SV, bosentan, troglitazone, erythromycin estolate, and glibenclamide have all been shown to inhibit Bsep in rats in a dose-dependent fashion.³³⁻³⁶ Sulindac also competitively inhibits canalicular bile acid transport.³⁷ Ethinylestradiol-17 β -glucuronide is secreted into bile by Mrp2 and then trans-inhibits Bsep from the luminal side of the canalicular membrane.³⁶

Cyclosporine, an MDR1 substrate, is a prototypical drug that can cause cholestatic liver injury through a number of different mechanisms: (1) competitive inhibition of ATP-dependent transporters,³⁸⁻⁴⁰ (2) inhibition of intrahepatic vesicle transport and targeting of ATP-dependent transporters to the canalicular membrane,⁴¹⁻⁴³ and (3) impairment of bile secretion partly by increasing canalicular membrane fluidity without affecting the expression of canalicular transporters.⁴⁴ Other studies suggest that cyclosporine reduces the expression of glutathione-synthesizing enzymes and the canalicular glutathione efflux system, MRP2, leading to reduced bile salt-independent bile flow. This cholestatic effect is enhanced when the drug is coadministered with sirolimus (rapamycin).⁴⁵ It remains uncertain whether these various mechanisms of toxicity also apply to patients who are chronically exposed to drugs such as cyclosporine. However, long-term impairment of hepatobiliary secretory mechanisms and their adverse consequences might be expected.

Other drugs that can be associated with cholestasis, such as the endothelin antagonist bosentan, also inhibit Bsep, an effect that is enhanced by coadministration of the oral hypoglycemic agent glibenclamide.³³ Troglitazone and troglitazone sulfate, the main troglitazone metabolite eliminated in bile, competitively cis-inhibit Bsep, which could lead to troglitazone-induced intrahepatic cholestasis and liver toxicity.^{34,35}

Male rats are more susceptible to liver injury than female rats, probably due to higher formation rates of troglitazone sulfate.⁴⁶ Troglitazone sulfate and troglitazone glucuronide (another important metabolite) are eliminated via MRP2 into bile, suggesting that canalicular elimination via MRP2 may be an important factor in the pathogenesis of troglitazone-induced cholestasis.⁴⁶ Direct competition of troglitazone metabolites with conjugated bilirubin at the level of MRP2 could result in conjugated hyperbilirubinemia.⁴⁶ Troglitazone also can produce mitochondrial

Table 4. Nomenclature, Location, and Function of the Major Hepatocyte Membrane Drug Transporters and Their Known Drug Substrates

Name	Abbreviation	Location	Function	Clinically Relevant Polymorphisms
Sodium-taurocholate cotransporter	NTCP (<i>SLC10A1</i>)	Basolateral membrane	Primary carrier for conjugated bile salt uptake from portal blood but can transport <i>estrone-3-sulfate</i> and <i>rosuvastatin</i> . ¹¹⁵	
Organic-anion-transporting polypeptides	OATPs (<i>SLCO, 1B1,1B3,2B1</i>)	Basolateral membrane	Broad substrate carriers for organic anions and other amphipathic organic solutes from portal blood, including a wide range of drugs as well as sodium-independent uptake of bile salts.	OATP1 *15 variant (1628T>G), associated with statin-induced myopathy. ⁶⁹
Organic cation transporter 1	OCT1 (<i>SLC22A1</i>)	Basolateral membrane	Transports organic cations such as <i>cimetidine</i> , <i>metformin</i> , <i>aclovir</i> , and <i>zidovudine</i>	
Organic anion transporter 2	OAT2 (<i>SLC22A7</i>)	Basolateral membrane	Transports organic anionic drugs like <i>para-amino-hippurate</i> , <i>salicylates</i> , and <i>prostaglandin E2</i> .	
Concentrative and equilibrative nucleoside transporters	CNT1 CNT2 (<i>SLC28A1 and SLC28A2</i>) ENT1 and ENT2 (<i>SLC29A1, SLC29A2</i>)	Basolateral Membrane	NTs transports nucleosides and nucleoside analogues such as <i>adenosine</i> , <i>uridine</i> , <i>didanosine</i> , <i>fialuridine</i> , <i>5-fluorouridine</i> , <i>ribivirin</i> . ¹¹⁸	
Multidrug resistance-1 P glycoprotein*	MDR1 (<i>ABCB1</i>)	Canalicular membrane	ATP-dependent excretion of various organic cations, xenobiotics, and cytotoxins into bile including cholestatic drugs like <i>cyclosporine</i> , <i>verapamil</i> , <i>erythromycin</i> , <i>chlorpromazine</i> , <i>ivermectin</i> , and anticancer drugs like <i>daunorubicin</i> and <i>vincristine</i> , cardiac glycosides, human immunodeficiency virus protease inhibitors.	
Multidrug-resistance protein-3*	MDR3 (<i>ABCB4</i>)	Canalicular membrane	Mediates ATP-dependent excretion of phosphatidylcholine into bile (a floppase, not a drug transporter); Polymorphisms predispose to drug-induced cholestasis.	Heterozygous p.1764L mutation associated with <i>risperidone</i> hepatocellular cholestasis. ⁴⁹ Progressive familial intrahepatic cholestasis mutation associated with oral contraceptive-induced cholestasis. ¹¹⁹
Multidrug resistance-associated protein (canalicular multispecific organic-anion transporter)*	MRP2 (<i>ABCC2</i>)	Canalicular membrane	Mediates ATP-dependent multispecific organic-anion conjugate efflux into bile (e.g., cholestatic drugs like <i>cephtriaxone</i> , <i>diclofenac</i> , <i>synthetic estrogens</i> ; also, <i>pravastatin</i> , <i>methotrexate</i> as well as <i>bilirubin diglucuronide</i>); contributes to bile salt-independent bile flow by glutathione transport	
Canalicular bile salt export pump*	BSEP (<i>ABCB11</i>)	Canalicular membrane	ATP-dependent bile salt transport into bile; stimulates bile salt-dependent bile flow but also can transport <i>taxol</i> ¹¹⁶ and <i>pravastatin</i> . ¹¹⁷	pV444A single-nucleotide polymorphism more frequently associated with drug cholestasis ⁴⁹ ; Heterozygous p.D676Y mutation in patient with hepatocellular cholestasis who is taking <i>fluvastatin</i> . ⁴⁹
Breast cancer resistance protein*	BCRP (<i>ABCG2</i>)	Canalicular membrane	ATP-dependent excretion of anticancer drugs (<i>anthracyclins</i> , <i>topotecan</i>), <i>estrogens</i> , <i>rosuvastatin</i> , <i>nitrofurantoin</i> into bile.	
Multidrug and toxin extrusion transporter	MATE1 (<i>SLC47A1</i>)	Canalicular membrane	Proton gradient-mediated efflux of cation drugs like <i>cimetidine</i> , <i>procaïnamide</i> .	
Concentrative and equilibrative nucleoside transporters	CNT1 and CNT2 (<i>SLC28A1 and SLC28A2</i>); ENT1 (<i>SLC29A1</i>)	Canalicular membrane	CNTs transport from bile into hepatocyte; ENTs are bidirectional. NTs transport nucleosides and nucleoside analogues such as <i>adenosine</i> , <i>uridine</i> , <i>didanosine</i> , <i>fialuridine</i> , <i>5-fluorouridine</i> , <i>ribivirin</i> . ¹¹⁸	
Multidrug resistance-associated protein-3*	MRP3 (<i>ABCC3</i>)	Basolateral membrane of hepatocytes	ATP-dependent efflux of drug glucuronide conjugates like <i>morphine glucuronide</i> . Expression induced in cholestasis.	
Multidrug resistance-associated protein-4*	MRP4 (<i>ABCC4</i>)	Basolateral membrane of hepatocytes	ATP-dependent efflux pump for sulfated drugs and bile acids. Expression up-regulated in cholestasis.	

The drug transporters that can produce cholestasis are shown in italics. *These transporters are members of the ATP-binding cassette family.

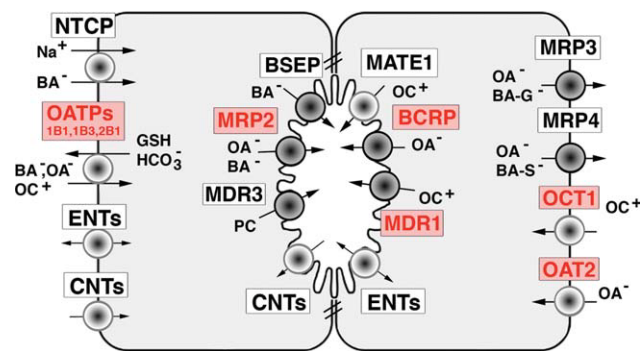


Fig. 1. Hepatocyte couplet illustrating location of major transporters that determine bile production and hepatic drug transport. The major drug transporters are indicated in red. ATP-dependent transporters are the dark circles. See Table 4 for their definition and function. BA⁻, bile acid; BA-G, bile acid glucuronides; BA-S, bile acid sulfates; CNT, concentrative nucleoside transporter; ENT, equilibrative nucleoside transporter; GSH, glutathione; Na⁺, sodium; OA⁻, organic anion; OC⁺, organic cation; PC, phosphatidylcholine.

toxicity and reactive oxygen species so that the pathogenesis may involve more than one mechanism. Fialuridine-induced hepatic toxicity with cholestasis also involves mitochondrial derangement.⁴⁷

Although not a drug transporter, MDR3 plays a key role in the biliary secretion of phosphatidylcholine. An aggressive form of progressive familial intrahepatic cholestasis, type III, results from mutations in MDR3. The inability to translocate this phospholipid across the canalicular membrane lipid bilayer results in its absence from bile, and this is thought to result in exposure of the biliary epithelium to the toxic, detergent effects of bile acids that lead to cholangiopathies.⁴⁸ Impaired expression of MDR3 can lead to development of cholangiolytic cholestasis and the VBDS. Genetic variants in MDR3 and BSEP may predispose individuals to drug-induced cholestasis (see section on genetic determinants below and Lang et al.).

Multiple drugs, particularly quaternary cationic amines, are transported into the bile via the MDR1 canalicular transporter. Although mutations in this transporter have not been described in humans, reduced expression or competition for binding sites with other drugs may cause drug accumulation and toxicity. Accumulation of a drug, like cyclosporine A, can subsequently inhibit Mrp2, resulting in direct toxicity to the cell. Studies in cell monolayers expressing human sodium-dependent taurocholate cotransporting polypeptide (NTCP) and BSEP suggest that many well-known cholestatic drugs like rifampicin, glibenclamide, and cyclosporine A reduce bile acid transport both into the hepatocyte and at the apical canalicular membrane.⁵⁰ Drug–drug competition for transporter-

binding sites may also play a role in drug-induced cholestasis in human liver, although these effects are not easily recognized and poorly understood mechanistically. See Fig. 2 for a possible example of drug–drug competition between two MDR1 substrates resulting in a transient cholestatic effect.⁵¹

Another potential mechanism for the development of drug-induced ductopenia and VBDS is the biliary excretion of toxic but stable metabolites that injure the bile duct epithelium. α -Naphthylisothiocyanate (ANIT) administration to rats results in chronic cholestatic injury characterized by bile duct injury and proliferation. ANIT forms a labile glutathione adduct in hepatocytes, which dissociates after concentrative transport into alkaline bile. Rats with mutated *Mrp2* are not able to pump the adduct into bile and thus are protected from ANIT-induced cholestatic injury.⁵²

Flucloxacillin is a beta-lactam semisynthetic antibiotic commonly used in Europe that causes cholestatic liver injury in ~8 per every 100,000 patients. It is an example of a drug that results in liver pathology consistent with VBDS.⁵³ The mechanism is not known, however, small amounts of the compound form metabolites involving the activity of CYP3A4, which itself may be under genetic control. Whether these metabolites are directly toxic to cholangiocytes after excretion into bile or might also be formed in cholangiocytes is not known. It is generally assumed that an immune-mediated response subsequently accounts for the development of the VBDS. Recent studies also support a role for genetic determinants (see below). Isolated reports of VBDS have been described for number of other drugs (Table 3).^{53–62}

Proinflammatory cytokines are also powerful down-regulators of CYP enzymes and biliary transporters,^{63,64}

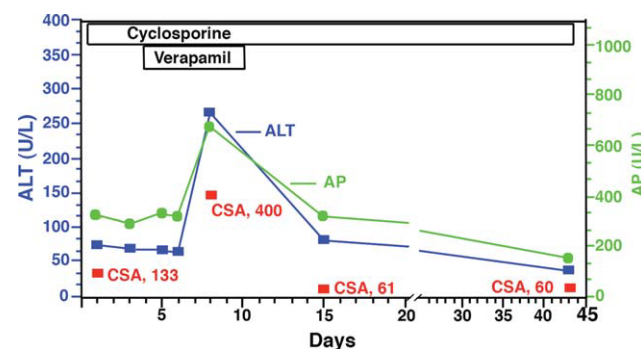


Fig. 2. Drug-induced cholestasis from interaction of cyclosporine and verapamil in a 46-year-old white male 5 days after liver transplant. Note rise in AP, ALT, and plasma cyclosporine A (CSA) levels during simultaneous administration of verapamil and cyclosporine, two MDR1 substrates. Drug-induced cholestasis resolved quickly after discontinuing the verapamil.

thereby lowering the threshold for liver injury and functioning as a critical “second hit”.⁶⁵ This phenomenon, also known as the “danger hypothesis”, is one of several explanations for the development of idiosyncratic drug toxicity.⁶⁵ Drug–drug interactions and genetic determinants of drug metabolism enzymes and transporters are other important variables.

Genetic Determinants

It is increasingly evident that genetic variants can determine an individual's susceptibility to develop cholestasis, particularly when the drug is a substrate for liver transporters and is excreted into bile.^{5,29} Very little information exists on the role of hepatic basolateral drug transporters in the development of drug-induced cholestasis although it has been speculated that increased expression of the organic anion transporting peptides (OATPs) and other drug uptake transport proteins might enhance the hepatic concentrations of certain drugs, thus predisposing the subject to cholestatic reactions. Oatp1b2 knockout mice are resistant to the hepatotoxic effects of the mushroom poison phalloidin, consistent with the role of OATPs in increasing the concentration of substrate drugs/toxins.⁶⁶ Although 14 nonsynonymous SLC1B1 single-nucleotide polymorphisms that encode OATP1B1 have been described by Tirona et al.⁶⁷ in African Americans and Europeans, six of these reduce rather than enhance the uptake of the OATP1B1 substrates estrone-3-sulfate and estadiol-17-glucuronide in *in vitro* studies. Genetic variants in OATP1B1 can influence the hepatic uptake of drugs like pravastatin and irinotecan. In one reported case, mutations in this transporter resulted in statin-induced myopathy,⁶⁸ which was explained by a decrease in turnover rate of this transporter.⁶⁹

More information is known about the functional and clinical impact of genetic variations in the canalicular transporters and their role in drug-induced cholestasis. One study using 110 healthy liver tissues demonstrated considerable variation in the expression of these proteins with 32% expressing low levels of at least one of the canalicular transport proteins, a feature that could predispose individuals to cholestatic drug injury.⁷⁰ Several common polymorphisms for canalicular ABC transporters have also been identified in healthy individuals by systematic genetic screening of their promoter and coding regions.^{71–73} Polymorphisms such as C1515Y in MRP2, V444A in BSEP, and C3435T in MDR1 were found to be associated with decreased hepatic expression of these proteins.^{70,74} These polymorphisms can influence the bi-

oavailability of drugs. For example, the C3435T polymorphism in MDR1 increases oral bioavailability of digoxin, but has no effect on the bioavailability of cyclosporine A.^{74,75}

However, considerable interindividual variability exists in the expression of the canalicular membrane ABC transporter proteins with 15%–20% of individuals being classified as low or very low expressors of at least one of these proteins in one study.⁷⁰ Differences in genetic variability of MDR3 and BSEP and haplotype structures in different healthy individuals may predispose different ethnic populations to drug-induced cholestasis.⁷⁶

Two nonsynonymous single-nucleotide polymorphisms in BSEP have been described for c.1331T→C (p.V444A) in exon 13 and c.2029A→G (p.M677V) in exon 17 with frequencies that are higher than 0.5% in different cohorts.⁷⁰ In another study, individuals with the p.V444A variant demonstrated lower BSEP expression levels.⁴⁹ This variant BSEP is now considered a risk factor for drug-induced cholestasis because it is found more frequently in such patients,⁴⁹ as well as in patients with intrahepatic cholestasis of pregnancy,^{77,78} than in controls. In the same Swiss study, full-length sequencing of BSEP and MDR3 also revealed a heterozygous p.D676Y mutation in BSEP in a patient taking fluvastatin, and a heterozygous p.I764L mutation in MDR3 in a patient taking risperidone.⁴⁹ Whether these mutations account for the cholestatic event remains uncertain. A recent study of contraceptive-induced cholestasis revealed an association with BSEP 1331T→C polymorphism as a susceptibility factor but not for MRP2.⁷⁹

Other examples of genetically determined drug-induced cholestasis involve susceptibility to diclofenac-induced toxicity. Allelic variants in the drug-metabolizing enzymes UGT2B7 and CYP2C8 and canalicular MRP2 presumably lead to an increase in the level of reactive metabolites and higher levels of protein–diclofenac adducts that then produce toxicity.⁸⁰ Other studies have identified a PXR polymorphism as a risk factor for flucloxacillin-induced liver injury. Flucloxacillin is a PXR agonist. The variant PXR (rs3814055; C-25385T) was found to be more common in patients who developed flucloxacillin drug-induced cholestasis, and reporter gene experiments demonstrated that the C allele had lower promoter activity than the T allele.⁸¹ These findings are a reminder that there is still much to be learned about the role of polymorphisms of nuclear receptors that regulate drug metabolism and transport in patients with drug-induced cholestasis.^{76,82}

Diagnosis

A detailed history is critical in the diagnosis of drug-induced cholestasis. The use of prescribed medications, over-the-counter medications, herbal drugs, and naturopathic substances as well as parenteral nutrition should always be explored.^{83,84} Temporal relationships between the initiation of the offending agent and development of the symptoms can provide the clue to the diagnosis. The period between drug ingestion and the onset of symptoms may provide a clue as to the offending agent. This latency period may be short (hours to days), intermediate or delayed (1-8 weeks), or long (1-12 months) depending on the agent. All drugs used by the patient within the last 3-6 months should be enumerated. This relationship may not be obvious in patients with chronic liver disease from other causes or when taking multiple medications that may lead to drug-drug interactions. Increase of serum AP activity (usually more than three times the upper limit of normal) is the most common laboratory finding in patients with drug-induced cholestasis. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels may be normal or minimally elevated.⁸⁵

International criteria for liver toxicity were established by the Council of International Organizations of Medical Sciences (CIOMS) in 1990. This group defined "cholestatic injury" as a patient with $AP > 2 \times$ upper limit of the normal range or $ALT/AP \leq 2$ whereas "mixed hepatocellular/cholestatic injury" was defined as an $ALT/AP \leq 2-5$ in contrast to "hepatocellular injury", where the ALT is $> 2 \times$ upper limit of normal or $ALT/AP \geq 5$.^{86,87} Diseases that can mimic drug-induced cholestasis, such as primary biliary cirrhosis or sepsis (bacterial or viral) should be ruled out. Evaluations should always include hepatitis and autoimmune serologies and appropriate imaging studies.

On rare occasions, patients may develop symptoms on reexposure to the same medication. However, rechallenge with the suspected drug is usually contraindicated, particularly if there is active liver injury, because severe or even fatal liver injury can occur.

The role of liver biopsy is controversial. Nevertheless, performing liver biopsy may be helpful when the diagnosis is not clear or when there are other complicating medical conditions. Occasionally, the pathologist will first suggest the possibility of a drug- or toxin-induced injury. A biopsy may also be useful in predicting prognosis (see a recent review⁸⁸ for a more comprehensive discussion of the role of liver pathology in drug-induced liver injury).

Management

Most cases of drug-induced cholestasis will resolve with withdrawal of the offending medication and not develop chronic liver disease. A Swedish adverse drug reaction advisory committee report concluded that AST and bilirubin levels are the most important predictors of death or liver transplantation in DILI.⁶ In another study, the persistent use of the offending agent for > 6 months after diagnosis of DILI, predicted the development of chronic liver disease and fibrosis in liver biopsies.⁸⁹ In addition to watchful waiting after stopping the suspected agent, it is important to treat pruritus when present. Severe pruritus may lead to sleep deprivation and psychological abnormalities especially in elderly patients. The pathophysiological mechanism of pruritus from cholestasis is still unknown. Suggested mechanisms include high tissue and serum bile salt concentrations, increased opioidergic tone, and alteration of serotonin neurotransmitters.⁹⁰⁻⁹² A recent study has suggested lysophosphatidic acid as a potential mediator.⁹³

Mild pruritus can often be managed by nonspecific measures such as emollients and warm baths and/or histamine-1-receptor blockers such as hydroxyzine and diphenhydramine due to their sedative properties. Bile acid resins (cholestyramine or colestipol) are the first-line agents in moderate to severe pruritus, particularly when associated with excoriations and disturbed sleep.⁹⁴ Based on the inference that the pruritogens are excreted in bile, they function to exchange organic anions such as bile acids with chloride anions in the intestine. To be effective, cholestyramine powder (4-g packets) or colestipol pills (1 g each), in doses of 4 g, must be taken 20 minutes before each meal in order to reach the small intestine when food induces bile flow.⁹⁵ Total doses range from 4-24 g/day. All other medications must be given at least 1-2 hours before or after administration of these binding resins to avoid interference with their absorption. Side effects such as constipation and hyperchloremia may occur.

Rifampicin (150-300 mg twice a day) may also be effective in patients intolerant to binding resins.^{96,97} Rifampicin is also a ligand for the nuclear receptor PXR, and ligand activation of this receptor induces expression of CYP isoforms that are capable of detoxification of hydrophobic bile salts.^{98,99} Side effects of rifampicin include hepatic toxicity. Phenobarbital (1-5 mg/kg/day divided in three doses) also induces hepatic microsomal enzymes via activation of constitutive androstane receptor¹⁰⁰ and therefore may facilitate detoxification and inactivation of putative peripheral

pruritogens.¹⁰¹ However, long-term administration of microsomal inducers may impair vitamin D metabolism.

Alternatives are limited but include the opiate antagonists naloxone and naltrexone.^{91,102,103} Invasive procedures, including plasmapheresis and extracorporeal albumin dialysis using the Molecular Adsorbent Recirculating System (MARS), are reported to relieve severe pruritus,¹⁰⁴ but both procedures may require hospitalization and significant input from renal dialysis staff. Severe pruritus can even be an indication for liver transplantation. Ursodeoxycholic acid (UDCA) is currently the only established drug for the treatment of cholestatic liver disease, and it has cytoprotective, immunomodulatory, antiapoptotic, and choleric effects.¹⁰⁵ UDCA is also a strong agonist of PXR, an important nuclear receptor that up-regulates CYP3A4.¹⁰⁶

Experimental studies in rats have demonstrated that UDCA improves cholestasis induced by phalloidin, 17 β -estradiol glucuronide, and endotoxins.¹⁰⁶⁻¹⁰⁹ UDCA increases the expression of canalicular export pumps for bile salts (BSEP) and other organic anions including bilirubin (MRP2) which stimulates bile secretion.^{110,111} UDCA also stimulates the insertion of these export pumps into the canalicular membrane in a protein kinase C and p38 mitogen-activated protein kinase-dependent fashion.^{111,112} UDCA stimulates targeting of P-glycoprotein (MDR1) to the canalicular membrane, which could prevent cyclosporine-associated cholestatic effects.¹¹³

Although the mechanism of UDCA's beneficial effect in cholestasis is not clearly understood, it is thought that its primary beneficial effect is decreasing the hydrophobicity of the bile acid pool by replacing toxic (e.g., hydrophobic) with nontoxic (e.g., hydrophilic) bile acids.¹⁰⁵ UDCA is best administered at bedtime to avoid inhibition of its absorption when administered with cholestyramine or colestipol. However, UDCA is approved by the US Food and Drug Administration only for use in patients with primary biliary cirrhosis, and its efficacy in patients with drug-induced cholestasis has not been clearly established. For a review of potential future therapeutic approaches for drug-induced and other forms of cholestasis, see Zollner et al.¹⁰⁶

Fat-soluble vitamins (vitamins A, D, and K) should be replaced via the parenteral route in patients with long-standing cholestasis. Patients at risk for developing biliary cirrhosis and liver failure should be promptly referred to liver transplant centers.

In the future, gene expression profile information, toxicogenomics, and proteomics may help to better understand the mechanisms of drug-induced cholesta-

sis and provide the technology to better identify individuals at risk.¹¹⁴

Conclusion

Drugs continue to be an important cause of cholestasis and always must be considered in the differential diagnosis of cholestatic syndromes. Prompt recognition and withdrawal of the offending agent is the mainstay of the management of cholestasis. Progress has been made in clarifying potential pathogenetic mechanisms and in establishing the role of genetic susceptibility for the development of drug-induced cholestasis.

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Supporting References 84 to 119 may be found in the online version of this article.