Drug-induced liver injury

Guruprasad P Aithal

Abstract

Lack of substantial advances in pre-clinical testing for hepatotoxicity has meant that drug-induced liver injury (DILI) remains an important issue during both the drug development and post-marketing phases. A number of drug-related, genetic and non-genetic host factors influence the risk of DILI in any individual. Demonstration of human leukocyte antigen genotype as a strong risk factor for the development of DILI from a range of drugs has highlighted the role of the adaptive immune system in its pathogenesis; there is accumulating evidence that drug metabolism genes also contribute to some forms of DILI. Early recognition and prompt withdrawal of the drug is essential in preventing serious hepatic failure and is the critical step in the management of adverse reactions. Diagnosis of DILI relies upon index of suspicion, careful evaluation of a temporal relationship between the exposure to a particular drug and the specific clinical event, and exclusion of potential alternative diagnoses. A high negative predictive value of genetic tests can be used to rule out DILI caused by particular drugs and to correctly identify the agent underlying DILI in a patient exposed to two concomitant medications.

Keywords Adverse drug reaction; drug-induced liver injury; hepatotoxicity; human leukocyte antigen; MRCP; pharmacogenetics

Key points

- The incidence of drug-induced liver injury (DILI) has been estimated to be 19 per 100,000 population; it is the second most common cause of hepatocellular jaundice presenting to secondary care
- The strong association between human leukocyte antigen (HLA) genotype and the development of DILI caused by a number of structurally dissimilar drugs indicates that the presentation of a drug–peptide complex to T cells is a key mechanism mediating liver injury
- Most HLA alleles associated with DILI have a very high negative predictive value of >0.95; this allows use of genetic tests to rule out DILI caused by particular drugs and to identify the correct agent underlying DILI in a patient exposed to two concomitant medications
- Accurate diagnosis with identification of the drug responsible is crucial to patient care so use of genetic tests and liver biopsy in selected cases is justified

*There is no evidence to support routine use of corticosteroids in the treatment of DILI

Introduction

Idiosyncratic drug-induced liver injury (DILI) is best described as an adverse hepatic reaction that is unpredictable on the basis of the pharmacological action of the drug administered; this is distinct and different from liver injury secondary to drug overdosage. 'Drug-induced liver disease' includes a wide variety of adverse effects such as drug-associated chronic liver diseases (drug-associated fatty liver disease, fibrosis, nodular regenerative hyperplasia, secondary sclerosing cholangitis), whereas the term 'DILI' refers to adverse events that are acute in onset.

Thresholds to define DILI include a 5-fold elevation above the upper limit of normal for alanine aminotransferase (ALT), a 2-fold elevation in alkaline phosphatase or a simultaneous 3-fold elevation in ALT concentration and 2-fold elevation of bilirubin concentration. Based on the associated biochemical abnormalities, DILI is subclassified as being of 'hepatocellular', cholestatic or mixed pattern.

Burden of hepatotoxicity

Because the liver is involved in the biotransformation of most drugs, hepatotoxicity is a potential complication of a large number of medications. Owing to their relatively low incidence, hepatic adverse reactions often remain undetected during clinical trials. Even after a drug has been marketed, hepatotoxicity can become apparent only when a large number of people have been exposed to it; for this reason, hepatotoxicity has been the second most common reason for withdrawal of drugs from the market worldwide, accounting for 32% of such cases between 1975 and 2007.

A prospective population-based study from Iceland estimated the crude incidence of DILI to be 19 per 100,000 population. The incidence of acute serious liver injury requiring hospitalization has been estimated to be 0.7–1 per 100,000 population per year. In a recent audit from the UK involving 881 consecutive patients presenting with jaundice in whom biliary obstruction had been ruled out by imaging, DILI was the underlying aetiology in 15% of cases; in this case-series, DILI was the second most common cause of hepatocellular jaundice after alcoholic liver disease. Idiosyncratic DILI contributes to 7–15% of cases of acute liver failure, highlighting the potentially serious consequences of this adverse reaction (Table 1).

Risk factors associated with DILI

Drug-related risk factors

Although idiosyncratic DILI is clearly distinguishable from hepatotoxicity related to overdose (e.g. paracetamol), serious DILI occurs more frequently with medications taken in a daily dose of ≥50 mg than in a daily dose of ≤10 mg. There is also a significant relationship between daily dose and reports of liver failure, liver transplantation and death caused by DILI. In addition, compounds in which >50% of elimination relies on hepatic

Guruprasad P Aithal *MB BS MD FRCP PhD is Head of Division, Faculty of Medicine and Health Sciences, University of Nottingham, NIHR Nottingham Biomedical Research Centre, and a Consultant Hepatobiliary Physician at Nottingham University Hospitals NHS Trust and Nottingham, UK. Competing interests: none declared.*

metabolism are more likely to be associated with DILI (fatal and non-fatal) and liver failure. Lengthy duration of exposure to the medication (flucloxacillin, co-amoxiclav) has also been associated with an increased risk of DILI.

Genetic susceptibility

A series of case–control studies in the last decade has demonstrated that allelic variants of UDP-glucuronosyltransferase-2B7, cytochrome P450 (CYP)–2C8 and the ATP-binding cassette, subfamily C -member 2 (*ABCC2*), which can promote the formation and accumulation of reactive diclofenac metabolites, are associated with susceptibility to diclofenac hepatotoxicity. With flucloxacillin, a promoter region polymorphism in gene coding for xeno-sensing pregnane X receptor (*PXR*, also known as *NR1I2*) has been associated with DILI; this receptor regulates the transcription of phase I and II drug-metabolizing enzymes such as CYPs and glutathione *S*-transferases (GSTs) as well as transporters.[3]

Several candidate gene and genome-wide association studies (GWAS) have confirmed the major influence of the human major histocompatability complex (MHC) on an individual's susceptibility to DILI. A seminal GWAS demonstrated that possession of the HLA-B*5701 allele was strongly associated with an 81-fold increased risk of flucloxacillin DILI. Several studies have confirmed association of DILI from co-amoxiclav with the DRB1*1501–DQB1*0602 haplotype. Human leukocyte antigen (HLA) variants have also been associated with DILI secondary to a number of currently used drugs. The most recent GWAS identified a variant in *PTPN22* to be associated with risk of liver injury caused by multiple drugs. This variant has been associated with increased risk of autoimmune diseases, providing support for the concept that alterations in immune regulation contribute to idiosyncratic DILI.

Pathogenesis

The development of idiosyncratic DILI involves a number of concurrent as well as sequential events determining the direction

Drugs associated with different forms of hepatotoxicity

Acute hepatocellular pattern of liver injury

- *Non-steroidal anti-inflammatory drugs*: diclofenac, naproxen, nimesulide, piroxicam
- *Anaesthetics*: enflurane, halothane, isoflurane
- *Antimicrobials*: ketoconazole, terbinafine, tetracyclines; antituberculosis drugs such as isoniazid, pyrazinamide, rifampicin; anti-HIV agents such as didanosine, nevirapine, zidovudine
- *Neuropsychotropics*: tricyclics (most), fluoxetine, paroxetine, sertraline; illegal compounds such as cocaine and ecstasy
- *Antiepileptics*: carbamazepine, phenytoin, valproate
- *Cardiovascular drugs*: bezafibrate, captopril, enalapril, lisinopril, lovastatin, simvastatin, ticlopidine
- *Antineoplastics*: cyclophosphamide, cisplatin, doxorubicin
- *Others*: herbal remedies

Acute cholestatic pattern of liver injury

- *Hormonal preparations*: androgens, anabolic corticosteroids, oral contraceptives, tamoxifen
- *Antimicrobials*: clindamycin, co-amoxiclav, co-trimoxazole, erythromycin, flucloxacillin, troleandomycin
- *Analgesics/anti-inflammatory drugs*: gold salts, sulindac
- *Neuropsychiatric drugs*: carbamazepine, chlorpromazine, tricyclic antidepressants
- *Immunosuppressants*: azathioprine, ciclosporin
- *Others*: allopurinol

Autoimmune hepatitis

Minocycline, nitrofurantoin, diclofenac, indometacin, statins, infliximab, halothane, herbal medicine (germander), methyldopa

Chronic drug-associated liver disease and/or cirrhosis

Methotrexate, tamoxifen, vitamin A

Chronic cholestasis and ductopenia

Carbamazepine, chlorpromazine, co-amoxiclav, co-trimoxazole, erythromycin, flucloxacillin, methyltestosterone, phenytoin

Granulomatous hepatitis

Allopurinol, carbamazepine, diltiazem, gold salts, methyldopa, nitrofurantoin, penicillin, penicillamine, phenytoin, sulfonamides, sulfonylureas

Macro and microvesicular steatosis

Amiodarone, corticosteroids, methotrexate, salicylate, tetracycline, valproate, zidovudine

Hepatic vascular lesion

- *Hepatic vein thrombosis/veno-occlusive disease*: azathioprine, combination chemotherapy (carmustine, cytarabine, mitomycin, thioguanine, urethane), oral contraceptives
- *Sinusoidal dilation/peliosis*: anabolic steroids, azathioprine, oral contraceptives

Tumours

Anabolic steroids, oral contraceptives

Table 1

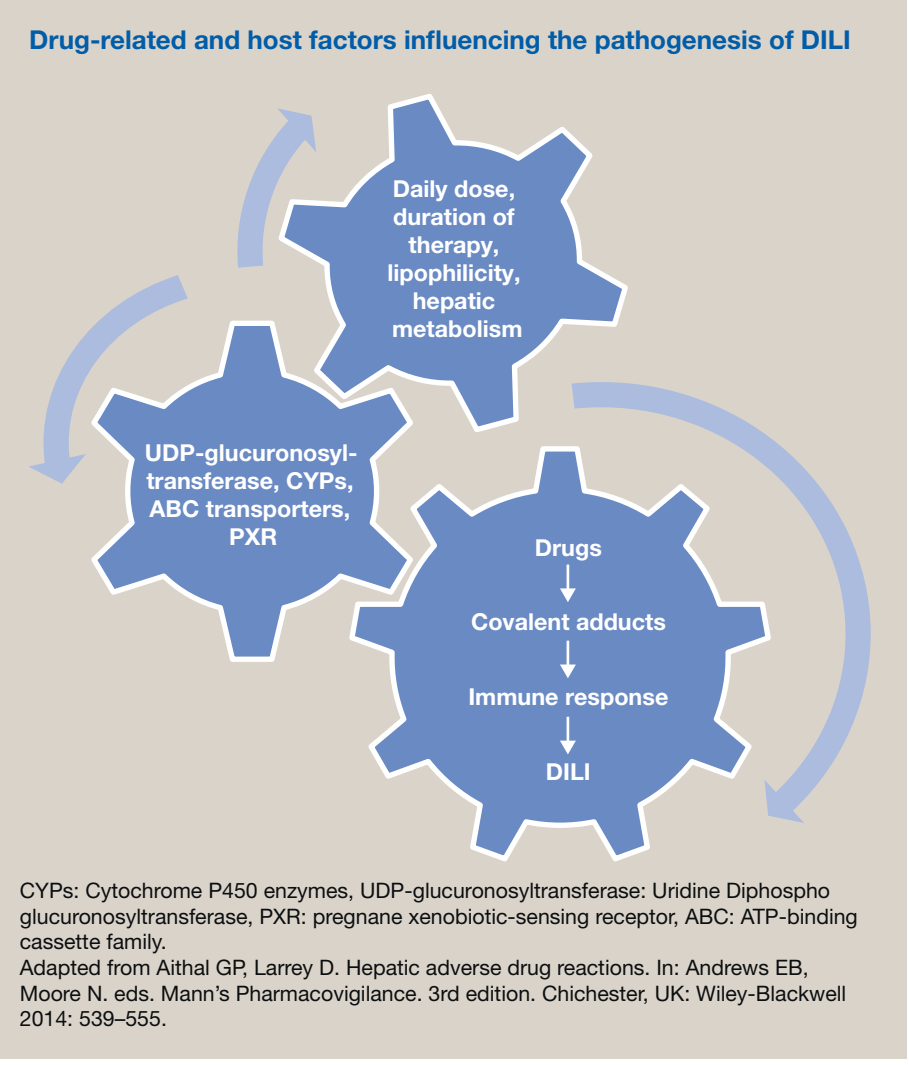
of the pathways, as well as the severity of liver injury and its manifestations (Figure 1).

The key upstream events include drug-specific pathways triggered by particular drugs or their metabolites. Genetic and environmental factors that influence the expression and activities of proteins involved in drug disposition determine the rate of formation and accumulation of reactive metabolites. These reactive metabolites induce the production of excessive reactive oxygen species, leading to lipid peroxidation and cell death. The cellular environment can modulate the threshold for hepatocyte death secondary to oxidative stress; thus dysregulation of the antioxidant pathway can contribute to propagation of DILI. In addition, the occurrence and extent of liver injury could be influenced by events downstream; the innate immune response can promote or inhibit the inflammatory process and thereby determine the progression and severity of DILI.

Although protein binding of reactive metabolites can impair cellular function to cause toxicity, drug metabolite adducts can interact with the immune system. In order for the drug metabolite adduct to be recognized by the immune system, it should be presented by antigen-presenting cells in conjunction with MHC molecules. Several candidate gene and GWASs have demonstrated that the human MHC plays a major role in increasing or decreasing susceptibility to DILI, highlighting the role of adaptive immunity in the pathogenesis. HLA variants associated with toxicity are thought to increase the specificity of the peptide-binding groove for the drug or drug–peptide complex, enhancing the presentation of these molecules as antigens to T cells, and ultimately leading to the immunological destruction of hepatocytes. Alternatively, drugs can act directly via non-covalent interactions with MHC proteins or T cell receptors in what is termed a 'pharmacological interaction' (pi concept), leading to the activation of T cells without the presence of an antigenic peptide.

Diagnosis

The importance of drugs as a cause of liver injury lies not just in the overall number of cases, but in the severity of some reactions and their potential reversibility provided the drug aetiology is promptly recognized. DILI can mimic a wide



CYPs: Cytochrome P450 enzymes, UDP-glucuronosyltransferase: Uridine Diphospho glucuronosyltransferase, PXR: pregnane xenobiotic-sensing receptor, ABC: ATP-binding cassette family.
Adapted from Aithal GP, Larrey D. Hepatic adverse drug reactions. In: Andrews EB, Moore N. eds. Mann's Pharmacovigilance. 3rd edition. Chichester, UK: Wiley-Blackwell 2014: 539–555.

Figure 1

spectrum of hepatobiliary diseases. The diagnosis relies on establishing the temporal relationship with the exposure to the drug; for most drugs, DILI manifests within the first year after exposure, usually between 4 days and 3 months of initial administration.

It is equally important to exclude alternative explanations based on the clinical manifestations; common missed diagnoses include ischaemic hepatitis, biliary obstruction, systemic sepsis and acute hepatitis E. Inaccurate diagnosis can lead to inappropriate withdrawal of an effective medication (e.g. antimicrobials, anticonvulsants) or a missed alternative diagnosis (e.g. autoimmune hepatitis) that merits prompt treatment.

Role of liver biopsy

DILI can cause any known pattern of liver pathology, although certain histological features are particularly suggestive of drug-induced aetiology. Hence, the benefits of performing a liver biopsy should be weighed against the disadvantages and its limitations. Liver biopsy is justified when:

- autoimmune hepatitis is one of the differential diagnosis under consideration
- the event does not resolve after discontinuing the drug
- the clinical/laboratory features are atypical (Figure 2)
- the patient appears to be suffering from an as yet unrecognized form of DILI.

Management

Early recognition and prompt withdrawal of the drug is essential in preventing serious hepatic failure and is the critical step in managing adverse reactions. When patients with DILI caused by a particular drug are re-exposed to the same medication, 11–30% develop recurrent DILI. Compared with the initial event, DILI after rechallenge occurs more rapidly (generally after days or weeks) than after initial exposure. DILI on rechallenge leads to jaundice in 64%, hospitalization in 52% and mortality in 13% of cases; all of these can be considered preventable, with the exception of the first-line antituberculosis treatment regimen, which is highly effective and inexpensive. The benefit of reintroducing these drugs once DILI has resolved generally outweighs the risks, and it is unwise to discard these drugs from the regimen.

There is no evidence to support DILI with corticosteroids except in circumstances where it is indistinguishable from autoimmune hepatitis even with liver biopsy and histogenetics. In such circumstances, immunosuppression withdrawal once liver enzyme concentrations return to normal, with careful monitoring, is recommended. The benefits and disadvantages of treatment with DILI secondary to cancer immunotherapeutics with extremely high-dose corticosteroids have not yet been established.

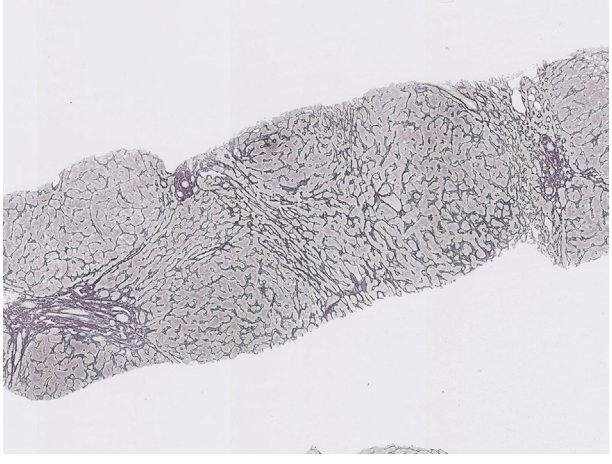


Figure 2 Reticulin stain demonstrating hypertrophic cell plates surrounded by atrophic cell plates typical of nodular regenerative hyperplasia attributed to azathioprine therapy.

Clinical applications of pharmacogenetic testing in DILI

Unlike the many studies that have explored association of genetic variants with complex traits, those investigating DILI have identified risk alleles that have substantially stronger associations with DILI. For example, possession of the HLA-B*5701 allele leads to an 80-fold increased risk of DILI after exposure to flucloxacillin. This might raise the possibility of pre-prescription genotyping to identify susceptible individuals. However, as DILI is a rare event when all individuals prescribed flucloxacillin are considered, the usefulness of this approach is limited by an inherently low pre-test probability of the adverse event. Nevertheless, a very high negative predictive value (>0.95) of HLA-B*5701 can be used to rule out flucloxacillin-induced DILI in a clinical scenario where DILI is one of the differential diagnoses, allowing an effective medication to be continued.

Although the positive predictive value of genetic tests has been shown to be low in the context of case–control studies, the performance characteristics of the genetic tests are expected to be similar to some tests that are used routinely in the diagnosis of autoimmune hepatitis. In addition, when the possibility of autoimmune hepatitis is raised, calculation of International Autoimmune Hepatitis Group score including appropriate HLA typing would assist accurate diagnosis. Genotyping for HLA-DRB1*1501, particularly in a patient recently exposed to amoxicillin–clavulanate, may be helpful in differentiating DILI from seronegative hepatitis by providing supportive (although not definitive) evidence for the diagnosis of DILI, and thus assist clinical decision-making; such patients should avoid further exposure to amoxicillin–clavulanate.

Combining both genetic and non-genetic risk factors that are associated with the development of DILI might generate better tools to pre-empt or diagnose DILI, thereby widening their clinical applicability. ◆

FURTHER READING

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TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 66-year-old woman presented with jaundice. She had had an episode of bronchitis 1 month previously and had been treated with amoxicillin–clavulanate for 7 days. Two weeks later, she developed jaundice. On clinical examination, there was jaundice, with no rashes, spider naevi or asterixis (liver flap), no organomegaly and no ascites on abdominal examination.

Investigations

- Bilirubin 7 micromol/litre(1–22)
- Alanine aminotransferase 446 U/litre (5–35)
- Alkaline phosphatase 302 U/litre (45–105)
- Prothrombin time 16 seconds (11.5–15.5)
- Antinuclear antibody positive 1:80 (negative at 1:20)
- Immunoglobulin (Ig) G 14 g/litre (6.0–13.0)
- Hepatitis A, B, C and E serology negative
- Ultrasound of the abdomen showed a normal liver, gallbladder and common bile duct

Which of the following is the most appropriate next step in the management?

A. Calculate the International Autoimmune Hepatitis Group score
B. Estimate serum bile acid concentration
C. Organize a lymphocyte transformation test
D. Perform liver biopsy and histological evaluation
E. Request magnetic resonance cholangiopancreatography

Question 2

A 70-year-old lady presented with a 2-week history of dark urine. She also had a dull ache in the right upper abdomen. Past medical history included hypothyroidism for 6 years treated with thyroxine 100 micrograms daily, and hypercholesterolaemia, for which she had taken simvastatin 20 mg daily initially for 3 years and then 40 mg daily for 2 years. She had had low backache for the past year and had been found to have Paget's disease, treated with diclofenac sodium 50 mg 12-hourly for the previous 4 months. She did not smoke, and drank about six glasses of wine per week.

On clinical examination, she was jaundiced, but no other signs suggestive of chronic liver disease were found. She had no flapping tremor. Abdominal examination was normal.

Investigations

- Haemoglobin 133 g/litre (115–165)
- White cell count 15.7 $\times$ 10^9/litre (4–11)
- Platelet count 183 $\times$ 10^9/litre (150–400)
- International normalized ratio 1.0 (<1.4)
- Total bilirubin 112 micromol/litre(1–22)
- Albumin 40 g/litre (37–49)
- Alanine aminotransferase 980 U/litre (5–35)
- Alkaline phosphatase 880 U/litre (45–105)
- γ-Glutamyl transferase 515 U/litre (4–35)
- IgG 11.5 g/litre (6–13)
- IgA 1.4 g/litre (0.8–3.0)
- IgM 1.2 g/litre (0.4–2.5)
- Antinuclear antibodies reactive 1:80 (negative 1:20)
- Anti-smooth muscle antibody negative
- Thyroid microsomal antibody reactive 1:800 (negative at 1:10)
- Antibody to hepatitis C virus negative
- Hepatitis B surface antigen not detected
- Hepatitis A IgM negative
- Ultrasonography of the abdomen showed normal liver echotexture with no intrahepatic duct dilation. The gallbladder, spleen and both kidneys were normal. The common bile duct was not visualized, and the pancreas was obscured by bowel gas

What would be the most appropriate next step in the management?

A. Organize a liver biopsy and histology
B. Reduce the dose of simvastatin
C. Request the human leukocyte antigen (HLA) genotype
D. Start corticosteroid treatment
E. Withdraw the diclofenac sodium

Question 3

A 31-year-old man presented with a 1-week history of jaundice and itching. He had been taking a body-building dietary supplement for 2 months. He had also previously suffered from an adverse reaction, with the development of jaundice, while taking anabolic steroids. He had had a skin infection 3 weeks previously for which he had been advised to take flucloxacillin for 1 week.

Investigations

- Total bilirubin 229 micromol/litre (1–22)
- Alanine transaminase 2548 U/litre (5–35)
- Alkaline phosphatase 144 U/litre (45–105)
- Prothrombin time 15 seconds (10–12)
- Hepatitis virus serology to A, B, C, E, Epstein–Barr virus and cytomegalovirus was negative
- Ceruloplasmin and α_1-antitrypsin were normal
- Antinuclear antibodies and anti-liver–kidney microsomal antibodies negative
- IgG 16.1 g/litre (6.0–13.0)
- Ultrasound of the abdomen showed a normal gallbladder; the common bile duct was obscured by duodenum

What test would be able to exclude flucloxacillin-induced liver injury?

A. Antibody to soluble liver antigen
B. Canalicular cholestasis on liver histology
C. High eosinophil count
D. HLA-B*5701 genotyping
E. Magnetic resonance cholangiopancreatography