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.**

96

ABSTRACT

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 observedto have hepatotoxic potential and drug-induced hepatotoxicity (DIH) is an important adverse effect with antituberculosis treatment. Idiosyncratic damage, dose-dependent toxicity, induction ofhepatic enzymes, drug-induced acute hepatitis and allergic reactionshave 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 involvingcytochrome P-450 system, activation of apoptotic pathways and programmed cell death, and inhibition ofmitochondrial 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 (CYPE21), 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 ofliver function derangement such as co-existent viral hepatitis mustbe 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.[1] 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 conjugationto 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.[1-3] 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 essentialcomponents of thedirectly observed treatment, short-course (DOTS) strategy for control of tuberculosis endorsed by theWorld Health Organization (WHO) [4,5] and all the three drugs have been observedto 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 withantituberculosis treatment.[6-8]

Recent developments especially in the field of molecular biology have expanded our views on the understanding of drug-induced liver damage. [9,10] In this paper, we have attempted to summarise the current understanding of the pathogenetic mechanismsof DIH as it has been viewed from the laboratory bench and the bed-sideimplications of these observations to the practicingclinician with particular reference toantituberculosis drugs.

MECHANISMS OF DRUG-INDUCED HEPATOTOXICITY

Several types of drug-induced liver damagehave been described. These include, (i) idiosyncratic damage;(ii) dose-dependent toxicity; (iii) induction ofhepatic enzymes; (iv) drug-induced acute hepatitis; and (v) allergic reactions; among others.[1-3,9]

Idiosyncratic reactions are the result of a "multihitprocess"due to the succession of unlikely events and are characterised by a variable latency periodfrom the initial time of ingestion of the drug.[1,9] 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 ofwhether

Table 1 : Specific patterns of hepatic damage

Disruption of intracellular calcium homeostasis
Cholestatic damage
Interruption of transport pumps and loss of villous processes
Reactions involvingcytochrome P-450 system
Activation of apoptotic pathways and programmed cell death
Inhibition ofmitochondrial function

the initial reaction was severe or mild.In the case of somedrugs such as acetaminophen, hepatic damage occurs in a dose-dependent fashion. Induction of hepatic enzymes by drugs such as ethanol, phenobarbitone andphenytoin, 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 andsevere hepatocyte injury constituting the "reactive metabolite syndrome".[1] 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).[1] Cell membrane bleb formation, rupture and cell lysis are the result ofdisruption of intracellular calcium homeostasisleading 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 ofthe excretion of bilirubin and other organic compounds.In the reactions involvingcytochrome P-450 system, covalent binding of the drug to the enzyme results inthe creation of non-functioning adducts that migrate to the cell surface and serve as targets for cytolytic attack by T-lymphocytes and triggering ofa multifaceted immune response. Activation of apoptotic pathways may trigger the cascade of intercellular caspases resulting inprogrammed cell death with loss of nuclear chromatin.Inhibition ofmitochondrial functioncan occur due to effect on enzymes ofoxidation and the respiratory chain leading to impaired metabolism of free fatty acids, lack of aerobic respiration, and theaccumulation of lactate and reactive oxygen species.Finally, toxic metabolites excreted in bile may damage bile-duct epithelium.[1-3]

ANTITUBERCULOSIS DRUGS AND HEPATOTOXICITY

The pathogenesis of DIH caused by isoniazidis not well-understood.[11] Histopathological evidence resembling that of viral hepatitis showing hepatocyte necrosis, ballooning degeneration and inflammatory infiltrates suggests dose-related toxicity.[12] However, lack of direct correlation between serum drug levels and hepatotoxicity argues against a direct toxic effect.[13] Given thedelayed 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,[14,15] hypersensitivity is considered unlikely. But, presence ofeosinophilic infiltrates on liver biopsy and recurrence of hepatotoxicityon re-challenge with the drug suggest hypersensitivity as a possible mechanism.[15]

Altered profile of antioxidant enzymes with increased lipid peroxidation indicated that isoniazid and rifampicin-induced hepatotoxicity appeared to be mediated through oxidative-stress.[16] Compared with isoniazid, DIH caused by rifampicin occurs earlier and produces a patchy cellular abnormality with marked periportal inflammation.[17] 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.[17]

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 enzymesconvert 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.[18] 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 itwas 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.[19]

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).[20-23] These issues have been discussed in earlier review.[11]

MOLECULAR MECHANISMS OF ANTITUBERCULOSIS DRUG-INDUCED HEPATOTOXICITY

Genetic Factors

Ethnic variations have been observed in subjects developing DIH. For example, ahigher risk ofDIH has been reportedin Indian patients than in patients from the West.[20,24] Sharma et al [20] recently reported the major histocompatibility complex (MHC) class II alleles and clinical risk factors for the development of

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

Advanced age
Female sex
Moderately/far advanced/extensive disease
Hypoalbuminaemia, malnutrition
Alcoholism
Underlying liver disease
Hepatitis B virus infection
Hepatitis C virus infection
HIV infection
Acetylator phenotype
N-acetyltransferase (NAT) activity
Glutathione S-transferase activity

Data from references 11, 20-23

hepatotoxicity in 346 North Indian patients with tuberculosis receivingantituberculosis treatment. Of these, 56 patients (16%) developed drug-induced hepatotoxicity, whereas the remaining 290 patients did not. Multivariate logistic regression analysis, older age [odds ratio (OR) 1.2], moderately/far advanced disease (OR 2.0), serum albumin less than 3.5 g/dl (OR 2.3), absence of HLA-DQA1*0102 (OR 4.0), and presence of HLA-DQB1*0201 (OR 1.9) were independent risk factors for the development of DIH.[20] Certain facts have to be kept in mind while interpreting studies relating to HLA. The number of subjects studied must be sufficient to allow for adequate power to detect a difference and to adjust the probability of no association with HLA by the number of tests performed. [21]

Acetylator Phenotype

There is considerable confusion in the literature regarding the acetylator phenotype and the hepatotoxicity.[20,22,25-28] 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. [29] However, theobservations 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 [30,31] 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 andthe proportion of drug metabolised through this "direct pathway" is greater in slow acetylators than in rapid acetylators.[32] Studies by Sarma et alassociates [33] showed that the hepatotoxic action of metabolites of isoniazid is due to the hydrazine formed from isoniazid. Rifampicin induces the metabolism of isoniazid by isoniazid hydrolase resulting in the formation of isonicotinic acid and hydrazine.[34] 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. [35] This hypothesis is supported by the finding of increased hepatotoxicity in slow acetylators.[35]

N-acetyl transferase

N-acetyltransferase (NAT) activity, one of the earliest pharmacogenetic traits to be recognized, was first identified as the genetically controlled step for the inactivation ofisoniazid. 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.[36] Human NAT1 is found in liver, gutand almost all tissues. It acetylatespara-amino salicylate and para-amino benzoic acid.In contrast, humans NAT2, found primarily in the liver andintestinal 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 intronless.[23,36] 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 isoenzymeNAT2 and cytochrome P450 2E1 (CYP2E1). However, the association of polymorphic NAT acetylator status and DIH induced by isoniazidis not clear.[23]Huang et al [37] reported that NAT2 slow-acetylator statusand agewere the onlyindependent risk factors for DIH due to ATT. Additionally; it was also observed that slow acetylators were prone to develop more severe hepatotoxicity than rapid acetylators.[37] Ohno et al[38] also reported thatNAT2 slow acetylator genotype significantly affected the development of DIH due to isoniazid and rifampicin. In another report,[39] even after adjustment for acetylator status and age, the CYP2E1 c1/c1 genotype remained an independent risk factor for hepatotoxicity suggesting thatCYP2E1 genetic polymorphism may be associated with susceptibility to DIH caused by antituberculosis drugs.

Glutathione S-transferase

In a case-control study[40] 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.[40]

CLINICAL IMPLICATIONS

Certain curious facts emerge when the published literature regarding antituberculosis drugsand DIH is reviewed. During the last 38 years of its use, it was observed thata large proportionof thesubjects who were treated for latent tuberculosis infection (LTBI) with isoniazid monotherapy developed asymptomatic elevation of hepatic transaminases. While the DIH rate in initial studies ranged from 1 per centto 10 per cent [41] recent observations where clinically relevant DIH was evaluated suggested that less than 1 per centsubjects receiving isoniazid for treatment of LTBI developed DIH[42]. Recently, in HIV-positive patients, the regimen of rifampicin and pyrazinamideadministered for two months was observed to be as efficacious as isoniazid administered for one year for the treatment ofLTBI and was found to be well tolerated.Even though the rifampicin and pyrazinamide regimen

Table 3: Clinical syndromes observed in patients with drug-induced hepatotoxicity

Abnormal liver function tests in asymptomatic patients
Acute viral hepatitis-like presentation
Acute (fulminant) hepatic failure
Subacute hepatic failure
Acute venous outflow obstruction
Cholestatic hepatitis, obstructive jaundice, chronic cholestasis
Liver disease with signs of hypersensitivity and/or disease in other organs
Auto-immune hepatitis-like injury
Cirrhosis
Primary hepatic neoplasms

Adapted from reference 9

was never tried outin them, this regimen was offered to HIV-negative patients also in the recently published American Thoracic Society (ATS) guidelines.[43] After these guidelines were published,severe liver injury including deaths were reported among5.8 per cent of 1311 patientstreated with the rifampicin and pyrazinamide regimen.[44] Revised guidelines[45] recommended that rifampicin and pyrazinamide regimen should generally not be offered topatients with LTBI and the clinicians should choose from the alternative regimens available. This regimen definitely should not be used in persons with underlying liver disease, history of alcoholism, or isoniazid-associated liver injury.[45]

During the period when diverse drug regimens were used to treat TB, the mean incidence of DIH in 1264 patients receiving rifampicin *without* isoniazid (1.1%) was found to besignificantly lower than the 2.6 per cent observed in patients receiving isoniazid *and* rifampicin in a meta-analysis published more than a decade ago.[24] These issues have been discussed in detail in an earlier review.[11]

Well recognized clinical syndromes described in patients with DIH are listed in Table 3.[9] While most of the patients with DIH caused by antituberculosis treatment have only asymptomatic elevation of transaminases, few manifest overt icterus. The onset of DIH usually resembles acute viral hepatitis. In majority of the patients, DIH caused by antituberculosis treatment resolves spontaneously following withdrawal of the offending drugs. However, substantial proportion of patients may develop severe liver damage leading to acute or subacute liver failure with subsequent death. Singh et al[46] reported that, overall mortality in patients with DIH caused by antituberculosis treatment was 12 per cent while it was 75 per cent in patients who developed acute and subacute liver failure.

DIAGNOSIS

Asymptomatic increase in aspartate aminotransferase (AST) has been observed in about 20% of patients receiving the standard four-drug regimen.[47] If the patient is asymptomatic, therapy should not be altered because of modest elevations of AST, but the patient should be more closely monitored. Antituberculosis drugs result in elevation of hepatic transaminases which may sometimes be accompanied by increase in serum bilirubin and serumalkaline phosphatase. Disproportionate increase in serum bilirubin and serum alkaline phosphatase along with increase in serum transaminases has been observed very often with rifampicin.[47]

Presence of at least one of the following criteria raises the possibility ofDIH dueto antituberculosis drugs. [20,22] 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 lessthan 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 theupper normal limit suggestssevere toxicity.[47]

MANAGEMENT

Ideally, antituberculosis treatment should be individualised according to the body weight and co-morbid illnesses present in the patient.[11] 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 [48] and the recent guidelines published by the American Thoracic Society, Centers for Disease Control and Prevention and the Infectious Diseases Society (ATS/CDC/IDSA)[47] form the basis for the diagnosis and management principles listed below.

Once the diagnosis ofDIH 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.[11]

After complete resolution of transaminitis, most antituberculosis drugs can be safely restarted in a phased manner. The British Thoracic Society guidelines[48] suggested that the first-line drugs can be reintroducedsequentially 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.

RECURRENCE 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 reintroduction 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[46,49] though a recurrence rate ofmore than 25 per cent has been cited in some studies.[50,51] In a study from New Delhi, Singh et al[46] 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[49], 55 of the cohort of 1036 patients (5.3%) developed DIH. Treatment was re-introduced in 48 patients and successfully completed in 45 patientsindicating that the recurrence rate ofDIH on reintroduction of antituberculosis treatment was 6.3 per cent. In a randomized prospectivestudy from Turkey [50] patients who developed DIH on antituberculosis treatment (n=45)were retreated with a drug regimen consisting of isoniazid, rifampicin, ethambutol and streptomycin administered by gradually increasing the number and dosage of the drugs (group I, n = 20). The remaining patients (group II, n = 25) were retreated with the same regimen (isoniazid, rifampicin, pyrazinamide and ethambutol) in the same dosages throughout. While none of the patients in group I developed recurrence of DIH, six (24%) of the patients belonging to group II developed recurrence of DIH (p = 0.021). The patients who developed recurrence of DIH while receiving group II regimen were then treated with the regimen used for group I patients and it was observed that all the patients recovered. The observations from this study suggest thatrecurrence rate ofDIH is higher when the full-dose regimen including pyrazinamide is used compared with gradual reintroduction of rifampicin and isoniazid in a regimen that does not contain pyrazinamide. These observations need to be confirmed in studies with a large sample size. In a study from Copenhagen,[51] 61 of the 752 patients with tuberculosis (8%) developed DIH. Recurrence of DIH was observed in 16 of these 61 patients (26.2%) on reintroductionof antituberculosis treatment and they required a modified regimen. Multicentric prospective randomized studies with a large sample size are required to clarify these issues.

According to the ATS/CDC/IDSA guidelines[47] the reintroduction protocol is somewhat different. According to these guidelines, suspected antituberculosis drugs can be started one at a time once the transaminase levels return to less than two times the upper normal. Rifampicin is to be restarted first. If the liver functions remain normal after one week, isoniazid can be added to the regimen. If the liver functions remain normal after one week, then pyrazinamide is added. If there is recurrence of symptoms or deterioration of liver functions, the last added drug should be stopped. Depending on the number of doses taken, bacteriological status and the severity of the disease, the treatment may have to beindividualized and extended.

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 reintroduction 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

1. Lee WM. Drug-induced hepatotoxicity. *N Engl J Med* 2003;349:474-85.
2. Farrell GC, Weltman M. Drug-induced liver disease. In: Gitnick G, editor. Current Hepatology. Vol.16. St. Louis: Mosby;1996.p.143-208.
3. Farrell GC. Drug-induced liver disease. Edinburgh: Churchill-Livingstone;1994.p.1-673.
4. World Health Organization.What is DOTS? AvailablefromURL: http://www.who.int/gtb/dots/whatisdots.htm. Accessed on 28 August, 2004.
5. Maher D, Chaulet P, Spinaci A, Harries A. Treatment of tuberculosis: guidelines for National Programmes. Geneva: World Health Organization;1997.
6. Girling DJ. The hepatic toxicity of antituberculous regimens containing isoniazid, rifampicin, and pyrazinamide. *Tubercle* 1978;59:13-32.
7. Schaberg T, Rebhan K, Lode H. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. *Eur Respir* J 1996;9:2026-30.
8. Mohan A, Sharma SK. Side effects of antituberculosis drugs. *Am J Respir Crit Care Med* 2004;169:882-3.
9. Goodman ZD. Drug hepatotoxicity. *Clin Liver Dis* 2002;6:381-97.
10. Kaplowitz N. Causality assessment versus guilt-by-association in drug hepatotoxicity. *Hepatology* 2001;33:308-9.
11. Sharma SK, Mohan A.Antituberculosis treatment induced hepatotoxicity. In: Venkataraman GS, editor.Medicine update. Vol.14. Mumbai: Association of Physicians of India; 2004.p.298-306.
12. Black M, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. *Gastroenterology* 1975;69:289-302
13. Mitchell JR, Long MWW, Thorgeirsson UP, Jollow DJ. Acetylation rates and monthly liver function tests during one year of isoniazid preventive therapy. *Chest* 1975;68: 181-90.
14. Meddrey WC, Boitmott JK. Isoniazid hepatitis. *Ann Intern Med* 1973;70: 1-12.
15. Gangadharam PR. Isoniazid, rifampin and hepatotoxicity (editorial). *Am Rev Respir Dis* 1986;133:963-5.
16. Sodhi CP, Rana SV, Mehta SK, Vaiphei K, Attari S, Mehta S. Study of oxidative-stress in isoniazid-rifampicin-induced hepatic injury in young rats. *Drug Chem Toxicol* 1997;20: 255-69.
17. Kenwright S, Levi AJ. Sites for competition in selective hepatic uptake of rifampicin, flavaspidic acid, bilirubin and bromsulphthalein. *Gut* 1974; 15:220-6.
18. Askgaard DS, Wilcke T, Dossing M. Hepatotoxicity caused by the combined treatment action of isoniazid and rifampicin. *Thorax* 1996;50: 213-4.
19. Devoto FM, Gonzalez C, Iannantuono R, Serra HA, Gonzalez CD, Saenz C. Risk factors for hepatotoxicity induced by antituberculosis drugs. *Acta Physiol Pharma Ther Latin Am* 1997;47:197-202.
20. Sharma SK, Balamurugan A, Saha PK, Pandey RM, Mehra NK. Evaluation of clinical and immunogenetic risk factors for the development of hepatotoxicity during antituberculosis treatment. *Am J Respir Crit Care Med* 2002;166:916-9.
21. Bothamley GH. Treatment, tuberculosis, and human leukocyte antigen (editorial). *Am J Respir CritCare Med* 2002;166:907-8.
22. Pande JN, Singh SPN, Khilnani GC, Khilnani S, Tandon RK. Risk factors for hepatotoxicity from antituberculosis drugs: a case-control study. *Thorax* 1996;51:132-6.

23. Sharma SK. Antituberculosis drugs and hepatotoxicity. *Infect Genet Evol* 2004;4:167-70.
24. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampicin. A meta-analysis. *Chest* 1991;99:465-71.
25. Parthasathy R, Sarma GR, Janardhanam B, Ramachandran R, Santha T, Sivasubramanian S, et al. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle* 1986;67:99-108.
26. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D.Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *Am J Respir Crit Care Med* 2003;167: 1472-7.
27. Gurumurthy P, Krishnamurthy MS, Nazareth O, Parthsarathy R, Sarma GR, Somasundaram PR, et al. Lack of relationship between hepatic toxicity and acetylator phenotype in three thousand South Indian patients during treatment with isoniazid for tuberculosis. *Am Rev Respir Dis* 1984; 129:58-61.
28. Singh J, Garg PK, Thakur VS, Tandon RK. Antitubercular treatment induced hepatotoxicity: Does acetylator status matter? *Indian J Physiol Pharmacol* 1995;35:43-6.
29. Mitchell JR, Thorgeirsson UP, Black M, Timbrell JA, Snodgrass WR, Potter WZ, et al. Increased incidence of isoniazid hepatitis in rapid acetylators, possible relation to hydrazine metabolites. *Clin Pharmacol Ther* 1975;18:70-9.
30. Ellard GA, Mitchison DA, Girling DJ, Nunn AJ, Fox W. The hepatic toxicity of isoniazid among rapid and slow acetylators of the drug. *Am Rev Respir Dis* 1978 118:628-9.
31. Lauterburg BH, Smith CV, Todd EL, Mitchell JR. Pharmacokinetics of the toxic hydrazine metabolites formed from isoniazid in human. *Pharmacol Exp Ther* 1985;235:566-70.
32. Ellard GA, Gammon PT. Pharmacokinetics of isoniazid metabolism in man. *J Pharmacokinet Biopharm* 1976;4: 83-113.
33. Sarma GR, Immanuel C, Kailasam S, Narayana AL, Ventakatesan P. Rifampin-induced release of hydrazine from isoniazid. A possible cause of hepatitis during treatment of tuberculosis with regimens containing isoniazid and rifampin. *Am Rev Respir Dis* 1986;133:1072-1105.
34. Blair IA, Mansilla Tinoco R, Brodie MJ, Clare RA, Dollery CT, Timbrell JA, et al. Plasma hydrazine concentrations in man after isoniazid and hydralazine administration. *Hum Toxicol* 1985;4:195-202.
35. Weber WW, Hein DW, Litwin A, Lower GM Jr. Relationship of acetylator status to isoniazid toxicity, lupus erythematosus, and bladder cancer. *Fed Proc* 1983;42:3086-90.
36. Sim E, Payton M, Noble M, Minchin R. An update on genetic, structural and functional studies of arylamine N-acetyltransferases in eucaryotes and procaryotes. *Hum Mol Genet* 2000;9:2435-41.
37. Huang YS, Chern HD, Su WJ, Wu JC, Chang SC, Chiang CH, et al.Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. *Hepatology* 2003;37:924-30.
38. Ohno M, Yamaguchi I, Yamamoto I, Fukuda T, Yokota S, Maekura R, et al. Slow N-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. *Int J Tuberc Lung Dis* 2000;4: 256-61.
39. Huang YS, Chern HD, Su WJ, Wu JC, Lai SL, Yang SY, et al. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis. *Hepatology* 2002;35:883-9.
40. Roy B, Chowdhury A, Kundu S, Santra A, Dey B, Chakraborty M, Majumder PP. Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. *J Gastroenterol Hepatol* 2001;16:1033-7.
41. Nolan CM. Isoniazid for latent tuberculosis infection. Approaching 40 and reaching its prime. *Am J Respir Crit Care Med* 2003;168:412-3.
42. Nolan CM, Goldberg SV, Buskin SE.Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. *JAMA* 1999;281:1014-8.
43. Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000;161:S221-47.
44. Jasmer RM, Daley CL. Rifampin and pyrazinamide for treatment of latent tuberculosis infection: is it safe? *Am J Respir Crit Care Med* 2003;167:809-10.
45. Centers for Disease Control and Prevention (CDC), American Thoracic Society. Update: adverse event data and revised American Thoracic Society/ CDC recommendations against the use of rifampin and pyrazinamide for treatment of latent tuberculosis infection-United States, 2003. *Morb Mortal Wkly Rep* 2003;52:735-9.
46. Singh J, Garg PK, Tandon RK. Hepatotoxicity due to antituberculosis therapy. Clinical profile and reintroduction of therapy. *J Clin Gastroenterol* 1996;22:211-4.
47. Blumberg HM, Burman WJ, Chaisson RE, Daley CL, Etkind SC, Friedman LN, et al. American Thoracic Society, Centers for Disease Control and Prevention and the Infectious Diseases Society. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med* 2003;167:603-62.
48. Joint Tuberculosis Committee of the British Thoracic Society. Chemotherapy and management of tuberculosis in the United Kingdom: recommendations 1998. *Thorax* 1998;53:536-48.
49. Teleman MD, Chee CB, Earnest A, Wang YT. Hepatotoxicity of tuberculosis chemotherapy under general programme conditions in Singapore. *Int J Tuberc Lung Dis* 2002;6:699-705.
50. Tahaoglu K, Atac G, Sevim T, Tarun T, Yazicioglu O, Horzum G, et al.The management of anti-tuberculosis drug-induced hepatotoxicity. *Int J Tuberc Lung Dis* 2001;5:65-9.
51. Dossing M, Wilcke JT, Askgaard DS, Nybo B. Liver injury during antituberculosis treatment: an 11-year study. *Tuber Lung Dis* 1996;77: 335-40.