Antituberculosis Treatment-Induced Hepatotoxicity: From Bench to Bedside

SK Sharma*, Alladi Mohan**

*Prof. & Head, Dept. of Medicine, Chief, Division of Pulmonary and Critical Care Medicine AIIMS, New Delhi 110 029; **Associate Prof. & Head, Department of Emergency Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati 517 507.

A B S T R A C T

Drug-induced liver injury is a common, but often unrecognized cause of liver damage that continues to fascinate and challenge clinicians. Isoniazid, rifampicin and pyrazinamide have been observed to have hepatotoxic potential and drug-induced hepatotoxicity (DIH) is an important adverse effect with antituberculosis treatment. Idiosyncratic damage, dose-dependent toxicity, induction of hepatic enzymes, drug-induced acute hepatitis and allergic reactions have all been implicated as the pathogenetic mechanisms of DIH. The pathological consequences manifest as disruption of intracellular calcium homeostasis, cholestatic damage, interruption of transport pumps and loss of villous processes, reactions involving cytochrome P-450 system, activation of apoptotic pathways and programmed cell death, and inhibition of mitochondrial function. Advanced age, female sex, history of alcoholism, underlying liver disease, acetylator phenotype, hepatitis B, C, and human immunodeficiency virus infection, extensive disease, hypoalbuminaemia; slow acetylator status of the N-acetyltransferase 2 (NAT2) gene, polymorphism of cytochrome P450 (CYP21), absence of HLA-DQA1*0102, and presence of HLA-DQB1*0201 have all been observed to be risk factors for the development of DIH. When patients develop clinical icterus and other manifestations of DIH, the offending hepatotoxic drugs must be stopped. Other causes of liver function derangement such as co-existent viral hepatitis must be ruled out. The patient must be closely monitored and non-hepatotoxic drugs such as streptomycin, ethambutol and fluoroquinolones can be used temporarily. Once the liver functions normalise, it is often possible to reintroduce the first-line drugs under close observation and supervision.

INTRODUCTION

The liver, referred to as the “metabolic factory” of the body, is central to the metabolism of virtually every foreign substance including antituberculosis drugs. Hepatic biotransformation mechanisms involving oxidative pathways, primarily by way of the cytochrome P-450 enzyme system are vital for rendering the drugs more hydrophilic. Further metabolic steps such as conjugation to a glucuronide, sulphate or glutathione result in the formation of hydrophilic metabolites that are exported into the plasma or bile and are excreted by the kidney or the gastrointestinal tract. Drug-induced liver injury is a common, but often unrecognized cause of liver damage that continues to fascinate and challenge clinicians.

Isoniazid, rifampicin and pyrazinamide are essential components of the directly observed treatment, short-course (DOTS) strategy for control of tuberculosis endorsed by the World Health Organization (WHO). Isoniazid, rifampicin and pyrazinamide have been observed to have hepatotoxic potential. Given the enormity of the burden of tuberculosis, the fact that DOTS is the most cost-effective life-saving measure ever conceived, and considering the phenomenal number of persons receiving DOTS world over, drug-induced hepatotoxicity (DIH) is an important and commonly encountered adverse effect with antituberculosis treatment.

Recent developments especially in the field of molecular biology have expanded our views on the understanding of drug-induced liver damage. In this paper, we have attempted to summarise the current understanding of the pathogenetic mechanisms of DIH as it has been viewed from the laboratory bench and the bedside implications of these observations to the practicing clinician with particular reference to antituberculosis drugs.

MECHANISMS OF DRUG-INDUCED HEPATOTOXICITY

Several types of drug-induced liver damage have been described. These include, (i) idiosyncratic damage; (ii) dose-dependent toxicity; (iii) induction of hepatic enzymes; (iv) drug-induced acute hepatitis; and (v) allergic reactions; among others. Idiosyncratic reactions are the result of a “multi-hit process” due to the succession of unlikely events and are characterised by a variable latency period from the initial time of ingestion of the drug. Idiosyncratic reactions are frequently fatal if the drug is continued once the reaction has begun. Re-challenge usually results in a more severe reaction irrespective of whether
the initial reaction was severe or mild. In the case of some drugs such as acetaminophen, hepatic damage occurs in a dose-dependent fashion. Induction of hepatic enzymes by drugs such as ethanol, phenobarbital and phenytoin, may alter plasma drug levels. Thus, enzyme inducers not only have a dynamic role in enhancing hepatotoxicity, they also result in extrahepatic adverse drug reactions and drug interactions. Allergic reactions manifest with fever, lymphadenopathy, rash and severe hepatocyte injury constituting the “reactive metabolite syndrome.” Phenytoin and halothane are often implicated in causing this type of injury. Depending on the intracellular organelles affected, specific patterns of hepatic damage have been described (Table 1). Cell membrane bleb formation, rupture and cell lysis are the result of disruption of intracellular calcium homeostasis leading to the disassembly of actin fibrils at the surface of the hepatocyte. Disruption of the actin filaments adjacent to the canaliculus indicates cholestatic damage. Interruption of transport pumps such as multidrug-resistance–associated protein 3 (MRP3) and loss of villous processes result in the prevention of the excretion of bilirubin and other organic compounds. In the reactions involving cytochrome P-450 system, covalent binding of the drug to the enzyme results in the creation of non-functioning adducts that migrate to the cell surface and serve as targets for cytolysis by T-lymphocytes and triggering of multifaceted immune responses. Activation of apoptotic pathways may trigger the cascade of intercellular caspases resulting in programmed cell death with loss of nuclear chromatin. Inhibition of mitochondrial function can occur due to effect on enzymes of oxi-ando and the respiratory chain leading to impaired metabolism of free fatty acids, lack of aerobic respiration, and the accumulation of lactate and reactive oxygen species. Finally, toxic metabolites excreted in bile may damage bile-duct epithelium.

**ANTITUBERCULOSIS DRUGS AND HEPATOTOXICITY**

The pathogenesis of DIH caused by isoniazid is not well-understood. Histopathological evidence resembling that of viral hepatitis showing hepatocyte necrosis, ballooning degeneration and inflammatory infiltrates suggests dose-related toxicity. However, lack of direct correlation between serum drug levels and hepatotoxicity argues against a direct toxic effect. Given the delayed onset of DIH, absence of symptoms usually associated with hypersensitivity such as rash, fever, arthralgia and eosinophilia, and no hepatotoxicity on re-challenge in most cases, hypersensitivity is considered unlikely. But, presence of eosinophilic infiltrates on liver biopsy and recurrence of hepatotoxicity on re-challenge with the drug suggest hypersensitivity as a possible mechanism.

Altered profile of antioxidant enzymes with increased lipid peroxidation indicated that isoniazid and rifampicin-induced hepatotoxicity appeared to be mediated through oxidative stress. Compared with isoniazid, DIH caused by rifampicin occurs earlier and produces a patchy cellular abnormality with marked periportal inflammation. Rifampicin-induced hepatitis has been postulated to occur as a part of systemic allergic reaction and due to unconjugated hyperbilirubinaemia as a result of competition with bilirubin for uptake at hepatocyte plasma membrane.

Whether the hepatotoxicity is due to the additive effect of isoniazid and rifampicin or due to their synergistic effect; whether the toxicity is due to direct toxic effect of drugs or is a hypersensitivity phenomenon is also being currently debated. The increased risk of hepatotoxicity with isoniazid and rifampicin combination has been attributed to the interaction between the metabolism of isoniazid and rifampicin. Acetyl-isoniazid, the principal metabolite of isoniazid, is converted to monoacetyl hydrazine. The microsomal p-450 enzmecnsocvert monoacetyl hydrazine to other compounds resulting in hepatotoxicity. Rifampicin is thought to enhance this effect by enzyme induction. The first human case of a proven hepatotoxic interaction between isoniazid and rifampicin has recently been reported by Askgaard et al.

A 35-year-old black Somalian patient with miliary tuberculosis developed hepatotoxicity after a few days of treatment with isoniazid, rifampicin, pyrazinamide and ethambutol. On withdrawing all the drugs, the liver profile normalised and remained so after isoniazid challenge. Hepatotoxicity recurred when rifampicin was added but it was well-tolerated when rifampicin was re-introduced without isoniazid.

The exact pathogenetic mechanism for the DIH caused by pyrazinamide has not been understood. In patients receiving a combination of isoniazid, rifampicin and pyrazinamide, two patterns of fulminant liver injury have been observed. Increase in serum transaminase activity which occurs late (usually after one month) has been attributed to pyrazinamide-induced hepatotoxicity while the early increase in transaminases (usually within first 15 days) has been attributed to rifampicin and isoniazid-induced hepatotoxicity.

**Factors Implicated in The Development of Antituberculosis Treatment-Induced Hepatotoxicity**

Advanced age, female sex, alcoholism, underlying liver disease, acetylator phenotype, N-acetyltransferase (NAT) activity, glutathione S-transferase activity, hepatitis B and C virus, human immunodeficiency virus (HIV) infection, extensive disease, malnutrition, have also been observed to be risk factors for the development of DIH (Table 2). These issues have been discussed in earlier review.

**MOLECULAR MECHANISMS OF ANTITUBERCULOSIS DRUG-INDUCED HEPATOTOXICITY**

**Genetic Factors**

Ethnic variations have been observed in subjects developing DIH. For example, a higher risk of DIH has been reported in Indian patients than in patients from the West. Sharma et al recently reported the major histocompatibility complex (MHC) class II alleles and clinical risk factors for the development of...
Studies by Sarma et al. showed that the hepatotoxic concomitant administration of rifampicin and isoniazid could be greater in slow acetylators than in rapid acetylators. A small portion of isoniazid is directly hydrolysed and the proportion of drug metabolised through this “direct pathway” contesting this theory. Other reports have suggested that products of hydrolysis rather than slow acetylators, it was suggested that rapid acetylators are more prone to hepatotoxicity. However, the observations that both rapid and slow acetylators excreted similar proportions of monoacetyl hydrazine suggested that, in rapid acetylators, the more rapid formation acetyl-isoniazid to monoacetyl hydrazine is compensated by its more rapid conversion to diacetyl hydrazone and its excretion contesting this theory.

Other reports have suggested that products of hydrolysis rather than acetylation are the critical toxic metabolites of isoniazid. A small portion of isoniazid is directly hydrolysed and the proportion of drug metabolised through this “direct pathway” is greater in slow acetylators than in rapid acetylators. Studies by Sarma et al showed that the hepatotoxic action of metabolites of isoniazid is due to the hydrazone formed from isoniazid. Rifampicin induces the metabolism of isoniazid by isoniazid hydrolase resulting in the formation of isonicotinic acid and hydrazine. It has been suggested that concomitant administration of rifampicin and isoniazid could result in increasing levels of hydrazine and this could provoke hepatotoxicity especially in slow acetylators. This hypothesis is supported by the finding of increased hepatotoxicity in slow acetylators.

### Acetylator Phenotype

There is considerable confusion in the literature regarding the acetylator phenotype and the hepatotoxicity. Because acetyl-isoniazid formation occurs in larger amounts in rapid rather than slow acetylators, it was suggested that rapid acetylators are more prone to hepatotoxicity. However, the observations that both rapid and slow acetylators excreted similar proportions of monoacetyl hydrazine suggested that, in rapid acetylators, the more rapid formation acetyl-isoniazid to monoacetyl hydrazine is compensated by its more rapid conversion to diacetyl hydrazine and its excretion contesting this theory.

### N-acetyl transferase

N-acetyl transferase (NAT) activity, one of the earliest pharmacogenetic traits to be recognized, was first identified as the genetically controlled step for the inactivation of isoniazid. Molecular genetic studies of NAT in humans revealed the presence of three loci, two of which encode distinct enzymes with similar action and the third is a pseudogene. Human NAT1 is found in liver, gut and almost all tissues. It acetylates para-aminosalicylate and para-amino benzoic acid. In contrast, humans NAT2, found primarily in the liver and intestinal epithelium, acetylates substrates such as isoniazid, dapsone and arylamine carcinogens. Gene mapping studies in humans have demonstrated that the NAT genes are located between 170 and 360 kb at 8p22. The coding region for both NAT1 and NAT2 is 870bp and is intron-less. Both NAT1 and NAT2 loci are highly polymorphic. The genotype-phenotype correlation study for human NAT2 has revealed alleles associated with rapid and slow acetylation. Isoniazid is metabolized to hepatotoxic intermediates by the isoenzyme NAT2 and cytochrome P450 2E1 (CYP2E1). However, the association of polymorphic NAT acetylation status and DIH induced by isoniazid is not clear. Huang et al reported that NAT2 slow acetylator status and DIH induced by isoniazid was not clear. Huang et al also reported that NAT2 slow acetylator genotype significantly affected the development of DIH due to ATT. Additionally, it was also observed that slow acetylators were prone to develop more severe hepatotoxicity than rapid acetylators. Ohno et al also reported that NAT2 slow acetylator genotype significantly affected the development of DIH due to isoniazid and rifampicin. In another report, even after adjustment for acetylator status and age, the CYP2E1 c1/c1 genotype remained an independent risk factor for DIH due to ATT. Moreover, the frequency of homozygous ‘null’ mutation at the CYP2E1 gene was significantly higher among cases suggesting that CYP2E1 genetic polymorphism may be associated with susceptibility to DIH caused by antituberculosis drugs.

### Glutathione S-transferase

In a case-control study of polymorphisms at the glutathione S-transferase (GST) loci (GSTM1 and GSTT1) and their relation to the development of DIH due to antituberculosis drugs, it was reported that the frequencies of mutations at GSTT1 and NAT2 genes did not differ significantly between cases and controls. However, frequency of homozygous ‘null’ mutation at the GSTM1 gene was significantly higher among cases suggesting that these mutations could predispose to the development of DIH due to antituberculosis drugs.

### Clinical Implications

Certain curious facts emerge when the published literature regarding antituberculosis drugs and DIH is reviewed. During the last 38 years of its use, it was observed that a large proportion of the subjects who were treated for LTBI with isoniazid monotherapy developed asymptomatic elevation of hepatic transaminases. While the DIH rate in initial studies ranged from 1 per cent to 10 per cent recent observations where clinically relevant DIH was evaluated suggested that less than 1 per cent subjects receiving isoniazid for treatment of LTBI developed DIH. Recently, in HIV-positive patients, the regimen of rifampicin and pyrazinamide administered for two months was observed to be as efficacious as isoniazid administered for one year for the treatment of LTBI and was found to be well tolerated. Even though the rifampicin and pyrazinamide regimen

---

**Table 2: Risk factors for the development of antituberculosis treatment-induced hepatotoxicity**

<table>
<thead>
<tr>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced age</td>
</tr>
<tr>
<td>Female sex</td>
</tr>
<tr>
<td>Moderately/far advanced/extensive disease</td>
</tr>
<tr>
<td>Hypoalbuminaemia, malnutrition</td>
</tr>
<tr>
<td>Alcoholism</td>
</tr>
<tr>
<td>Underlying liver disease</td>
</tr>
<tr>
<td>Hepatitis B virus infection</td>
</tr>
<tr>
<td>Hepatitis C virus infection</td>
</tr>
<tr>
<td>HIV infection</td>
</tr>
<tr>
<td>Acetylator phenotype</td>
</tr>
<tr>
<td>N-acetyltransferase (NAT) activity</td>
</tr>
<tr>
<td>Glutathione S-transferase activity</td>
</tr>
</tbody>
</table>

Data from references 11, 20-23
and pyrazinamide regimen. 
Revised guidelines recommended among 5.8 per cent of 1311 patients treated with the rifampicin published, severe liver injury including deaths were reported acute and subacute liver failure. 

12 per cent while it was 75 per cent in patients who developed in patients with DIH caused by antituberculosis treatment was bilirubin and serum alkaline phosphatase along with increase serum alkaline phosphatase. Disproportionate increase in serum bilirubin and serum alkaline phosphatase sometimes be accompanied by increase in serum bilirubin and serum alkaline phosphatase which may result in elevation of hepatic transaminases which may sometimes be accompanied by increase in serum bilirubin and serum alkaline phosphatase. Disproportionate increase in serum bilirubin and serum alkaline phosphatase along with increase in serum transaminases has been observed very often with rifampicin. 

Presence of at least one of the following criteria raises the possibility of DIH due to antituberculosis drugs. These include: (i) a rise of five times the upper limit of normal levels (50 IU/L) of AST and/or alanine aminotransferase (ALT); (ii) a rise in the level of serum total bilirubin 1.5 mg/dl; and (iii) any increase in AST and/or ALT above pre-treatment levels together with anorexia, nausea, vomiting, and jaundice. Some workers have suggested that if the transaminase levels are less than five times the upper normal limit, the toxicity was considered mild. When the transaminase levels were increased to five to ten times the normal, the toxicity was considered to be moderate. Elevation of transaminases more than 10 times the upper normal limit suggests severe toxicity. 

**MANAGEMENT**

Ideally, antituberculosis treatment should be individualised according to the body weight and co-morbid illnesses present in the patient. Whenever feasible, baseline liver function testing must be done. When drug-induced hepatotoxicity is suspected, the patient receiving antituberculosis-treatment should be systematically investigated for other causes such as viral hepatitis. Consensus guidelines for the management of patients with antituberculosis treatment-induced hepatotoxicity are yet to be evolved. The Joint Tuberculosis Committee of The British Thoracic Society recommendations and the recent guidelines published by the American Thoracic Society, Centers for Disease Control and Prevention and the Infectious Diseases Society (ATS/CDC/IDSA) form the basis for the diagnosis and management principles listed below. 

Once the diagnosis of DIH is established, it is essential to first stop all potentially hepatotoxic drugs till complete clinical and biochemical resolution of hepatotoxicity occurs. In the interim period, at least three non-hepatotoxic drugs such as ethambutol, streptomycin and quinolones such as levofloxacin or ofloxacin or ciprofloxacin can be used after appropriate evaluation of renal function and visual acuity. 

After complete resolution of transaminosis, most antituberculosis drugs can be safely restarted in a phased manner. The British Thoracic Society guidelines suggested that the first-line drugs can be reintroduced sequentially in the order isoniazid, rifampicin and pyrazinamide. With daily monitoring of the patient's condition and liver function. Isoniazid should be introduced at 50 mg/day, gradually increasing sequentially to 300 mg/day over two to three days if it is well tolerated and continued thereafter. After a further period of two to three days, rifampicin is introduced at a dose of 75 mg/day increasing to 300 mg/day after two to three days and then increased to 450 mg (<50 kg) or 600 mg (>50 kg) as appropriate for the patient's weight after a further period of two to three days. If this is tolerated, it is then continued. Finally, pyrazinamide can be added at 250 mg/day increasing to 1000 mg after two to three days and then to 1500 mg (<50 kg) or 2000 mg (>50 kg) as appropriate for the patient's body weight. If these drugs are well tolerated, they are continued and the alternative drugs introduced temporarily can be withdrawn.
RECURRENT OF DIH ON RE-TREATMENT

The re-introduction of antituberculosis drugs has seldom been systematically studied and a great deal of controversy exists regarding sequence in which the drugs are to be reintroduced, whether the re-introduction should be done in full dosage or in gradually escalating dosages. Usually, it is possible to safely re-introduce the same drugs that have been implicated in the causation of DIH in a majority of the patients. Review of published literature suggests that, the recurrence rate of DIH when antituberculosis drugs are re-introduced was less than 7 per cent, though a recurrence rate of more than 25 per cent has been cited in some studies. In a study from New Delhi, Singh et al. reported that, after resolution of DIH, reintroduction of isoniazid and rifampicin was possible in 41 of 44 patients suggesting that the recurrence rate of DIH on reintroduction was 6.8 per cent. In the study reported by Telman et al., 55 of the cohort of 1036 patients (5.3%) developed DIH. Treatment was re-introduced in 48 patients and successfully completed by 46, though a recurrence rate of more than 25 per cent has been published. Literature suggests that, the recurrence rate of DIH is causation of DIH in a majority of the patients. Review of published literature suggests that, the recurrence rate of DIH when antituberculosis drugs are re-introduced was less than 7 per cent. Though a recurrence rate of more than 25 per cent has been cited in some studies. In a study from New Delhi, Singh et al. reported that, after resolution of DIH, reintroduction of isoniazid and rifampicin was possible in 41 of 44 patients suggesting that the recurrence rate of DIH on reintroduction was 6.8 per cent. In the study reported by Telman et al., 55 of the cohort of 1036 patients (5.3%) developed DIH. Treatment was re-introduced in 48 patients and successfully completed by 46, though a recurrence rate of more than 25 per cent has been published. Literature suggests that, the recurrence rate of DIH is causation of DIH in a majority of the patients.

ISSUES TO BE RESOLVED

Consensus guidelines for the management of patients with antituberculosis treatment-induced hepatotoxicity are yet to be evolved. The re-introduction of antituberculosis drugs has seldom been systematically studied and a great deal of controversy exists regarding sequence in which the drugs are to be reintroduced, whether the re-introduction should be done in full dosage or in gradually escalating dosage. Since there is no consensus on these issues, large multicentric studies are required to provide answers to these questions.

REFERENCES


ANTITUBERCULOSIS TREATMENT-INDUCED HEPATOTOXICITY: FROM BENCH TO BEDSIDE

483