molecular pharmaceutics

reviews

Clinical Hepatotoxicity. Regulation and Treatment with Inducers of Transport and Cofactors

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Received December 27, 2006; Revised Manuscript Received August 2, 2007; Accepted September 13, 2007

Abstract: Drug-induced liver injury (DILI) is a rare condition. This review presents a clinical update on the incidence and prevalence of DILI, cellular targets and histopathological features of liver injury, and basic diagnostic approaches. At present, with the exception of acetaminophen poisoning where timely administered *N*-acetylcysteine serves as the antidote, treatment usually is supportive. Since most biotransformation processes in the liver are under the control of nuclear receptors (NR), treatment modalities aiming to stimulate or inhibit NR expression are at present tested in animal models of APAP toxicity or cholestasis. Some NR ligands have also been used in human cholestatic liver disease, but studies on the efficacy in DILI are lacking. This review describes possible future options for the treatment of DILI.

Keywords: Nuclear receptors; conjugation; hydroxylation; detoxification; drug-induced liver disease; acetaminophen; cholestasis

The liver is central to the metabolism of virtually any foreign substance. Hepatotoxicity is defined as injury to the liver by drugs or other foreign, noninfectious agents. When injury impairs liver function, hepatotoxicity becomes clinically overt.[1–3]

Most drug-induced liver injury (DILI) is considered idiosyncratic, with a variable delay or latency period, ranging from 5 to 90 days from the initial ingestion of the drug. Continued use once the reaction has begun frequently has a fatal outcome. Only with a few drugs, such as isoniazid[4] or statins,[5] mild injury may disappear despite continued use. Rechallenge is typically met with a more severe reaction regardless of whether the initial reaction was severe or mild.[6] A few drugs injure the liver in a dose-dependent manner. The prototype for this kind of reaction is acetaminophen (APAP).

Idiosyncratic DILI is the most problematic form of drug toxicity. The underlying mechanisms are poorly understood. It refers to a combination of genetic and nongenetic factors that makes rare patients susceptible to drug injury.[7] In order to advance understanding and research on DILI, in 2003,

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10.1021/mp060133c CCC: $37.00
Published on Web 11/15/2007

the Drug-Induced Liver Injury Network (DILIN; http://dilin.dcri.duke.edu) was established at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The goals of the network are to establish a retrospective registry of patients who have experienced clinically significant DILI after treatment with isoniazid, valproate, phenytoin, and amoxicillin/clavulanate and to prospectively study of all cases of DILI presenting at the participating clinical centers and collaborative sites. Prescription medications, over-the-counter agents, and herbal medications are included.[8,9]

1. Epidemiology

Adverse drug reactions causing clinical hepatotoxicity are rare events. The reported incidence varies between 1 in 10000 and 1 in 100000 patients.[2,10] It was not even listed among the diagnoses causing hospitalization for adverse reactions in a recent large prospective study from the UK including 18820 patients.[11] However, the true incidence certainly is much higher due to difficulties in detection and diagnosis. The rarity of drug-related hepatotoxicity explains why it is commonly not detected in clinical trials that do not involve more than 2000–5000 participants. Generally, drug-related liver disease is first detected during the first two years after the beginning of marketing. Then, however, liver injury is the most common reason for the withdrawal from the market of an approved drug.

In a systematic investigation of hepatic adverse drug reactions in outpatients in France, an incidence of 14 cases per 100000 inhabitants (i.e., 0.014%) was found, suggesting that the true incidence would be 16 times greater than the number noted by spontaneous reporting.[12] In US hospitals, an incidence of 6.7% serious and 0.32% fatal adverse drug reactions was found.[13] A Swiss study in medical inpatients found DILI in 1.4% of patients; however, the majority of incidences were not mentioned in the physician's discharge letter.[14] DILI cases constituted 6% of all out-patients and 3% of referrals in a Swedish university out-patient hepatology clinic.[15]

In a prospective study in the US on the short-term outcomes of 308 cases with acute liver failure, idiosyncratic drug reactions were the presumptive cause in 13% of cases. Since 53% of these patients were liver transplanted, overall short-term survival reached 73%, whereas transplant-free survival was only 25%.[16] APAP accounted for 39% of all cases of acute liver failure.[16] Recent data from the US indicate that the number of APAP-induced acute liver failure cases has steadily increased to about 50% of cases.[17]

Of 461 cases of drug-induced liver disease submitted to the Spanish registry, 58% presented with jaundice, which was associated with 11.7% chance of progressing to death or transplantation.[18] In Sweden, drugs accounted for 17% of causes of acute liver failure.[19] Nonsteroidal anti-inflammatory drugs, analgesics, and antibacterials were the most common drugs likely to be responsible for acute liver disease in the Spanish and Swedish registries.[18–22] The most common drugs associated with fatalities reported from 1968 to 2003 to the WHO Collaborating Centre for International Drug Monitoring in Uppsala, Sweden, were acetaminophen, troglitazone, valproate, stavudine, halothane, lamivudine,

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didanosine, amiodarone, nevirapine and sulfamethoxazole/trimethoprim.[22]

2. Patterns of Liver Injury

Drug-induced liver injury covers a broad range of illnesses, which differ in severity level, temporal operational sequence and clinical-pathological modifications. The severity level reaches from clinically irrelevant biochemical and ultrastructural modifications, which may represent a metabolic adjustment of the liver to the drug, up to liver necroses with frequently fatal outcome, cirrhosis, or liver tumors. However, acute liver injuries, characterized by abnormalities lasting less than 3 months, predominate (about 90% of cases).[10,23]

Drug-induced pathology mimics nearly all known hepatobiliary diseases. Some histologic features are listed in Table 1. Although some drugs almost always produce the same liver disease, there are no pathognomonic features. In rare instances, dihydralazine and tienilic acid trigger liver disease resembling immune mediated hepatitis, including the development of anti-LKM2 and anti-LM autoantibodies.[24] Some drugs can produce many different hepatobiliary diseases varying from one patient to another.

3. Clinicopathological Classifications

In 1989, a panel of 12 European and American experts under the auspices of the Council for International Organizations of Medical Sciences (CIOMS) proposed standard designations of drug-induced liver disorders and criteria of causality assessment.[25,26] When liver biopsy has been performed, the lesion should be named according to the histological findings, for example, cirrhosis, acute hepatitis, chronic hepatitis, or hepatic necrosis. In the absence of histological data (and this corresponds to the majority of cases), the preferred term is *liver injury*.

By consensus, liver injury was defined as an increase of more than twice the upper limit of the normal (ULN) in the levels of serum alanine aminotransferase (ALT) or conjugated bilirubin or a combined increase of aspartate aminotransferase (AST), alkaline phosphatase (AP), and total bilirubin, provided one of them is >2 ULN. The international panel classified acute liver injuries in three groups using biochemical criteria based on ALT, AP, and the ratio between them.[6,25,26]

Table 1. Histological Features of Drug-Induced Liver Disease

histology in drug-induced liver disease	examples
abnormal liver function tests without morphology	
microsomal enzyme induction (γGT)	phenytoin, warfarin
hyperbilirubinemia	rifampicin
steatosis	
acute fatty liver	tetracyclins
steatohepatitis	amiodarone
acute hepatocellular necrosis	
focal necrosis	isoniacid, halothane (mild)
bridging necrosis	α-methyldopa
zonal necrosis	acetaminophen, halothane (severe)
massive necrosis	valproinic acid, halothane (fatal)
acute cholestasis	
without hepatitis	anabolic steroids, oral anticonceptives
	cytarabin, azathioprin
with hepatitis and/or bile duct lesions	chlorpromazine, flucloxacillin, erythromycin, amoxicillin/clavunalate
chronic cholestasis	
"vanishing bile duct syndrome"	chlorpromazine, flucloxacillin
sclerosing cholangitis	fluorouracil intra arterially (a. hepatica)
chronic parenchymal liver injury	
chronic active hepatitis	α-methyldopa
fibrosis and cirrhosis	methotrexate, hypervitaminosis A
granulomatous reactions	hydralazine
vascular lesions	
sinusoidal dilatation	oral anticonceptives
peliosis hepatis	anabolic androgenic steroids
noncirrhotic portal hypertension	hypervitaminosis A
liver vein occlusion, Budd–Chiari	6-thioguanine, pyrrolizidine alkaloids
nodular regenerative hyperplasia	thiopurines, antineoplastic agents
tumors	
hemangioma	oral anticonceptives (?)
focal nodular hyperplasia	oral anticonceptives trophical effects
hepatocellular adenoma and carcinoma	oral anticonceptives, androgenic steroids
rare carcinoma	oral anticonceptives, androgenic steroids
epitheloid hemangio-endothelioma	oral anticonceptives, androgenic steroids

3.1. Acute Drug-Induced Liver Injury. *Acute hepatocellular injury*, defined by ALT >2 ULN or ALT/AP ≥ 5, is the most common form of hepatic damage caused by drugs. It may be related to an overdosage, to an

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idiosyncratic drug reaction, or to drug hypersensitivity or autoimmune manifestations. Hundreds of compounds can produce acute hepatocellular injury, including herbal medicines.[27] Acute hepatocellular injury generally conveys no specific clinical features and mimics acute viral hepatitis. The major pathological finding is liver cell necrosis generally associated with inflammatory infiltration. The presence of eosinophils in the infiltrate and the centrilobular predominance of the lesions argue for drug hepatotoxicity and may, in contrast to hepatocyte drop-outs, be associated with more favorable outcome, as recently shown for disulfiram-induced liver injury.[28]

Discontinuation of the treatment is usually followed by complete recovery within 1–3 months. Subfulminant or fulminant courses occur that are associated with a 90% mortality rate unless emergency liver transplantation can be performed. The risk of developing fulminant hepatitis varies from one drug to another but is promoted by continuation of treatment despite the occurrence of jaundice.[10]

The presence of jaundice is of particular prognostic importance. A FDA working group paper of the year 2000 (http://www.fda.gov/cder/livertox/clinical.pdf) suggested ALT $\geq$3 ULN and total bilirubin levels $\geq$2 ULN as indicators of clinically significant abnormalities for use in the assessment of the hepatotoxicity of newly developed drugs. Whereas elevations of serum enzyme levels (ALT and aspartate aminotransferase, AST) were taken as indicators of liver injury, increases in total bilirubin were measures of overall liver function. The concept of combining these measures as prognostic markers has been named "Hy's Law"[29] after Hyman Zimmerman who in 1978 already described drug-induced hepatocellular jaundice as a serious condition with mortality rates of 10–50%.[30] Two recent studies from Spain[18] and Sweden[21] confirmed that DILI with jaundice is associated with greater mortality or the need for transplantation than is cholestatic or mixed injury.

Acute cholestatic liver injury is characterized by an isolated increase of serum AP above 2ULN or by an ALT/AP ratio $<$2. There are two subtypes: pure cholestasis and cholestatic hepatitis.

Pure cholestasis is characterized by pruritus and jaundice. Transaminases are generally normal or only slightly increased (although transient higher levels may occur early in the course because of bile salt-induced injury to hepatocytes). A liver biopsy may show bilirubin deposits in hepatocytes and dilated biliary canaliculi containing biliary pigments. These lesions predominate in the centrilobular area. Pure cholestasis is observed with a few drugs, mainly sex steroid derivatives, cytarabine, and azathioprine.

Acute cholestatic hepatitis associates cholestasis with clinical features such as pain and hepatic tenderness that frequently mimic features of acute biliary obstruction and cholangitis. Acute cholestatic hepatitis represents the second most frequent type of drug-induced liver injury and involves several hundred of agents. A liver biopsy reveals cholestasis with inflammatory infiltration in portal tracts. Hypersensitivity manifestations such as eosinophilia are often present. The prognosis is much better than that of hepatocellular hepatitis. After drug withdrawal, symptoms rapidly disappear and full recovery occurs within 3 months after drug withdrawal. Rarely, chronic cholestasis may follow, particularly when cholangiolitis is present and destruction of numerous small bile ducts has occurred, a picture mimicking primary biliary cirrhosis.[10,23]

Mixed pattern acute liver injury is characterized by an ALT/AP ratio between 2 and 5.[25] It represents a frequent picture of liver injury[10,23] with a generally very good prognosis. Clinical and pathological manifestations correspond to a mixture of those observed with hepatocellular and cholestatic hepatitis but also include granulomatous reactions. A mixed pattern liver injury is frequently associated with immunoallergic manifestations.

3.2. Chronic Drug-Induced Liver Injury. Chronicity is observed only in a minority ($<$10%) of cases with DILI. By consensus, the term chronic drug-induced liver injury is applied to liver diseases with a course longer than 3 months after withdrawal of the drug, without any reference to the underlying lesion.[25] A recent study from Spain defined chronic outcome as a persistent biochemical abnormality of hepatocellular pattern more than 3 months after drug withdrawal or more than 6 months after cholestatic/mixed damage.[31] In this study, cholestatic/mixed injury became chronic in 9% of patients which was significantly more likely than in patients with hepatocellular injury that developed chronic liver disease in 4%.[31]

Although being the cause of less than 1% of cases of chronic hepatitis and cirrhosis, drugs are an important cause for some rare lesions. Such is the case for estrogens and hepatic adenoma, and for the contribution of thiopurines and antineoplastic agents to hepatic vascular disorders such as nodular regenerative hyperplasia and peliosis hepatis (Table 1). Cholangio-destructive lesions in chronic cholestasis can progress to the vanishing bile duct syndrome[32] resembling primary biliary cirrhosis but without serum antimitochondrial antibodies.[33]

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4. Drug Metabolism and Excretion

Most drugs that enter the body, irrespective whether via the gastrointestinal tract, through the lungs or skin or by a parenteral route, are lipophilic and rendered more hydrophilic by enzyme reactions in the liver or kidney so that they can be filtered by the glomerulus or excreted in bile. Biotransformation from a nonpolar to a polar compound takes place in several steps, grouped as phase I reactions (oxidation, reduction or hydrolysis) and phase II reactions (mainly glucuronidation by UDP-glucuronosyltransferases, UGTs; sulfation by sulfotransferases, SULTs; and conjugation with glutathione by glutathione-*S*-transferases, GSTs).

Phase I reactions are mainly performed by cytochrome P450 enzymes belonging to a gene family consisting of about 300 members. Cytochrome P450 enzyme reactions result in aliphatic and aromatic hydroxylation; *O*-, *N*-, or *S*-dealkylation; or dehalogenation. Typically, a hydroxyl group is generated which then can participate in phase II conjugation reactions. Of intrinsic compounds, bilirubin is glucuronidated whereas steroid compounds and bile acids are glucuronidated and/or sulfated.

Native or by phase I and II reactions modified drugs are then excreted via specific transport proteins which is considered as phase III of drug metabolism and disposition. Transport proteins for xenobiotics are either ATP-binding cassette (ABC) proteins, i.e., ABCB (multidrug-resistance proteins, MDR1 and MDR3), ABCC (multidrug resistance-associated proteins, MRP1–4; putatively, MRP5–9)[34–37] and ABCG transporters[38,39] or organic anion-transporting polypeptides (OATPs) and organic anion transporters (OATs)[40,41] (Figure 1).

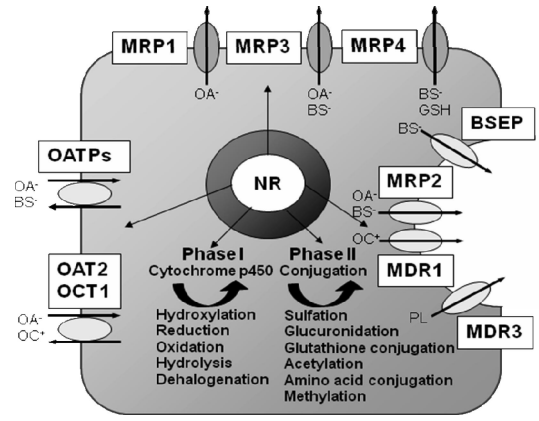

Figure 1. Hepatobiliary drug metabolism and transport systems. Hepatic drug uptake (mainly via organic anion transport polypeptides (OATPs), organic anion transporter 2 (OAT2), and organic cation transporter 1 (OCT1)) and hepatic drug export (mainly via multidrug resistance-associated proteins (MRPs) at the basolateral hepatocyte membrane and at the canalicular membrane via the bile salt export pump (BSEP), the phospholipid flippase MDR3, MRP2, and the multidrug resistance protein 1 (MDR1)) is mediated by specific transport systems which are able to transport drugs in addition to their physiological substrates. Biotransformation from a nonpolar to a polar compound takes place in several steps, grouped as phase I reactions (via cytochrome p450 enzymes) and phase II reactions (conjugation reactions). Distinct, partially overlapping nuclear receptor (NR) pathways are critically involved in the regulation of phase I, phase II, and transport processes.

4.1. Regulation by Nuclear Receptors. All phases of hepatic drug metabolism and disposition are under the control of nuclear receptors (NR). These are small proteins of similar structure despite variations in ligand affinity. In general, NR contain an amino-terminal ligand-independent transactivation domain, a core DNA-binding domain, a hinge region providing protein flexibility to allow simultaneous receptor dimerization and DNA binding, and a large carboxy-terminal region containing the ligand-binding domain, dimerization interface, and a ligand-dependent activation function.[42,43] Upon ligand binding, nuclear receptors undergo a conformational change that coordinately dissociates corepressors and facilitates recruitment of coactivator proteins to enable transcriptional activation.[44,45] NRs bind to their DNA response elements in a sequence-specific manner either as

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homodimers or as heterodimers with the retinoid X receptor (RXR) as partner.

The constitutive androstane receptor (CAR), the pregnane X receptor (PXR) and the arylhydrocarbon receptor (AhR) are the major sensors for lipophilic xenobiotics, including drugs, and thus the main NRs regulating expression of biotransforming enzymes and xenobiotics transporter proteins[46–48]. Side effects may be caused by NR (PXR/CAR) mediated CYP induction with subsequent alterations in drug metabolism. Conjugation enzymes are specifically induced via the antioxidant response pathway mediated by the transcription factor nuclear-related factor 2 (NRF-2).[49] NRF-2 knockout mice show significantly enhanced sensitivity to APAP hepatotoxicity associated with reduced expression of UGTs, SULTs, GSTs, as well as the cofactor synthesis enzymes.[50–52]

Many other orphan and adopted orphan NRs have recently been identified as key regulators of drug disposition genes. Indeed, nuclear receptors including farnesoid X receptor (FXR), peroxisome proliferator-activated receptor (PPAR), and hepatocyte nuclear factors (HNR1α, 3 and 4α) exhibit overlapping ligand specificities and regulate multiple gene targets, resulting in tissue- and organ-specific expression of drug disposition genes.[53] HNF4α is of particular importance. In fetal mice with conditional deletion of *HNF4α* it was shown that HNF4α critically is involved in the PXR- and CAR-mediated transcriptional activation of *Cyp3a4*.[54] As cross-talk between distal CAR/PXR binding sites and HNF4α binding sites was also found at the *Cyp2c9* promoter.[55] An exceptional member of the mammalian NR family is short heterodimer partner (SHP). SHP lacks an own DNA binding domain but modulates the activities of almost half-of all conventional nuclear NRs, which in turn regulate the expression of multiple target genes in a tissue-specific manner.[56]

Due to the idiosyncratic nature of most DILI, studies of NR-regulated biotransformation mechanisms are hard to perform. Exceptions are the cholestatic form of DILI with accumulation of cytotoxic bile acids and APAP toxicity, the latter being reviewed below. In fact, lithocholic acid is a rare example of a toxic endobiotic.[57] The role of NRs in the adaptive response to bile acid and cholestasis and NR regulated bile acid oxidation and conjugation has recently been reviewed in detail in this journal.[58,59]

5. Mechanisms and Targets of Cell Injury

Phase I biotransformation, mainly catalyzed by cytochromes P450, may not only detoxify xenobiotics but also activate drugs to reactive species that cause liver injury.[60] For APAP, basic mechanisms of injury and protection were described already in 1973.[61–65]

APAP primarily undergoes sulfation and glucuronidation (phase II reactions) but is metabolized by CYP2E1 (in a phase I reaction) to the reactive intermediate metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI) if the capacity of the phase II reactions is exceeded or if

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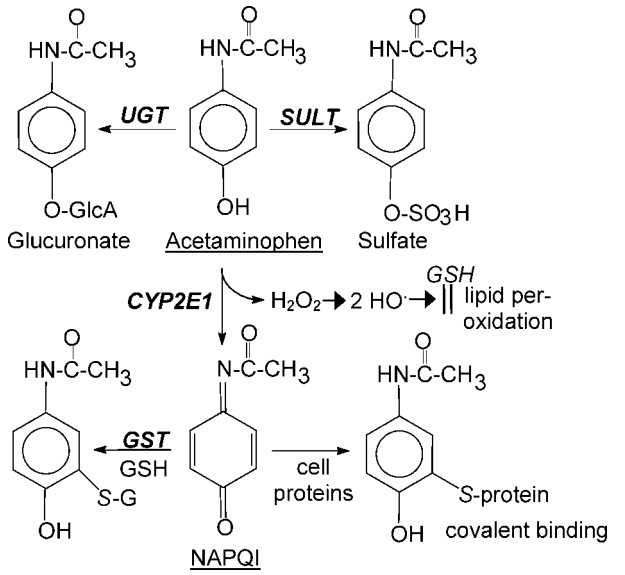

Figure 2. Metabolism of acetaminophen (APAP). Under normal conditions, APAP is conjugated in a phase II reaction with glucuronidate (GlcA) or sulfate by UDP-glucuronosyl- (UGT) and sulfotransferases (SULT), respectively. *N*-Acetyl-*p*-benzochinonimin (NAPQI), formed in a phase I reaction by CYP2E1, is conjugated with glutathione (GSH) by glutathion-*S*-transferase (GST). In the case of diminished GSH pools as in APAP poisoning, protein adducts are formed. GSH also inactivates H_2O_2 and hydroxyl radicals that are formed by CYP2E1.

CYP2E1 synthesis is induced,[66] as shown in Figure 2. Ethanol is a typical inducer of CYP2E1 and paradoxically enhances liver injury of APAP after cessation of alcohol consumption when it no longer competes with the drug at the enzyme.[67] This is consistent with the finding that patients hospitalized with APAP toxicity related to accidental misuse had higher rates of morbidity and mortality than those who attempted suicide, even though the latter had taken more APAP. A higher frequency of chronic alcohol abuse among the patients with accidental overdoses may be one explanation.[68]

GST is capable of detoxifying NAPQI to yield mercapturic acid and its derivatives, if glutathione is available or reconstituted by *N*-acetylcysteine. In the absence of glutathione substrate, covalent binding to cell proteins occurs.[66]

Hepatocytes are the major targets of DILI but other cells within the liver may also be the target of drug injury (Table 2) or aggravate incipient reactions.

A number of mechanisms triggered by high-energy intermediate or side products such as free radicals or activated oxygen species have been described that primarily involve the hepatocyte.[2] These reactive species may damage mitochondria and disrupt fatty acid oxidation and energy production, resulting in oxidative stress, lactic acidosis and triglyceride accumulation which morphologically is seen as microvesicular fat in liver cells.

Adducts formed by covalent binding of drugs to cellular proteins or enzymes may initiate and propagate tissue damage by still poorly understood mechanisms.[69] Protein–drug adducts may cause loss of ionic gradients or disruption of calcium homeostasis leading to disassembly of actin fibrils at the cell surface which results in cell swelling and lysis. Enzyme–drug adducts may result in loss of function. Furthermore, these adducts may migrate in vesicles to the cell surface. They are large enough to induce antibody formation and may also serve as target immunogens for cytolytic T-cell responses.[70] This in turn activates inflammatory reactions with the production of interferon γ (IFNγ)[71] and further hepatotoxicity. Innate immune systems seem to play a critical role. It was shown the depletion of natural killer NK/NKT-cells that are the major source of IFNγ protects against APAP toxicity.[71,72] Intracellular signaling mechanisms of APAP-induced liver cell death have recently

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Table 2. Cells and Subcellular Localization of Toxic (Drugs, Xenobiotics) Liver Lesions

cells and compartments	structural abnormalities	pathological consequences	examples
hepatocytes	plasma membrane damage	loss of membrane potentials	CCl_4
		ion leakage, cell rupture	$FeCl_3$
	mitochondrial toxicity	loss of ATP and energy	valproic acid, aspirin, Hg
	oxidative stress or alkylation	mitochondrial damage	acetaminophen
	multiple lesions	DNA damage, SH oxidation	
	disruption of cytoskeleton	1. cholestasis	1. phalloidine, cyclosporine
		2. apoptosis or necrosis	2. TCDD, chinones
tight junctions	loss of canalicular integrity	cholestasis	BCNU, leukotrienes
cholangiocytes	chemical or immunological cytotoxicity	cholestasis with bile duct lesions, chronic cholestasis	chlorpromazin, flucloxazillin
Kupffer cells	free radical oxygen metabolites	hepatocellular damage	endotoxins (?)
adherent leucocytes	ischemia/reperfusion damage (?)	leukotrienes	cholestasis, vascular damage, tumor necrosis factor (TNF)
	galactosamine (leucocytes)		
stellate cells	hypertrophia and hyperplasia	fibrosis	vitamin A, methotrexate
hepatic vessels, endothelial cells	damage of terminal arterioles, portal venoles, sinusoids, liver veins	ischemia, ischemia/reperfusion, veno-occlusive disease, portal hypertension, peliosis hepatis	thiopurine (azothioprin, 6-thioguanin, 6-mercaptopurin), androgenic/ anabolic steroids

been reviewed.[73] Fas and FasL deficient mice are protected against APAP[72] and silencing of Fas has also been shown to decrease toxicity.[74] The threshold for cell death by apoptosis or necrosis[75] can be modulated by intrahepatocyte signal transduction and transcription factors for protective or injurious pathways such as NrF2 and JNK.[76] The Fas system may also promote an anti-inflammatory responses, as shown in IL-6 and IL-10 knockout mice that where more susceptible to APAP injury.[77,78]

Impairment of hepatobiliary transport proteins at the canalicular membrane results in cholestasis.[79–81] A recent review summarized drug–transporter interactions and genetic risk factors predisposing to drug-induced cholestasis.[82]

Of interest, 13 out of 21 drugs withdrawn from the U.S. market due to hepatotoxicity or have a black box warning for hepatotoxicity have been shown to produce reactive metabolites.[83] Newer *in vivo* and *in vitro* approaches to detect potential toxicity early in the drug development process include higher-throughput screens for covalent binding as well as metabolomics, proteomics, transcriptonomics, in silico strategies,[84] and toxicogenomics.[85]

6. Diagnosis

DILI is difficult to diagnose with certainty. A drug reaction must be considered in any patient with liver dysfunction using prescription or nonprescription medication or dietary supplements. Symptoms range from nonspecific anorexia, nausea, and fatigue to obvious jaundice.

Aiming to improve the certainty of the diagnosis of DILI,

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causality-assessment methods have been developed.[6,25,26,86–90] The principles of these methods are similar and based on chronological and clinical criteria (reviewed in refs 10 and 90). DILI is suggested when symptoms occur between 1 and 12 weeks after the initial exposure; when symptoms improve within about a week after the patient stopped taking the drug, with normally complete recovery after a few weeks; and when a relapse occurs after accidental readministration. However, reexposure should not be performed on purpose due to the risk of life-threatening hypersensitivity reactions and liver failure.

Clinically, DILI is diagnosed after exclusion of other causes that might explain liver injury and on the presence of features favoring a drug etiology. A careful drug history should be taken, which includes the patient's use of prescription, over-the-counter, herbal, or alternative medications. A thorough medical history taking and appropriate serologic tests must exclude other causes of liver dysfunction, such as viral hepatitis (hepatitis A, B, C, E; cytomegalovirus, Epstein–Barr virus, herpes viruses), Wilson's disease, hemochromatosis, autoimmune liver disease, hypotension, and in particular liver disease related to alcohol abuse. Biliary tract disease causes cholestasis with elevations of AP and bilirubin and is diagnosed by ultrasonography, computed tomographic scanning or magnetic resonance imaging. The use of endoscopic retrograde cholangiopancreatography allows for simultaneous diagnosis and interventions to relieve obstruction.

Nevertheless, the diagnosis of DILI frequently remains doubtful. Diagnostic difficulties are due to nonspecific clinical features, previous or undiagnosed chronic liver disease, or due to the fact that the treated (e.g., infectious) disease itself affects the liver. However, the major problems are the intake of several possible or established hepatotoxic compounds at the same time and the underreporting of alternative, over-the-counter, or illicit medications.

DILI is excluded when another cause such as viral hepatitis is established or when the chronology is incompatible, i.e., when treatment started at a time when symptoms already where present or when the onset of DILI is later then 2 weeks after the end of treatment. An important exception is amoxicillin/clavulanate toxicity, which may typically occur 2–4 weeks after the end of treatment.

7. Treatment

7.1. Specific and Supportive Therapy. In most cases, there is no effective treatment other than stopping the drug and providing general supportive care. Intravenous carnitine for valproate-induced mitochondrial injury[91] and prompt use of *N*-acetylcysteine after APAP overdose[92] are exceptions. Activated charcoal seems the best choice to reduce APAP absorption. No *N*-acetylcysteine regime has been shown to be more effective than any other.[93]

The US Acute Liver Failure Study Group (ALFSG) is presently conducting a randomized, double blind, controlled trial of *N*-acetylcysteine given IV in persons with non-APAP ALF.[9] As yet, intravenous acetylcysteine cannot be recommended for routine treatment of non-APAP-induced ALF.[94]

Liver transplantation as the ultimate alternative for the treatment of ALF in DILI cures the majority of patients[16] but is limited by serious shortfall of donors. Furthermore, some patients may recover spontaneously without liver transplantation. Alternative nonbiological, biological and hybrid hepatic extra-corporeal support methods have been developed to provide extra-corporeal hepatic support in ALF.[95] A systematic review indicated that artificial support systems might reduce mortality in acute-on-chronic liver failure. However, artificial and bioartificial support systems did not appear to affect mortality in ALF.[96,97]

Ursodeoxycholic acid (UDCA) is a nontoxic bile acid (3α,7β-dihydroxy-5β-cholanic acid), which is normally present in human bile albeit in a low concentration of about 3% of total bile acids. UDCA is widely used for the treatment of a variety of chronic cholestatic liver diseases,[98] and at present, it is the only drug approved by the United States Food and Drug Administration for the treatment of primary

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biliary cirrhosis (PBC). However, the use of UDCA in drug-induced cholestasis is unproven.[99,100]

7.2. Treatment with Inducers of Transport and Cofactors. Whereas a number of drugs targeting heterodimers with RXR have been approved for treatment of metabolic diseases,[101] similar concepts for the treatment of DILI are still experimental, with the possible exception of rifampicin for the treatment of cholestatic pruritus.[102,103]

Corticosteroids are of unproven benefit for most forms of drug hepatotoxicity, although they may have a role for treating patients with hypersensitivity reactions.[2] However, in acute cholestasis (e.g., "steroid whitewash" in inflammation-induced cholestasis) as well as in chronic cholestatic disorders such as primary biliary cirrhosis (PBC)[104–106] the beneficial effects of steroids could be attributed to their anti-inflammatory and immune-modulatory actions and to effects mediated via enzyme and transport protein alterations. It is well established that glucocorticoids are required for the maintenance of cytochrome P450 expression and induction in hepatocytes. Moreover, induction of rat, mouse, or human CYP2B by phenobarbital either requires or is strengthened by glucocorticoids.[107] These effects are most likely indirectly mediated by PXR and CAR. As such, CAR appears to be a primary glucocorticoid receptor (GR)-response gene, since the CAR gene promoter harbors a GR response element.[108] Dexamethasone also induces CAR nuclear translocation.[109] Furthermore, glucocorticoids also induce PXR expression and nuclear translocation and thus induce target gene expression such as CYP3A4.[110,111] Corticosteroids were also reported to induce Mrp2 and Bsep and counteract its downregulation after endotoxin treatment *in vitro*.[112–114] These effects could possibly mediated by targeting GR, although this has so far only been shown for the bile salt transport proteins ASBT and NTCP.[115]

7.3. APAP Toxicity. In experimental animals, models have been developed to specifically study NR-regulated biotransformation pathways in APAP poisoning. The NRs under investigation are CAR, NrF2, and PPARα.

CAR is a key regulator of APAP metabolism and hepatotoxicity. Known CAR activators as well as high doses of APAP induced expression of three APAP-metabolizing enzymes in wild-type but not in $Car^{-/-}$ mice, and the $Car^{-/-}$mice were resistant to APAP toxicity. Inhibition of CAR activity by administration of the inverse agonist ligand androstanol 1 h after APAP treatment blocked hepatotoxicity in wild-type but not in,[116] $Car^{-/-}$ mice,[117] suggesting CAR inhibition as an innovative therapeutic

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approach for treating the adverse effects of APAP and potentially other hepatotoxic agents.

Keap1 is an adaptor molecule for the rapid degradation of Nrf2. The effects on Nrf2 were studied in mice bearing a hepatocyte-specific disruption of the *keap1* gene. The mutants with chronic activation of Nrf2 were significantly more resistant to toxic doses of APAP than control animals without affecting the morphological and physiological integrity of hepatocytes.[118]

PPARα controls genes encoding proteins involved in fatty acid transport and metabolism, including the fatty acid β-oxidation systems of peroxisomes and mitochondria. Activation of PPARα by clofibrate resulted in resistance to APAP-induced toxicity; this effect was not found in *Ppar*α-null mice.[119] Thus, in the event of APAP toxicity, peroxisomal fatty acid oxidation induced by activation of PPARα could compensate for mitochondrial damage by production of NADPH and ATP required for cell survival.

The latter concept may be of particular importance since agonists of PPARα such as fibrates and statins, which are inhibitors of the key enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), have also been discussed for the treatment of cholestasis (see below).

7.4. Drug-Induced Cholestasis. Drug-specific animal models for drug-induced cholestasis are lacking. Thus, the following general treatment options for cholestatic diseases are presented. Treatment strategies should be aimed at NRs and their target genes that, by affecting phase I and II biotransformation systems as well as phase III biliary and renal elimination systems, ameliorate cholestatic liver injury.

UDCA stimulates the expression and function of hepatobiliary transporters and enzymes involved in bile acid synthesis and detoxification at multiple transcriptional and posttranscriptional levels.[98–100,120] While UDCA proves to be effective in the treatment of human cholestatic liver diseases, most of the knowledge on its mechanisms of action was obtained from experiments in rodents. As such, UDCA stimulates the overall gene expression of both canalicular (Mrp2, Bsep) and alternative basolateral efflux pumps (Mrp3, Mrp4) in mouse liver.[121–124] Moreover, UDCA also stimulates renal (Mrp2, Mrp4) and intestinal (Mrp2, Mrp3) efflux pumps in mice, changes that may coordinately result in an increased overall elimination capacity for potentially toxic biliary constituents from the body.[123] Induction of CYP3A4/Cyp3a11 in primary human hepatocytes and in mouse liver by UDCA has recently been demonstrated.[122,125] However, *in vivo*, UDCA is only a weak inducer of human CYP3A4, as indicated by the formation of 1β-hydroxy DCA and 4β-hydroxycholesterol,[126] in particular when compared to rifampicin.[127] In contrast to these studies in healthy humans, in patients with PBC, no significant effects of UDCA on CYP3A-dependent steroid metabolism could be found.[128] While having only moderate effects on CYP3A4 expression in otherwise healthy human gallstone patients, UDCA markedly enhanced expression of BSEP, MDR3, and

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MRP4.[127] Although UDCA changes gene expression at a transcriptional level, no definite nuclear receptor has been elucidated. FXR, PXR, and the glucocorticoid receptor GR may mediate some of the UDCA actions,[125,129,130] but most of the transcriptional transporter effects are independent of FXR.[122,123]

Pharmaceutical compounds targeting FXR have been proposed as promising therapeutics[131–136] since studies in rodents revealed that FXR is an important factor determining liver injury in cholestasis.[137–139] Expression of FXR is reduced in the endotoxin-challenge model of cholestasis.[140] Moreover, hereditary forms of cholestasis are associated with reduced FXR expression, and mutations in FXR target genes such as *BSEP* lead to progressive familial intrahepatic cholestasis (PFIC type 2).[141,142] Experiments with synthetic FXR agonists GW4064 and 6α-ethylchenodeoxycholic acid (6-ECDCA) in cholestatic rodent models have been promising and resulted in reduced biochemical and histomorphological markers of liver injury in some models.[131,132,135] Reduced basolateral bile acid uptake via repression of Ntcp and Oatp 1 and 4, increased canalicular bile acid secretion via Bsep and Mrp2, and reduced bile acid biosynthesis through downregulation of Cyp7a1 and Cyp8b1 can be achieved by treatment with synthetic and naturally occurring (i.e., bile acids) FXR ligands; however, synthetic FXR ligands do not display bile acid toxicity. The plant sterol guggulsterone (GS) is the active agent of the guggul tree extract guggulipid that has been used to treat hyperlipidemia in humans. GS is a farnesoid X receptor antagonist in coactivator association assays but acts to enhance transcription of bile salt export pump.[143]

FXR also regulates the human phospholipid export pump MDR3, which may protect bile ducts by increasing phospholipid concentration in bile.[144] Besides modulation of bile acid transport and metabolism, FXR ligands have recently been suggested to have antifibrotic properties. Activation of the FXR/SHP cascade negatively regulates hepatic stellate cells leading to resolution of liver fibrosis.[145,146] In addition, FXR might also exert its antifibrotic properties via activation of PPARγ, which reduces hepatic stellate cell activation.[147] FXR was also proposed as a novel therapeutic target for treating or preventing cholesterol gallstone disease, since pharmacological activation of FXR increases cholesterol solubility by enhancing biliary bile acid and phospholipid concentrations.[148] Most of these compounds have been tested in animal models of cholestasis, and the experience with FXR agonists in humans is still very limited. Clinical studies with 6-ECDCA in the treatment of PBC have started recently, but no data are avialbale as yet.

Long before knowing their exact mode of action, ligands for CAR (phenobarbital[149–153] and Yin Chin,[154] a traditional Chinese herbal decoction) and PXR (rifampicin[149,155–158]) have been used for treatment of jaundice and pruritus in cholestatic liver diseases. In rodents, activation of PXR has

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been demonstrated to counteract LCA-induced liver toxicity. This was attributed to increased phase I and II biotransformations of LCA (i.e., hydroxylation via Cyp3a11 and sulfation via Sult2a1 and 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (PAPSS2), an enzyme that generates the donor cofactor (PAPS) for the reaction)[159–161] and to downregulation of bile acid biosynthesis via repression of Cyp7a1. Moreover, PXR also induced the export pump MRP2/Mrp2.[162,163] Besides increasing the urinary excretion of 6α-hydroxylated and glucuronidated bile acids,[127,164] administration of rifampicin also led to MRP2 overexpression in otherwise healthy gallstone patients.[127] Of clinical importance, Mrp2 induction together with increased glucuronidation of bilirubin via PXR-induced UGT1A1/Ugt1a1[127,165] enhances bilirubin detoxification.[166] This concept is supported by the opposite observation in a patient with Dubin-Johnson syndrome, bearing a nonsense mutation of the MRP2 gene, where rifampicin administration resulted in further increases of conjugated bilirubinemia.[167] Of note, ArH[168,169] and GR[170] were also found to regulate and induce human *UGT1A1*.

Recently, 6,7-dimethylesculetin, the main active compound present in a herbal decoction (Yin Chin) used in traditional Chinese medicine to treat and prevent neonatal jaundice, has been identified as a CAR activator.[154] This substance and other CAR agonists coordinately regulate the bilirubin clearance pathway including uptake (Oatp1a4/Sclo1a4),

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glucuronidation (Ugt1a1), and excretion (Mrp2)[163,171] . In addition to its effects on bilirubin metabolism, CAR stimulates expression of CYP3A4/Cyp3a11, SULT/Sult2a1 and PAPSS2 similarly to PXR, suggesting a coordinated regulation of this important bile acid detoxification pathway by both of these nuclear receptors.[162,172–175] Is has to be kept in mind that HNF4α is an important regulator of coordinate CAR- and PXR-mediated response to drug and xenobiotic effects on CYPs.[54,55] Phenobarbital was recently found to regulate murine *Hnf4α* independent of CAR and PXR.[176]

Besides stimulation of bile acid detoxification, CAR also stimulates expression of the alternative export pumps Mrp3 and Mrp4,[173–175,177–179] which are able to transport bile acids and their glucuronidate and sulfate conjugates.[169,180–183] The functional relevance of these pathways is reflected by reduced liver injury in LCA-fed mice after CAR activation.[162,174] However, these *Mrp3*$^{-/-}$ mice were more tolerant to APAP toxicity compared to wild-type mice, presumably due to a faster repletion of hepatic GSH and a redirection of APAP-glucuronide to bile.[183] Hepatic induction of murine *Mrp1–9* was also found to various extends after stimulation of AhR, PXR, PPARα, and Nrf2.[179]

Future studies are needed in other models to determine the safety of agents that activate FXR, CAR, HNF4α and potential other NRs. It has to be kept in mind that stimulating these receptors may interfere with various other metabolic pathways. Despite overall safety,[184] use of rifampicin in patients with cholestasis can be associated with liver toxicity in rare cases eventually causing liver failure,[185] and chronic CAR activation may promote liver tumors.[186,187]

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Another therapeutic strategy in the treatment of cholestasis may represent the use of PPARα agonists such as cholesterol-lowering fibrates and statins.[188,189] Administration of these substances to patients with primary biliary cirrhosis improved liver function tests.[190–195] This could, at least in part, be explained by stimulation of the biliary phospholipid excretion pump Mdr2, since phospholipids protect the bile duct epithelium from detergent bile salts by formation of mixed micelles.[196–200] Fibrates also repress cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme of bile acid synthesis.[201–204] For statins, pleiotropic antiinflammatory effects may contribute to improvements of surrogate parameters of cholestasis.[205,206] PPARγ agonists might also be of use in cholestasis, since these agonists inhibit hepatic stellate cell activation and counteract liver fibrosis in cholestasis.[147,207,208]

8. Summary and Outlook

Drug-induced liver injury (DILI) is a rare, largely unknown condition. With the exception of acetaminophen poisoning where timely administered *N*-acetylcysteine serves as an antidote, treatment usually is supportive. Since most biotransformation processes in the liver are under control of nuclear receptors (NR), treatment modalities aiming to stimulate or inhibit NR expression are tested in animal models of APAP toxicity or cholestasis. Some NR ligands have also been used in human cholestatic liver disease but studies on the efficacy in DILI are lacking. Our increasing understanding of the molecular regulation of transport and detoxification systems should lead to the development of more specific and powerful therapies targeting NRs for the treatment of DILI.

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Abbreviations Used

ABC, ATP-binding cassette; AhR, arylhydrocarbon receptor; ALF, acute liver failure; APAP, acetaminophen; BSEP, bile salt export pump; CAR, constitutive androstane receptor; NR, nuclear receptor; DILI, drug-induced liver disease; FXR, farnesoid X receptor; GR, glucocorticoid receptor; GST, glutathione-*S*-transferase; GSH, reduced glutathione; HNR, hepatocyte nuclear factor; LCA, lithocholic acid; MDR, MRP, multidrug resistance/multidrug resistance-associated protein; OAT/ OATP, organic anion transporter/transporting polypeptide; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RXR, retinoid X receptor; SULT, sulfotransferases; UDCA, ursodeoxycholic acid; UGT, UDP-glucuronosyltransferase; ULN, upper limit of normal.

Acknowledgment. This work was supported by grants from Karolinska Institutet and the Swedish Medical Association (to H.U.M.) and by Grant No. P18613-B05 from the Austrian Science Foundation and a GEN-AU project grant from the Austrian Ministry of Science (to MT).

MP060133C