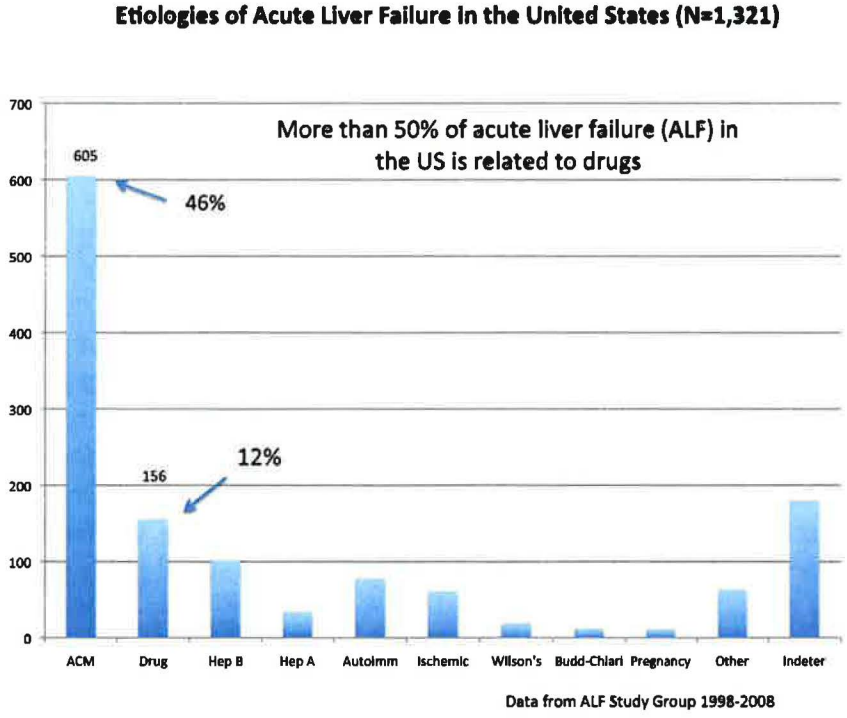
Drug-Induced Liver Injury 2009



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Brief biography

Dr. William M. Lee was educated at Amherst College, Columbia University College of Physicians and Surgeons (AOA), and completed his house staff training at the Presbyterian Hospital in New York City and Kings College Hospital, London. He has held faculty positions at Columbia and the Medical University of South Carolina prior to coming to UT Southwestern as Professor of Internal Medicine in 1990. Since 2003, he has held the Meredith Mosle Chair in Liver Diseases. He leads a large clinical trials group at UT Southwestern performing basic and clinical studies in hepatitis B and C, including the NIH-sponsored HALT-C Trial. In 1998, Dr. Lee launched the Acute Liver Failure Study Group (ALFSG) that has been continuously funded by NIDDK and based at UT Southwestern since that time. Two new clinical networks as part of the UTSW portfolio since 2008 are the Drug-Induced Liver Injury Network (DILIN: the subject of today's talk), and the Hepatitis B Research Network.

This is to acknowledge that William M. Lee, MD has disclosed financial interests with commercial concerns related indirectly to this program. Dr. William M. Lee will be discussing off-label uses in his presentation.

Glossary of Terms

DILIN: Drug-Induced Liver Injury Network, a group of 8 US academic centers including UTSW, engaged in studying DILI, its causes, genetics and treatment.

RUCAM: Roussel-Uclaf Causality Assessment Method. A standardized tool to assess the likelihood of a drug causing an acute liver injury. RUCAM was designed 20 years ago to yield a score that places drug-related events in definite, probable, possible or unlikely categories. RUCAM does not work very well and should be replaced.

GWAS: Genome wide association study. GWAS uses the broadest possible approach to attach associations to specific nucleotide polymorphisms. As the name implies, it is not targeted but analyzes millions of genes to see if there are specific ones associated with a disease pattern (e.g., acute liver failure or even elevated aminotransferases after receiving a drug).

SNP: Single nucleotide polymorphism. One change in host DNA seems unlikely to characterize many examples of DILI; however, we may be pleasantly surprised that certain instances of increased susceptibility to a drug are related to a single SNP which would be relatively easy to test for.

Introduction

Drug-induced liver injury is a frequent cause of liver necrosis of exceptional severity, comprising more than 50% of all cases of acute liver failure (ALF) in the United States[1,2] (Fig. 1, cover). Hepatotoxicity has been described for a large number of drugs,[3] although the overall number of cases of each is quite low, given the number of prescriptions written.

Different agents cause liver injury in different ways and at different rates. The majority of reactions appear directed against hepatocytes but biliary injury as well as combined hepatocyte/biliary injury or damage to specific organelles (e.g., mitochondria) produce the different disease patterns observed. While some agents such as isoniazid cause liver injury in as many as 1 in 100 people and fatality in 1:10,000, other agents result in liver damage in only 1:50,000 or may never cause liver injury.

Few data on the epidemiology of drug-induced liver disease are available. A population-based study from France between 1997 and 2000 demonstrated an annual incidence of ~13.9 per 100,000 with significantly more cases found in those over 50 years of age.[4] Those with a fatal outcome are more limited and most studies are subject to under-reporting.[5] In developing parts of the world, drug-induced liver disease is much less common and related to very few drugs.[6] However, probably only a small fraction (< 10%) of actual cases are reported, and a true estimate of the incidence of drug-induced liver disease may be impossible to obtain. This is due in part to the difficulty in establishing the diagnosis, as well as inadequate reporting systems.

The exact number of drug-induced liver injuries per year in the United States is unknown, but the severity of many of these cases and the tragedy involved in a presumed preventable injury makes it imperative that all sensible precautions be taken to avoid such incidents. Recent research is beginning to identify the genetic signature of liver injury due to specific drugs. The Drug-Induced Liver Injury Network (DILIN) is a new initiative by NIH to address the problem of idiosyncratic drug toxicity, and is intended to accelerate genetic research by providing well-characterized patient groups specific for certain drug reactions, so that we can better understand their pathogenesis and move to prevent these reactions via screening prior to drug use.

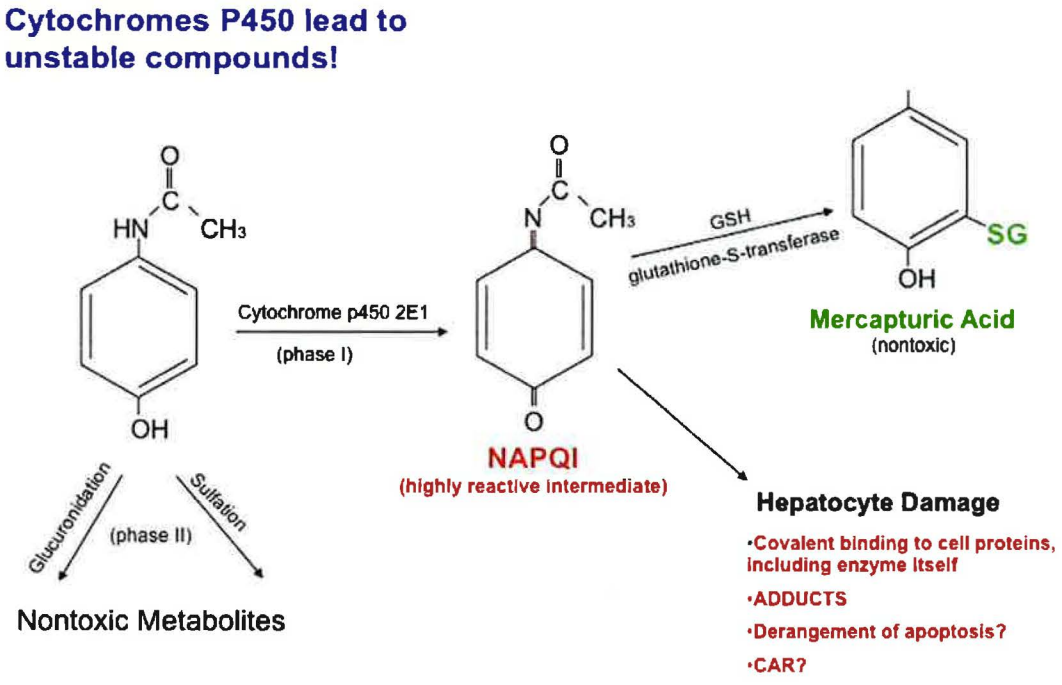
DRUG METABOLISM AND MECHANISMS OF HEPATOTOXICITY

The liver, situated between the absorptive surface of the gastrointestinal tract and target of drug effects throughout the body, is central to the metabolism of foreign substances. Since hepatocyte metabolism is required for virtually every drug, it is remarkable how seldom injury to liver cells occurs. Most drugs and xenobiotics cross the intestinal brush border because they are lipophilic. *Biotransformation* is the process whereby lipophilic therapeutic agents are rendered more hydrophilic by the hepatocyte, resulting in drug excretion in urine or bile. In most instances, biotransformation changes a nonpolar to a polar compound through several steps. Foremost is an oxidative pathway (e.g., hydroxylation) mediated by the cytochromes P450 (CYPs).[7] This is typically followed by esterification to form sulfates and glucuronides, which results in addition of highly polar groups to the

hydroxyl group. These two enzymatic steps are referred to as phase I (P450 oxidation) and phase II (esterification). Other important metabolic pathways involve glutathione S-transferase, acetylating enzymes, and alcohol dehydrogenase, but the principal metabolic pathways for most pharmacological agents involve P450 and subsequent esterification.

The exact details of the pathogenesis of liver injury remain unclear for most drugs. A single drug may cause its toxic effects in several ways. An oversimplified approach suggests that high-energy unstable metabolites of the parent drug, the result of P450 activation, bind to cell proteins or DNA and disrupt cell function. Perhaps the best example is acetaminophen. Although used universally for non-narcotic pain relief, acetaminophen taken in large quantities causes profound centrilobular necrosis.[8] The metabolic pathway for acetaminophen involves both phase I and phase II reactions, glutathione detoxification, and the formation of reactive intermediates (Fig. 2). It has served as a template for understanding drug metabolism more globally.

Figure 2. Metabolic pathway of acetaminophen.



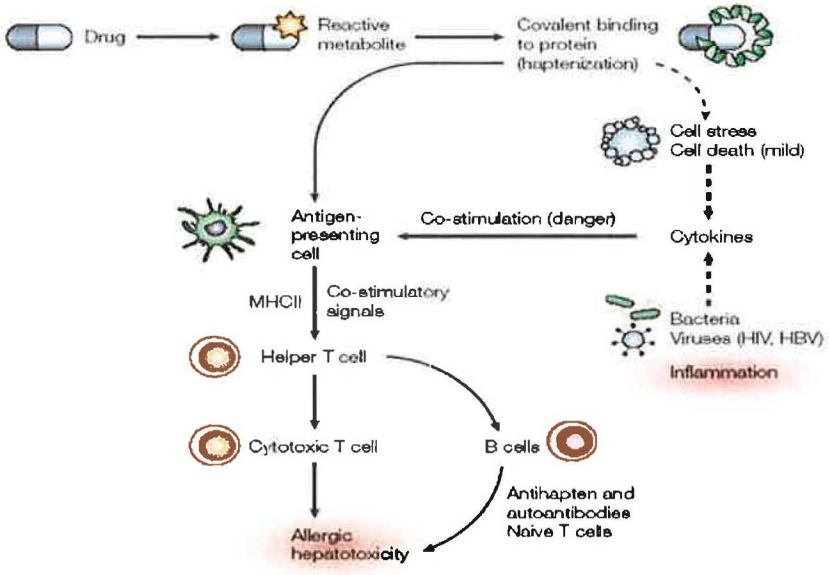
Glucuronidation and sulfation occur as the initial detoxifying step since the parent compound contains a hydroxyl group. Since glucuronidation and sulfation capacity greatly exceeds daily needs, even patients with far-advanced liver disease continue to have adequate glucuronidation capacity, which explains why no obvious enhancement of toxicity is observed in patients with cirrhosis taking acetaminophen.[9]

Enzyme Polymorphisms

The rarity of drug toxicity begs the question of how an infrequent event (1:10,000) occurs. Genetically variant CYP iso-enzymes, such as are observed with metabolism of debrisoquine, partially explain observed individual variation in responses to drugs. Debrisoquine is an antihypertensive drug marketed in Europe which is hydroxylated by CYP2D6, an iso-form that is totally lacking in 5% of healthy individuals, greatly prolonging the half-life of the parent compound in affected individuals.[10] Fast and slow acetylator patterns are observed to affect whole races, and have been implicated in isoniazid metabolism, which includes an acetylation step.[11] Genetic variants, which occur relatively frequently, cannot explain the formation of a toxic intermediate in only a rare individual. While there might be other metabolic variant P450 species that are even rarer, little evidence for these has been found in affected patients. Other explanations are necessary.

Most drugs are small organic compounds that are unlikely to evoke an immune response by themselves. The very products of CYP metabolism, the highly reactive intermediates formed within the microsomes, can covalently bind to the metabolizing enzyme itself to form a drug-hapten adduct (a larger, more immunogenic molecule) that disables the enzyme and also may injure the cell.[12] Haptenization then evokes an immune response directed against the newly formed antigen. The Danger Hypothesis proposes that activation of the immune response in this way leads to cell stress with augmentation of cytokines and invocation of the innate immune response (Fig. 3).

Figure 3. Proposed process by which drug hepatotoxicity develops



Adapted from: *Kaplowitz, Nat Rev Drug Disc 2005*

Whether adducts or smaller peptides processed and presented via the major histocompatibility complex (MHC) class I and class II schemes are the targets remains unclear. Recent evidence outlined below suggests that this is highly likely.

With or without cell necrosis, the formation of P450-drug adducts can evoke an immune response. Any subsequent P450-drug adduct present on the hepatocyte surface would evoke a further response. Responses may be antibody-mediated or occur from direct cytolytic attack by primed T cells[12,13] (Fig. 4).

Combined toxic/immunologic mechanisms are involved in the liver injury caused by halothane. Halothane was a widely used fluorinated hydrocarbon anesthetic, now largely abandoned, that caused severe, often fatal liver injury particularly after multiple exposures.[13] Other fluorinated hydrocarbons still in use occasionally yield the same response.[14–16] Both direct cytotoxicity and immune-mediated toxicity are observed in keeping with the clinical observations that severe halothane toxicity occurs only with repeated exposure. Still, some evidence of injury can usually be identified within a week of the first exposure. As befits an immune reaction, the interval to toxicity is shortened and the damage more severe with each successive exposure.

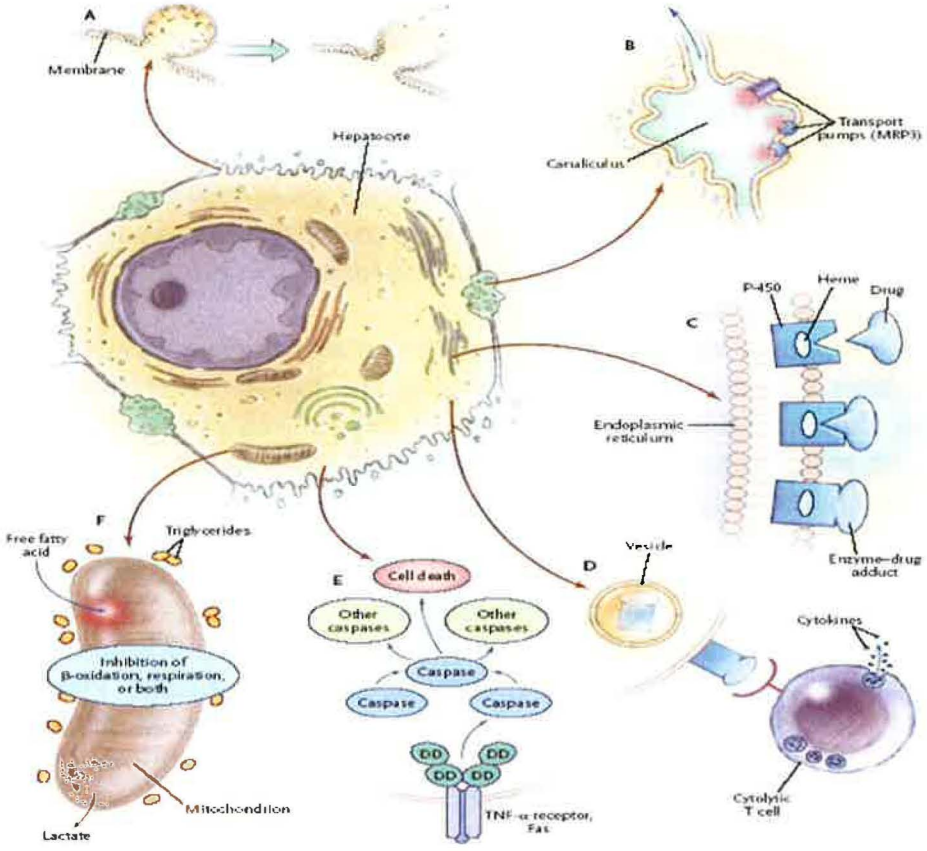
Specific genetically determined components of the immune response may be important. For example, the binding of peptides for antigen presentation depends on human leukocyte antigen (HLA) configurations that are genetically determined (see below for specific HLA haplotypes associated with drug toxicity). This variation among individuals is thought to mirror the diverse responses observed in patients encountering the hepatitis B virus. The highly variable severity of reactions observed depends on the fit of antigen peptides in the HLA groove. A specific HLA haplotype has been associated with amoxicillin-clavulanate–induced hepatitis, being found in 57.1% of patients versus 11.7% of controls.[17] Polymorphisms have also been identified for the interleukin 10 (IL-10) promoter and for tumor necrosis factor-α (TNF-α). These variations in immune responsiveness could modulate the severity of the downstream responses observed, once injury begins. For example, different IL-10 promoter phenotypes are recognized. A C-to-A substitution at position 627 is linked to severe asthma and, by inhibiting IL-10 secretion, an up-regulation of immune reactions of the Th2 type. This same phenotype has been linked to hepatitis C-related liver injury and to the severity of alcoholic liver injury.[18,19] Variant TNF-α phenotypic expression has been implicated in determining the severity of drug reactions related to acetaminophen.[20] A multistep, immune-based mechanism would best explain both the rarity of idiosyncratic reactions, and their severity, as well as the findings of mild, non-progressive liver injury in some patients—those with "protective" phenotypes. Recent studies suggest that, for specific agents, genetic markers can be identified that associate with the toxic reaction. Such pharmacogenomic observations may bear fruit, particularly for high risk drugs.[21] While an immunologic explanation for many reactions is plausible, the exact mechanism to account for most drug reactions remains obscure. Both cell necrosis and apoptosis have been recognized, and hepatic sinusoidal epithelial cells (SECs) as well as Kupffer cells are part of the process.[22] Recent studies have implicated SEC injury in several forms of venocclusive disease (see below, ref. 92).

There should be little doubt that the metabolic fate of any compound is a complex process. Often multiple factors are at play simultaneously, including drug interactions, either induction or competition. Common inducing agents include ethanol, phenobarbital, and phenytoin, but cigarette smoke is also a potent inducer of certain P450 species. Induction or substrate competition for available enzyme may not result in hepatotoxicity but strongly impacts plasma drug levels. For example, the effect of ketoconazole on enhancing cyclosporin levels is the result of induction,[23] while competitive inhibition by ketoconazole increases serum levels of astemizole (Hismanal) with resulting torsades de pointes.[24]

Other Mechanisms

In addition to direct hepatocyte injury, other mechanisms are at play (Fig 4).

Figure 4. Several different mechanisms are at play in hepatotoxicity.



In drug-induced cholestasis, disruption of or binding to specific transport proteins such as bile salt export pump (BSEP) or processes in hepatocytes or cholangiocytes may be the event that results in cholestasis. Bile salt transport from plasma into the hepatocytes is provided for by two basolateral (sinusoidal) transport systems: the sodium-taurocholate cotransporter (NTCP), and the organic-anion transporting polypeptide (OATP), whereas several canalicular export pumps have been identified.[25] Estrogen may cause multiple canalicular membrane transport changes,[26] affecting, among others, the canalicular bile salt pump.[27]

Uncoupling or inhibition of mitochondrial respiration may in some instances lead to microvesicular steatosis.[28] Mitochondrial β-oxidation of fatty acids is

impaired and this may decrease cellular energy supply leading to severe liver dysfunction. The mitochondrial β-oxidation may be affected either directly or by impairment of mitochondrial respiration.[29]

Drug-induced liver injury may be modulated and enhanced by inflammatory mediators that may trigger hepatocyte apoptosis.[30] Hepatocyte apoptosis is complex and may be controlled by the intracellular energy status[31,32] and by the redox state of the cell.[33] How the specific drugs involved in hepatotoxicity affect hepatocyte apoptosis remains to be studied.

As noted above, new techniques of pharmacogenomics may be helpful in predicting an individual's risk of hepatotoxicity for a given drug based on discovery of genetic susceptibility profiles associated with liver injury.[34] Recent genetic surveys have implicated a single HLA haplotype in causing liver injury due to flucloxacillin. Still, it seems unlikely that most drug reactions will be found to represent a single nucleotide polymorphism (SNP), though it certainly would make things easier.[35,36]

CLASSIFICATION OF HEPATOTOXIC AGENTS

Two main categories of drugs can produce liver disease. One group consists of intrinsic (predictable) drugs, whereas a second group consists of idiosyncratic (unpredictable) drugs. Unfortunately, the vast majority of drugs involved in liver disease belong to the idiosyncratic, and thus unpredictable, group.

Intrinsic (Dose-Dependent) Agents

Hepatotoxins of this group produce liver disease in most patients in a dose-related fashion if toxic amounts of the drug are ingested. Furthermore, similar lesions can often be found in animal models.[37] Hepatotoxicity may be caused by the drug itself or, most frequently, by toxic effects of its metabolites.

Acetaminophen (paracetamol) has emerged as the most dominant intrinsic hepatotoxic drug. Taken in small doses (4 g or less per day) acetaminophen is an extremely safe drug. However, its therapeutic index is low since only as little as 10 to 12 g may cause extensive hepatic necrosis.[38,39]

In acetaminophen metabolism, the phase II reactions predominate, with only a small fraction of acetaminophen metabolized by cytochrome P450, until the quantity of acetaminophen exceeds phase II capacity, at which point significant amounts of a toxic intermediate, *N*-acetyl-p-benzoquinoneimine (NAPQI), are formed primarily via CYP2E1 (Fig. 2).[40,41] NAPQI binds covalently to cell macromolecules disrupting mitochondrial and nuclear function.[35] Antibodies to nitrotyrosine residues can be detected as evidence of oxidative stress in livers of patients (or experimental animals) demonstrating toxicity.[42] These residues are formed by the rapid reaction of superoxide and nitric oxide formed by Kupffer cells reacting to form peroxynitrite, unless covalent bonding of NAPQI is prevented by its conjugation (via glutathione-S-transferase) to form mercapturic acid, a harmless water-soluble product excreted by the kidney.[43] Depletion of glutathione lowers this last defense against the formation of NAPQI-related intracellular adducts. Thus, starvation and chronic alcohol intake by depleting glutathione enhance toxic injury,[44–48] while *N*-acetylcysteine, by replenishing glutathione, protects against acetaminophen-induced injury.[49] This direct toxic reaction occurs predictably in all

individuals. The final step leading to cell death remains unclear but appears to involve altering the cytoskeleton and membrane integrity.[50] Apoptotic pathways are also implicated. A finding that peroxisomal proliferator activation prevents the liver injury associated with acetaminophen links this liver damage to apoptosis but does not preclude a combined necrosis/apoptosis effect.[51,52] Thus, even a dose-related toxin appears to involve responses of the innate immune system.

Acetaminophen overdose is the most common cause of acute liver failure with hepatic encephalopathy in several Western countries including the United Kingdom and the United States.[39,53,54] Although the prognosis for acetaminophen-induced acute liver failure is relatively good, with a spontaneous survival (i.e., survival without liver transplantation) of approximately 57%.[1] It is still the leading cause of acute liver failure death of in the United States.[2] Except for acetaminophen-induced liver disease, intrinsic drug cases are rare.

Idiosyncratic Reactions

While acetaminophen is a dose-related toxin, most drug reactions are idiosyncratic, occurring from 1 in 1000 to 1 in 50,000 patients. The etymology of "idiosyncratic" from the Greek loosely translated is "the unique composite of the self"—the particular features of a given individual. This places the emphasis appropriately on the patient's characteristics rather than on the drug itself. Idiosyncratic reactions are not due to the drug itself, since almost everyone can tolerate them, but to something unique about the patient who ingests them and gets a toxic reaction. Theories abound to explain these reactions. Features of idiosyncrasy suggest a role for the innate and adaptive immune response systems (Table 1).

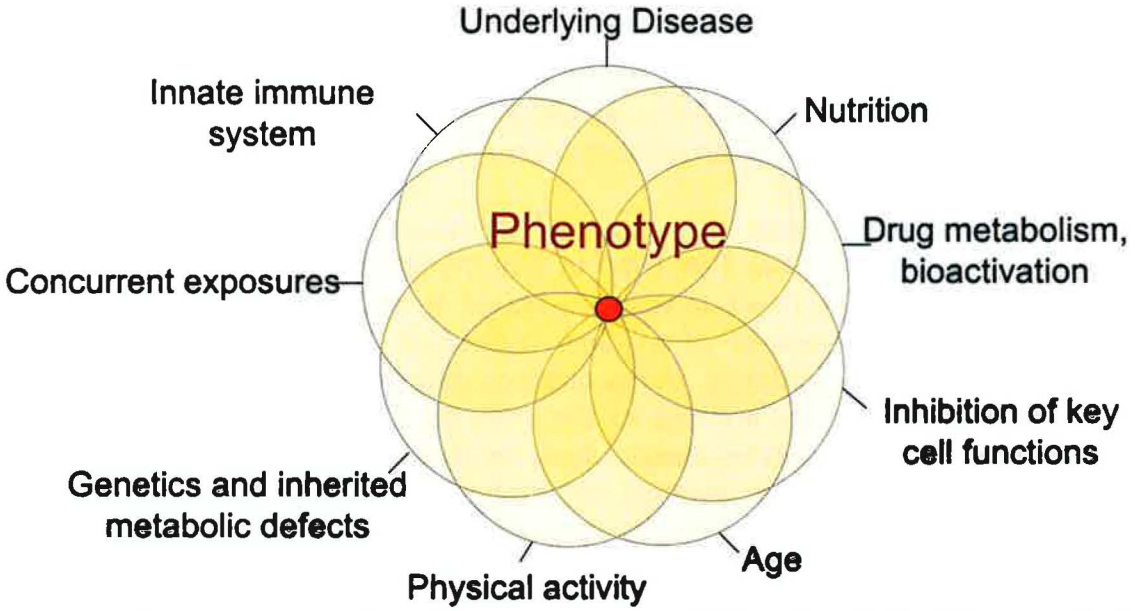
Table 1. Idiosyncratic Drug Reactions

1. Occur rarely
2. Similar consistent pattern for each drug
3. Similar drugs exhibit similar features called "class effects"
4. Individual drugs in a class still vary considerably
5. Reactions occur at varying time intervals after ingestion (3 days to one year)
6. Reactions vary in severity, but typically severe and fatal if drug continued
7. Mild injury often disappears with continued use (adaptation)
8. Rarity of most reactions suggests possibility of multiple hits
9. Re-challenge is virtually always met with greater severity, shorter latency
10. Most idiosyncratic drugs are given at doses > 100 mg/day[55]

The proportion of drug-induced liver disease varies greatly among drug classes as evidence of class effect. Antibiotics and anticonvulsants are highly associated with drug-induced liver disease (see DILIN data below), whereas hormones, antihypertensive drugs, digoxin, and anti-arrhythmic drugs (some exceptions) are very rarely associated with DILI. Idiosyncratic reactions occur in small numbers such that some drugs continue to be used when usefulness or uniqueness makes the risk acceptable. Isoniazid is such a drug, virtually the only drug implicated in DILI when it occurs in developing countries. Some 15% to 20% of

individuals receiving isoniazid as a single agent for tuberculosis prophylaxis may develop increased transaminases, but these usually stabilize or improve, so that less than 1% may develop severe hepatic necrosis.[56] More recently, a lower estimate of severe liver injury of 1:1000 has been given for isoniazid from a large tuberculosis public health clinic,[57] since 11 of 11,141 patients developed isoniazid-induced liver disease. This is still a high rate of injury compared to other idiosyncratic drug reactions, yet the usefulness of the drug has precluded its withdrawal.

Figure 5. Drug reactions are the result of multiple complex interactions.



Aside from isoniazid, antibiotics, non-steroidals and anti-convulsants are the most commonly associated drug class capable of inducing idiosyncratic reactions.[58]

Adaptation

It has recently been recognized, or described more carefully, that many drugs that cause rare idiosyncratic reactions are associated with more frequent aminotransferase elevations that are limited and resolve even with continuing the suspected agent.[61] This was known to be true for isoniazid but is now recognized as a common feature of the cholesterol-lowering 'statin' drugs. How this nascent liver injury is aborted or modulated by the host remains unclear. An interesting correlate is that acetaminophen appears to cause significant aminotransferase elevations in up to 40% of users within the first two weeks, if they take the recommended maximum of 4 gm per day.[62]

TYPES OF DRUG REACTIONS, CLINICAL PICTURES

While most liver injury involves direct hepatocyte necrosis/apoptosis, some drugs primarily injure bile ducts or canaliculi, causing cholestasis without significant hepatocyte damage. Others affect sinusoidal cells or present a particular pattern of liver injury affecting multiple cell types (mixed type). In a rough way, drug reactions can be grouped as hepatocellular, cholestatic or mixed, but these are only very general terms and do not apply to all circumstances. An additional way to categorize drug reactions emphasizes the histological changes involved as well as the cell type (Table 2).

Table 2. Types of Drug Reactions, With Examples

Hepatocellular: isoniazid, trazodone, diclofenac, nefazodone, venlafaxine,
Cholestatic: chlorpromazine, estrogen, erythromycin
Mixed: amoxicillin/clavulanate, carbamazepine, herbs, cyclosporin, methimazole
Immunoallergic: halothane, phenytoin, sulfamethoxazole
Granulomatous: allopurinol, diltiazem, nitrofurantoin, quinidine, sulfa drugs
Steatohepatitis: amiodarone, perhexiline maleate, tamoxifen
Autoimmune: nitrofurantoin, methyldopa, lovastatin
Fibrosis: methotrexate, vitamin A excess
Vascular collapse: nicotinic acid, cocaine, ecstasy

The distinction between hepatocellular and cholestatic is based on the R value, calculated by comparing two liver enzyme levels: alanine aminotransferase (ALT) and alkaline phosphatase (AP).[63] Liver injury is considered *hepatocellular* when ALT alone is more than two times above upper limit of its normal (ULN) range, or when the ratio (ALT/ULN)/(AP/ULN), the R (ratio) value is ≥ 5. Liver injury is termed *cholestatic* if AP alone is more than two times above ULN and the R value is ≤ 2; the term *mixed* designates a situation when both ALT and AP are above two times ULN and the ALT/ULN:AP/ULN ratio is between 2 and 5.

Hepatocellular Reactions

Hepatocellular reactions are the most common type of drug-induced liver disease, constituting up to 90% of cases.[64] They are characterized by a hepatocellular pattern of serum liver tests, as defined above. Many drugs have been implicated in hepatocellular type drug-induced liver disease. Usually, improvement is quick after discontinuation of the drug (1–2 months), and only a few patients develop fulminant, acute liver failure with hepatic encephalopathy.[65]

Histological findings include necrosis and cellular infiltration. Necrosis may be zonal (e.g., acetaminophen- or CCl_4-induced) or diffuse (e.g., halothane-induced), and the inflammatory response consists of lymphocytes and/or eosinophils. Massive necrosis may cause acute liver failure and death—the exact quantity of remaining hepatocytes necessary to support life has not been established.[8]

Cholestatic Reactions

Cholestatic reactions have been described for a number of drugs, some of which are listed in Table 3.

Table 3. Drugs Involved in Cholestatic Drug Reactions

Pure cholestasis	Anabolic steroids
	Tamoxifen
	Estrogens
Cholestatic hepatitis	Allopurinol
	Amoxicillin/clavulanate
	Azathioprine
	Barbiturates
	Captopril

Cholestasis is best defined as failure of bile to reach the duodenum,[67] and common symptoms are jaundice and pruritus. *Pure cholestasis* with no signs of hepatocellular necrosis is seen with use of oral contraceptives, anabolic steroids, or sex hormone antagonists such as tamoxifen.[68] *Cholestatic hepatitis* indicates a mixed picture with cholestasis (dilated canaliculi, brown granules in cytoplasm of hepatocytes) and liver cell necrosis, bile duct injury and polymorphonuclear leukocyteic infiltration. Drugs in this category include carbamazepine,[69] trimethoprim-sulfamethoxazole,[70] captopril,[71] and ticlopidine.[72]

Generally, drug-induced cholestasis resolves more slowly than the hepatocellular reactions.[3] In some instances progressive destruction of segments of the intrahepatic biliary tree may occur, the so-called *vanishing bile duct syndrome*[73] that occurs after a protracted course (more than 6 months) of drug-induced cholestasis. The result is a state of chronic cholestasis, resembling primary biliary cirrhosis.[74] Approximately 30 drugs have so far been implicated in the vanishing bile duct syndrome, including among them chlorpromazine[75] and ajmaline.[76]

A sclerosing cholangitis-like syndrome with jaundice caused by intra- and extrahepatic strictures of the bile ducts is sometimes observed in patients receiving intraarterial floxuridine chemotherapy for hepatic metastases of colorectal cancer.[77]

Immunoallergic Reactions

Drugs may be associated with reactions that are definitely allergic in nature. Hypersensitivity reactions such as fever, eosinophilia, or rash are common. Halothane induces fever, eosinophilia, and antimitochondrial antibodies.[13] Halothane was formerly widely used as an inhalation anesthetic. However, it has been implicated in a high number of very severe cases of liver disease.[78] Halothane causes a hepatocellular injury as evidenced by findings of necrosis—ranging from spotty necrosis to bridging hepatic necrosis and multilobular necrosis—in liver biopsies.[79]

Phenytoin (Dilantin) induces the simultaneous onset of fever, rash, lymphadenopathy, or eosinophilia.[80] The mechanisms responsible for the combined allergic and hepatotoxic reaction are unknown, but the slow resolution of the illness suggests that the allergen remains on the hepatocyte surface for weeks or months. With phenytoin, a mononucleosis-like picture may also be seen and frequently is confused with a viral illness or streptococcal pharyngitis.[81] When the offending agent is not discontinued promptly, despite signs of developing hepatitis, a severe Stevens-Johnson drug eruption and prolonged fever may result.[82] As with any therapeutic agent, rapid recognition of the presence of a toxic drug reaction and immediate discontinuation of the compound are the keys to limiting hepatic damage. It is important to remember that features of an allergic reaction may not be obvious. Even in the absence of systemic signs of allergy, eosinophilia or granulomas may be present on liver biopsy.

Steatohepatitis

Steatosis in the liver can be present either in a microvesicular or in a macrovesicular pattern. Macrovesicular steatosis is the most common form and is histologically characterized by hepatocytes containing a single vacuole of fat filling up the hepatocyte and displacing the nucleus to the cell's periphery.[28]

Macrovesicular steatosis is typically caused by alcohol, diabetes, or obesity. Sometimes drugs such as corticosteroids or methotrexate may cause these hepatic changes.[83]

In microvesicular steatosis hepatocytes contain numerous small fat vesicles, not displacing the nucleus,[28] Disruption of mitochondrial DNA with resulting anaerobic metabolism leads to lactic acidosis in the most severe cases.[29] Acute fatty liver of pregnancy[84] and Reye syndrome[85] are two examples of severe liver diseases caused by microvesicular steatosis.

Drugs causing microvesicular steatosis include valproate,[86] tetracycline,[87] fialuridine,[88] and others. Aspirin use in children has been associated with Reye syndrome,[89] and the incidence of Reye syndrome has virtually disappeared in recent years. A relatively new situation is that of considering the impact of drugs such as the cholesterol-lowering 'statins' in the setting of known fatty liver disease due to the metabolic syndrome. Although these agents may be associated with elevations in aminotransferase levels, they appear to be remarkably safe and can be used without monitoring liver enzyme levels in those with as well as without fatty liver disease.[90]

Other Drug Reactions

There are several other types of drug reactions involving the liver, which are of lesser importance in terms of number and severity. These include granulomatous reactions, fibrosis, ischemic injury, and chronic autoimmune liver injury. The type of reaction observed can be helpful in determining the likely agent, since most drugs have a specific injury profile.

A pattern of veno-occlusive disease (VOD) with obliteration of small intrahepatic veins, sinusoidal congestion, and necrosis is observed frequently in patients receiving chemotherapy (e.g., cytoxan, busulfan) following bone marrow transplant.[91-93] Symptoms include rapidly accumulating ascites, painful hepatomegaly, and jaundice occurring shortly after the conditioning regimen has been instituted. Rarely, herbal medicines may cause VOD.[94]

TREATMENT

Prompt discontinuation of a suspected drug is mandatory. General supportive therapy may be necessary according to the state of the patient, ranging from none to intravenous fluid replacement to the very intensive monitoring and treatment of patients with acute liver failure with hepatic encephalopathy.[63,95,96] Corticosteroids appear to have no place in management, even of patients who manifest allergic IgE-mediated responses.

The ALF Study Group recently completed a study of N-acetylcysteine for non-acetaminophen-related ALF. The results showed significant benefit for NAC in patients with early coma grades who were treated with NAC. The overall cohort included more than ¼ DILI case and their responses were even better although the overall number in the study was too small to make statistical conclusions.[97] Liver transplantation is performed in more than 50% of patients with idiosyncratic drug-induced acute liver failure since spontaneous survival in this setting is less than 20%.[1]

CRITERIA FOR CAUSAL ASSESSMENT OF DRUG-INDUCED LIVER INJURY

How do we decide whether a certain drug is responsible for the liver disease encountered in a certain patient? It is important to understand that we are always trying to assess a possible association from the *past* unlike most other scoring models that try to predict the *future*.[98] A standardized reporting form developed by an international panel working in France provides a worthwhile causality assessment scoring system,[61] called the RUCAM (*R*oussel *U*claf *c*ausality *a*ssessment *m*ethod These guidelines outline the steps an experienced clinician uses to assess likelihood of drug reactions.[99] Causality assessment methods typically include temporal relationship, course after cessation of drug, risk factors, concomitant drugs, a search for nondrug causes (viral hepatitis), previous information concerning the drug, and response to re-challenge, which is usually not available. Validation of the RUCAM scoring system suggests that a RUCAM score could be classified as *highly probable* (RUCAM score >8), *probable* (score 6–8), *possible* (score 3–5), *unlikely* (score 1–2), or *excluded* (score ≤0).[100]

A newer model called the clinical diagnostic scale (CDS) has been developed [101] Features from the CDS include most of the features from the RUCAM plus extrahepatic manifestations such as rash, fever, arthralgia, cytopenia, and eosinophilia. However, a recent Spanish comparison study in 215 patients suggested that the RUCAM system is more accurate than the CDS model.[102] The DILI Network is developing its own scoring system (see below).[103]

LESSONS FROM DRUGS WITHDRAWN FROM THE MARKET

An example of a drug withdrawn due to severe and fatal liver injury was troglitazone. It is an example of the difficult issues surrounding drug-induced liver injury and its regulation. Troglitazone (Rezulin) was approved by the FDA in January 1997, the first of a new class of compounds, the thiazolidinediones. As an agonist of the nuclear regulatory factor peroxisomal proliferator activator receptor-γ (PPAR-γ), troglitazone reduces insulin resistance and increases insulin-stimulated glucose disposal, resulting in improved glycemic control for patients with type II diabetes. In clinical trials, reversible elevations of aminotransferase levels were observed, occasionally reaching more than eight times ULN, but, again, no examples of ALF. However, once the drug was approved, several reports of severe and fatal liver injury appeared.[104-106] After more than 3 years on the market and millions of prescriptions troglitazone was withdrawn from the market. A factor in the FDA decision was the arrival of two new PPAR-γ agonists, rosiglitazone (Avandia) and pioglitazone (Actos), approved, respectively, in May and July 1999. Although of the same class, these agents do not seem to have the same degree of toxicity. Thus far, neither drug has shown the 1:30,000 incidence of severe hepatotoxicity seen with troglitazone. Two drugs recently found to have significant toxicity were ximelagatran (Exanta®) and telithromycin (Ketek®). The first was licensed in Europe as a thrombin inhibitor that would work similarly to warfarin. Unfortunately, aminotransferase elevations were observed in 7.9% of patients in pre-approval trials and examples of toxicity were observed as long as several weeks after the drug had been discontinued—approval was denied in the US and the

sponsor withdrew the drug from the European market.[107] Ketek was approved in the US and Europe in 2004 as the first in a new class of antibiotics, the ketolides, that have activity against penicillin and erythromycin resistant pneumococci. After more than 50 reports of serious liver injury, the drug has now been given more severe restriction on its use and is to be used only as a 'second tier' drug.[108,109]

THE DRUG APPROVAL PROCESS

How can new drugs get through the drug approval process and still lead to so many deaths that they are ultimately withdrawn? In brief, drug development is divided into three stages: initial research and development, clinical testing for new drug application (NDA), and the post-marketing experience.

Research and Development

The initial stage of drug development includes drug discovery and initial testing for efficacy, or toxicity in animals or in vitro model systems. Most new compounds fail to make it through this stage, either because of toxicity or lack of efficacy. Compounds may be "discovered" in several ways: synthesized to resemble previous compounds, discovered in the field by purification of naturally occurring peptides (e.g., cyclosporin A), or generated by computer modeling. A compound shown to have a desirable effect *in vitro* or *in vivo*, then undergoes extensive preclinical testing in a variety of animals using doses up to 50 times that predicted to be useful in humans to ascertain the types of toxicity that might be expected. While metabolic pathways differ in some specific aspects, the similarities between lower mammals and humans are quite notable. Animals are euthanized after short-term experiments and all organs examined; those dying during experiments undergo necropsy to determine cause of death. Long-term exposure studies are performed looking for carcinogenicity or other delayed effects. Preclinical testing, which may take 5 to 6 years to complete, is still a crude technique and no substitute for clinical trials in humans. The use of massive dosing in animals may in part compensate for metabolic differences between species, but human trials are ultimately needed. Toxicogenomics offers some promise of early identification of "toxicity" gene expression profiles (i.e., signatures).

Clinical Trials

In phase I testing, progressively larger doses of the test medication are given to well-paid healthy volunteers. Routine monitoring includes vital signs, electrocardiogram (ECG), assessment of reported side effects, and blood measurements including serum aspartate aminotransferase/alanine aminotransferase (AST/ALT), amylase, and creatine phosphokinase (CPK). In phase II testing, patients are exposed for the first time and the emphasis shifts from safety alone to safety *and* efficacy. Depending on the intended use of the medication and the prevalence of the disease to be treated, from 500 to 5000 study patients may test the medication for periods of up to a year. In early phase II trials, a progressive dosing scheme identifies the maximal dose that is effective and still safe. If a given dose is effective, it is then determined if there are any short- or long-term side effects. This stage is where safety concerns regarding idiosyncratic reactions are identified and drugs scrapped. A 'signal' of aminotransferase elevations often is associated with more severe toxicity as can be observed with isoniazid and possibly

ximelagatran, but most aminotransferase elevations do not lead to severe injury. Ximelagatran was suspect because of the 7.9% experiencing ALT elevations of 3X ULN.

Dr. Hyman Zimmerman, long recognized as the founder of the study of drug-induced liver injury, stated that if a drug causes enough liver injury to lead to jaundice, even rarely, then 10% of affected patients will develop acute liver failure ("Hy's rule").[83] Put another way, any drug that in phase II or III testing demonstrates not only aminotransferase elevations, but increases in bilirubin or jaundice will likely lead to ALF when larger numbers of patients are exposed. This sounds like a very imprecise "rule" but it has served quite well over the years, and there does not seem to be anything better.

How certain can we be that clinical studies identify instances of liver injury? First, all studies are conducted according to previously established guidelines of good clinical practice. FDA has published a guidance recently to indicate to the pharmaceutical industry what constitutes a strong new drug application (NDA) submission.[110] In each NDA, a detailed assessment of liver biochemical parameters provide comparisons with the incidence of abnormalities in a placebo or a comparator group. Aminotransferases exceeding three times the ULN generally require discontinuation of the drug. Increased aminotransferase levels *without* bilirubin elevations may not lead to discontinuation during a phase III trial, but frequent or more severe aminotransferase increases (>8 times ULN) or accompanying increases in bilirubin will likely bring a new drug trial to a halt.

The FDA approves approximately 50 new drugs each year. The approval process takes between 6 months to a year, once the NDA is filed. Approval brings with it instant widespread, intense marketing efforts and the necessity for all U.S. pharmacies to stock the drug. As noted previously, the number of prescriptions frequently rises rapidly into the millions, within a year or less. This fact explains why some drugs only demonstrate problems once they receive FDA approval. Idiosyncratic events occurring in only 1:50,000 patients are not going to be recognized in a study of 4500 patients. The "rule of threes" applies: to reliably identify a single case of liver injury due to a drug with 95% confidence, there must be three times the number of patients studied as the incidence of the drug reaction. In other words, a 1:1500 reaction requires 4500 patients to reliably detect a single case; a 1:50,000 reaction would require 150,000 patients. No clinical trial will reliably pick up rare drug reactions. Approval by the FDA provides a wider experience than the limited exposure of the carefully controlled clinical trial. Thus, it should not be surprising that drug reactions are observed in the post-marketing period and not before. However, post-approval drug recall still takes time to evolve while the drug continues to be prescribed despite the recognition of adverse events.

Post-Marketing Surveillance

The greatly increased number of patients receiving a new drug ensures that untoward or unusual drug effects will be observed. In addition to increased numbers, a wider range of patients than the defined clinical trial population is exposed. For example, most studies do not include patients with renal failure, heart failure, HIV/AIDS, pregnant women, the elderly, or children. Any of these groups

may show enhanced toxicity. Even the best-randomized controlled clinical trial is not a "real-life" experience. The difficulty is in identifying these drug reactions quickly and accurately, once the product is released. During clinical trials and the after market period, pharmaceutical companies must report serious adverse events (SAEs) to the FDA within 24 hours. An SAE is any unexpected medical occurrence that at any dose results in death, is life threatening, requires hospitalization, or results in permanent disability or a birth defect. Pharmaceutical manufacturers maintain a safety monitoring force that gathers reports, assesses likelihood of the reaction being attributed to their product, and issues a report to the FDA and to clinical investigators if there is still an ongoing trial. However, there is bound to be a bias toward any new product, just as there is bias built in to the design of clinical trials.[111]

In general, post-marketing surveillance has failed to provide adequate protection of the consumer. The MedWatch system is passive; physicians and pharmacists are under no obligation to report adverse events. Physicians and pharmacists are to report all drug reactions they observe on a standardized form. However, it is estimated that less than 10% of severe adverse drug reactions are reported to the company or the FDA. Reasons for under-reporting include: failure to recognize "hepatitis" as being due to a drug, concern about malpractice implications, reluctance to get involved, complacency ("too busy"). Reports received seldom contain full clinical information. Privacy issues may preclude further inquiries and raise concern regarding possible legal implications. Nevertheless, the main source of information is the Medwatch system, plus case reports.[112] DILIN is one effort to remedy this problem. An additional DILIN program underway is to provide a detailed educational component on the National Library of Medicine website.

HEPATOTOXICITY IN THE PATIENT WITH CHRONIC LIVER DISEASE

Hepatologists are frequently asked "Is the patient with liver disease more susceptible to liver injury?" Intuitively, this makes sense, until we realize that hepatotoxic reactions represent the culmination of hepatic enzyme activity. If liver function is impaired, one might predict diminished activity of certain enzyme systems. Patients with liver disease do not appear to be at increased risk for hepatic injury compared to their counterparts without underlying liver problems. Dr. Zimmerman put it best: "A stubborn [misconception] has been the view that patients with pre-existing hepatic disease are more likely than others to suffer hepatic injury on exposure to drugs that cause liver damage. There is virtually no evidence for this view."[83] What do we know of the liver function of patients with cirrhosis? Many enzyme systems are well preserved even in advanced disease. For example, patients with terminal alcoholic hepatitis still are able to conjugate most of their bilirubin. Therefore, enzyme activity in many instances exceeds the daily requirement, so that even severe liver injury would not be expected to lead to an adverse drug reaction. In general, phase I reactions may be diminished but this is not uniformly so. In severe liver disease the activity of CYP2C19 is greatly decreased while that of CYP2D6 is intact.[113] In nonalcoholic steatohepatitis (NASH) enzyme cytochrome CYP2E1 is increased, particularly in the centrilobular region, so that acetaminophen toxicity should be enhanced in patients with NASH.[114] Thus far, this

has not been appreciated clinically; further support in an animal model of NASH has recently been presented.[115]

Drug metabolism in patients with cirrhosis can be reduced as much as 50%. Whether the cells in a patient with cirrhosis are sick or simply reduced in number but functioning normally is not clear. Neither answer is exactly correct. It appears that the physiological changes seen with fibrosis along the hepatic sinusoids results in a widening of the barrier between the bloodstream and the hepatocyte. In support of this, patients with cirrhosis with comparably diminished metabolism of acetaminophen and theophylline normalize theophylline disposal, but not acetaminophen, with oxygen supplementation.[116] The metabolism of theophylline uses CYP1A1 and CYP1A2, which requires oxygen as substrate, unlike acetaminophen conjugation (phase II). The limitation to metabolism is the barrier to oxygen absorption. These studies support the "intact hepatocyte/sick membrane" hypothesis. In summary, dosage adjustments may need to be made in patients with cirrhosis, but these individuals do not appear to have an abnormally sensitive hepatic metabolic system, just less reserve if an hepatotoxic insult were to occur.[116,117]

Avoiding further liver injury in the patient with preexisting liver disease is a difficult task. Anti-tuberculous therapy cannot be withheld from patients just because they have alcoholic cirrhosis. In these instances, frequent monitoring appears to be helpful, but the value of this monitoring has not been proven in controlled trials, is seldom adhered to and can prove very expensive. Despite surveillance using liver enzyme levels, acute liver failure has developed in patients treated with isoniazid. In many instances, the presence of preexisting liver disease is subclinical, (e.g., in patients with NASH). Whether the diabetic population is more at risk for troglitazone hepatotoxicity due to their diabetic fatty liver is still debated. Statins are associated with increased aminotransferases but may actually improve liver enzyme levels in the presence of fatty liver.[118,119] Chronic hepatitis C has been associated with increased likelihood of hepatottoxicity due to chemotherapy and due to HIV drugs.[120,121] In summary, one should maintain a healthy regard for the possibility of increased hepatotoxic reactions in patients with preexisting liver disease, instruct the patient if you are using an agent with known risk, and use periodic surveillance during treatment to avoid continuing drugs that are showing evidence of hepatic injury. There is no firm threshold, but an AST or ALT of 5X ULN usually requires holding or permanently discontinuing an agent. Re-challenge is seldom performed for the reasons outlined above.

The DILI Network (DILIN)

The NIH and in particular, NIDDK has over the past decade launched a number of new networks to enhance the study of relatively rare conditions in hepatology. These include the Acute Liver Failure Study, headquartered at UT Southwestern, HALT-C (which we also have participated in),[123,124] ViraHep-C,[125] and the NASH Clinical Research Network.[126] Two relatively new initiatives are the Drug-Induced Liver Injury Network (DILIN) and the Hepatitis B Research Network. DILIN was initially proposed and the Request for Applications put forth in 2003. Five participating sites were chosen. The aims were to identify and carefully study patients with presumed DILI and to obtain serum, plasma and DNA from such

patients. The emphasis was on carefully identifying cases and adjudicating them as to causality while acquiring the DNA to perform pharmacogenomics studies that would identify those genetic polymorphisms that led to the injury. As noted above, it is assumed that the signature for most drugs will involve not a single altered CYP 450 enzyme, but a series of abnormalities to explain the low frequency, and varying severity in different individuals. Over the first 5 years of the study, approximately 400 patients were enrolled and a descriptive paper of the first 300 patients was recently published.[127] The most common drugs implicated were antibiotics, followed by neurological medications. More than 100 different medications were implicated.

UT Southwestern successfully competed in 2008 to be a part of an expanded DILIN, now with 8 sites. We are just beginning to enroll patients at our site and encourage referrals for this important endeavor. Suspected cases will be enrolled at our site with a one time visit, that includes detailed history of all medications and co-morbid conditions, directed physical examination and collection of blood and urine for routine testing if needed, plus storage of DNA, serum and plasma in the NIDDK repository. Clinicians need only inform the patient that they are suspected to have drug-induced liver injury, get their approval to be contacted, then contact us with information so that we may arrange for the visit. There is no cost to the patient and we will pay travel expenses. A follow-up visit usually takes place 6 months after the initial visit and a one- year visit is scheduled if there are persistent abnormalities at 6 months. Once the case history is complete, it is entered into a detailed reporting form and forwarded to the data-coordinating center at Duke. Cases are then adjudicated for causality by 3 investigators including the principal investigator using an online review system. Once the reviews are in, these are analyzed and those that are in agreement are finalized, while those where disagreements occur are discussed on a monthly causality conference call. All cases have RUCAM scores applied as well. One of the main goals of DILIN is to identify the genetic signatures for individuals susceptible to specific agents. This might eventually lead to using genetic analysis prior to treatment to avoid the catastrophic injury that can develop. While large genome wide analyses are incredibly expensive, focused single nucleotide polymorphism analyses, though still quite expensive, will undoubtedly become cheaper in the future.

Other initiatives of DILIN include developing a more robust causality methodology, one that would be of practical use for clinicians and easy to apply. N-acetylcysteine has shown apparent benefit in early stage acute liver failure (ALF) and in particular appeared to improve outcomes in DILI ALF. We have proposed that the group conduct a blinded treatment trial of NAC for all levels of DILI. Since most cases resolve (8% were fatal in the DILIN 300 patient series) but are quite prolonged, a shortening of the length of illness would be a reasonable outcome measure. Oral NAC could be given to outpatients. A third direction of DILIN is to gain a better understanding of herbal and supplement hepatotoxicity. An entire Grand Rounds could be given on this topic alone. Problems include the use of many CAMs at the same time, the lack of quality control and supervision by FDA of these products as a group. Efforts are underway targeting some particularly egregious offenders. Case examples from Parkland include hepatotoxicity due to Herbalife

(which contains a large number of ingredients but remains proprietary)[128] and Hydroxycut, a weight loss supplement containing, among others, green tea extract (Camellia sinensis) which is known to be hepatotoxic.[129,130]

THE FUTURE

New data is coming forth daily regarding the identification of the genetic patterns of hepatotoxicity due to drugs. Using both genome wide association studies (GWAS) as well as more targeted single nucleotide polymorphism (SNP) analyses, a number of specific HLA haplotypes have now been identified for a number of drugs as shown in Table 5. Of interest, some of these are remarkably close or identical with each other. It is supposed that these HLAClass I and II haplotypes relate to display of drug-containing haptens on the cell surface, and provide a remarkably good binding groove for display to evoke a cytolytic T-cell response. Studies begin utilizing either all patients with a 3X increase in liver enzymes, or those cases fulfilling Hy's Law.[131]

Table 4. Haplotypes strongly associated with specific drug-related diseases

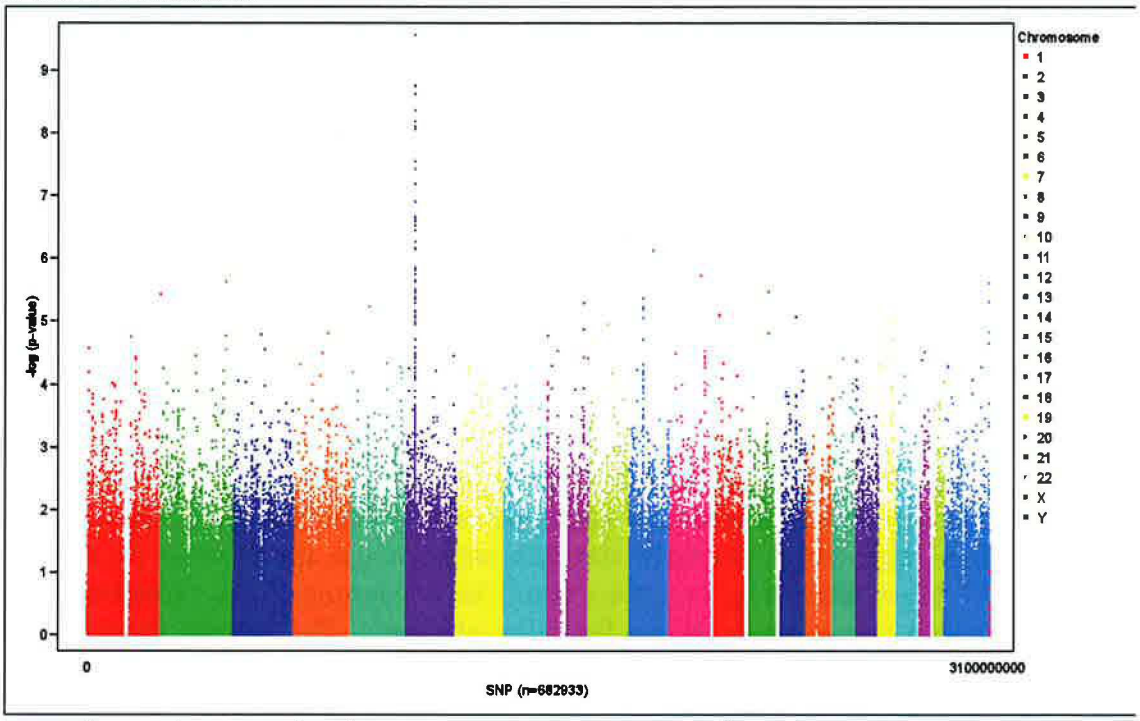
HLA-B*1502	Stevens Johnson/TEN	carbamazepine
DRB1*0701/DQA1*02	Hepatotoxicity	ximelagatran
DRB1*1501-DQA1*0102-DQB1*0602-DRB5*0101	Mixed hepatotoxicity	amoxicillin/clavulanate
HLA-B*5701	Hypersensitivity/hepatotoxicity	abacavir
HLA DRB1*0701 and HLA B*5701	Hepatotoxicity	flucloxacillin
DRB1*1302 and DQB1*0604	Hepatotoxicity	ticlopidine
DQA1*0102	Hepatotoxicity	lumiracoxib

For example, in a study from the United Kingdom, 84% of patients experiencing flucloxacillin-related liver injury were positive for the HLA Class I haplotype B*5701, as compared to 6% of controls, giving an odds ratio of 80.6. This is remarkably strong.[132] What is of interest is that there may be less complexity to these associations than we originally suspected. For example, HLA B*5701 is the same for abacavir and flucloxacillin. Other associations are apparent as well. Still, the role of downstream modulators will likely be important. For example, a specific haplotype associated with the promoter for IL 10 that results in low IL-10 levels is associated with worse absence of eosinophilia and worse clinical outcomes after DILI.[133] DILIN will help to facilitate the gathering of important DNA samples to allow careful characterization of these important drug phenotypes. Once established, simplified genetic testing for disease phenotypes is sure to follow.

Another striking example is lumiracoxib. This COX-2 inhibitor showed excellent anti-inflammatory activity like other NSAIDs without any ulcerogenic potential. However, 2.6% developed ALT elevations > 3x ULN as well as Hy's law cases. Once approved, cases of ALF appeared. The drug was never approved for use I the US by FDA. Using a GWAS approach, those patients with 5X ULN ALT levels were analyzed for SNPs using appropriate comparators. Strong signals were detected for

6 MHC SNPs and these haplotypes were further analyzed using the 3X ULN group. The very strong association was for DQA1*0102 (Figure 6).

Figure 6. Manhattan plot showing one extremely strong signal in MHC region on chromosome 6.



Again, this is the same haplotype as is seen in amoxicillin/clavulanate toxicity and is associated with an increased risk of multiple sclerosis. GWAS and SNP analyses will prove to be valuable tools in the very near future!

WORKING WITH DILIN

Prospective study: A main aim of the study is to prospectively enroll well-characterized cases of DILI associated with the following features: Subjects must be > 2 years at the time of enrollment; have evidence of liver injury that is known or suspected to be related to consumption of a drug or CAM product in the 6-month period prior to enrollment; and, have documented clinically important DILI defined in terms of serum AST (at least 5X ULN), and/or Alk Phos (at least 2X ULN) as described below. Subjects will be excluded if there is acetaminophen hepatotoxicity, a competing cause of acute liver injury, or liver transplant prior to the development of drug- or CAM-induced liver injury. Candidate cases that are not certain should be evaluated. An initial visit is arranged at our study site for history, directed physical examination, blood draw for routine labs and DNA. There is also a 6 month followup visit and, if labs are still abnormal, a one year visit.

Retrospective study: A group of common agents were targeted for retrospective enrollment for individuals that have had in the past (as long ago as 1994) typical cases of DILI. The drugs of interest are isoniazid, amoxicillin/clavulanate, valproate and phenytoin. Recently, an additional group has been added: sulfa drugs including TMP/SMX, nitrofurantoin, as well as minocycline and the quinolones. The aim for this study is a single DNA sample and appropriate clinical history to establish the likelihood of DILI in these instances. No follow-up

visits are required. Any expenses occurred such as travel will be reimbursed. Clinicians simply need to make the patient aware of, and get their consent to consider participation in the study so that we can initiate contact with the patient. A consent that includes DNA handling verbiage is administered at the time of the face-to-face visit. We will travel to enroll patients if this is feasible with the patient and the site.

CLINICIAN'S GUIDE TO HANDLING NEW DRUGS

The best advice in prescribing new pharmaceutical agents is not to prescribe them. It is wise to defer embracing new drugs during their first year of introduction particularly if they demonstrate no unique advantages over accepted formulations. Marketing hype exceeds real-life experience with any new agent. Physicians must strive to instill in their patients a healthy level of alertness with regard to drug-induced liver injury, particularly for agents with known hepatotoxicity. Physicians and pharmaceutical companies should strike a careful balance between alerting patients to the potential for severe reactions without frightening them so that they avoid needed medications. Monitoring aminotransferase levels is suggested for known hepatotoxins such as isoniazid or diclofenac on a monthly basis but is unlikely to be cost-effective when an adverse reaction occurs in only one in 50,000 patients. Since many drug reactions develop within days, monitoring provides no guarantee.[133] Most fatal drug reactions could be prevented if the offending agent were withdrawn immediately, at the first sign of illness. The patient most likely to be harmed is the one who believes in the complete safety of drugs, doesn't realize that drug-induced injury is possible, or is encouraged to be compliant when signs of toxicity are beginning.

New drugs should be prescribed with caution, keeping an eye out for case reports. Some of the newer agents implicated in acute liver necrosis are listed in Table 9. The diagnosis of drug-induced liver injury necessitates determining the precise timing of the drug ingestion, making a careful record of all drugs ingested, and being particularly suspicious of known hepatotoxic agents begun within 3 months of the onset of illness. After withdrawal of the offending agent, improvement should be rapid, within days. Cautious re-challenge may be made *only* if the toxicity observed was highly questionable and if no other drug is available for a serious problem. If jaundice, coagulopathy, or any degree of encephalopathy is present initially, then hospitalization is required since drug reactions worsen quickly, and fatal outcomes are common.

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Figure 7. Structural comparison of 3 compounds having similar haplotypes.