Serum Transaminase Elevations as Indicators of Hepatic Injury Following the Administration of Drugs

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During the preclinical, early clinical, late-stage clinical, and postmarketing phases of the pharmaceutical discovery and development process, one important aspect of drug safety assessment involves monitoring for possible drug-induced hepatic injury. Hepatic injuries vary in nature from direct, intrinsic effects that are observed in most recipients and more than one species to rare idiosyncratic responses seen only in a few clinical subjects. Histological types of injuries vary from hepatocellular to hepatobiliary with multiple cellular effects characteristic of each type. Of the various clinical laboratory markers for hepatic injury, serum transaminases, especially alanine aminotransferase (ALT), are the most universally important indicators for studies ranging from early preclinical animal testing to postmarketing patient monitoring. This review examines the characteristics of hepatic toxicity that result in serum ALT changes, the differences in the etiology of hepatic responses which govern when liver injury is most likely to be detected during the four phases of the drug discovery and development process, and those modulating factors which affect the utility of ALT as a dependable marker of hepatic injury in clinical populations. The paper concludes with a summary of some ancillary methods for early preclinical screening such as in vitro metabolism and toxicity assays, gene and protein expression analysis, and some strategies for enhancing the probability for the early detection of idiosyncratic hepatotoxic responses which are infrequent but significant factors in the safety assessment process. © 1998 Academic Press

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1. DRUG HEPATOTOXICITY AND THE PHARMACEUTICAL DEVELOPMENT PROCESS

Because of its central role in drug metabolism, the liver is particularly susceptible to injury following systemic exposure to xenobiotics. Accordingly, liver toxicity as suggested by slightly elevated serum enzymes of hepatic origin is one of the more frequently encountered adverse effects in early clinical trials of therapeutic agents, although the actual incidence of hepatic injury is extremely low. During the pharmaceutical discovery, development, and marketing processes, drug-associated hepatotoxicity can be encountered at any of four stages.

The first stage involves the detection of hepatotoxic phenomena in preclinical safety assessment studies using rats, dogs, monkeys, or other mammals at dose levels in excess of the intended therapeutic dose. A significant incidence of drug-associated hepatocellular or hepatobiliary injury detected by clinical chemistry procedures and confirmed by histological methods in one or more species generally precludes further development unless there is clear evidence that a similar effect is not anticipated in humans. An exception could be the demonstration that animal toxicity is due to a specific metabolite that would not be a major metabolite in humans. Most intrinsically hepatotoxic (type A) compounds are detected in preclinical animal trials and are not advanced to clinical trials. The proportion of compounds causing some degree of hepatic effects such as hepatomegaly, serum enzyme elevations, hepatobiliary effects, or necrosis at high doses in animals can reach as high as 50% of drugs tested in preclinical studies.

The next level where evidence of hepatotoxicity may be evaluated is in early clinical development. At therapeutic doses, untoward findings could include, for example, the reporting of serum transaminase elevations that are 2 times the upper limit of normal, jaundice, or 1.5 times the normal alkaline phosphatase (Alk P; EC 3.1.3.1) activity in a small treated population of healthy volunteers during early clinical trials (Batt and Ferrari, 1995). While these clinical chemistry changes are usually transient and not accompanied by clinical evidence of hepatic toxicity, drugs eliciting these changes are typically not continued in development unless there is a compelling reason to do so.

The third stage typically involves the reporting of a few patients within a controlled clinical population who are found to have transaminase levels two to three times the upper limit of normal but who are otherwise...
asymptomatic. A fairly large number of drugs fall within this category. These are not typically discontinued for this phenomenon alone. For example, the U.S. Physicians’ Desk Reference indicates that liver enzyme elevations may be found in up to 15% of patients taking nonsteroidal anti-inflammatory drugs (NSAIDs). One NSAID in particular, sulindac, actually accounts for most of the risk (Fry and Seeff, 1995; García Rodríguez et al., 1994). Another example is the 12% incidence of asymptomatic hepatic enzyme abnormalities reported in ketoconazole recipients (Mosca et al., 1985).

The disease under treatment may also affect the liver adversely leading to elevated hepatic enzymes. Most of these reactions are host-dependent, idiosyncratic responses which are not easily predicted in preclinical testing or observed in early clinical trials because of the small number of subjects.

The final stage involves similar findings in a large postmarketing population. These cases involve very-low-frequency, idiosyncratic (type B) events that are not detectable in controlled clinical populations of 500–1000, for example, due to mathematical probability. These result from a novel aspect of the patient, not the drug, and are more uncertain in etiology because they are typically reported in an uncontrolled nonclinical setting. Thus, drug-induced hepatic injury ranges from frequent, dose-related effects in multiple species to rare but profound hepatic effects only detectable in susceptible individuals at therapeutic doses. Serum transaminase activities are the single diagnostic indicator for all of these effects and are the subject of this review.

2. HISTOLOGICAL TYPES OF TOXIC HEPATIC INJURY

In general, the type and degree of acute toxic liver injury following drug administration depend upon the dose and duration of exposure. In addition, many chemicals preferentially affect certain cell populations but not others in the same host. Major histologic lesions of the liver due to chemical toxicity include fatty change, hepatocellular death, and hepatobiliary lesions. Granulomatous and mixed histological changes may also be seen (Pirmohamed et al., 1992) or venoocclusive effects, especially with some antineoplastic drugs (McDonald and Tirumali, 1984). Steatosis or fatty change refers to an increase in hepatic lipid content greater than 5% by weight (Moslen, 1996). Lipid accumulates as intracytoplasmic vacuoles in either a zonal or diffuse pattern. Microvesicular and macrovesicular steatoses, differing in the lipid vacuole size and the mechanism for their formation, are distinguishing characteristics of specific toxicants. Macrovesicular steatosis is characterized by a single large fat droplet which displaces the nucleus to the cell periphery, but in microvesicular steatosis there are a number of small lipid droplets that do not displace the nucleus (Stricker, 1992a). Steatosis is reversible unless it is persistent enough to cause secondary changes or so severe that cell death results. In humans, the incidence of fatty liver disease noted at autopsy for accident victims ranges from 40 to 80% (Hodgson et al., 1989) suggesting a high incidence from unknown causes in the general adult population. Necrotic cell death is accompanied by a disruption of cell membrane integrity and the loss of liver-specific enzymes which forms the basis of the serum chemistry analyses used systematically for its detection. Clinical manifestations of hepatic necrosis and degeneration range from jaundice to fulminant hepatic failure. Cholestatic injury occurs when there is a disruption or impairment of bile flow. Canaliculcholangitis can be caused by several mechanisms including leaky paracellular junctions, a diminished contractility of canaliculus, diminished transcytosis, impaired transporters, and toxin concentration in the paracanicular area (Moslen, 1996). Toxins or their metabolites can also damage the intrahepatic bile ducts. Chlorpromazine, oral contraceptives, and androgens used to increase muscle mass can produce a cholestatic response. Hepatobiliary lesions lead to elevated serum levels of bile constituents or enzymes localized in the hepatobiliary tree such as Alk P or gamma glutamyltranspeptidase (GGT; EC 2.3.2.2). In humans, most hepatic drug reactions appear within 3 months of starting therapy (Davis, 1989).

3. BIOCHEMICAL TESTS USED FOR THE DETECTION OF HEPATIC INJURY

3.1. Categories of Laboratory Tests

Three categories of noninvasive laboratory tests are used to identify the type and extent of drug hepatotoxicity based on the presence or absence of specific markers in the blood of exposed individuals. The first category of clinical assays is that used to assess hepatocellular damage leading to liver cell necrosis. Evidence of this type of injury is based on the detection the hepatic transaminases, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). These are not normal components of blood and serve no known function outside the organ of origin. A third specific marker of hepatocellular damage, serum F protein, has been recently described (Foster et al., 1989), although it is not yet as widely used as the transaminases. Based on liver biopsy specimens, chronic ALT elevations in asymptomatic patients have also been associated with fatty liver (Hulteranz et al., 1986). In fact, nonalcoholic steatosis has been cited as a very common cause of chronic ALT elevations in the general population (Craxi and Almasio, 1996). Thus, transaminase elevations are indicative of hepatocellular death and fatty degeneration in particular, but can also be used in conjunction with other serum enzymes distinguishing non-hepatocellular injury as described below.

The second group of tests includes markers for hepatobiliary (cholestatic) effects. This type of injury in-
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includes biliary obstruction or hepatic infiltrative processes resulting in the retention of bile acids in liver and leading to drug-induced jaundice if severe. Serum markers include Alk P from the cell canalicular membrane, 5′ nucleotidase (5′NT), and GGT. Neither Alk P nor GGT, which is also inducible by alcohol and certain drugs (see below), is liver-specific. However, marked serum Alk P elevations, especially when accompanied by 5′NT or GGT elevations, suggest mechanical bile duct obstruction, primary sclerosing cholangitis, primary biliary cirrhosis, or drug-induced hepatitis (Herlong, 1994). Thus, serum enzyme profiles as opposed to individual enzyme changes are used for diagnostic purposes, especially for monitoring hepatobiliary effects.

The final category of diagnostic procedures is based upon altered liver function. These are methods that monitor serum albumin, cholesterol, prothrombin time, or serum bilirubin as general indicators of the synthetic and general metabolic capacity of the liver (Fregia and Jensen, 1994) as opposed to marking some specific toxic injury. The serum bilirubin assay will indicate liver injury; however, elevated serum enzyme assays as described above usually reflect hepatotoxicity earlier (Herlong, 1994). Although drug-associated hepatic dysfunction is uncommon in general, severely altered liver function, especially those processes leading to coagulation disorders or bilirubin encephalopathy, are indicative of severe hepatic injury.

3.2. Aminotransferases

Aminotransferases are a group of enzymes that catalyze the reversible transfer of the amino group from an α-amino acid to an oxo acid. ALT and AST shunt their amino acid and oxo acid substrates into several intermediate pathways. Cytosolic ALT is associated with the utilization of pyruvate in glycolysis, mitochondrial ALT is involved in the conversion of alanine to pyruvate for gluconeogenesis, and AST plays an important role in the transport of reducing equivalents across the mitochondrial membrane (Sakagishi, 1995; Rej, 1989). Hepatocellular damage with the subsequent disruption of the plasma membrane allows leakage of intracellular enzymes such as ALT or AST into the bloodstream. With some hepatotoxins, increased hepatic synthesis of aminotransferases has also been suggested as a source of increased serum enzyme levels in hepatocellular injury (Pappas, 1986). The range of normal is determined by either cutoff values of ±2 SD or 97.5 percentile cutoffs in a population without known disease. Due to half-lives of approximately 17 h for AST and 47 h for ALT, the presence of these enzymes in serum is considered an indicator of recent hepatocyte injury (Scheig, 1996). Some drugs actually decrease the activities of serum ALT and AST. Examples include oxodipine which causes hepatic damage and yet decreases ALT activities in dogs and rats (Waner and Nyska, 1991), cefazolin which significantly depresses ALT and AST activities in rat liver (Dhrami et al., 1979), and isoniazid which significantly inhibits hepatic and serum AST in rodents (Yamada et al., 1984a,b). In addition to inhibitory effects by antivitamin B6 compounds, transaminase levels are effected by other nutritional events. For example, healthy human volunteers ingesting a choline-deficient diet for 4 weeks showed significantly elevated levels of ALT compared their cohorts (Zeisel et al., 1991). A discussion of the characteristics of each of these important enzymes follows.

3.2.1. ALT. ALT (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) is a pyridoxal enzyme which catalyzes the reversible interconversion of L-alanine + α-ketoglutarate to pyruvate + L-glutamate with pyridoxal phosphate as a coenzyme. The presence of low levels of ALT in the peripheral circulation represents normal cell turnover or release from nonvascular sources. Drug-related increases in aminotransferase activity are typically transitory with values returning to within normal reference limits within a few weeks (Rej, 1989).

ALT is widely distributed. Human isozymes are found in the cytosol and mitochondria of liver, kidney, and skeletal and cardiac muscles (Sakagishi, 1995). Mitochondrial ALT comprises only a small portion of the tissue activity and has not been demonstrated in normal human serum. The largest pool of ALT is in the cytosol of hepatic parenchymal cells (Sherman, 1991). Considerable differences in both the organ distribution and intracellular compartmentalization of ALT have been found among species (Hoffmann et al., 1989). Some nonhuman primates, for example, show little or no ALT organ specificity (Clampitt and Hunt, 1978). However, overall, serum ALT is one of the most universal markers for hepatic injury across species.

In the clinical laboratory, the measurement of ALT is a routine part of serum chemistry panels used to assess hepatic injury. ALT values in healthy blood donors, however, can be influenced by age, sex, dietary change, geographical location, ethnicity, obesity, long-term acetaminophen use, alcohol use, and marital status (Sherman, 1991), a complicating factor when monitoring human populations for transaminase elevations following drug exposure. Even the difference in mean ALT values between males and females in a donor population can be significant (Mijovic et al., 1997). ALT activities are elevated for a few days following major abdominal or thoracic surgery (Stricker et al., 1992a) and can also be elevated by the disease being treated. The incidence of hepatic damage due to cotrimoxazole, for example, is around 20% higher in AIDS patients (Westphal et al., 1994) compared to other diseases. Cognizant of these modulating factors in clinical populations, ALT is the single most important indicator of...
hepatocellular injury for preclinical animal studies, clinical trials, and postmarketing monitoring.

3.2.2. AST. AST (EC 2.6.1.1) is found in both the cytosol and mitochondria of hepatocytes (Herlong, 1994), but high tissue levels are also found in heart, skeletal muscle, kidney, brain, and pancreas (Rej, 1989). Accordingly, muscle trauma and surgery by itself can lead to AST serum elevations (Clermont and Chalmers, 1967). An estimated 60–70% of AST activity in human hepatocytes is localized within mitochondria (Schmidt and Schmidt, 1990). Hence, when found in blood, AST is considered to be a sensitive indicator of mitochondrial damage, especially in the hepatic centrilobular regions which are particularly sensitive to toxic and hypoxic liver injury (Schmidt and Schmidt, 1990). It should be noted, however, that depending upon the assay used a number of drugs can reportedly produce spurious elevations in AST (Davis, 1989). Serum AST is affected to a greater degree by alcohol consumption than ALT (Lewis, 1984). Within these limitations, AST in conjunction with ALT is a very important marker of hepatic injury.

4. NON-DRUG-RELATED TRANSAMINASE ELEVATIONS

The term transaminitis has been applied to mild elevations of serum aminotransaminases in the absence of other clinical laboratory abnormalities in asymptomatic individuals (Hodgson et al., 1989; Rej, 1989). When observed after drug exposure, these small elevations are often considered as being uninterpretable. A second difficulty in monitoring transaminase levels in patient populations is the underlying incidence of mildly abnormal results in a classical panel of liver tests used in routine monitoring of asymptomatic, unaffected individuals. These may be associated with population characteristics previously discussed and are not usually encountered in preclinical animal toxicity studies. Liver injury tests are abnormal in up to 8% of a healthy population (Hodgson et al., 1989). Liver enzyme elevations noted during hospital admission have been estimated at 5.7–7.6% (Grohmann et al., 1984; Van Dijke et al., 1986). In one study, 25–30% of asymptomatic, normal workers had serum chemistries on a five-test liver panel in excess of the established normal range in the absence of risk factors for liver disease (Wall et al., 1988). Of course, specific estimates of the incidence of abnormal ALT values alone are lower in donor populations. In one study, the frequency of elevated ALT in a donor population was reported to be 4.6% above 40 IU/liter in 1986 and appeared to be increasing with time (Mijovic et al., 1987). In the absence of any intentional drug exposure, extremely high transaminase levels (>8- to 10-fold normal) in a patient population may indicate acute viral hepatitis, drug- or toxin-mediated liver necrosis, or other damage; persistent mild elevations (2- to 8-fold) are characteristic of chronic hepatitis, steatosis, metabolic diseases (Fregia et al., 1994), alcoholic liver disease, and, much less frequently, drug-induced liver disease and nonalcoholic steatosis (Renner and Dallenchbach, 1992). In addition, some disease states, especially malignancies, produce hepatic effects culminating in elevated transaminases.

5. GUIDELINES FOR SERUM TRANSAMINASE ELEVATION AS AN INDICATOR OF LIVER INJURY

By international consensus, liver injury, as might be detected by liver tests, has been defined as an increase greater than two times the upper limit of normal range (ULN) in ALT or conjugated bilirubin or a combined increase in AST, Alk P, and total bilirubin provided that one of them is greater than two times the ULN (Benichou, 1990). This injury is further defined as hepatocellular when (1) there is an increase of greater than two times the ULN for ALT alone or (2) there is a ratio of serum ALT activity/serum Alk P activity greater than five or cholestatic when there is an increase of greater than two times the ULN of Alk P alone or a ratio equal or less than two. Acute liver injury is distinguished from chronic liver injury when the increases have lasted less or more than 3 months, respectively. Severe liver injury and fulminant liver injury are defined by (1) the presence of jaundice, prothrombin <50% (or equivalent), and hepatic encephalopathy and (2) the rapid development of hepatic encephalopathy and severe coagulation disorders, respectively.

6. SERUM ENZYME ELEVATIONS DUE TO HEPATIC ENZYME INDUCTION

Enzyme induction is one reported iatrogenic effect leading to elevated hepatic serum enzyme levels in patient populations that are not directly indicative of hepatic injury. This has been well documented, especially for anticonvulsants, but also for some other drugs. In one such study, 56 children of 63 receiving phenobarbital and/or diphenylhydantoin had elevated GGT; 6 had elevated ALT and AST for more than 20 weeks in the absence of specific histopathology (Aiges et al., 1980). Further evidence of hepatic enzyme induction by antiepileptics in asymptomatic patients was cited by Von Dijke et al. (1992) in a study of 206 adults and children. Of these, serum GGT was elevated in 74.6%, Alk P in 29.7%, and ALT in 25.2%. Of 242 patients administered antiepileptic drugs, 40 exhibited high levels of serum GGT and nearly all cases indicated hepatic microsomal enzyme induction as measured by antipyrine half-life, leading Hirayanagi et al. (1991) to conclude that, in these patients, elevated serum GGT did not necessarily indicate hepatocellular damage. Elevated circulating levels of GGT are commonly observed in patients taking cytochrome P450 enzyme-inducing drugs such as rifampin (Davis, 1989). Similar studies with co-antiep-
ileptic drug therapy (Haidukewych and John, 1986) indicated ALT elevations up to three times and AST elevations up to two times the upper limit of normal in more than one-quarter of the patient population. These were not considered clinically significant but instead were attributed to enzyme induction. Liver biopsies in similar patients undergoing long-term anticonvulsant therapy showed no signs of chronic liver damage (Jacobsen et al., 1976). Both ethanol and glucocorticoids have been shown to induce GGT activity in rat liver (Nishimura et al., 1981; Billon et al., 1980). In studies with both man and rat, the primary cause for increased GGT associated with prolonged alcohol exposure has been attributed to hepatic enzyme induction rather than liver cell injury (Tescké et al., 1983), although it is clear that ethanol is hepatotoxic in general. In developing rats, cortisol enhances hepatic activities of ALT (Herzfeld and Raper, 1979).

7. TYPES OF DRUG HEPATOTOXICITY

7.1. Intrinsic Hepatotoxicity

One of two major categories of chemicals that can produce dose-dependent, hepatic injury, either directly or indirectly via a metabolite, includes the intrinsic or "direct" (type A) hepatotoxicants. These responses often can be anticipated from the known pharmacology of the drug, are generally detectable in animal models, and occur more frequently or with greater severity when exposure is increased, i.e., with increasing dose levels or duration of dosing. Salicylates (Lewis, 1984) and acetaminophen (Fry and Seef, 1995) are examples of analgesics that produce intrinsic liver toxicity. At high blood levels, aspirin can produce hepatocellular injury, confirmed by liver biopsy studies (focal necrosis), with 10- to 40-fold elevations for serum transaminases noted (Zimmerman, 1978). Chemotherapeutic agents often possess predictable, dose-dependent hepatotoxicity (King and Perry, 1995), even though they do not intentionally target slowly dividing hepatocytes. Tetracycline, methotrexate, mercaptopurine (Finlayson, 1973), and possibly sulindac (Boelsterli et al., 1995) are other examples of direct hepatotoxins. Intrinsic hepatotoxins produce injury in a large percentage of exposed individuals after a short fixed latent period either by direct, nonselective physicochemical distortion or disruption of hepatocytes or by indirect interference with specific metabolic processes leading to structural damage (Lewis, 1984). This type of toxicity can be alleviated by dose reduction in patient populations.

7.2. Idiosyncratic Hepatotoxicity

Although a number of drugs, as for example some antineoplastic agents (McDonald and Tirumali, 1984), exhibit intrinsic toxicity in man or animals, most hepatotoxic drug reactions in humans are considered to be idiosyncratic (King and Perry, 1995), i.e., due to unusual susceptibility of an individual. Occurring at therapeutic doses after a variable latent period, these responses are characterized by an incidence of hepatic injury that is very low in frequency within a population, dose-independent, and not reproducible in experimental animals (Zimmerman, 1978). Due to their low incidence, idiosyncratic responses are generally not detected until late in the drug development process after a large number of patients have been treated. Although rare, serious adverse liver responses may include fulminant hepatitis and cholestasis. These account for many drug-induced deaths worldwide. Drugs withdrawn from the market due to idiosyncratic drug reactions, including or due solely to hepatotoxicity, include benoxaprofen, ibufenac, temafloxacin, tenilic acid (Park et al., 1992), nomifensine, and perhexilene (Breckenridge, 1996).

Etiologically, these reactions are of two major types: (1) aberrant metabolism-based responses leading to the accumulation of toxic metabolites in susceptible individuals and (2) hypersensitivity or immune-based toxicity. Other mechanisms may include abnormal receptor sensitivity, latent biochemical abnormalities, or multifactorial causes (Park et al., 1992). Almost all hepatotoxic reactions to antibacterials (George and Crawford, 1996) or NSAIDs (Boelsterli et al., 1995), especially of the indole, pyrazole, and propionic acid classes (Lewis, 1984), are idiosyncratic. Mechanisms may be primarily metabolite-dependent (isoniazid, diclofenac, hypersensitivity-mediated (beta-lactams, sulindac), or both (sulfonamides, erythromycin derivatives) (Westphal et al., 1994; Miyamoto et al., 1997; Boelsterli et al., 1995). Some notable histamine (H2)-receptor antagonists and a number of antidepressants are associated with hepatic idiosyncratic reactions, presumably mediated via chemically reactive metabolites (Black, 1987; Pirri Mohamed et al., 1992).

7.2.1. Metabolism-based toxicity. Interindividual variations of drug effects are most frequently explained by genetic variation or polymorphism of drug metabolism. Based on immunogenicity and catalytic activities, approximately 20 human cytochrome P450 isozymes have been identified (Larrey and Pageaux, 1997) comprising at least 10 distinct P450 gene families (Watkins, 1990). Of these, cytochromes CYP1A2, CYP2C9, CYP2C19, CYP2E1, and, in particular, CYP2D6 and CYP3A are especially important for the hepatic metabolism of drugs (Brockmoller and Roots, 1994). While bioactivation of a drug to a toxic metabolite may represent <1% of the overall metabolism of that drug (Pirmohamed et al., 1996), specific reactive metabolites may directly lead to hepatic injury or may act via the immune system. Genetic deficiencies in hepatic cytochrome CYP2D6, CYP2C19, N-acetyltransferase 2 (NAAT2), and glutathione synthetase have been cited to explain individual variations in drug hepatotoxicity (Larrey and Pageaux, 1997; Meyer, 1996). Seven muta-
tions of the NAT2 gene are known to define numerous alleles associated with decreased function, and two mutant alleles of CYP2C19 have been described in poor metabolizers (Meyer and Zanger, 1997; Goldstein and de Morias, 1994). Oxidative polymorphism for cytochrome CYP2D6, which metabolizes drugs typically having a positively charged nitrogen atom (Pirmohamed et al., 1996), includes poor, intermediate, and ultrarapid metabolizers (Meyer and Zanger, 1997) leading to a wide range of interindividual variability in CYP2D6-based response in a healthy population (Spina et al., 1994). For example, patients receiving the antiganglionic agent perhexiline maleate who are deficient in CYP2D6 can develop hepatotoxicity due to perhexiline accumulation (Morgan et al., 1984). Although it is not subject to genetic polymorphism (Ereshefsky, 1996), both genetic and nongenetic factors lead to marked (5- to 20-fold) individual variability in metabolic clearance via CYP3A (Wilkinson, 1996) which comprises 25% of the total hepatic cytochrome system. This leads to wide variations in individual exposure levels to drugs such as cyclosporine (Watkins, 1990). Polymorphism has also been described for CYP2C9 and CYP2E1 (Meyer and Zanger, 1997). The latter is important for the metabolism of general anesthetics such as halothane which causes hepatitis in 1 in 35,000 patients on first exposure and in 1 in 3700 patients upon multiple exposures (Pirmohamed et al., 1996).

7.3. Mixed or Species-Relevant Toxicity

Some drugs appear to cause idiosyncratic hepatic injury in humans and yet can also produce hepatotoxicity, perhaps by a different mechanism, in animal models at high doses. Thus, the same drug can be an intrinsic hepatotoxicant in one or more animal models and yet cause an idiosyncratic liver response in some humans. Some of the angiotensin-converting enzyme inhibitors used for the treatment of hypertension and congestive heart disease fall into this category. Captopril, for example, has been associated with the rare incidence of hepatotoxicity in humans (Rahmat et al., 1985; Tabibian et al., 1987). In mice, acute high doses of captopril cause moderate increases in ALT, decreases in hepatic GSH, and histological evidence of hepatic necrosis with 24 h (Helliwell et al., 1985). Enalapril maleate which has been reported to cause a rare but potentially serious hepatotoxicity in humans, was demonstrated by Urima-Romet and Huang (1992) to produce centrilobular necrosis and significant moderate increases in ALT and AST 24 h after acute exposure in Fisher 344 rats.

8. THERAPEUTIC CLASS AND DRUG-INDUCED HEPATIC INJURY

The frequencies of drug-induced hepatitis at therapeutic doses vary among drug classes with some classes more consistently demonstrating low levels of hepatic injury than others. These can vary from 1/100 for a few compounds, to 1/10,000 for some intermediate compounds, to 1/100,000 for others (Larrey and Pageaux, 1997). For one antitumor antibiotic, elevations of aminotransferases can occur in up to 100% of patients (King and Perry, 1995). In one large study (Sameshima et al., 1984) based on 8156 case histories of drug-induced hepatic injury over a 70-year period in Japan, the greatest number of cases was due to antibiotics (34%), followed by CNS drugs (15%), chemotherapeutic drugs (14%), cardiovascular drugs (11%), carcinostatic drugs (7%), hormones and hormone antagonists (6%), diagnostic aids (4%), and other drugs (9%). A smaller study based on 46 patients representing 20% of patients admitted for acute or chronic hepatitis during a 3-year period cited anti-inflammatories, antibiotics, antihypertensives, and diuretic drugs as being most frequently implicated (Grippon et al., 1985). In the U.S. Physicians Desk Reference, transaminase elevations, albeit usually minor and infrequent, are not uncommon for drugs classified as antineoplastic compounds, neurotrophic drugs, and antibiotics/antibacterials. Table 1 summarizes available information for some of these drug categories.
TABLE 1
The Relative Incidence of Liver Injury and Elevated Transaminase Levels for Various Drug Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Hepatic effects</th>
<th>Mechanism of injury</th>
<th>References</th>
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<tbody>
<tr>
<td>Nonsteroidal anti-inflammatory drugs</td>
<td>Incidence of acute liver injury is about 3.7 per 100,000 NSAID users. Transaminase levels abnormal in 5–15% of patients taking a particular drug.</td>
<td>Mostly idiosyncratic responses.</td>
<td>Garcia Rodriguez et al. (1994), Boeckstiele et al. (1995)</td>
</tr>
<tr>
<td>Anticancer drugs</td>
<td>Hepatotoxic manifestations include venooclusive disease, hepatocellular necrosis, and fatty change. Incidence of 1.3% in 980 cases of hepatic injury in one study.</td>
<td>Diverse injuries, both intrinsic and idiosyncratic.</td>
<td>King and Pery (1995), McDonald and Tirumalii (1984), Jean-Paston and Jouglard (1984)</td>
</tr>
<tr>
<td>Antibacterials</td>
<td>Estimated frequency of hepatotoxic reactions is between 1 and 10 per 100,000 drug prescriptions. Antibiotics accounted for one-third of all suspected drug-induced hepatic injury in one study. Mild transient transaminase elevations reported in up to 30–50% of recipients for some antibiotics.</td>
<td>Injuries include necrosis, cholestasis, cholangitis, and other. Mostly idiosyncratic except intrinsic for tetracyclines and some macrolides.</td>
<td>George and Crawford (1996), Stricker et al. (1992b), Stricker (1986)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>In one study, cardiovascular agents were implicated in 27.3% of 980 cases of liver injury. Diuretics accounted for 13.9% in another study. Mild ALT/AST elevations are common at the onset of treatment.</td>
<td>Autimmune or hypersensitivity reactions with antiarrhythmic agents; hepatocellular pattern with antihypertensive agents.</td>
<td>Jean-Paston and Jouglard (1994), Stricker et al. (1992b), Stricker (1986)</td>
</tr>
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9. DRUGS FREQUENTLY ASSOCIATED WITH HEPATOTOXICITY

Some marketed drugs have been associated with a high, predictable incidence of suspected hepatotoxicity in human populations, as, for example, they may cause asymptomatic, transient elevations in serum transaminase levels in <10% of the patient population. It should be noted that the actual frequency of isolated, asymptomatic elevations of transaminases in patient populations may actually be understated because liver function tests are not routinely ordered during routine clinical practice (Jick, 1995). Some examples are shown in Table 2. Likewise, drug interaction, disease complications, viral infections, or combinations of drugs may be responsible, in part, for some of these effects.

10. DISCUSSION

Drug-induced liver toxicity can be detected at various stages of drug development depending upon the mechanism responsible for hepatic injury. Intrinsic hepatotoxins are effectively identified in the preclinical testing stage using animal models at doses much higher than the intended human dose while, at the other extreme, rare but sometimes fatal idiosyncratic responses can only be discovered after the exposure of thousands of patients to therapeutic levels of the drug. Elevated serum transaminases, especially ALT, are the single most important laboratory indicators of hepatic effects from early preclinical testing to the postmarket monitoring of patients responding to a variety of histologic effects. Baseline serum transaminase levels in human populations are affected by a variety of both genetic and environmental factors. In addition, some serum enzymes of hepatic origin used for assessment of liver injury or disease can be increased by hepatic enzyme induction instead of or in addition to membrane leakage caused by hepatic damage. Within these limitations, guidelines for the detection of hepatic injury via these biochemical markers have been established.

The hepatic metabolism of drugs to reactive toxicants or protein-binding moieties is the single most important, but by no means the only mechanism leading to hepatic injury. Unlike preclinical laboratory animals which are relatively homogenous in terms of their constitutive hepatic cytochrome P450 isozymes, human populations are remarkably diverse with cytochrome P450 enzyme polymorphisms and other individual differences in drug-metabolizing enzyme systems being prevalent. Subtle individual differences in the hepatic detoxification processes or the formation of minor but highly reactive metabolites in susceptible individuals can lead to rare but unpredictable consequences as an increasing proportion of the population is exposed to a drug at therapeutic doses. Frequently, these idiosyncratic responses are caused by an aberrant immune response to the drug or a drug-altered self-antigen. For both direct, intrinsic toxic responses in humans or laboratory animals and isolated idiosyncratic responses in a clinical setting, early evidence of hepatic injury is obtained via biochemical methods including the monitoring of serum transaminases.

While knowledge of the typical human metabolism of experimental drugs will not predict idiosyncratic hepatic toxicity, it can be useful in determining similari-
<table>
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<tr>
<th>Drug name</th>
<th>Description of transaminase response</th>
<th>Hepatic histological responses</th>
<th>Mechanisms</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Amiodarone (antiarrhythmic)</td>
<td>Approximately 25% of users develop transaminase elevations $&gt;2$ times normal during treatment.</td>
<td>Phospholipidosis.</td>
<td>Metabolic idiosyncratic.</td>
<td>Lewis et al. (1989), Sticker et al. (1992)</td>
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<tr>
<td>Chlorpromazine (anticonvulsant)</td>
<td>Mild liver enzyme elevations in $10$--$42%$ of recipients; serum transaminases typically elevated first week of treatment.</td>
<td>Cholestatic or mixed.</td>
<td>Reactive metabolite responsible, immunoallergy.</td>
<td>Davis (1989), Pessayre (1992), De Mol et al. (1984), Stricker et al. (1992b)</td>
</tr>
<tr>
<td>Cognex (tacrine-HCl) cholinesterase inhibitor (cognition activator)</td>
<td>Elevated transaminases ($&gt;3$ times upper limit of normal) in $25%$ of patients.</td>
<td>Consistent with hepatocellular injury.</td>
<td>Mechanism of hepatotoxicity is unclear.</td>
<td>Watkins et al. (1994)</td>
</tr>
<tr>
<td>Iproniazid (antidepressant)</td>
<td>Abnormal transaminases in up to $20%$ of recipients.</td>
<td>Hepatocellular necrosis.</td>
<td>Reactive metabolite</td>
<td>Sticker et al. (1992b)</td>
</tr>
<tr>
<td>Isoniazid (antibacterial)</td>
<td>Serum transaminases elevated in $10$--$20%$ of patients.</td>
<td>Liver injury reported in $0.5%$ of adults; higher in children.</td>
<td>Reactive metabolite responsible for metabolic idiosyncratic response.</td>
<td>Davis (1989), Timbrell et al. (1986), Stricker et al. (1992b), PDR</td>
</tr>
<tr>
<td>α-Methyldopa (antihypertensive)</td>
<td>Transient increases in transaminases, Alk P, and bilirubin in $2.8%$ of patients.</td>
<td>Acute hepatitis in about $6%$ of patients within the first few weeks.</td>
<td>Reactive metabolite responsible, hypersensitivity.</td>
<td>Davis (1989), Finlayson (1973), Dybing et al. (1976)</td>
</tr>
<tr>
<td>Naltrexone-HCl (Revia) (opioid antagonist)</td>
<td>Peak ALT levels $3$--$19$ times baseline values in some patients after $3$--$8$ weeks of treatment.</td>
<td>No cases of hepatic failure reported.</td>
<td>Direct hepatotoxin.</td>
<td>PDR</td>
</tr>
<tr>
<td>Precose (acarbose) α-glucosidase inhibitor (diabetes)</td>
<td>ALT and/or ALT elevated $&gt;3$ times upper limit of normal in $15%$ of patients.</td>
<td>Hepatic abnormalities improved or resolved upon discontinuation.</td>
<td>Not reported.</td>
<td>PDR</td>
</tr>
<tr>
<td>Precose (acarbose) α-glucosidase inhibitor (diabetes)</td>
<td>ALT and/or ALT elevated $&gt;3$ times upper limit of normal in $15%$ of patients.</td>
<td>Hepatic abnormalities improved or resolved upon discontinuation.</td>
<td>Not reported.</td>
<td>PDR</td>
</tr>
<tr>
<td>Proleukin (aldesleukin; interleukin-2 product) (anticancer)</td>
<td>Elevated ALT in $10%$ and AST in $14%$ of patients at $100$ mg/kg.</td>
<td>Dose-related (intrinsic).</td>
<td>Bryson et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Riluzole (Rilutek) (amyotrophic lateral sclerosis treatment)</td>
<td>Elevated ALT in $10%$ and AST in $14%$ of patients at $100$ mg/kg.</td>
<td>Dose-related (intrinsic).</td>
<td>Bryson et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Tienilic acid (uricosuric diuretic)</td>
<td>AST and ALT elevated.</td>
<td>1 in $10,000$ patients develop hepatitis of the immunoallergic type. Withdrawn from the market.</td>
<td>Reactive metabolite responsible, immunoallergy.</td>
<td>Dansette et al. (1991), Stricker (1986)</td>
</tr>
</tbody>
</table>
ties between species that may lead to intrinsic toxicity or to explain tolerance or sensitivity to the hepatic toxicity of a compound in one species versus another. Current in vitro laboratory procedures utilize isolated microsomal preparations or specific recombinant human cytochrome P450 products in conjunction with chromatographic procedures for the detection and identification of drug metabolites. In vitro methods have been used to predict the in vivo drug metabolism of verapamil, loxoribine, diazepam, lidocaine, phenacetin, and some other compounds in the human liver (Iwatsubo et al., 1997). In a similar manner, in vitro comparisons of drug toxicity in metabolizing rat, dog, monkey, and, where possible, human hepatocyte cultures can be used for the preclinical assessment of drug hepatic toxicity using the same clinical chemistry end points, namely the transaminases, that are used in vivo. Primary hepatocytes cultured on an extracellular collagen matrix in time form bile canaliculi (LeCluyse et al., 1994) providing the opportunity to study the effects of drugs on the hepatobiliary system in vitro. Examples of the successful use of hepatocyte cultures from humans and other species to study hepatic effects include the metabolism of adinazolam, the cytotoxicity of trospectomycin, and the human-specific hepatotoxicity of panadiplon (Ulrich et al., 1995).

Recent developments that may have application in preclinical testing for potential liver toxicity include procedures that have evolved in conjunction with combinatorial chemistry and pharmacological screening methods (see Lam, 1997). Screening methods of particular interest include genomic and proteomic assays that are sensitive and plenary by nature, although they may be of limited value in revealing specific toxicities. These methods depend heavily upon automation, the availability of specific probes or libraries for pattern recognition, and advanced computer systems. The ultimate advantage of these techniques would be the detection of early cellular events, as, for example, markers of oxidative damage, at therapeutic dose levels that may predispose the organism to frank toxicity with sustained exposure. Examples of gene expression in liver after toxic injury include heat shock and oxidative stress-inducible genes (Schiaffonati and Tiberio, 1997). Both induction and suppression of gene expression can occur with some gene families (Runge-Morris, 1997). Reactive intermediates of xenobiotics often bind covalently to proteins as conjugates or adducts leading to either direct toxicity or immune-mediated toxicity (Pumford et al., 1997). In addition to the modification of cellular proteins, xenobiotics may modify protein expression (Richardson et al., 1994). Two-dimensional gel electrophoresis techniques have been described that allow the resolution and quantitation of hundreds of liver proteins that may be altered by the toxic effects of xenobiotics (Anderson et al., 1996). If a clear association between either altered gene expression or specific changes in protein structure and abundance can be established with specific liver injury, these techniques may be useful in detecting hepatotoxic potential preclinically, even at therapeutic doses.

Idiosyncratic responses remain difficult to assess at a preclinical or early clinical level. Using separate cultures from several donors, it may be possible to expose human hepatocytes to higher concentrations of a drug than would be found in clinical practice in order to forcibly detect idiosyncratic responses due to minor reactive metabolites that do not involve the immune system. Because most compounds that have been tested do cause observable changes in liver gene expression (Anderson et al., 1996) and idiosyncratic hepatotoxins such as diclofenac, halothane, tienilic acid, and valproic among others are known to form protein adducts (Pumford and Halmes, 1997), protein analysis by two-dimensional gel electrophoresis may provide a useful preclinical screening too. Future developments in assays may include determinations of drug effects on transgenic animals expressing human MHC class I and II and associated genes (Campbell and Milner, 1993), utilization of the sensitive reporter antigen/popliteal lymph node assay (Albers et al., 1997), the comparative testing of a compound at high doses or concentrations in transgenic animals or cell lines expressing various constitutive human cytochrome P450s, or the testing of preclinical candidates in rodent strains genetically diverse in terms of the hepatic cytochrome P-450 system.

11. CONCLUSIONS

- The incidence of drug-induced hepatic injury varies among the different drug classes as well as within a drug class. Intrinsic responses are generally detected early in the discovery and development process, while host-dependent, idiosyncratic responses are usually not detectable during the early phases of development due to their rarity.

- Of the various clinical laboratory markers for hepatic injury, serum transaminases, especially ALT, are the most universally important indicators for studies ranging from early preclinical animal testing to postmarketing patient monitoring. Serum ALT is elevated in response to several categories of hepatic lesions in multiple species. However, slight transaminase elevations following drug exposure in humans must be interpreted with care due to the many extrinsic factors known to elevate transaminases.

- The hepatic metabolism of drugs to reactive toxicants or protein-binding moieties is a frequently encountered mechanism leading to hepatic injury. Subtle individual differences in the hepatic detoxification processes or the formation of minor but highly reactive metabolites in susceptible individuals can lead to rare but unpredictable consequences as an increasing proportion of the population is exposed to a drug at therapeutic doses. Improvements in the metabolic profiling of drugs using in vitro microsomal systems expressing
multiple human cytochromes during the preclinical testing phase may enhance our ability to anticipate both intrinsic and idiosyncratic toxic responses.

- Although the monitoring of serum transaminases will continue to be an important clinical laboratory procedure for the assessment of preclinical hepatotoxicity, new developments involving comparative toxicity assays in human and animal hepatocyte cultures, techniques to detect altered gene expression or changes in protein structure and abundance, or improved methods for the detection of immunogenicity will augment the safety assessment process.

REFERENCES


