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DILI: New Insights into Diagnosis and Management

Thanh Tran, MD and William M. Lee, MD

Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Dallas, TX

Abstract

Drug-induced liver injury continues to have a significant impact. With over 1000 drugs now registered as causing DILI, this form of liver injury is the most cited reason for withdrawal of a drug from the marketplace. Despite this, the diagnosis of DILI continues to rely on subjective measures and expert opinion with results that are both difficult to verify and reproduce. However, recent developments in DILI research may provide new insights to improve diagnosis and treatment in the future.

Keywords

biotransformation; acetaminophen; herbal and dietary supplements; genome wide association study; genetic polymorphism

Introduction

Drug-induced liver injury (DILI), or drug-induced hepatotoxicity, is an under-recognized and relatively rare cause of acute liver failure (ALF) that remains an important topic of discussion in hepatology for several reasons. In the 1970s and 1980s hepatitis B was reported to be the most common cause of acute liver failure [1]. By the late 1990s, a study conducted through the U.S. Acute Liver Failure Study Group found that acetaminophen overdose and idiosyncratic drug reactions had replaced viral hepatitis as the most common cause of acute liver failure and were now responsible for more than 50% of all cases of ALF in the United States [1]. The reported transplant-free survival was only 27.1% in patients with acute liver failure from hepatotoxicity [2]. Although many drug reactions are infrequent, in the range of 1 in 10–50,000 exposures, the significant morbidity and mortality of hepatotoxicity has made it the most common reason for withdrawal of a medication from the marketplace and a prominent reason for cessation of drug testing during clinical trials [3, 4]. Currently more than 1,100 drugs, including prescription, natural products, herbal remedies, vitamins, have been reported to cause DILI [2]. This article will review the basics of DILI and recent developments in our understanding of the pathogenesis, diagnosis and treatment of DILI.

Background

Hepatotoxic agents are broadly classified as either intrinsic (meaning directly toxic) or idiosyncratic (rare and infrequent) drugs. Intrinsic drugs are predictable and related to dose administered, the prime example being acetaminophen [5]. Acetaminophen, referred to as

Corresponding Author: William M. Lee, MD, Digestive and Liver Diseases, Division UT Southwestern Medical Center at Dallas, 5959 Harry Hines Blvd. Ste 420, Dallas TX 75390-8887, Phone: 214 645 6111, Fax 214 645 6114.

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paracetamol in Europe, is the most common cause for ALF, accounting for 46% of all reported cases in the United States and well above 50% in some European countries [1, 6, 7]. By contrast, most reactions to prescription drugs are considered idiosyncratic; they are unpredictable, vary greatly in severity, and occur at varying time intervals after ingestion (anywhere from a few days to one year) [3]. Their toxicity is usually unrelated to dose, route or duration of drug administration (though a review of drugs withdrawn from the market in the United States have found that most were prescribed at daily doses exceeding 50 mg per day) [8]. Instead, the Greek root of idiosyncrasy emphasizes that it is the unique characteristics of the individual that predispose them to develop a toxic reaction, whereas many others can use the drug safely [3, 8]. It is assumed that the adverse reactions to these xenobiotic compounds have a strong genetic basis [4]. DILI, as a result, has become an important catalyst for pharmacogenomics research [4].

Idiosyncratic reactions are further classified according to their clinicopathological presentations: hepatocellular, cholestatic or mixed (hepatocellular and cholestatic). Hepatocellular reactions are the most common reaction and constitute up to 90% of cases [9], and feature high aminotransferase levels and relatively little alkaline phosphatase change. The R value is used to determine whether an agent should be classed as hepatocellular or cholestatic. R is the ratio of ALT/alkaline phosphatase. An R value >5 indicates hepatocellular liver injury; $R = 2-5$ denotes mixed liver injury; and $R <2$ is indicative of cholestatic liver injury. Rarer types of reactions include steatohepatitis, autoimmune, immunoallergic, oncogenesis, veno-occlusive disease, etc. These classifications are important as they constitute a drug's phenotype, or the typical clinical and histopathological presentation of a drug's toxicity [8]. However, there are occasions when a drug may display varying phenotypes, their presentation mimicking other causes of acute liver injury, such as viral hepatitis, making the diagnosis of DILI a difficult task.

Diagnosis

A diagnosis of hepatotoxicity must always be considered in the differential for acute liver injury or failure. However, making a definitive and accurate diagnosis of DILI is often difficult, particularly when full clinical and serological data is unavailable. Currently there are no objective tests or markers for drug-induced liver injury. Instead, expert opinion is regarded as the gold standard [10]. Diagnosis relies on subjective summation of clinical, biochemical and histological information. Establishing the start of the drug use and the onset of injury, evidence of improvement upon withdrawal (dechallenge), effect of rechallenge, and exclusion of other conditions as potential causes are some of the clinical data points required to proceed toward a causality assessment and therefore diagnosis [10]. Excluding other causes of acute liver injury, unfortunately, may be difficult. DILI may be erroneously diagnosed when other rarer causes are not effectively excluded. For example, acute Hepatitis E infection, a disease endemic in developing countries, is often misdiagnosed as DILI in developed countries since testing for Hepatitis E is not routinely done in the United States or in Europe [11, 12]. Currently, epidemiological studies in the UK are showing higher incidences of hepatitis E infections than previously thought, with 329 cases reported, 33 of which were indigenous cases, among UK subjects [13]. Another study from the UK retrospectively tested 47 patients with suspected DILI for Hepatitis E and found that six of those patients were HEV positive [12].

The Roussel-Uclaf Causality Assessment Method (RUCAM) was established by the Council for International Organizations of Medical Scientists in 1989 in an attempt to provide a more objective and uniform approach to the diagnosis process [10, 14, 15]. RUCAM is a scoring system that categorizes each scenario into "definite or highly probable" (score >8), "probable" (score 6–8), "possible" (score 3–5), "unlikely" (score 1–2) and "excluded" (score

0) based on an additive scoring of seven different domains including: the time to onset, risk factors, clinical course, concomitant drugs, non-drug-causes, published information on hepatotoxicity, and the response to any re-administration. Unfortunately, calculating RUCAM is time-consuming and provides confusing and insufficient information on how to score some of the domains. Even though it was designed to be objective, a recent study found that the RUCAM had poor reproducibility, even when repeated by the same reviewers [10, 16]. As a result, it has not been widely used in general clinical practice by gastroenterologists and hepatologists.[10] Other diagnostic scales, such as the Maria and Victorino method and the Naranjo scale have been put forward, but in studies comparing these instruments, even RUCAM has been found to have better reproducibility and relatively more agreement with specialist opinion [10, 17–20]. In 2010, the Drug-Induced Liver Injury Network (DILIN) developed a causality adjudication process that relied on a structured expert opinion process. They compared it to the RUCAM, and found that the DILIN scale produced higher agreement rates and likelihood scores [10]. However, there was still significant disagreement between the experts even with the DILIN scale, occurring in almost 1/3 of the cases [10]. In addition, the DILIN scale is not a clinically viable option as it requires performance by multiple experts, a luxury that is not routinely available.

The difficulty of diagnosing DILI has prompted many studies to identify reliable and sensitive biomarkers for liver injury, with most of these studies being done with acetaminophen toxicity. Elevated levels of NAPQI-protein adduct (a product of the covalent bonding of the reactive acetaminophen intermediate, NAPQI, with cell proteins) correlate well with known acetaminophen liver injury with high specificity and good sensitivity, making adducts assays an attractive biomarker of acetaminophen toxicity [21, 22]. In addition, the long elimination half-life of the adducts (levels were detectable 12 days after overdose) suggests that it could be more reliable than measuring acetaminophen (parent compound) concentration for diagnostic purposes [22]. As of now, protein adducts are measured by high-performance liquid chromatography with mass spectrometry, which assays are not viable in clinical settings [21]. Clinical trials are underway to provide a more accessible point-of-care test and these should soon be available.

MicroRNAs (miRNAs) have also emerged as potential biomarkers of hepatotoxicity, showing even stronger correlations with acetaminophen induced liver injury. MicroRNAs are small regulatory, noncoding RNAs that are believed to regulate specific cellular proteins by regulating messenger RNA. It has been shown that tissue levels of miRNA correlate well with development of several different cancers, so it was postulated that miRNAs specific to the liver might be used to detect and monitor the development of drug-induced liver injury [23]. A study with mouse models showed that levels of miR-122 and miR-192, both abundant within the liver, were dependently elevated based on the dose of acetaminophen and duration of exposure [23]. The elevations not only paralleled serum aminotransferase levels and histopathology of liver degeneration, but their changes could also be detected significantly earlier. Recently, a cohort study in patients showed significant elevations of mir-122 in acetaminophen-induced acute liver injury and concluded that circulating miRNAs can provide a novel addition to the diagnosis of DILI [24].

Unfortunately these biomarkers are still in research development and are not yet available for clinical use. The diagnosis of DILI still relies on subjective methods, which remain unreliable. This has resulted in the under-recognition and under-diagnosis of DILI, which has made it difficult to truly assess the incidence of DILI.

Mechanism of Hepatic Injury

The metabolism of acetaminophen has been taken as a paradigmatic example of all drug toxicity and no agent has been more fully studied to date. About 5% to 10% of acetaminophen is metabolized by the cytochrome P450 to the toxic *N*-acetyl-*p*-benzoquinoneimine (NAPQI) [6]. In normal circumstances, glutathione conjugates to the NAPQI and detoxifies it to mercapturic acid, which is then excreted into the urine [8]. In overdoses, the glucuronidation pathways are overwhelmed and the stores of glutathione become depleted. The NAPQI, a highly reactive, electrophilic molecule, forms covalent bonds with other intracellular proteins to form NAPQI-protein adducts. This bonding disrupts the cellular proteins' structure and function, which leads to a decrease in calcium ATPase activities and an increase in levels of cytosolic calcium. Abnormal cellular calcium homeostasis alters the permeability of the cell and the disassembly of actin fibrils that results in blebbing of the cell membrane, rupture and cell lysis [5, 6]. NAPQI also binds to mitochondrial proteins, which induces mitochondrial oxidant stress and the eventual release of mitochondrial proteins into cell cytoplasm [6]. Apoptosis and activation of the JAK/STAT pathway are also part of this very complex hepatic injury picture [25].

Most idiosyncratic reactions are thought to be immune based. Evidence for this includes the typical delay (latency) between starting a new drug and the appearance of the hepatotoxic injury, the shorter latency with re-exposure and the frequent allergic manifestations that are not always present but indicate that activation of the IgE allergic pathways are also occurring. Like acetaminophen, some drugs such as halothane and tienilic acid form drug-protein adducts that act as haptens, leading to immune responses to the haptenated proteins [5]. These haptens are presented on cell surfaces and elicit an immune response through either the formation of antibodies, induction of direct cytolytic T-cells responses, or activation of apoptotic pathways by tumor necrosis factor α receptor or Fas [5, 26].

In cholestatic diseases, drugs either bind to or disable transport proteins at the canalicular membrane or directly damage cholangiocytes by excretion of a toxic metabolite into the bile [5, 8]. The impaired bile flow allows toxic bile acids to accumulate that then directly injure hepatocytes [5].

Drugs can also directly damage and inhibit mitochondrial DNA. This disrupts fatty-acid oxidation and energy production, which in turn causes oxidative stress, production of lactic acidosis through anaerobic metabolism, and the accumulation of triglyceride that produces the characteristic microvesicular fat [5]. This type of injury is common with tetracycline, amiodarone, valproate and various antiviral nucleoside analogues [8, 27]. In oncogenic reactions, hormone administration induces a hepatocyte dedifferentiation that results in benign adenomas, and rarely, carcinomas [5]. As one can see, there are multiple pathways and mechanisms of hepatic injury, with each drug inducing a different mechanism.

Susceptibility to DILI

There has always been immense interest in uncovering the reason why a small population of people is susceptible to idiosyncratic drug reactions. Many genome-wide association studies have indicated that polymorphisms within the genome hold the key.

Variations within the host immunity appear to play an important role. Two recent studies of patients with amoxicillin-clavulanate toxicity, the most common idiosyncratic reaction, have found an association of the HLA-DRB1*1501 with cholestatic liver injury [8, 28, 29]. Though another study did not find the same association with HLA-DRB1 *1501, it did find correlation with the HLA-DQB1*06 allele [8]. Though the correlations have been found, the precise mechanism of increased susceptibility is still unknown. An association between the

HLA-DRB1*1501 allele and lumiracoxib induced hepatotoxicity was also found. However, only 6–8% of individuals with this HLA haplotype developed liver injury, so, though the study showed a correlation, it did not necessarily show causation [30]. Many other associations with different HLA haplotypes have been found that are beyond the scope of this review.

Polymorphisms within the genes encoding bile acid transporters have been found to cause cholestatic hepatotoxicity. The bile salt export pump (BSEP; also known as ABCB11) is either directly or indirectly inhibited by drugs such as cyclosporin and estradiol. A study in 36 patients with drug-induced cholestasis found mutations in the genes encoding the ABC family of transporters [8, 31].

A study in 17 patients with valproate hepatotoxicity found that heterozygous genetic variation within the *POLG* gene correlated significantly with valproate-induced liver injury [32]. *In vitro* analyses confirmed these correlations. They showed that therapeutic concentrations of valproate suppressed the proliferation of hepatocytes and the administration of high doses led to non-apoptotic cell death [8, 32].

In addition to idiosyncratic drugs, there has also been further insight into acetaminophen hepatotoxicity. Though the mechanism and metabolism of acetaminophen is relatively similar in most individuals, differential sensitivity to acetaminophen hepatotoxicity has been demonstrated among healthy volunteers [4, 33]. Differences in age, polymorphisms in the cytochrome isoenzymes, nutritional status, and alcohol intake have all been postulated to alter the susceptibility to liver injury, though the validity of this has been questioned by some [34, 35].

Within the past few years, emerging evidence suggests that differences within the host immune system and not within the metabolism and detoxification mechanisms, may be responsible for the susceptibility to acetaminophen toxicity. A whole-genome association analysis and targeted sequencing determined that polymorphisms within the CD44 human gene were associated with increased susceptibility to acetaminophen toxicity in two independent cohorts [4]. The CD44 gene encodes a cell adhesion molecule expressed on lymphocytes and is involved in cell-matrix interactions [8]. Mouse strains with certain polymorphisms and knockout of the CD44 gene were considerably more susceptible to acetaminophen hepatotoxicity as compared to wild-type mice [4]. Like the mouse models, polymorphisms in the human CD44 were also associated with increased levels of serum ALT following acetaminophen administration [4]. Interestingly, CD44 knockout mice demonstrated less severe liver injury in the early stages (within 6 hours) after carbon tetrachloride administration secondary to reduced leukocyte migration and cytokine production in the early phase [36]. However, this also caused severe liver inflammation with increased numbers of apoptotic hepatocytes at the late phase, owing to the inability of the host to clear the apoptotic cells [8]. Therefore, CD44 not only correlated with susceptibility to acetaminophen toxicity but also with the course and severity of the hepatotoxicity.

While the current basic science research for susceptibility to drug-induced liver injury is still far from translation into clinical use, there is already an example of the clinical use of pharmacogenetic testing to prevent a specific toxic effect of a drug. At present, screening for an allele called HLA-B*5701 is recommended in HIV patients who are to start the anti-retroviral abacavir. For many years, a hypersensitivity syndrome characterized by fever, nausea, vomiting, diarrhea and a range of other constitutional symptoms (but not liver injury), was associated with abacavir. The syndrome led to significant morbidity and even some reported deaths. Research into the syndrome found a strong association between the HLA-B*5701 allele and a predisposition to the hypersensitivity syndrome [37]. Though the

allele is only present in about 5.6% of white populations, screening for the allele has been shown in multiple cohort studies to have significantly reduced the incidence of hypersensitivity syndrome [37]. This is the first time that an immunogenetic marker has been used in clinical practice to prevent a specific drug toxicity. Hopefully further pharmacogenomics research in DILI can also allow for future susceptibility testing. This would allow tailoring of drug therapy to individual patients according to their unique genetics, which, theoretically, would minimize hepatotoxicity while maximizing drug therapy. Interestingly, susceptibility to DILI in flucloxacillin, a β -lactam antibiotic widely used in Europe, was also found to correlate with HLA-B*5701 [8, 38].

Treatment

The mainstay for the treatment of DILI continues to be prompt removal of the offending drug and prevention of re-exposure. Re-exposure in idiosyncratic reactions is virtually always met with greater severity and with a shorter latency period to evidence of injury. In mild to moderate cases of DILI, discontinuation of the drug is sufficient for a relatively rapid and full recovery. In cholestatic liver injury, however, the signs of liver injury may linger with complete resolution potentially taking weeks and even months [39]. Interestingly, a process known as adaptation can also occur where even with continuing the drug, the aminotransferase elevations are limited or even resolve. Discontinuation of the drug is then not necessarily required. Isoniazid and statin drugs are examples of this phenomenon [39].

In the case of acetaminophen, prompt administration of *N*-acetylcysteine (NAC) within 8 to 10 hours, and even up to 24 hours, of ingestion, regardless of the initial acetaminophen concentration, can treat and prevent severe hepatotoxicity [34]. NAC is a glutathione precursor that metabolizes the toxic NAPQI metabolite into a non-toxic conjugate that can be excreted into the urine [34]. If the patient presents within 4 hours of ingestion, gastric lavage can decrease the pill load and activated charcoal can reduce the serum acetaminophen concentration by decreasing absorption by 50% to 90% [34].

In addition to increasing glutathione stores, NAC also has anti-inflammatory, antioxidant, inotropic and vasodilating properties that provide favorable effects on the acutely injured liver [34, 40]. As such, there has been recent interest in using NAC for non-acetaminophen hepatotoxicity. Recently a prospective, randomized, double blind placebo controlled trial of NAC for cases of acute liver failure not due to acetaminophen found that intravenous NAC significantly improved transplant-free survival (40% for NAC patients versus 27% in placebo) [40].

Besides NAC, there have been other implicated treatment modalities for non-acetaminophen hepatotoxicity. L-carnitine has been proven effective in valproate-induced hepatotoxicity [41]. Ursodeoxycholic acid can be used in cholestatic DILI to potentially hasten the resolution of jaundice and pruritus [39]. The use of cholestyramine is also recommended in severe toxicity from leflunomide [42]. The most controversial is the use of corticosteroids. Corticosteroids are particularly used in DILI with hypersensitivity features such as rash, fever and eosinophilia. However, the efficacy of corticosteroids is unproven and may even be reportedly ineffective [39]. In addition, they also carry the risks of mood disturbances, glucose intolerance and infection [39].

Rarely, hepatotoxicity is so severe that it progresses to acute liver failure characterized by rapid onset of encephalopathy and often death. In these instances, liver transplantation may be the only definitive therapy.

Prognosis

Mild cases of DILI are expected to completely recover without any significant morbidity or long-term consequences. In fact, more than 90% of all patients with acetaminophen poisoning can be expected to recover completely [34]. However, in the 10% of cases that appear severe and progressive, morbidity and mortality is high.

Final Thoughts

DILI is the most frequent reason for a drug to fail to achieve licensing by FDA or to be withdrawn following approval. Attempts to recognize hepatotoxicity even during pre-approval clinical trials of new therapeutic agents are limited because infrequent reactions are unlikely to be detected unless the number of patients studied is enormous. For example, to reliably detect a 1:10000 reaction that would be considered relatively common, 30,000 patients would need to be given the new agent and most phase III trials employ less than 5,000 patients. As a result, to support a claim of significant safety or toxicity manufacturers have relied on indirect measures, such as aminotransferase elevations. Based on review of early FDA data, Hyman Zimmerman postulated that drugs that caused any degree of jaundice would be likely in a larger cohort to lead to patients having acute liver failure. He estimated that for every 10 jaundiced patients, one would develop ALF, and this has been codified in FDA parlance as Hy's Law. According to Hy's law, patients with an elevated serum ALT >3 times the upper limit of normal *with concurrent jaundice* thought secondary to drug toxicity, mortality without a liver transplant ranges from at least 10% to 50% [39]. 'Hy's Law cases' are reported as such in assessing phase III drug trials where hepatotoxicity becomes an issue.

In patients who develop acute liver failure from acetaminophen toxicity and do not receive a liver transplant, mortality approaches 30% [34]. In patients with acute liver failure from idiosyncratic drug reactions, the mortality is even higher; short-term transplant-free survival in these cases is only 25% [1], meaning that 75% will die, absent a transplant [1]. Infection and cerebral edema remain the leading cause of death in patients progressing to ALF [1].

Conclusions

Though the incidence of DILI remains rare, it still possesses significant importance because of its high morbidity and mortality. The relative rarity of DILI has made it difficult to perform large-scale research studies but as this review article has shown, modern research methods within the past few years has significantly increased our understanding of the pathogenesis of DILI and changed our methods of diagnosis and treatment of DILI. The DILI Network has moved the field forward to an extent but more needs to be done. A recent welcome addition is the livertox website (livertox.nih.gov), which is a joint project between NIH and the National Library of Medicine. This extensive and carefully developed registry presents detailed information, references and case examples for virtually all known more than 650 prescription and many non-prescription drugs, providing a ready reference for clinicians and patients alike. In the future, with further research into pharmacogenomics, we may one day be able to tailor drug therapy according to a patient's unique genetic composition.

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*Important article

**Of major importance article

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