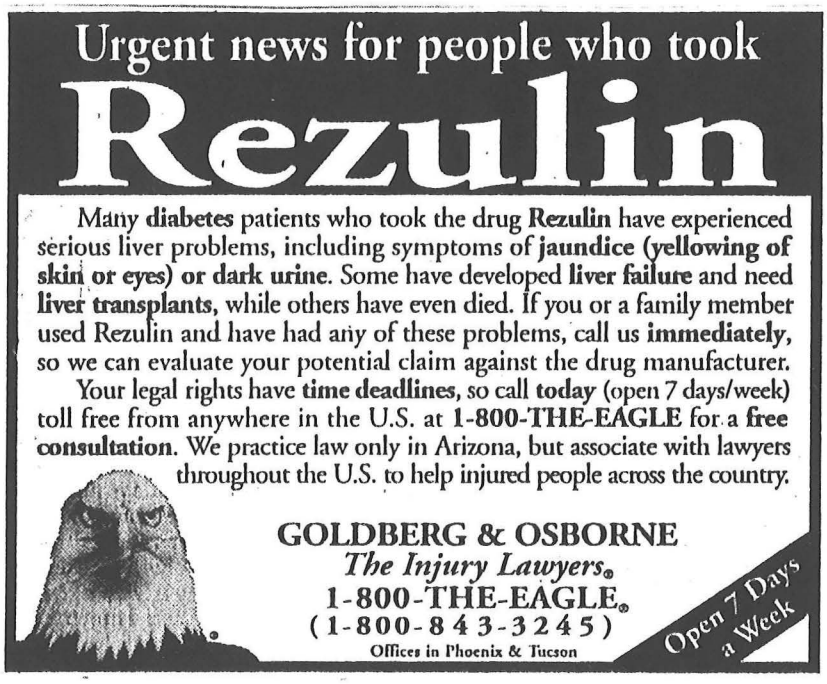
Drug-Induced Liver Injury: The Threat Continues



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Dr. Lee will not be discussing "off-label" uses in his presentation.

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The Clinical Center for Liver Diseases is the largest site in Texas performing liver disease clinical trials including the NIH- and FDA-sponsored multi-center Acute Liver Failure Study Group, the forthcoming NIH HALT-C Trial and numerous other viral hepatitis trials.

Introduction

Recent events have placed drugs, the pharmaceutical industry and the Food and Drug Administration (FDA) under increased scrutiny. In the past 2 years, two drugs were withdrawn in the U.S. as a result of their causing severe liver injury. Drug-induced liver injury is frequently severe and leads to death or liver transplantation and, inevitably, lawsuits. During this same time period, 3 drugs were recalled for induction of fatal arrhythmias. A worried public greets reports of drug withdrawals with increasing fear and skepticism toward the pharmaceutical industry as a whole and toward FDA as the agency charged with industry oversight.

Drug hepatotoxicity was the topic of my Grand Rounds presentation in 1994. Some things have changed in 6 years: the pathogenesis of drug-induced liver damage is better understood and the list of drugs that cause significant liver injury has been updated. The questions posed then remain the same: How do idiosyncratic drug reactions develop? What are the common clinical scenarios in which drug reactions occur? How can we identify serious adverse drug reactions and avoid them? This year I have added some new questions: What is the process that leads to approval of a new drug application (NDA)? Why does it fail to detect such severe adverse drug reactions? How could the approval process be improved? Can improvements in post-marketing surveillance improve outcomes? As shown in Figure 1, drug-related liver injury constitutes more than 50% of acute liver failure (ALF) cases in the United States today and appears to be on the increase.[1]

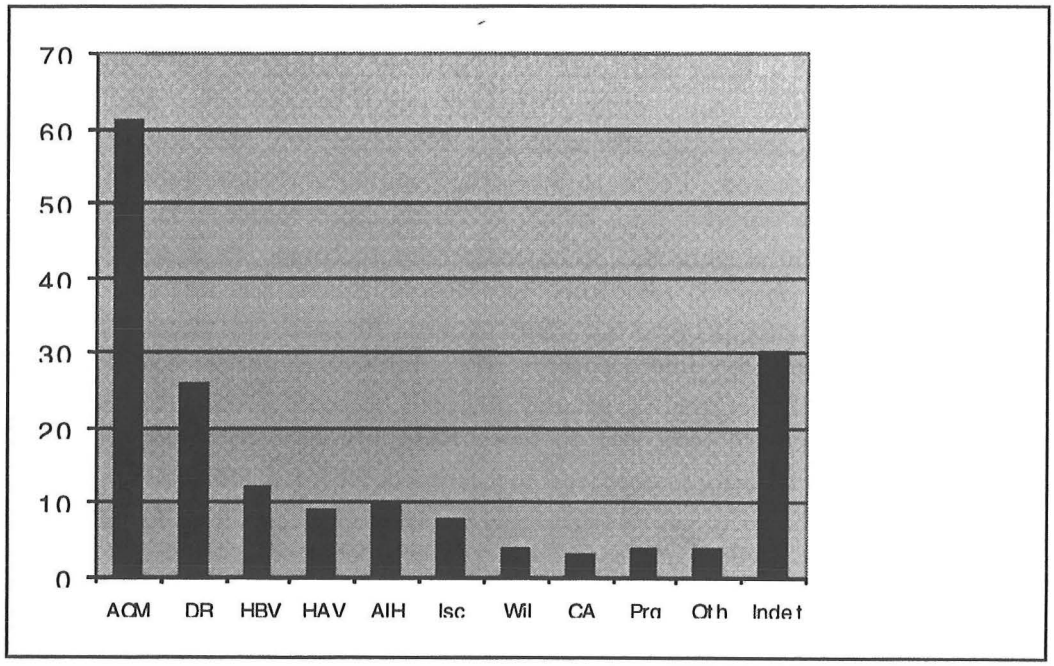

Figure 1. Number of cases of acute liver failure among 171 patients grouped according to etiology in a multi-center study between 1998-2000 at 14 sites around the United States participating in the Acute Liver Failure Study Group. Acetaminophen (ACM) and idiosyncratic drug reactions (DR) were the presumed cause in 87 (51%) of cases of ALF in this series.

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. The prudent physician can guard against these outcomes. The purpose of this review is to highlight some of the ways to do so.

Background

The liver, situated between the absorptive surface of the gastrointestinal tract and targets of drug effect throughout the body, is central to the metabolism of every foreign substance. Most drugs and xenobiotics cross the intestinal brush border because they are lipophilic. Biotransformation is the process whereby lipophilic therapeutic agents are made more hydrophilic by the hepatocyte, resulting in drug excretion in urine or bile. In most instances, biotransformation changes a non-polar to a polar compound through several steps. Foremost is an oxidative pathway (typically yielding hydroxylation of a benzene ring) mediated by the cytochromes P 450.[2] 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 principle metabolic pathways for most pharmacologic agents involve P450 and subsequent esterification.

Pathogenesis of hepatoxicity

Since hepatocyte metabolism is required for virtually every drug, it is remarkable how seldom injury to liver cells occurs! The exact details of the pathogenesis of liver injury remain unclear. 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. The metabolic pathway for acetaminophen involves both phase I and phase II reactions, glutathione detoxification, and the formation of reactive intermediates (Figure 2). Glucuronidation and sulfation occur as the initial detoxifying step since the parent compound contains an 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 little toxicity is observed in cirrhotics with acetaminophen.

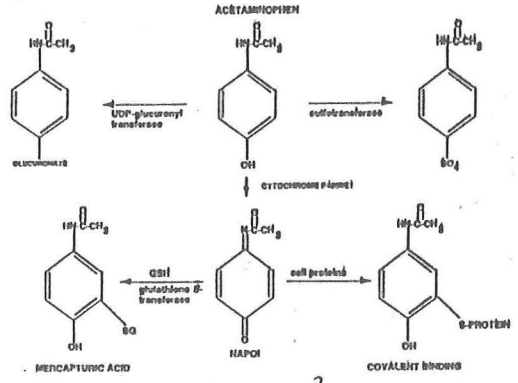

Figure 2. Metabolic pathway for acetaminophen[3].

In acetaminophen metabolism, the phase II reactions predominate, with only a small fraction of acetaminophen metabolized by cytochrome P 450, 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 P450 IIE1. NAPQI binds covalently to cell macromolecules disrupting mitochondrial and nuclear function. Antibodies to nitro-tyrosine residues can be detected as evidence of this covalent binding in livers of patients (or experimental animals) demonstrating toxicity.[4] These residues are formed by the rapid reaction of super-oxide 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. Depletion of glutathione lowers this last defense against the formation of NAPQI-related intracellular adducts. Thus, starvation and alcohol intake by depleting glutathione enhance toxic injury, while N-acetylcysteine by replenishing glutathione protects against acetaminophen-induced injury. This direct toxic reaction occurs predictably in all individuals and is not an allergic reaction. The final step leading to cell death remains unclear but may involve an increase in levels of cytosolic calcium altering the cytoskeleton and membrane integrity, leading to "blebbing" of the cell membrane and loss of its integrity. Dose-related necrosis (lysis) of hepatocytes occurs but apoptotic pathways are also implicated. A recent 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.[5,6]

Idiosyncratic reactions

While acetaminophen is a dose-related toxin, most drug reactions are idiosyncratic, occurring from 1 in 1,000 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. Any theory of pathogenesis must 'explain' the features shown in Table 1.

Table 1. Idiosyncratic drug reactions:

1. Occur rarely in a pattern consistent for each drug
2. Similar drugs exhibit similar features called 'class effects'
3. Individual drugs in a class still vary considerably
4. Reactions occur at varying time intervals after beginning ingestion
5. Reactions vary in severity, but typically severe and fatal if drug continued
6. Mild injury can sometimes disappear with continued use
7. Rarity of most reactions suggests possibility of multiple hits
8. Re-challenge is virtually always met with greater severity, shorter latency

Enzyme polymorphism

Genetically variant P 450 iso-enzymes such as are observed with metabolism of debrisoquine partially explain observed individual variation in responses to drugs. Debrisoquine is an anti-hypertensive marketed in Europe which is hydroxylated by P450 IID6, an iso-form that is totally lacking in 5% of normal individuals, greatly prolonging

the half-life of the parent compound in affected individuals. Fast and slow acetylator patterns are observed to affect whole races, and have been implicated in isoniazid metabolism which includes an acetylation step. Genetic variants which occur relatively frequently cannot explain the formation of a toxic intermediate in only 1:50,000 individuals. 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. While some toxic drug reactions are associated with an obvious allergic response, most are not. Nevertheless, immune mechanisms not associated with systemic allergic IgE reactions or skin hypersensitivity might be involved. Recent studies suggest that the very products of cytochrome P450 metabolism, the highly reactive intermediates which are formed within the microsomes covalently bind to the enzyme itself to form a drug-hapten adduct that disables the enzyme and injures the cell. Haptenization then evokes an immune response directed against the newly formed antigen or neo-antigen. P450s have recently been shown to traffic to the plasma membrane that would allow the drug-P450 adduct to become the target of a subsequent cytolytic attack (Figure 3). Whether these adducts or smaller peptides processed and presented via the MHC class I and class II schemes are the targets remains unclear. Still, the association of neo-antigens, autoantibodies and hepatotoxic drugs implicates an immunologic mechanism.

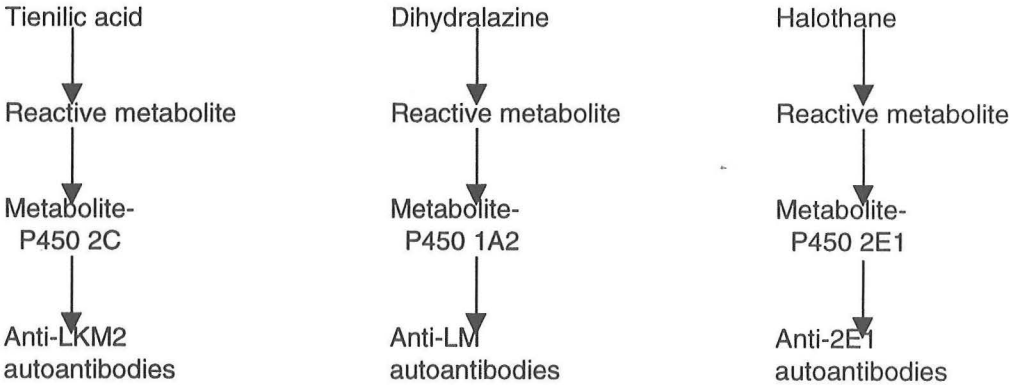

Figure 3. Modification of host P450 enzymes renders them immunogenic. Autoantibodies can be detected which recognize the enzyme-metabolite adducts.[7]

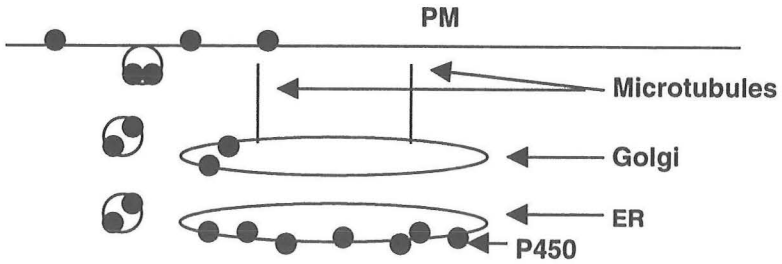

Figure 4. Vesicular transport of P450 to the plasma membrane. P450 is inserted in the endoplasmic reticulum (ER), and can then follow a vesicular route to the plasma membrane (PM). Adapted from ref 7.

Whether the drug causes significant cell necrosis or not, the P450-drug adducts can evoke the immune response. Any subsequent P450 drug-adduct present on the hepatocyte surface would evoke a further response (Figure 4). In this model, responses may be antibody-mediated or occur from direct cytolytic attack by primed T-cells.[7,8]

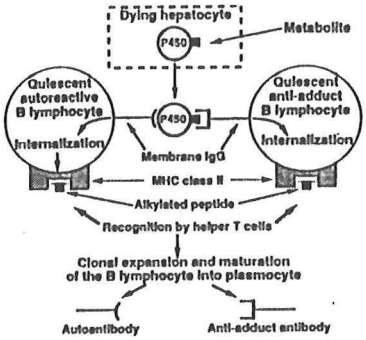

Figure 5. Possible models of liver injury due to drugs. Direct toxicity may play a role but immune mechanisms are also operative. In this model, re-challenge or continuing to receive the same drug would simply elicit a pre-formed cytolytic response.[7]

Such a combined toxic/immunologic mechanism is involved in the liver injury caused by halothane. Halothane was a widely used fluorinated hydrocarbon anesthetic which causes severe an often fatal liver injury after multiple exposures.[8] Other fluorinated hydrocarbons also occasionally result in the same response.[9] While halothane has never been withdrawn, its use has been limited by the advent of safer agents. Both direct cytotoxicity and immune-mediated toxicity are observed in keeping with the clinical observations that severe halothane toxicity occurs with repeat exposures, but 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. Most idiosyncratic drug reactions occur after an interval of 2-8 weeks, suggesting immune-mediated injury, which requires weeks to evolve. An immune response against target neo-antigens (drug-enzyme adducts) which appear on the hepatocyte surface makes intuitive sense.[7]

Specific genetically determined components of the immune sequence may be important. For example, the binding of peptides for antigen presentation depends on HLA configurations that are genetically determined. This variation among individuals is thought to underlie 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 Augmentin-induced hepatitis, being found in 57% of patients and 11% of controls.[10] Polymorphisms have also been identified for the IL-10 promoter and for TNF alpha. These variations in immune responsiveness could modulate the severity of the responses observed. 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 upregulation of immune reactions of the Th2 type. This same phenotype has recently been linked to hepatitis C-related liver injury, the severity of alcoholic liver injury and to

diclofenac toxicity.[11-13] Variant TNF alpha phenotypic expression has been implicated in determining the severity of drug reactions related to acetaminophen.[14] A multi-step, immune-based mechanism would best explain both the rarity of idiosyncratic reactions, their severity as well as the findings of mild, non-progressive liver injury in some patients—those with 'protective' phenotypes. While an immunological 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 sinusoidal lining cells as well as Kupffer cells are part of the process.[15] Figure 6 provides a model for a multi-hit mechanism of liver injury which would 'explain' the rarity of idiosyncratic cases, as well as their severity.

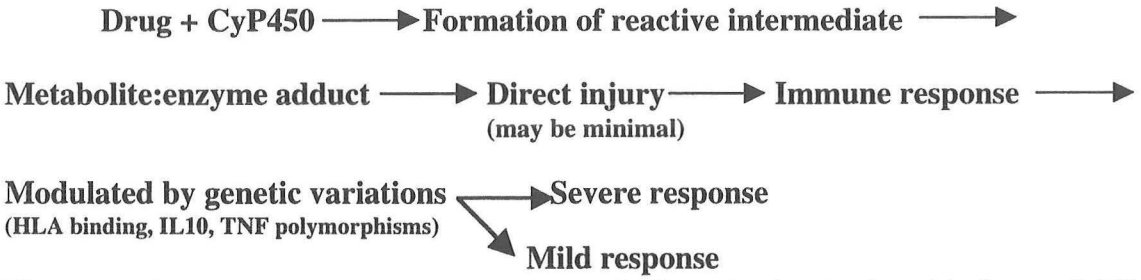

Figure 6. Proposed events sequence after metabolic activation leads to binding to P450.

There should be little doubt that the metabolic fate of any compound is a complex process. There are other important environmental and host variables outlined in Table 2.

Age: drug reactions appear to impact the elderly more often, P450s vary.[16]
Gender: women are more prone to drug reactions statistically, mechanism unknown.[17]
Size/weight: effects on women relate to intrinsic gender differences but also to size.
Pregnancy: effects of drugs in pregnancy have been poorly studied.
Liver Disease: see p. 19. Hepatic disease may protect against certain reactions.
Renal Disease: slowed disappearance of parent compound yields higher concentrations and affects P450.[18]
Certain Foods: grapefruit has an unknown substance that interferes with metabolism.[19]
Concomitant Drugs: drug-drug interactions are common causes of adverse effects.
Genetic Factors: enzyme polymorphisms, HLA phenotypes

Table 2. Factors influencing the metabolic fate of drugs.

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 and grapefruit juice are also potent inducers of certain P 450 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, while

competitive inhibition by ketoconazole increases serum levels of astemizole (Hismanal) with resulting *torsades de pointes*.[20]

Types of drug reactions

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 hepatic, 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 histologic changes involved as well as the cell type, granulomatous, e.g. (Table 3).

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

Table 3. Types of drug reactions.

Clinical pictures of drug-induced liver injury: hepatocellular reactions

Acetaminophen has been the best understood example of direct hepatocyte toxicity. Liver injury occurs predictably after intentional suicidal overdose,[21] and during the 'therapeutic misadventure,' in which acetaminophen used in therapeutic or excessive doses for pain relief leads to severe liver injury.[22,23] Enhanced toxicity occurs due to the enzyme induction and glutathione depletion by alcohol as well as fasting as outlined previously.[24] Acetaminophen toxicity is the most common form of acute liver failure observed in the United States.[1] In a review from Parkland Hospital, we found that 71 patients were admitted in a 39 month period with actual or potential hepatotoxicity due to acetaminophen.[25] Those who ingested acetaminophen accidentally, without suicidal intent fared worse because they appeared late, did not realize that they had done anything harmful and were more likely to develop severe liver injury and to die from the episode. By contrast, those with suicidal intent took larger doses, presented to hospital earlier and received N-acetylcysteine, an effective antidote. One fifth of the suicidal cases had severe injury and the potential for a fatal outcome should not be underestimated. Key features in the accidental group were excessive chronic alcohol intake (usually more than 6 drinks/day), and the use acetaminophen for a specific pain problem, but generally in excess of package recommendations. The extremely elevated aminotransferase values (mean value, ca. 9,000 IU/L in one study) observed in suicidal and accidental acetaminophen ingestion help distinguish these cases from viral hepatitis or other drug

injury. N-acetylcysteine should be given by nasogastric tube on admission, and for the ensuing 72 hrs, to provide glutathione substrate. Expected survival is greater than 80%, although transplantation is occasionally indicated. The incidence of acetaminophen poisoning varies widely throughout the world, but is becoming more frequent and widespread as indicated by a recent report from Taiwan.[26] Acetaminophen poisoning is an increasing cause of ALF in children.[27-30] As in adults, two forms are seen: teenagers may overdose with suicidal intent, but more ominous are instances of inappropriate dosing of small infants with catastrophic results.

The Acute Liver Failure Study Group recently reviewed our experience with 79 patients who developed ALF defined as altered mentation and coagulopathy, due to acetaminophen toxicity. The majority (77%) were women, although the gender breakdown for other etiologic categories of ALF is similar. Accidental cases comprised 58% compared with 85% of the Parkland series reaching hepatic encephalopathy. A third of each group was on anti-depressant medications at the time of admission, suggesting that the line between accidental and suicidal may be somewhat blurred. Another observation in this series was that of the 46 accidental patients, 22 had been using acetaminophen for >7 days. There has been little suggestion previously that chronic acetaminophen toxicity was a significant problem. These cases will need further investigation to confirm this finding. Chronic alcohol abuse had been present in 63% of the accidental cases vs. 25% of the suicidal patients in the Parkland study, but was less frequent (54%) and almost as common in the suicidal patients (45%) in this larger US multi-center study. This is an evolving story, but the overall number of acetaminophen cases continues to increase.

Other dose-related reactions

While acetaminophen is clearly a dose-related toxin, the majority of drug injuries are not considered dose-related. Nonetheless, dose-related effects are important for certain medications. Dose-related effects are observed with the agents listed in Table 4.

Drug	Response
Acetaminophen	Total dose, single vs. multiple time points
Amiodarone	Total dose over time
Bromfenac	Toxicity only occurs after extended use
Cocaine	Dose-related vascular collapse
Cyclophosphamide	Dose related, worse with previous ALT elevations
Cyclosporine	Cholestasis with toxic blood levels
Methotrexate	Aminotransferase/fibrosis; single dose/total dose
Niacin	Large doses yield vascular collapse
Oral contraceptives	Prolonged usage yields hepatic adenomas

Toxins including phosphorus, tetrachlorethylene, amanita toxin, bacterial toxins

Table 4. Drugs/toxins in which a dose-response effect is observed.

Idiosyncratic reactions

Idiosyncratic reactions occur in small numbers such that some drugs continue to be used when usefulness or uniqueness make the risk acceptable. Isoniazid is such a drug, virtually the only drug implicated in developing countries, where drug-induced liver injury is otherwise unheard of.[30] Fifteen 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 as many as 1% may develop severe hepatic necrosis.[31] More recently, a lower estimate of severe liver injury of 1:1000 has been given for isoniazid from a large tuberculosis public health clinic.[32] This is still a high rate of injury compared to other idiosyncratic drug reactions, yet the usefulness of the drug has precluded its withdrawal.

Aside from isoniazid, non-steroidal analgesics may be the most commonly associated drug class capable of inducing idiosyncratic reactions.[33] The newer COX-2 inhibitors have recently been implicated.[34] It is important to recognize that certain classes are known to be associated with toxic injury while others are much less likely.

Highly Associated (toxicity listed in package insert, usually a case series)
Non-steroidal agents
Antibiotics (fluoroquinolones, penicillins, sulfa, isoniazid)
Seizure medications (dilantin, valproate, carbamazepine)
Intermediate (case reports)
Statins (highly variable)
Psychotropic drugs (highly variable)
Anti-thyroid drugs
Not associated (rare case report only, or none)
Anti-arrhythmics
Hormones
Anti-hypertensives
Digoxin
Theophylline

Table 5. Classes of drugs generally associated with idiosyncratic liver injury, and those that have not been associated with these reactions.

Allergic reactions

Drugs may be associated with reactions that are definitely allergic in nature. Halothane induces fever, eosinophilia and anti-mitochondrial antibodies. Phenytoin (Dilantin®) induces the simultaneous onset of fever, rash, lymphadenopathy, or eosinophilia.[35] 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 illnesses or streptococcal pharyngitis. When the offending agent is not discontinued promptly, despite signs of developing hepatitis, a severe "Stevens-Johnson" drug eruption

and prolonged fever may result. As with any therapeutic agent, rapid recognition of the presence of a toxic drug reaction and immediate discontinuation of the compound is the key 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.

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 cholestatic reactions, in which primary injury is directed at cholangiocytes, granulomatous reactions, alcoholic hepatitis-like reactions, 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.

More than one case report:
Amoxicilliin/clavulanate
Carbamazepine
Erythromycin esters
Flucloxacillin

Less frequent:
Azathioprine
Barbiturates
Captopril
Allupurinol
Clindamycin
Methyltestosterone
Phenytoin
Prochlorperazine
Trimethoprim/sulfa

Table 6. Drugs causing cholestatic reactions. Except for hormonal preparations, most drugs will cause a mixed hepatocyte-cholangiocyte damage.[36] Liver injury may be permanent with a poor outcome.[37]

Granulomatous reactions in the liver, resembling sarcoidosis are seen, and the list of drugs causing this particular allergic pattern is long. A partial list will be found below.

allopurinol
aspirin
carbamazepine
cephalexin
diazepam
diltiazem
halothane
hydralazine
isoniazid
metahydrin
methyldopa
metolazone
nitrofurantoin
penicillin
phenytoin
procainamide
procarbazine
quinidine
sulfonamide
sulfonylurea

Table 7. Drugs implicated in granulomatous reactions in the liver. Adapted from Maddrey and Zimmerman, ref 38.

Lessons from recent drugs withdrawn from the market

The two drugs recently withdrawn due to severe and fatal liver injury were bromfenac and troglitazone. There are important lessons from each one, illustrating the difficult issues surrounding drug-induced liver injury. Bromfenac was a non-steroidal anti-inflammatory drug marketed as Duract®, and introduced in 1997 as a short-term analgesic for orthopedic pain. Non-steroidals as a class have been associated with considerable hepatotoxicity.[39,40] Aminotransferase elevations had been noted during initial testing of bromfenac, but no instances of severe toxicity had been reported. However, increased transaminases were associated with longer use, although the total number of patients undergoing long-term treatment was small. No instances of ALF were observed, but less than 1,000 patients were studied. Because of concerns that longer term use might be hazardous, approval was given with the limitation that the drug should only to be used for intervals of 10 days or less. Once released, bromfenac was associated with more than 50 cases of severe liver injury and the drug was withdrawn in June 1998. Bromfenac was deemed safe if only used for short intervals. Indeed, all cases of toxicity observed had been taking the drug for more than 8 weeks.[41] With hindsight, a drug that effectively relieved pain would not be prescribed or used reliably for only 10 days periods. The ALF Study Group identified 4 bromfenac cases in the first 6 months of 1998. Characteristics of these cases and other cases are shown in Table 8.

Variable	Bromfenac (n = 4)	Troglitazone (n = 4)	INH (n = 4)	Other Drugs (n = 10)
Female (%)	100	50	50	70
Median Age (yrs)	45	58	25	49
Transplanted (%)	75	75	50	50
Spontaneous survival (%)	0	0	25	20
Overall survival (%)	75	50	75	50

Table 8. Clinical features of drug-induced liver injury from the ALF Study Group.[1,42]

In retrospect, another non-steroidal that carried any limitation at all was a recipe for trouble, given that other similar compounds were available with proven safety profiles. Risk/benefit ratio must be an important consideration in the approval process for any new drug—bromfenac offered little that was new and on that basis should have met an even stricter test for safety.

A second agent withdrawn more recently is troglitazone. Approved by FDA in January 1997, troglitazone (Rezulin®) was the first of a new class of compounds, the thiazolidinediones. As an inhibitor of the nuclear regulatory factor PPARgamma, 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 > 8X upper limit of normal (ULN), but, again, no examples of ALF. However, once the drug was approved, reports began to surface of severe and fatal liver injury.[43,44] By the end of 1997, 500,000 new prescriptions were being filled each month. During the early months of 1998, the ALF Study Group reported 4 cases of troglitazone toxicity,[45]

others have been observed at our own institution.[46] Troglitazone has a variable time frame for demonstrating toxicity, from a few days to several months.[47] The pathogenesis is not understood. Unlike bromfenac, troglitazone was not immediately removed. As a new agent, its benefits were initially thought to outweigh the risks. Two 'Dear Dr.' letters were sent in July 1998 and January 1999. The drug was recalled in the United Kingdom in 1999. US data were collected by the manufacturer and presented to FDA in March 1999, and the agency issued a letter of caution suggesting limited use and "black box labeling." The renewed warning in the package insert (in a black box), mandated monthly monitoring during initial 6 months usage of the drug. However, the number (>90) and severity of these cases (68 fatal, 10 transplanted) as well as doubts over the efficacy of monitoring led to drug withdrawal earlier this year, 3 years after its approval. A factor in the FDA decision was the arrival of 2 new PPAR-gamma inhibitors, rosiglitazone (Avandia®) and pioglitazone (Actos®), approved in May and July 1999.

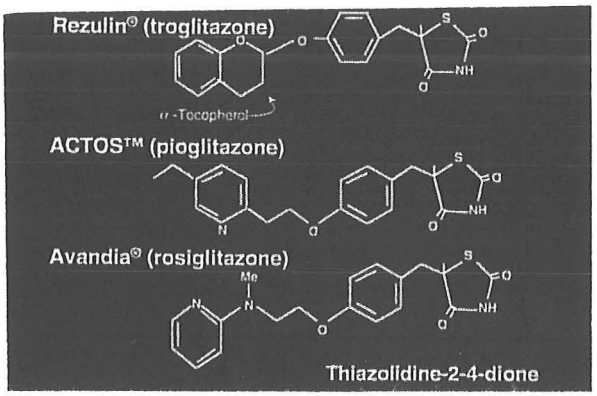

Figure 7. Comparison of the chemical structures of troglitazone, rosiglitazone and pioglitazone. Only troglitazone contains an intact tocopherol ring.

Although of the same class, these agents do not seem to have the same degree of toxicity. Thus far, neither drug has shown the 1:50,000 incidence of severe hepatotoxicity seen with troglitazone. While a launch to launch comparison has its limitations, the table below shows the comparison of the first 9 months use for each drug.

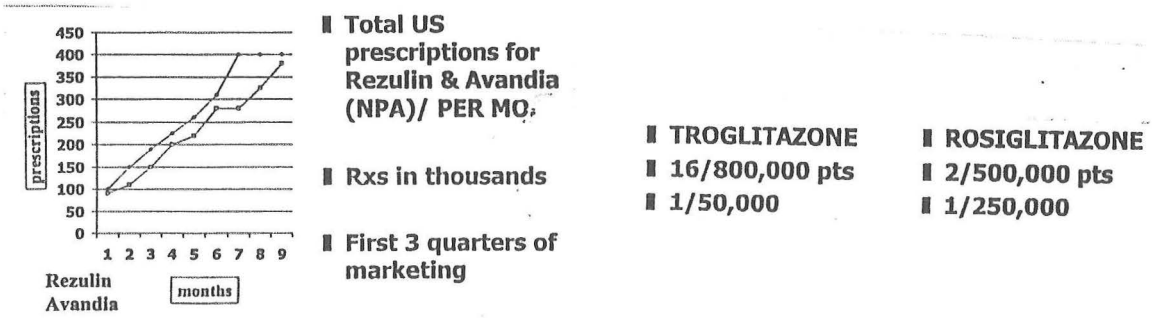

Figure 8. Comparison of early marketing and safety for the first 2 thiazolidinediones.[48]

The number of cases of liver failure observed was less with rosiglitazone. Reasons for the difference include intrinsic differences in the drugs, more careful

screening of patients by more alert physicians, monitoring of ALT levels, possible early discontinuation of moderately severe cases because of increased awareness of hepatotoxicity.

Still, it will be necessary for these new agents to prove themselves after a larger exposure. Two recent reports[49,50] suggested that rosiglitazone may be capable of causing similar catastrophic liver injury but the interpretation of these reports has been contested by the manufacturer.[51] Several other new agents are under suspicion, but the number of cases thus far has not warranted drug recall, although the FDA is recommending aminotransferase monitoring for both agents. Of interest, all agents in this class cause fluid retention and can lead to pulmonary edema in patients with severe underlying heart disease.[52] The controversy surrounding at least one of the rosiglitazone cases is whether the patient actually suffered liver injury in the context of severe heart failure possibly exacerbated by the fluid retentive properties of the drug, a different problem at least than the random development of severe direct liver injury. Whether the lesser incidence of toxicity with the newer agents is due to closer monitoring or to greater intrinsic safety of these closely-related compounds remains to be seen.

Understanding the drug approval process

How could two promising drugs get through the drug approval process and still lead to so many deaths that they were ultimately withdrawn? What is wrong with the screening process that this sort of thing can happen? To answer these questions, it is necessary to understand the overall process of drug development and approval. 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.

I. 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 pre-clinical testing in a variety of animals using doses up to 50 times that predicted to be useful in man to ascertain the types of toxicity that might be expected. While metabolic pathways differ in some specific aspects, the similarities between lower mammals and man are quite notable. Animals are sacrificed 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. Pre-clinical testing, which may take 5-6 years to complete, is still a crude technique and no substitute for clinical trials in man. The use of massive dosing in animals may in part compensate for metabolic differences between species, but human trials are ultimately needed.

II. 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, EKG, assessment of reported side effects and blood measurements including serum AST/ALT, amylase,

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 5,000 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, does it have any short- or long-term side effects? In one recent example, adefovir dipivoxil, a nucleotide analogue was being given for HIV infection. Unanticipated renal failure was noted at 60 and 120 mg daily doses, and the dose was then lowered to 30 and finally to 10 mg/day. However, adefovir was not effective at 10 mg and only partially so at 30 mg, so the drug did not gain approval for HIV. Adefovir is still undergoing trial for hepatitis B where its efficacy appears to be better than that observed for HIV infections. Such an example of frequent renal or hepatotoxicity is easy to spot—serum creatinine levels rose in many patients taking adefovir within the first few months on treatment. Several non-steroidal drugs have made it to Phase II trials only to be withdrawn due to too many aminotransferase level increases. However, this is where safety concerns regarding idiosyncratic reactions founder. It is easy to pick up common reactions but hard to pick up the truly rare drug injury during the pre-approval process.

Dr. Hyman Zimmerman, who died recently, was the guiding light of the field of drug hepatotoxicity for more than 40 years. His experience both as a clinician and as a student of the problem has been recognized in a named lecture at the American Association for the Study of Liver Diseases (AASLD) Annual Meeting and in the high respect he commanded at FDA. Dr. Zimmerman proposed in 1978 what has become known as "Hy's Rule," regarding severe drug reactions: if a drug causes enough liver injury to lead to jaundice, even rarely, then 10% of affected patients will develop acute liver failure.[53,54] Put another way, any drug that in phase II-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.[55] In each clinical study, a detailed assessment of liver biochemical parameters are part of every company's NDA filing. Data supporting the safety of the drug include placebo-controlled trials where the incidence of abnormalities must be shown to be similar to that observed in the placebo group. Aminotransferases exceeding 3 times the upper limit of normal (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. If any case of ALF occurs, the trial is discontinued as was the case with fialuridine.

Approximately 50 new drugs are approved by FDA each year, the approval process taking between 6 months to a year, once the NDA is filed. Approval brings with it instant activation of intense marketing campaigns and the necessity for essentially all U.S. pharmacies to stock the drug. As noted above, the number of prescriptions written may be enormous. This fact explains why 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 4,500 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 3 times the number of patients studied as the incidence of the drug reaction.[55] In other words, a 1:1,500 reaction requires 4,500 patients to detect reliably a single case, and a 1:50,000 reaction would require 150,000 patients! No clinical trial will reliably pick up rare drug reactions. Approval by 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.

III. 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, patients with HIV/AIDS, pregnant women, the elderly or children, any of which may enhance 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.[55] An SAE is any untoward medical occurrence that at any dose results in death, is life-threatening, requires hospitalization, or results in permanent disability or a birth defect.[56] This obligation is variably executed and enforced. Pharmaceutical manufacturers maintain a safety monitoring force which gathers reports, assesses likelihood of the reaction being attributed to their product, and issues a report to 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.[57]

There are several additional shortcomings to the effectiveness of post-marketing surveillance. First, the reporting system is passive. Physicians and pharmacists are under no strong obligation to report adverse events. FDA introduced the Medwatch program to improve surveillance, asking physicians and pharmacists to report on a standardized form all drug reactions they observe. However, it is estimated that <10% of severe adverse drug reactions are reported to the company or FDA.[56] 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"), etc. Reports received from pharmacists or drug representatives (or physicians for that matter) seldom contain full clinical information. Privacy issues may preclude further inquiries and raise concern regarding possible legal implications. For all these reasons, a passive reporting system is inadequate. Nevertheless, the main source of information is the Medwatch system, plus case reports.[58] These were certainly part of the firestorm that eventually sunk troglitazone and bromfenac.

There is no national surveillance system in place, and no immediate plans for one. Groups such as the ALF Study Group provide a quick, albeit limited, reporting system

around the country. We correctly identified early cases of both bromfenac and troglitazone and reported these findings to FDA, but the system needs to be speeded up and made more global. By the time of our reports, other cases had surfaced, and the process of reviewing the approval had begun. However, spontaneous reporting still took nearly 3 years and 100 deaths or transplants before troglitazone was withdrawn.[59]

A standardized reporting form developed by an international panel provides a worthwhile causality assessment scoring system.[60,61]

Criteria for Causal Assessment of Drug-induced Liver Injury

Temporal relationship of start of drug to start of illness	
Initial treatment 5-90 days; subsequent treatment course: 1-15 days	+2
Initial treatment <5 or >90 days; subsequent treatment course: > 15 days	+1
From cessation of drug: ≤ 15 days	+1
Course	
ALT decreases ≥ 50% from peak within 8 days	+3
ALT decreases ≥ 50% from peak within 30 days	+2
If the drug is continued, inconclusive	0
Risk factors	
Alcohol	+1
No alcohol	0
Age ≥ 55 years	+1
Age ≤ 55 years	0
Concomitant drug	
Concomitant drug with suggestive time of onset	-1
Concomitant drug known hepatotoxin with suggestive time of onset	-2
Concomitant drug with further evidence of involvement (rechallenge, e.g.)	-3
Non-drug causes: 6 are primary: Hepatitis A, B, or C, biliary obstruction, alcoholism (AST ≥2x ALT), recent hypo-tension (heart disease).	
Secondary group: Underlying other disease; possible CMV, EBV or HSV infection	
In this category, all causes ruled out:	+2
4 or 5 causes ruled out	+1
Less than 4 causes ruled out	-2
Non-drug cause highly probable	-3
Previous information on hepato-toxicity of the drug in question	
Package insert mention	+2
Published case reports but not in package label	+1
Reaction unknown	0
Re-challenge	
Positive (ALT doubles with drug alone)	+2
Compatible (ALT doubles, compounding features)	+1
Negative (Increase in ALT but ≤2 X ULN)	-2
Not done	0

Table 9. Scoring system for assessing causality. Adapted from reference 63. A positive score of 6 or more represents a strong causal relationship.

These guidelines in effect outline the steps an experienced clinician uses to assess likelihood of drug reactions.[62,63] Causality assessment methods must include temporal relationship, course after cessation of drug, risk factors, concomitant drugs, a search for

non-drug causes (viral hepatitis), previous information concerning the drug, and response to rechallenge, which is usually not available.

Hepatotoxicity in the chronic liver disease patient

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. Liver disease patients 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."[53] 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 fully conjugate bilirubin. Therefore, enzyme activity in many instances exceeds the daily requirement, and therefore even severe liver injury would not be expected to impact the likelihood of 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 Cyp 2C19 is greatly decreased while that of 2D6 is intact.[64,65] In non-alcoholic steatohepatitis enzyme Cyp 2E1 is increased, particularly in the centri-lobular region, so that acetaminophen toxicity should be enhanced in NASH patients.[66-68] Thus far, this has not been appreciated clinically.

Drug metabolism in patients with cirrhosis can be reduced as much as 50%. Whether in cirrhosis, the cells are sick or simply reduced in number but functioning normally is not clear. Neither answer is exactly correct. It appears that the physiologic changes seen with fibrosis along the sinusoids results in a widening of the barrier between the blood stream and the hepatocyte. In support of this, cirrhosis patients with comparably diminished metabolism of acetaminophen and theophylline normalize theophylline disposal but not acetaminophen with oxygen supplementation.[69] The metabolism of theophylline utilizes P 4501A1 and 1A2, which requires oxygen as substrate, unlike acetaminophen conjugation (phase II). These studies support the "intact hepatocyte/sick membrane" hypothesis. In summary, dosage adjustments may need to be made in cirrhosis patients, but patients do not appear to have an abnormally sensitive hepatic metabolic system, just less reserve if an hepatotoxic insult were to occur.[69,70]

In general, patients with liver disease suffer more renal than hepatic insults. They are particularly prone to nephrotoxicity due to the altered renal circulation of the cirrhotic patient. Nephrotoxicity of aminoglycosides, radiocontrast and prostaglandin inhibitors such as indomethacin are a frequent problem for cirrhotic patients, but doses of antibiotics, anti-psychotics, etc. are seldom adjusted, although any medication with sedating effects may be a problem if metabolism is slowed.

Avoiding further liver injury in the patient with pre-existing liver disease is a difficult task. Anti-tuberculous therapy cannot be witheld 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 and is very expensive. Despite surveillance using liver enzyme levels, acute liver failure has

developed in patients treated with isoniazid. In many instances, the presence of pre-existing liver disease is sub-clinical, e.g., in NASH patients. Whether the diabetic population is more at risk for troglitazone hepatotoxicity due to their diabetic fatty liver is still debated. Nevertheless, a healthy regard for the possiblity of increased hepatotoxic reactions in patients with pre-existing liver disease, and the use of periodic surveillance during treatment should allow the maximum chance to avoid harmful prescribing.

Clinician's Guide to Handling New Drugs

The best advice in prescribing new pharmaceutical agents is abstinence! "Just wait." 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. Next time around, say 'no' to the next bromfenac, say 'maybe' to the next troglitazone. 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 must strike a careful balance between alerting patients to the potential for severe reactions without frightening them so that they avoid needed medication. 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. 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 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 12.

Atorvastatin [72,73]	Norfloxacin [90.91]
Agent orange [74]	Paroxetine [92]
Carbamazepine [75]	Pemoline [93]
Celecoxib [34]	Pravastatin [94]
Clarithromycin [76]	Ranitidine [95]
Coumarin compounds [77,78]	Risperidone [96]
Cyproterone acetate [79]	Terbinafine [97]
Fluconazole [80]	Ticlopidine [98]
Fluoxetine [81-83]	Tolcapone [99,100]
Indinavir [84]	Trazodone [101]
Losartan [85]	Trovafloxacin [102,103]
Mesalazine [86]	Valproate [104]
Metformin [87]	Venlaxafine [105]
Nefazodone [88,89]	And don't forget herbs! [106,107]

Table 12. Current list of drugs reported to cause severe toxicity.

General diagnostic and therapeutic measures

The diagnosis of drug-induced liver injury necessitates determining the precise timing of the drug ingestion, making a careful record of all drugs ingested, being particularly suspicious of known hepatotoxic agents begun within three 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 frequent.[1]

The Future

Two national conferences are planned for later this year on the topic of Drug Hepatotoxicity, one sponsored by the National Institutes of Health and another by FDA, the Pharmaceutical Research Manufacturers Association, and the AASLD. These workshops are aimed at updating current state of knowledge of the mechanism of adverse drug reactions and re-examining the process of pre-clinical and clinical drug testing, the FDA approval mechanism and the role of post-marketing surveillance. The hope is that more can be done in the future to avoid unnecessary drug-induced liver injury.

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