

Xenobiotic-induced hepatotoxicity: mechanisms of liver injury and methods of monitoring hepatic function

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Xenobiotic-induced liver injury is a clinically important etiology of hepatic disease that, if not recognized, can lead to hepatic failure. In this article we discuss the mechanisms of xenobiotic-induced liver injury, various factors that can alter the risk and severity of injury, the clinical and laboratory manifestations of injury, and the methods used to detect the presence of injury and (or) functioning liver mass.

INDEXING TERMS: xenobiotic metabolism • cytochrome P450 enzymes • cytotoxic/cholestatic liver injury • liver function tests

Many xenobiotics (drugs and environmental chemicals) are capable of causing some degree of liver injury. In the US, xenobiotic-induced liver toxicity is implicated in 2–5% of hospitalizations for jaundice, an estimated 15–30% of the cases of fulminant liver failure, and ~40% of the acute hepatitis cases in individuals older than 50 [1, 2]. Fortunately, most drug-induced liver injuries resolve once the offending agent is withdrawn, but morbidity may be severe and prolonged as recovery ensues. The overall mortality rate for drug-induced liver injury is ~5% [3].

The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure [4]. The liver is divided into multiple lobules, each centered around a terminal hepatic (central) venule and surrounded peripherally by six portal triads. Afferent blood is supplied by the portal venules

and hepatic arterioles of the portal triads, flows through the hepatic venous sinusoids, and empties into the terminal hepatic venule. The regional pattern of hepatocellular necrosis observed with some xenobiotic-induced liver injuries can be understood by dividing the liver into functional subunits referred to as acini [4, 5]. Each liver acinus is divided into three concentric zones of hepatocytes radiating from a portal triad and terminating at one or more adjacent terminal hepatic venules. Hepatocytes closest to the portal triad (zone one) receive blood most enriched with oxygen and other nutrients and are most resistant to injury. Hepatocytes more distal to the blood supply receive a lower concentration of essential nutrients, making them more susceptible to ischemic or nutritional damage. Most important for xenobiotic-induced hepatic damage, the centrilobular (zone three) hepatocytes are the primary sites of cytochrome P450 enzyme activity, which frequently makes them most susceptible to xenobiotic-induced liver injury [6], as discussed below.

Contribution of Metabolism to Xenobiotic-Induced Liver Injury

Most drugs are not intrinsically toxic to the liver but can cause injury secondary to the production of an hepatotoxic drug metabolite, a process known as bioactivation [7, 8]. Because gastrointestinal absorption is enhanced by lipid solubility, most xenobiotics are highly lipophilic compounds, which are poorly excreted by the kidneys [7]. The liver plays a critical role in promoting excretion of these compounds by transforming them into metabolites of greater water solubility.

Metabolic reactions are of two types, phase I and phase II [7, 8]. Phase I (oxidation, reduction, or hydrolysis) reactions typically occur first, and enhance water solubility by generating hydroxyl, carboxy, or epoxide functional groups on the parent compound. These functional groups in turn facilitate phase II reactions, conjugation with glucuronate, sulfate, acetate, or glutathione moieties. Conjugation reactions generally serve to further enhance water solubility and renal excretion [7]. Phase II reactions

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also play a role in the prevention of xenobiotic-induced liver injury because most conjugates are biologically inactive [7–9]. Disruption of normal phase II processes can lead to accumulation of hepatotoxic phase I metabolites.

Phase I oxidation and reduction reactions are primarily catalyzed by cytochrome P450 enzymes, a supergene family of heme-containing, mixed-function oxidase enzymes found in greatest concentration in the smooth endoplasmic reticulum of centrilobular hepatocytes [7, 10, 11]. These enzyme reactions have the potential to induce cellular injury via several mechanisms of toxicity. The cytochrome P450 enzyme-catalyzed oxidation of xenobiotics such as bromobenzene or acetaminophen generates a highly electrophilic intermediate capable of forming covalent adducts with critical cellular macromolecules such as thiol-containing membrane proteins that regulate calcium homeostasis [7, 8, 12]. The induction of increased intracellular calcium concentrations may be the common pathway leading to cell death. Cytochrome P450 enzyme-mediated reduction of halogenated hydrocarbons such as carbon tetrachloride or halothane can also generate free radical intermediates, which can directly damage cell membranes via lipid peroxidation, or can target nucleophilic DNA residues [8, 13–15]. Similar cellular damage can result from the generation of reactive oxygen species such as hydrogen peroxide and hydroxyl free radical during a process known as redox cycling [8, 16]. Redox cycling occurs when a reduced quinone substrate such as menadione or doxorubicin spontaneously reoxidizes in the presence of oxygen, thereby reducing the oxygen molecule [17].

Determinants of Host Susceptibility to Xenobiotic-Induced Liver Injury

Xenobiotic-induced liver injuries can be broadly classified as intrinsic or idiosyncratic in nature, as outlined in Table 1 [1, 18]. Intrinsic injuries are predictable, in that a threshold dose exists in all individuals, typically leading to zonal liver cell necrosis accompanied by little or no signs of inflammation. These injuries are generally the result of phase I bioactivation reactions, with damage mediated by reactive drug metabolites as previously discussed. In contrast, the nature of idiosyncratic liver injuries suggests that most of these are mediated by an immune mechanism [18, 19].

Idiosyncratic liver injuries are usually associated with classic signs of hypersensitivity, including fever or rash, and liver biopsy specimens reveal evidence of monocytic or eosinophilic infiltrates [9, 20]. These reactions tend to occur only after repeated exposure, suggesting the need for initial sensitization, and drug rechallenge generally elicits prompt reappearance of symptoms. Both humoral and cellular immune mechanisms have been implicated in these types of liver injuries [1]. One proposed explanation is the formation of a metabolite that is either haptogenic or alters an hepatocyte macromolecule to generate a neoantigen [19]. The idiosyncratic hepatotoxicity of non-steroidal antiinflammatory drugs such as diclofenac have been linked to such a mechanism [21]. The phase II glucuronidation of these compounds can also produce reactive acyl glucuronides, which may bind irreversibly to nucleophilic amino acid side chains in hepatocyte membranes, potentially inducing a cell-mediated or humoral immune response [21]. T lymphocytes or immunoglobulin molecules targeted against a variety of presumed neoantigens have been identified. Some of these immunoglobulin molecules recognize the cytochrome P450 isoenzyme responsible for metabolism of the offending drug compound. Examples of anti-microsomal antibodies include anti-liver/kidney microsome (LKM)³ antibodies directed against cytochrome P450IID6 isoenzymes (anti-LKM1 antibodies), and anti-LKM2 antibodies, which recognize P450IIC9 [22, 23]. Anti-microsomal antibodies targeting P450IA2 and P450III A1 isoenzymes have also been reported in idiosyncratic liver injuries associated with dihydralazine and aromatic anticonvulsants, as well as anti-LKM3 antibodies directed against uridine diphosphate glucuronyltransferase (UDPGT) enzymes [24–26].

Idiosyncratic drug-induced liver injuries can also exhibit a regional pattern of cellular damage and necrosis or a mixed type of pattern. A centrilobular pattern is associated with the use of halothane and other volatile halogenated anesthetic agents [27]. As would be expected with idiosyncratic injury, halothane hepatitis is non-dose-related and exceedingly rare [28]; however, it exhibits characteristics of both idiosyncratic and intrinsic liver injury [29]. Halothane is extensively metabolized by cytochrome P450 enzymes, with the corresponding oxygen tension present during these reactions determining whether oxidation or reduction pathways predominate [30, 31]. Conditions of relative hypoxia favor reduction and the generation of various hepatotoxic phase I intermediates capable of forming protein adducts. This process takes place in all individuals exposed to halothane, but

Table 1. Characteristics of intrinsic and idiosyncratic xenobiotic-induced liver injuries [8, 18].

	Intrinsic	Idiosyncratic
Predictable	Yes	No
Dose-dependent	Yes	No
Predominant pattern of injury	Zonal hepatocellular necrosis	Diffuse necrosis and cholestasis
Inflammatory infiltrates	Rare	Common
Systemic signs of hypersensitivity	Rare	Common

³ Nonstandard abbreviations: anti-LKM, liver/kidney microsome antibodies; UDPGT, uridine diphosphate glucuronyltransferase; NAPQI, *N*-acetyl-*p*-benzoquinone imine; UDPGA, uridine diphosphate glucuronic acid; NAT, *N*-acetyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ -glutamyltransferase; PT, prothrombin time; and MEGX, monoethylglycinebutyrate.

antibodies directed against these presumed neoantigens are detectable only in individuals with severe liver injuries, suggesting individual host susceptibility to injury [32]. The neoantigens include adducts with proteins in the endoplasmic reticulum (reticuloplasmins) and the pyruvate dehydrogenase enzyme complex of the mitochondria [33, 34].

However, some agents classified as idiosyncratic hepatotoxins cause milder forms of dose-related liver damage that cannot be explained by an immune mechanism [1, 2]. These injuries are generally not accompanied by the usual signs of hypersensitivity and are slow to reappear on drug rechallenge. In addition, the threshold dose required to induce liver injury with many intrinsic hepatotoxins displays a great deal of individual variability. These observations have led to use of the terms "immunologic" and "metabolic" idiosyncrasy [18]. Metabolic idiosyncrasy appears to be the result of inherent individual variability in the activity of hepatic drug-metabolizing enzymes. A variety of host-related factors are thought to contribute to metabolic idiosyncrasy.

VARIABILITY IN PHASE I ENZYMATIC ACTIVITY

Three *CYP* gene families, designated *CYP1*, *CYP2*, and *CYP3*, encode the cytochrome P450 enzymes that play the major role in human xenobiotic metabolism (Table 2) [7, 10, 35]. Genetic, physiologic, pathophysiologic, and xenobiotic-induced factors that affect cytochrome P450 enzyme activity may help to account for the increased susceptibility of certain individuals to drug-induced liver injury, as outlined in Table 3 [1, 8, 36–43]. As a general rule, women are at increased risk of drug-induced liver

injuries, particularly chronic ones. Oral contraceptives are known inducers of cytochrome P450 enzyme activity, whereas pregnancy has been shown to induce certain isoenzymes, such as P450III A4, and inhibit others [42–46]. Cytochrome P450IA2 activity is gender-related, with males consistently exhibiting higher enzyme activity [47]. However, parity may be an important determinant of P450IA2 activity. Parous females who lactated appear to exhibit activity similar to that of males [47]. Genetic polymorphisms, characterized by poor and extensive metabolizer phenotypes, have been identified in the P450IIC18, P450IID6, P450IIE1, and possibly the P450IIIA4 isoforms and can alter susceptibility to xenobiotic-induced liver injury (Table 3) [11, 39, 40, 48]. For example, the risk of perhexiline maleate-induced liver injury is higher in individuals with the P450IID6 poor-metabolizer phenotype [49].

Many drug-induced liver injuries are clearly age-related [50, 51]. The activity of some cytochrome P450 isoenzymes (such as P450IA2 and P450IID6) is reduced by ~70% in neonates, followed by a rapid increase in activity during the first few weeks to months after birth to an amount two- to threefold more (for P450IA2) than that of adults. The activity of other P450 isoforms, e.g., P450IIIA enzymes, can be higher in newborn infants than in adults, and certain P450IIIA isoforms are primarily expressed only in the developing fetus [52–54].

The classic example in which altered activity of a cytochrome P450 isoenzyme can increase the risk of liver injury is acetaminophen toxicity. Ordinarily, >90% of an acetaminophen dose undergoes phase II glucuronidation and sulfation, yielding inactive conjugates that are excreted in urine and bile [9, 55]. About 5% of a dose is oxidized by cytochrome P450IIE1 isoenzymes, and to a lesser degree by other P450 isoenzymes, to the hepatotoxic intermediate *N*-acetyl-*p*-benzoquinone imine (NAPQI). Hepatocellular damage is ordinarily prevented by phase II glutathione conjugation, which converts NAPQI to the inactive metabolite mercapturic acid. Acute ingestion of >~10 g of acetaminophen saturates the normal glucuronidation and sulfation pathways, leading to increased production of NAPQI, which rapidly depletes available glutathione stores. The risk of damage is increased, and the threshold dose lowered, with concomitant use of compounds such as alcohol or phenobarbital that are capable of inducing P450IIE1 activity [56].

Table 2. Characteristics of the major cytochrome P450 enzymes involved in human xenobiotic metabolism [7, 10, 35].

Gene family	Cytochrome P450 isoenzymes	Representative substrates
<i>CYP1</i>	P450IA2	Caffeine, theophylline
<i>CYP2</i>	P450IIC19	Mephenytoin, phenylbutazone
	P450IID6	Debrisoquin, metoprolol
	P450IIE1	Ethanol, acetaminophen
<i>CYP3</i>	P450IIIA4	Erythromycin, cyclosporine

Table 3. Determinants of individual variability in cytochrome P450 enzyme activity.

	Genetic polymorphism	Enzyme inducers
P450IA2	No	Smoking, cruciferous vegetables, charbroiled beef [37, 38]
P450IIC19	Yes [11]	None
P450IID6	Yes [39]	None
P450IIE1	Yes [40]	Chronic ethanol use, diabetes, isoniazid [41]
P450IIIA4	No	Glucocorticoids, barbiturates, rifampin, pregnancy [42, 43]

VARIABILITY IN PHASE II ACTIVITY

Perhaps equally important to host susceptibility is the functional capacity of phase II detoxification pathways. The most common type of phase II reaction is glucuronidation, where glucuronic acid is transferred from uridine diphosphate glucuronic acid (UDPGA) to a drug or phase I metabolite by the enzyme UDPGT [7]. UDPGT enzymes are produced by two gene families, *UGT1* and *UGT2* [57]. At least six isoenzymes are encoded by *UGT1* genes and four isoforms by *UGT2* [58, 59]. The potential

for individual variability is illustrated by the fact that inducing agents such as phenobarbital do not affect the activity of these isoforms equally [60]. The capacity of the glucuronidation process can be inhibited by the temporary depletion of available UDPGA stores by drugs such as acetaminophen and chloramphenicol [61]. Other drugs, including naproxen, ethinyl estradiol, and certain benzodiazepines have been shown to directly inhibit UDPGT enzyme activity [62, 63]. Age can also alter UDPGT activity, which is low at birth but increases steadily to nearly adult values by age 1–3 months [64]. Nutritional deficiencies are another potentially relevant cause of deficient UDPGA stores [65].

Sulfation reactions catalyzed by three families of cytosolic sulfotransferase enzymes represent important detoxification pathways for alcohols and phase I intermediates containing phenol groups [7, 66]. The efficiency of sulfation reactions can be compromised by temporary depletion of inorganic sulfate pools by ingestion of drugs such as salicylamide [67].

Glutathione conjugation is critical in preventing liver injury from several agents, including acetaminophen and bromobenzene epoxide, by acting as a free radical scavenger [7]. Acetaminophen overdose causes liver injury secondary to the temporary depletion of glutathione stores in the liver. Administration of the antidote *N*-acetylcysteine prevents further injury by stimulating glutathione synthesis, thereby replenishing liver stores [68]. Glutathione stores are also sensitive to fasting and alcohol ingestion and, as in most phase II pathways except sulfation, glutathione conjugating activity is depressed in neonates, even though glutathione transferase enzyme activities are apparently within the reference interval [69].

Amine or hydrazine-containing drugs or phase I metabolites are detoxified primarily by phase II acetylation reactions, catalyzed by cytosolic *N*-acetyltransferase (NAT) enzymes [7]. *NAT-1* and *NAT-2* represent the two gene families currently known to exist in the human liver [70]. Polymorphism in *NAT-2* results in the rapid or slow acetylator phenotype, which has been implicated in host susceptibility to liver damage by drugs such as isoniazid [71, 72]. Isoniazid undergoes extensive *NAT-2*-catalyzed acetylation to acetylisoniazid, which is then hydroxylated by cytochrome P450 enzymes to the hepatotoxic intermediate acetylhydrazine, a metabolite capable of forming covalent cellular adducts [73]. The risk of liver toxicity is higher in slow acetylators, in the elderly, and in association with concomitant use of cytochrome P450 inducers such as alcohol or rifampin [74].

Diagnosis of Xenobiotic-Induced Liver Injury

CLINICAL SUSPICION

Because most xenobiotic-induced liver injuries are reversible, it is critical that the developing injury be recognized promptly and the offending agent withdrawn [9]. Unfortunately, many injuries of this type present insidiously with nonspecific symptoms attributable to several poten-

tial etiologies. Once the classic signs of hepatic injury such as jaundice or coagulopathy are established, the injury is often severe and the risk of mortality increased [75]. The use of any agent known to cause liver injury should involve an extra measure of caution and vigilance [9]. Regular monitoring of hepatic aminotransferase enzymes may be warranted in some cases, particularly during the initial months of therapy with agents such as phenytoin or isoniazid. Before the institution of therapy, the patient's medical history should be carefully reviewed to identify potential risk factors for injury, including concomitant medications. Patient education is equally important, for many liver injuries are preceded by relatively mild prodromal symptoms. Severe injuries are often preventable if these early signs and symptoms are brought to the attention of healthcare providers and the offending agent is withdrawn.

A key element in establishing the cause of injury is the temporal relationship between drug administration and appearance of symptoms, as well as the withdrawal of therapy and resolution [9]. Because many drug-induced liver injuries are much more severe with reexposure, rechallenge with the suspected agent is generally not recommended. A complete medication history, including all prescribed and over-the-counter agents, as well as potential exposure to occupational or environmental chemicals, should be carefully documented and screened.

CLINICAL, BIOCHEMICAL, AND HISTOLOGIC PATTERNS OF XENOBIOTIC-INDUCED LIVER INJURY

Xenobiotic-induced liver injuries can be broadly classified as cytotoxic (necrotic or steatotic), cholestatic, or mixed (Table 4) [1]. The presence of an injury can be clearly established on the basis of the clinical and biochemical evidence. These indices can also be utilized in establishing a tentative classification of the injury [76]. However, histologic examination of a liver biopsy specimen remains the only means of definitively diagnosing the type of injury present [77].

Hepatocellular necrosis. Clinically, hepatocellular necrosis can range in severity from asymptomatic increases of aminotransferase enzymes to jaundice to overt hepatic failure [78]. With intrinsic hepatotoxins, nonspecific gastrointestinal symptoms such as nausea or vomiting typically begin within a few hours of exposure. These symptoms often resolve within 48 to 72 h, followed by a 1- to 2-day period of relative well-being. With severe injuries damage is ongoing, however, and biochemical evidence (increased hepatic aminotransferase enzymes) of damage, often accompanied by oliguria, begins to appear. Overt liver failure is generally established within 3–5 days, characterized by jaundice, coagulopathies, neurologic symptoms, and acute renal failure. The degree of aminotransferase enzyme increase, hyperbilirubinemia, and prolongation of the prothrombin time have prognostic

Table 4. Histologic patterns of xenobiotic-induced liver injury.

Pattern of liver injury	Proposed mechanism of injury	Representative xenobiotics
Zonal necrosis	Formation of cellular adducts, free radicals, neoantigens	Acetaminophen, halothane [27, 55]
Toxic hepatitis	Hapten formation	Isoniazid, phenytoin, sulfonamides [82, 83]
Microvesicular steatosis	Deficient β -oxidation of fatty acids	Tetracycline, valproic acid, tamoxifen [90, 93]
Macrovesicular steatosis	Increased triglyceride synthesis, decreased egress	Chronic ethanol use [87]
Phospholipidosis	Formation of lysosomal inclusions	Perhexiline maleate, amiodarone [98, 99]
Hepatocanalicular cholestasis	Inflammation, damage to bile canaliculi	Chlorpromazine [103, 104]
Canalicular cholestasis	Decreased membrane fluidity, Na^+/K^+ -ATPase activity	Steroid hormones [106]
Veno-occlusive disease	Thrombosis of terminal hepatic venules	Radiation, azathioprine, 6-thioguanine [113]
Peliosis hepatitis	Damage to sinusoidal membranes	Steroid hormones, tamoxifen [117, 118]
Hepatic tumors	Unknown	Steroid hormones [119, 122]

significance, as does the appearance of any manifestation of hepatic encephalopathy [79, 80].

Toxic hepatitis. Hepatocellular necrosis is a hallmark of these injuries, but the associated symptoms and histologic pattern of injury are nearly identical to those observed with acute viral hepatitis, as outlined in Table 4 [1, 18, 81–83]. Histologically, these injuries typically reflect diffuse hepatocellular necrosis, which may be associated with cholestasis. Lobular structure is generally maintained, and even in severe cases, areas of necrosis are usually surrounded by viable hepatocytes that reveal various degrees of degenerative changes [18]. Prominent monocyte or eosinophilic inflammatory infiltrates are common [20]. These injuries are thought to result from bioactivation to toxic metabolites [18].

Symptoms of toxic hepatitis range from asymptomatic increases of hepatic aminotransferase enzymes to signs of overt liver failure. With drugs such as phenytoin, these injuries often present with abrupt onset of fever and nausea, which may be accompanied by diffuse rash and arthralgias [1, 84]. Evidence of severe liver injury (jaundice, coagulopathies, neurologic symptoms) appears 3–4 days after the onset of fever. The prodromal symptoms are usually mild or even absent with drugs such as isoniazid [85]. As with hepatocellular necrosis induced by intrinsic hepatotoxins, clinical and biochemical markers have prognostic value [86]. Extreme increases of hepatic aminotransferase enzymes, jaundice, and coagulopathies are associated with a mortality rate >10%.

Steatosis. Steatosis results from the abnormal accumulation of triglycerides within the hepatocyte, as summarized in Table 4 [18, 87]. Macrovesicular steatosis is characterized by a single large cytoplasmic vacuole of triglyceride within the hepatocyte that displaces the nucleus peripherally. The etiology of macrovesicular steatosis is multifactorial, including increased mobilization of fatty acids, increased hepatic synthesis of fatty acids, increased synthesis of triglyceride from fatty acids, and deficient removal of triglyceride from the hepatocyte via defective VLDL synthesis [78, 88]. Microvesicular steatosis is a less common but more severe variant, resulting primarily

from deficient mitochondrial β -oxidation of fatty acids and characterized by the presence of multiple small droplets of triglyceride within the hepatocyte, which do not displace the nucleus [89, 90]. The β -oxidation of fatty acids is a critical process, because the resulting acetyl-coenzyme A moieties are the primary source of ATP in most cells. Disruption of this process promotes the esterification of fatty acids in the cytoplasm to triglyceride, robs the cell of energy, and leads to hyperammonemia via inhibition of ureagenesis [91, 92]. Microvesicular steatosis can exhibit a diffuse or regional pattern and in severe cases is accompanied by inflammation and hepatocellular necrosis [1, 89].

Valproic acid is an established cause of microvesicular steatosis, which resembles Reye syndrome and is in fact more likely to occur in young children [93, 94]. The lesion is accompanied in severe cases by inflammation, necrosis, and bile duct injury. Valproic acid-induced liver injury is thought to result from phase I bioactivation [1, 95]. Cytochrome P450 enzymes mediate the production of Δ^4 -valproic acid, an oxidative metabolite capable of generating coenzyme derivatives. Production and accumulation of these derivatives may inhibit mitochondrial β -oxidation via depletion of free coenzyme A and carnitine concentrations [18, 89, 96]. Early symptoms of injury are insidious in nature, and include gastrointestinal complaints and mental status changes; they usually appear during the first 4 months of drug exposure [94, 97].

A related condition termed phospholipidosis is produced by perhexiline maleate and the antiarrhythmic agent amiodarone [18, 98, 99]. This dose-related liver injury is characterized by the accumulation of phospholipid–drug complexes within the lysosomes of hepatocytes, bile ductal cells, sinusoidal Kupffer cells, and various other tissues, including peripheral nerves, skeletal muscle, cornea, heart, lungs, and skin [100]. The amphiphilic nature of these agents is thought to promote the formation of drug–phospholipid complexes, called lysosomal inclusions. Both amiodarone and its *N*-desethyl metabolite readily form these complexes, subsequently promoting the accumulation of phospholipids via inhibition of normal phospholipase activity [101]. These inclusions are

present in virtually all patients treated with amiodarone, although overt liver injury is rare [102].

Cholestasis. Xenobiotic-induced cholestasis results from the disruption of bile production or flow and exhibits one of two patterns, as outlined in Table 4 [1, 18]. Hepatocanalicular (hypersensitivity) cholestasis is characterized by prominent monocyte portal inflammation and secondary damage to bile canaliculi, as seen with chlorpromazine. Chlorpromazine and its 7,8-dihydroxy and 7-hydroxy metabolites interfere with bile acid secretion via disruption of canalicular membrane fluidity and Na^+/K^+ -ATPase activity [103, 104]. Inhibition of phase II sulfation pathways increases the risk of liver injury [105]. Evidence of immunologic idiosyncrasy includes the relatively rare occurrence of this reaction (in <2% of the population), the rapidity of onset (typically within the initial month of therapy), the prompt reappearance of symptoms upon drug rechallenge, and the usual presence of peripheral eosinophilia [18]. Metabolic idiosyncrasy as a cause of toxicity is supported by the observation that the sulfate conjugate is not hepatotoxic, coupled with the fact that formation of oxidative phase I metabolites would be expected to exhibit individual variability. Nonspecific flu-like and gastrointestinal symptoms often precede the development of jaundice, which is associated with intense pruritis [18]. Overt jaundice is generally accompanied by extreme increases of alkaline phosphatase and conjugated serum bilirubin. Hepatic aminotransferase enzymes are generally only mildly increased in the absence of significant necrosis. Most patients recover completely within 3 months of drug withdrawal.

In contrast, the use of estrogen or 17α -substituted steroids is associated with canalicular or bland cholestasis, a less severe variant [18, 106]. Generally appearing within the first 2 months of drug therapy, bland cholestasis is associated with an insidious onset of jaundice and pruritis, and less commonly with gastrointestinal complaints. Systemic signs of hypersensitivity are absent. Bile stasis is most prominent in the canaliculi, without accompanying inflammation or necrosis [107]. The injury typically resolves completely within 2 months of drug withdrawal. Although asymptomatic mild increases of alkaline phosphatase or aminotransferase enzymes are relatively common, jaundice is rare [108]. The influence of individual susceptibility is illustrated by the fact that the risk of steroid jaundice is greater in patients with a history of jaundice of pregnancy and in populations of Scandinavian or Chilean descent [108-110]. Bland cholestasis appears to result from drug-induced alterations in sinusoidal membrane fluidity and Na^+/K^+ -ATPase activity with no apparent effect on canalicular membranes, resulting in a decreased hepatocellular uptake of bile acids from sinusoidal blood [18]. Host susceptibility may relate to differences in the production and excretion of presumably nontoxic methylated metabolites and glutathione activity [111].

Hepatic vascular injury. Veno-occlusive disease is a severe form of drug-induced liver injury characterized by thrombosis of efferent hepatic venules, leading to centrilobular necrosis and liver outflow obstruction, which can progress to congestive cirrhosis, as outlined in Table 4 [18, 112, 113]. The condition presents clinically with abrupt onset of severe abdominal pain, hepatomegaly, and jaundice, accompanied by extreme increases of hepatic aminotransferase and alkaline phosphatase enzymes [114]. Veno-occlusive disease can progress rapidly to overt hepatic failure, with manifestations of congestive cirrhosis such as ascites, coagulopathies, and hepatic coma.

Oral contraceptive use is associated with hepatic venous thrombosis, a condition that can also lead to congestive cirrhosis resembling Budd-Chiari syndrome [16, 115]. This injury is reportedly twice as common in oral contraceptive users as in nonusers and is thought to result from the thrombogenic properties of these agents [116]. Oral contraceptives can also produce another type of vascular lesion called peliosis hepatitis, in which weakening of sinusoidal membranes leads to the development of blood-filled sacs within the hepatic parenchyma [117, 118].

Hepatic tumors. Chronic use of oral contraceptives is associated with the development of hepatic adenomas, benign tumors typically observed only in women of childbearing age and which were exceedingly rare before the widespread use of these agents, as outlined in Table 4 [18, 119]. These tumors usually resolve completely with drug withdrawal, and risk of development is highly correlated with duration of drug exposure [120]. Hepatocellular carcinomas have been associated with the chronic use of anabolic, androgenic, and contraceptive steroids [121, 122]. The underlying mechanism by which these agents produce tumors is not well understood, but malignant tumors may be an extension of the same process, presenting initially as benign adenomas [123, 124].

Classification of Injury and Evaluation of Residual Liver Function

A variety of static and dynamic markers of liver injury or function are widely used in the detection of injury, assessment of injury type and severity, determination of functioning liver mass, prognosis, and response to medical management (Table 5) [77]. Each marker has inherent deficiencies in sensitivity or specificity, and no single method appears capable of completely discerning the etiology, severity, and prognosis associated with a given injury [77, 126].

BIOCHEMICAL EVALUATION

Serum aminotransferase enzymes. Serum activity concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most commonly used biochemical markers of hepatocellular necrosis [77, 121]. These enzymes are localized in periportal hepatocytes, reflecting their role in oxidative phosphorylation and

Table 5. Biochemical markers used to assess the degree of liver injury [77].

Type of liver injury	Marker	Characteristics
Cytotoxic	Serum aspartate aminotransferase	Low specificity for liver injury
	Serum alanine aminotransferase	High specificity for acute hepatocellular injury
	Serum albumin	Reliable marker of chronic hepatocellular injury
	Prothrombin time	Reliable marker of acute or chronic hepatocellular injury
Cytotoxic or cholestatic	Serum alkaline phosphatase	Highest increases occur with cholestatic injuries; specificity poor
	Serum γ -glutamyltransferase	Correlates with alkaline phosphatase (can exclude bone disease)
	Serum bilirubin	High increase (at least 50% of the direct fraction) indicates liver injury
	Serum bile acids	Higher sensitivity and specificity for liver injury than serum bilirubin
Cholestatic	Serum 5'-nucleotidase	Highly specific for cholestasis
	Urinary bilirubin	Higher sensitivity than serum bilirubin; specific for cholestasis

gluconeogenesis. ALT is highly specific for the liver, whereas AST is also located in the heart, brain, kidney, and skeletal muscle, making this enzyme less specific for liver injury [127]. Serum activities in generally healthy individuals are $<0.58 \mu\text{kat/L}$. These serum activities presumably increase as a result of cellular membrane damage and leakage [126].

Serum aminotransferase activities are increased in all types of hepatic injury, but they provide only a static estimate of the amount of recent damage and no indication of residual functional capacity [128]. The highest increases (often >20 -fold) are observed with acute hepatocellular injuries, such as xenobiotic-induced necrosis or acute viral hepatitis [129]. Cholestasis or chronic liver disease rarely cause increases $>8.3 \mu\text{kat/L}$, and serum activities are generally within the reference interval or only slightly increased in alcoholic liver disease [130]. The degree of increase does not correlate well with the extent of liver injury or prognosis [126]. A decline in serum activity concentrations usually indicates recovery but in fulminant injury may be a poor prognostic sign, reflecting a major loss of functional hepatocytes [126, 127].

Serum alkaline phosphatase. Four separate genes encode this family of isoenzymes that catalyze the hydrolysis of phosphate esters, generating inorganic phosphate [127, 131]. Sources of alkaline phosphatase include the liver, bone, leukocytes, kidneys, and first-trimester placenta. Serum values ordinarily range from 0.5 to $2 \mu\text{kat/L}$ but are affected by several physiologic variables [132]. Alkaline phosphatase activities are markedly increased in children and adolescents, as well as the third trimester of pregnancy [133]. In young to middle-aged adults serum activities are usually higher in men; in elderly individuals the activities are often higher in women [133].

Serum alkaline phosphatase increases to some extent in most types of liver injury. Bile acids account for this increase: They induce alkaline phosphatase synthesis and exert a detergent effect on the canalicular membrane, allowing leakage into serum [126, 134]. Mild to moderate increases (less than threefold) are not specific for the type of liver injury [135]. The highest concentrations are observed with cholestatic injuries [77]. Alkaline phosphatase activity concentrations cannot be used to differentiate between intrahepatic or extrahepatic etiologies, because similar increases are observed with each type of bile stasis. The specificity of alkaline phosphatase for the liver is poor, for several other conditions (particularly bone diseases, growth spurts, or pregnancy) also increase serum values [77, 126].

Serum γ -glutamyltransferase (GGT). GGT enzymes are located in a variety of tissues, including the heart, brain, kidney, pancreas, spleen, and the biliary ductule cells of the liver [77]. These enzymes catalyze both the transfer of γ -glutamyl groups from peptides to amino acids and the metabolism of glutathione conjugates. Serum activity concentrations are ordinarily $<0.5 \mu\text{kat/L}$ but may be higher in neonates and the elderly [126]. Serum concentrations correlate well with alkaline phosphatase. The primary utility of monitoring serum GGT is in the exclusion of bone disease as a cause of increased serum alkaline phosphatase, a condition that does not affect GGT concentrations [136]. However, the enzyme is inducible by chronic alcohol use and by drugs such as phenytoin [137].

Serum 5'-nucleotidase. These enzymes are located in a variety of tissues, including the brain, heart, blood vessels, intestine, pancreas, and sinusoidal/canalicular membranes of the liver, where they catalyze the hydrolysis of

Table 6. Dynamic markers of residual hepatic functional mass [77].

Test	Metabolizing enzyme	Reliable uses
Caffeine clearance	N-Demethylation (cytochrome P450IA2)	Evaluation of chronic hepatocellular injury
Galactose elimination capacity	Phosphorylation (galactokinase)	Evaluation of acute or chronic hepatocellular injury
Lidocaine metabolite formation	N-Demethylation (cytochrome P450IIIA4)	Evaluation of acute or chronic hepatocellular injury

nucleotides, generating inorganic phosphate [77]. Serum activity concentrations ordinarily range from 17 to 183 nkat/L and correlate well with serum alkaline phosphatase concentrations [126]. However, despite their wide distribution, an increased serum value is highly specific for cholestatic liver injury, given that the detergent action of bile acids on the canalicular membrane is thought to be the only mechanism by which the enzyme can gain access to the circulation [138]. The primary utility of 5' nucleotidase activities is in the diagnosis of cholestatic liver injury in childhood or pregnancy, because neither condition affects the serum values of this enzyme [139, 140].

Serum bilirubin. Free bilirubin is not water soluble and must be bound to albumin to facilitate transport to the liver [77]. This indirect or unconjugated bilirubin fraction therefore does not enter urine. Once taken up by hepatocytes, bilirubin is conjugated by UDPGT enzymes, with the glucuronide conjugates (the direct bilirubin fraction) being excreted in bile. Intestinal bacteria metabolize direct bilirubin to urobilinogen, which is mainly excreted in feces. A minor portion undergoes enterohepatic circulation, with small quantities excreted in urine. Total serum bilirubin concentrations (reference interval, 3–15 $\mu\text{mol/L}$) indicate the functional transport capacity of the liver. The direct fraction typically accounts for ~5% of the total serum value, and total bilirubin concentrations are consistently higher in males.

Mild to moderate isolated, indirect hyperbilirubinemia is generally associated with hemolysis, although neonatal jaundice or inherited defects in hepatic uptake or conjugation can cause a similar pattern [77]. Total bilirubin concentrations rarely exceed 70 $\mu\text{mol/L}$ in such cases. Higher isolated increases or associated abnormalities of other liver enzymes indicate a hepatic etiology. When hepatic injury is present, the direct bilirubin fraction typically is at least 50% of the total serum value, but the total concentration rarely exceeds 500 $\mu\text{mol/L}$ regardless of severity, because of renal excretion of the direct fraction. Urinary bilirubin is a more sensitive indicator of liver injury than is serum bilirubin. An increase in urinary bilirubin is nearly always indicative of a corresponding increase in the serum direct fraction attributable to intrahepatic or extrahepatic cholestasis. The degree of increase in serum bilirubin values has prognostic significance in chronic liver injuries, but not in acute injuries [141].

Serum bile acids. Cholic acid and chenodeoxycholic acid are the primary bile acids in humans [142]. These organic anions are synthesized in hepatocytes from cholesterol, conjugated to glycine or taurine, and excreted into the canaliculus [77]. Measurement of serum bile acid concentrations is a more specific indicator of functional hepatic excretory capacity than serum bilirubin is [143]. Serum bile acid concentrations are also more sensitive to subtle excretory abnormalities, in that the quantity of bile flow is so much greater than that of bilirubin.

An increase in serum bile acid concentrations in fasting is highly specific for liver injury and serves to exclude congenital or hemolytic causes of hyperbilirubinemia [144]. The greatest increases are observed in acute viral hepatitis or extrahepatic cholestasis [77]. The ratio of cholic to chenodeoxycholic acid (reference interval, 0.5–1.0) decreases with chronic injuries such as cirrhosis and increases with extrahepatic bile obstruction [145].

Serum albumin. Serum albumin, the major plasma protein synthesized in the human liver, is a clinically useful marker of hepatic synthetic function [77]. The relatively long elimination half-life (~20 days) and ample storage pool, however, limit the utility of this index in evaluation of chronic liver injuries. In addition, several factors other than liver injury can disrupt albumin synthesis, including nutritional deficiencies and alterations in plasma oncotic pressure (hypergammaglobulinemia) [146, 147]. Alcoholic cirrhosis with or without accompanying ascites generally lowers serum albumin concentrations, although hypoalbuminemia in the setting of ascites may reflect dilution rather than decreased synthesis [126, 148].

Prothrombin time (PT). PT provides an index of hepatic synthetic capacity that applies to both acute and chronic liver injuries [77]. An indicator of the extrinsic clotting cascade, the PT provides an indirect measure of the hepatic synthesis of clotting factors I, II, V, VII, IX, and X [149]. Other causes of a prolongation of PT include vitamin K deficiency, warfarin therapy, and acquired or congenital clotting factor deficiencies [77, 150]. The administration of vitamin K can be helpful in distinguishing liver injury from vitamin K deficiency, because a relative normalization of PT would be expected with the latter etiology (which can result from obstructive jaundice), in contrast to a lack of normalization when hepatocellular necrosis is present [151].

PT has prognostic value in both acute and chronic liver injury. An extreme or worsening prolongation of the PT in the setting of acute hepatocellular necrosis is associated with an increased risk of fulminant injury [152]. Similarly, a poor prognosis is associated with PT prolongation in the setting of chronic liver injury and after portal-systemic shunt surgery [153].

QUANTITATIVE MEASURES OF LIVER FUNCTION

Biochemical measurements provide a static assessment of the degree of liver injury, but give little information about residual liver function. In contrast, the administration of a compound metabolized by the liver affords a dynamic evaluation of residual metabolic capacity (functional liver cell mass) [77]. To rule out confounding factors such as alterations in hepatic blood flow or plasma protein binding, suitable agents should have a low hepatic extraction ratio and not be highly protein bound. Marker compounds should also be relatively nontoxic and, if admin-

istered orally, should be both rapidly and completely absorbed.

Caffeine clearance. Caffeine elimination depends highly on cytochrome P450IA2 isoenzyme-mediated *N*-demethylation, which leads to a variety of urinary methylxanthine metabolites [154]. Several methods are used to assess liver function with caffeine [155, 156]. The [¹³C]caffeine breath test is highly specific for P450IA2 isoenzyme activity, which catalyzes caffeine 3-*N*-demethylation [157]. Breath samples are collected over 1 h after oral administration, and the enzyme activity is quantified via analysis of the ratio of ¹³CO₂ to ¹²CO₂ by differential mass spectroscopy. Alternatively, caffeine or caffeine metabolites (or both) can be analyzed in fasting plasma, urine, or saliva by HPLC, but this also monitors metabolites that are produced by several P450 isoforms, including P450IA2, P450IIIA4, and P450IIA6 [158–160].

Caffeine clearance is a reliable indicator of global hepatic function but is less sensitive for assessing mild injury [158, 160]. It is most reliable in the assessment of cirrhosis but appears to be no better than the Child–Pugh classification scheme with respect to prognosis [161]. Confounding factors may include smoking (P450IA2 isoenzyme induction), gender, age, or concomitant exposure to drugs that alter enzyme activity [162–164].

Galactose elimination. Galactose is metabolized by hepatic galactokinase enzymes rather than by the cytochrome P450 system [77]. Accordingly, less individual variability confounds interpretation of test results [165]. Multiple methods can be used in measuring galactose elimination, including a [¹⁴C]galactose breath test and intravenous administration of nonradiolabeled drug, followed by serial blood sampling at 5-min intervals between 20 and 45 min postdose [166, 167]. The accuracy of this approach may be improved by eliminating the initial 20-min blood sample, which has been shown to display the greatest amount of individual variability [168].

Galactose elimination is defined by hepatic metabolism (thereby reflecting functional hepatocyte mass) so long as plasma galactose concentrations exceed 500 mg/L [169]. At lower concentrations, hepatic blood flow becomes the primary determinant of clearance. Clearance is decreased by acute or chronic hepatocellular necrosis but is typically unaffected by cholestatic liver injury [170]. However, most studies suggest that measurement of galactose elimination provides no prognostic advantage over the Child–Pugh classification [171]. In addition, serum albumin concentrations appear to be just as effective as galactose clearance in distinguishing cirrhotic patients from healthy controls [166]. In contrast, the redox tolerance test has been shown to accurately depict functional hepatic mass and provide prognostic information in patients with obstructive jaundice [172]. Preoperative results correlate significantly ($P < 0.01$) with serum bilirubin half-life. This test is based on an oral glucose load followed by 2-h serial

arterial blood sampling to determine the cumulative enhancement of the ratio of acetoacetate to β -hydroxybutyrate.

Formation of monoethylglycinexybutyrate (MEGX). Lidocaine undergoes *N*-demethylation to MEGX via a phase I reaction mediated by cytochrome P450 enzymes, including the P450IIIA4 isoform [77, 173]. Serum concentrations of MEGX are analyzed via fluorescence polarization immunoassay [174]. After baseline blood sampling and administration of a single intravenous lidocaine dose, formation clearance can be measured from serial blood samples obtained for 1–2 h postdose or a single 15-min postdose MEGX concentration can be determined [175]. Factors that may confound test results include gender (formation clearance is consistently higher in males), age (the rate of MEGX formation declines with age), and concomitant exposure to drugs that alter cytochrome P450IIIA4 activity [176].

Some studies suggest that MEGX formation has better prognostic value than the Child–Pugh classification in patients with end-stage liver disease, including selection of appropriate transplant candidates [177, 178]. The serial use of MEGX formation clearance has also been shown to accurately predict hepatic failure in patients with multiple trauma, with the assessment on day 3 providing the greatest predictive value [179]. However, other studies imply that MEGX formation clearance is less effective than galactose elimination capacity in assessing the severity of cytotoxic liver injury [180].

Antipyrine clearance. The elimination of antipyrine almost totally depends on metabolism by hepatic cytochrome P450 enzymes [77, 181]. In addition, antipyrine is nearly completely absorbed after oral administration, is not bound to plasma proteins, and is distributed into total body water, so that alterations in protein binding do not interfere with clearance assessment. Antipyrine clearance is measured after oral administration, followed by collection of multiple blood samples.

Antipyrine clearance is most accurate in the assessment of chronic liver injury [182]. In such instances the increase in elimination half-life correlates strongly with the degree of PT prolongation, hypoalbuminemia, and severity of hepatocellular damage [183]. Antipyrine clearance is less sensitive in the assessment of acute liver injury. Limited evidence also suggests that antipyrine clearance may be a reliable predictor of clinical outcome in patients with obstructive jaundice [184].

The major problem with the use of antipyrine clearance in the evaluation of liver injury is the individual variability inherent with cytochrome P450 enzyme activity [185]. As discussed previously, multiple patient-specific factors other than liver injury determine the metabolic capacity of the various cytochrome P450 isoforms. A “cocktail” approach with multiple markers, each primarily metabo-

lized by a separate P450 isoform, has been proposed to overcome this problem [186, 187].

[¹⁴C]Aminopyrine breath test. This noninvasive test utilizes aminopyrine, an agent eliminated via cytochrome P450 enzyme-catalyzed *N*-demethylation [77]. Serial breath sampling after oral administration of ¹⁴C-labeled aminopyrine permits assessment of metabolic efficiency via analysis of the percentage of ¹⁴CO₂ in expired air [188]. A single 2-h postdose breath sample appears to be equally accurate, with presumably healthy subjects excreting 6.6% ± 1.3% of the dose by this time.

The rate-limiting step in the formation of CO₂ involves a folic acid-dependent enzyme. Several factors therefore affect aminopyrine metabolism, including folate or vitamin B₁₂ deficiency, glutathione deficiency, protein deficiency, infection, and thyroid disease [189]. Both acute and chronic hepatocellular injury are associated with a decrease in aminopyrine elimination, but the sensitivity of the test is poor, there being a great deal of overlap between healthy and diseased subjects [190, 191]. Aminopyrine excretion generally remains within the reference interval in the presence of cholestatic injuries, regardless of etiology, but most studies imply little advantage of this approach over routine biochemical measurements in the differentiation of cholestatic from cytotoxic injury [192, 193]. Limited evidence suggests that the aminopyrine breath test is a better predictor of survival in patients with alcoholic cirrhosis than are the Child–Turcotte and Child–Pugh classification schemes, although this conclusion has been disputed by other studies [194–197].

Future Directions

The development of safe, noninvasive methods such as the caffeine breath test for monitoring hepatic function permits an indirect assessment of hepatic functional mass at the time of the test. In contrast, classical liver function tests can provide only an assessment of recent hepatocellular damage. The capacity of noninvasive tests to specifically monitor the activity of a single P450 isoform, such as cytochrome P450IA2 in the case of caffeine, can increase the utility of these tests beyond that of just monitoring hepatic function. For example, such tests can be used to monitor exposure to potentially toxic chemicals and possibly (by governments) to identify permissible/safe quantities of environmental chemicals in the workplace or in the general environment. An example is the caffeine breath test, where animal data and preliminary data in humans indicate that the more some environmental chemicals increase P450I activity, the more adverse effects they have on select organ systems and the more susceptible the individual is to the toxic effects [198–200]. Preliminary human studies comparing two cohorts of individuals, one exposed to very high concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and another exposed to very high concentrations of a mixture of polychlorinated biphenyl and dibenzofuran congeners, indicate

similar findings [198]. The first group had little adverse effects and little induction of P450IA2, whereas the second cohort had multisystem dysfunction and a very great induction of P450IA2 [198]. Clearly, such knowledge about chemicals in the workplace or general environment will allow more cost-effective and safe regulatory environmental standards by industry and government.

Currently, the erythromycin breath test can monitor cytochrome P450IIIa activity in humans, and other substrates are under development to monitor specific liver enzymes [201]. As more enzyme-specific substrates allow the *in vivo* examination of critical hepatic enzymes, the more we will learn about the roles of these enzymes in human development, birth defects, cancer, drug metabolism, and a host of human diseases.

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