REVIEW

Isoniazid metabolism and hepatotoxicity

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Abstract Isoniazid (INH) is highly effective for the management of tuberculosis. However, it can cause liver injury and even liver failure. INH metabolism has been thought to be associated with INH-induced liver injury. This review summarized the metabolic pathways of INH and discussed their associations with INH-induced liver injury.

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Abbreviations: AcHz, acetylhydrazine; AcINH, acetylisoniazid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; DiAcHz, diacetylhydrazine; GSH, glutathione; GST, glutathione S-transferase; Hz, hydrazine; INA, isonicotinic acid; INH, isoniazid; MPO, myeloperoxidase; NAD, nicotinamide adenine dinucleotide; NAT, N-acetyltransferase; P450, cytochrome P450; R.M., reactive metabolite; TB, tuberculosis

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1. Introduction

Tuberculosis (TB) is a global health issue. The standard therapies for TB include a combination treatment of isoniazid (INH), rifampicin, pyrazinamide, and ethambutol. INH can also be used alone for TB prevention. Despite the beneficial effects of INH, severe adverse effects especially peripheral neuropathy and hepatotoxicity are associated with INH therapies. About 10%–20% of patients consuming INH have a transient elevation of serum alanine aminotransferase (ALT) level. Most of the patients can adapt to it and their serum ALT levels return to normal without discontinuation, while some patients (less than 1%–3%) develop severe liver injury and even liver failure. The most current report from the Drug-Induced Liver Injury Network (DILI) indicates that the true incidence of INH-induced liver injury is largely under-reported in the United States, and it is the second-ranking drug that causes liver injury in spite of under-reporting.

Clinically, INH-associated treatments usually cause a hepatocellular-type of liver injury, as characterized by a marked elevation of ALT levels (> 10 times upper limit of normal [ULN]) but minimal increases in alkaline phosphatase (ALP) levels (usually < 2 times the ULN). Even though INH-induced liver injury has been known and extensively studied, its underlying mechanisms are still poorly understood. Different experimental animal models have been used to study the hepatotoxicity of INH, including rats, mice, and rabbits. Unfortunately, there is no validated animal model to recapitulate the human patterns of INH-bound proteins in mice, rat, and human liver microsomes. Furthermore, INH was found to induce microvesicular steatosis in different animal models, including mice, rabbits, and rats, but these phenotypes are usually not observed in patients with INH-induced liver injury.

INH metabolism is thought to be associated with INH-induced liver injury. Acetylhydrazine (AcH), hydrazine (Hz), and acetylisoniazid (AcINH) are the major metabolites of INH. Studies of INH hepatotoxicity in rats showed that AcINH and AcHz can cause hepatic necrosis; however, treatment with INH directly even at high dose and long-term treatment did not cause toxicity. These results suggested INH metabolites are responsible for INH hepatotoxicity. Covalent binding of acetyl group to liver proteins were observed after treating rats with [14C]-labeled AcINH but not with aromatic ring [13C] labeled AcINH, indicating that AcHz is responsible for INH hepatotoxicity in rats. Studies carried out in mice showed different results. When Hz or AcHz was administrated at a dose of 300 mg/kg to mice, Hz produced hepatic necrosis, macrovesicular degeneration, and steatosis, whereas AcHz did not, suggesting that Hz is responsible for INH-induced liver injury in mice. In a rabbit model of INH-induced liver injury, the plasma level of Hz is correlated with the extent of INH-induced necrosis and steatosis, but plasma levels of INH and AcHz are not. In addition, Hz inhibits mitochondrial complex II and affects the function of electron transport chain and ATP production in mouse primary hepatocytes. Co-treatment with Hz and a complex I inhibitor can cause hepatocyte death. Recent studies also found INH itself can bind to liver proteins and cause immune-mediated hepatotoxicity.

In summary, despite extensive studies in INH metabolism and its role in INH-induced liver injury, the observations and conclusions are inconsistent and even controversial. This review summarized and updated the pathways of INH metabolism. We also discussed and provided novel insight into the association of INH metabolism with INH-induced liver injury.

2. The metabolic map of INH

INH is a low-molecular weight and water-soluble compound that can be rapidly absorbed from the gastrointestinal tract. Pharmacokinetic properties of INH are affected by various patient-specific factors, like genetic status, age, comorbidities, and the co-administered food or drugs. The peak plasma concentration is achieved around 1–3 h after administration of the drug. Meals containing high fats can decrease absorption of INH as revealed by the reduction of Cmax by 51% and the increasing of Tmax to 2 times. Hence, it is recommended to consume INH on an empty stomach. After absorption, INH diffuses into all tissues and body fluids rapidly, including cerebrospinal fluid, saliva, pleural and peritoneal exudates, bronchi and pulmonary alveoli. INH also can be excreted into breast milk.

The major pathways of INH metabolism (Fig. 1) include: (1) Acetylation to form AcINH through N-acetyltransferase (NAT) 2; and (2) Hydrolysis to produce isonicotinic acid (INA) and Hz through amidase. AcINH can also be hydrolyzed to form INA and AcHz. Hz and AcHz are thought to be further oxidized to reactive metabolites and involved in INH hepatotoxicity, which was proposed to be mediated by microsomal P450s, especially CYP2E1.

Besides these major metabolic pathways, INH can also conjugate with several endogenous metabolites, including ketone acids, vitamin B6 (pyridoxal and pyridoxal 5-phosphate), and NAD+. In addition, INH was found to disturb the homeostasis of endogenous metabolites, such as vitamin B6, bile acids, cholesterol, and triglycerides. The major metabolic pathways of INH are enzymatic-dependent reactions, including acetylation and hydrolysis of INH by NAT and acyl amidase, respectively. Catalase-peroxidase (KatG) of mycobacterium tuberculosis (Mtb) and human neutrophil myeloperoxidase can catalyze the formation of INH-NAD+ adducts. Nevertheless, conjugation of INH with ketone acids and vitamin B6 are non-enzymatic reactions. We illustrated these metabolic pathways of INH in details in the following sections and discussed their associations with INH hepatotoxicity.

3. Role of NATs in INH metabolism and hepatotoxicity

NATs (EC 2.3.1.5, N-acetyltransferase, arylamine N-acetyltransferases) are a class of enzymes that catalyze the acetylation of arylamines from acetyl-CoA. It is widely found in different species, both in eukaryotes and prokaryotes. NATs are responsible for acetylation of hydrazide drugs and carcinogenic aromatic amines, as well as endogenous molecules, such as serotonin. NAT1 and NAT2 are the major NATs that are involved in the biotransformation of xenobiotics. The NAT genes are located in close vicinity in the genome and share high
sequence identity69, but their expression profiles have distinct tissue distribution patterns and the enzymes have different substrate preferences70,71. NAT1 is widely expressed in all tissues, including endocrine tissues, blood cells, neural tissue, liver, and the gastrointestinal tract, whereas NAT2 expression is limited to the liver and gastrointestinal tract72. p-Aminobenzoate and p-aminoisalicylate, are prefer substrates of NAT1, whereas NAT2 preferentially metabolizes sulfamethazine, procainamide72.

NAT2 is the dominant enzyme that catalyzes the acetylation of INH, Hz, and AcHz46,73,74. NAT2 is involved in three steps of INH biotransformation, including deactivation (formation of AcINH), bioactivation (formation of AcHz), and detoxification (formation of DiAcHz)6,16,68. The role of NAT2 in INH hepatotoxicity is complicated and still controversial13,75,76. NAT2 is highly polymorphic and has been thought to be involved in INH hepatotoxicity68,76–78. Rapid acetylators have been proposed to have a higher risk of INH-induced liver injury than slow acetylators, which is based on the proposition of an increased rate of AcHz formation in rapid acetylators75. This proposition is supported by early clinical observations13,75,76. In one study, 86% of patients with probable and 60% with possible liver injury were rapid acetylators5. In another study with 143 patients who received INH-containing regimens for anti-TB therapies, 18 patients with abnormal elevated levels and 18 patients with normal levels of serum aminotransferase were investigated. They found out that 14 patients with abnormal serum aminotransferase were rapid acetylators, while only 7 were rapid acetylators in patients with a normal serum aminotransferase level59. These results suggest that rapid acetylators have a higher risk of liver injury with INH therapies.

However, the later clinical studies found that the presence of slow acetylator alleles has a higher risk of INH hepatotoxicity72,80,81. In a study of 224 patients that received anti-TB treatment, slow acetylators had a much higher risk of liver toxicity than rapid acetylators (26.4% vs. 11.1%, P = 0.013)77. Another study reported the risks of different acetylator status in INH hepatotoxicity in Brazilian patients. The risk of developing hepatitis was 22% for slow acetylators, while only 9.8% for intermediate acetylators and 2.9% for rapid acetylators81. The plasma levels of INH and AcHz are higher in slow acetylators than those in rapid acetylators, which contradicts previous findings75. Even though the acetylation rate of INH is slow in slow acetylators, the acetylation of AcHz is also slow72, thus leading to a higher accumulation of AcHz in slow acetylators37,46,83. The clearance rate of INH is also slower in slow acetylators than in rapid acetylators38, which also contributes to the accumulation of INH in slow acetylators. A higher level of free INH might be the cause of the high incidence of liver injury directly as INH can bind to liver proteins and cause immune-mediated liver injury15.

Elevation of INH can also lead to an increase in Hz formation, which is supported by an increased rate of hydrolysis process of INH in slow acetylators than in rapid ones77,83,84. Furthermore, decreasing the dose of INH in slow acetylators can reduce the incidence of INH hepatotoxicity85. In a multicenter, paralleled, randomized, and controlled clinical trial with Japanese patients, treatment with a lower dose of INH (2.5 mg/kg) used for anti-TB therapy in slow acetylators significantly decreased the incidence of INH-induced liver injury than the standard treatment (5 mg/kg for all patients)85. In addition, NAT2 status also plays an important role in hepatotoxicity caused by INH and rifampicin combination therapies78. In a study with 77 Japanese patients with INH + rifampicin treatment, the risk of liver injury was much higher in slow acetylators than in intermediate and rapid acetylators78.

Even though these clinical reports showed that slow acetylators have a higher risk of INH-induced liver injury with INH treatment in different populations77,80,81,83, several other clinical observations showed modest or no significant difference of incidence of INH hepatotoxicity between different acetylators status85–89. In addition, the positive prediction value of the NAT2 genotypes for identification of patients with risk of liver injury is low77. Furthermore, the incidence of INH hepatotoxicity did not show significant correlation with NAT2 status in different ethnic populations6,84. The frequency of slow acetylators in the Asian populations (10%–20%) is much lower than that in Caucasians and Africans (more than 50%)77,92, but the incidence of INH hepatotoxicity does not show such dramatic ethnic differences84,91.
Overall, the causal role of NAT2 in INH hepatotoxicity remains controversial and the detailed mechanism is still unknown.

4. Role of amidase in INH metabolism and hepatotoxicity

Amidases (EC 3.5.1.4, acylamidases, acylases, or acylamide amidohydrolases) are a class of enzymes that catalyze the hydrolysis of amides and they usually have carboxylesterase activities that can hydrolyze carboxylic esters. Carboxylesterases also can hydrolyze amides. Amidases and carboxylesterases have similar catalytic mechanisms and share some substrates. Both enzymes can add water to esters or amides and form the corresponding acids and alcohols or amines, without any co-factors. Amidases and carboxylesterases play important roles in maintaining homeostasis of endobiotics. They are also of great importance in hydrolysis of drugs and environmental toxicants. Several different types of amidases have been identified in mammals, like trypsin-like acidic arginine amidases, anandamide amidases (fatty acid amide hydrolase), and N-acylethanolamine hydrolyzing acid amidases.

Amidases can directly hydrolyze INH to INA and Hz, and they also can hydrolyze AcINH and AcHz (Fig. 1). Pretreatment with an amidase inhibitor, bis-p-nitrophenyl phosphate (BNPP), can inhibit both the hydrolysis of INH and AcINH and decrease the formation of Hz and AcHz. Both Hz and AcHz are considered as hepatotoxic metabolites of INH, thus a higher level of amidase activity can lead to the increasing formation of Hz and AcHz and result in a high incidence of INH hepatotoxicity. Rabbits are more sensitive to INH-induced liver injury as they have higher amidase activities. Around 40% of INH is directly hydrolyzed to INA and Hz in rabbits, while less than 10% of INH is hydrolyzed in man. The hydrolysis rate of AcINH is also faster in rabbit than the rate in rats. A higher amidase activity in rabbit can also be reflected by the formation of higher levels of acetyl-bound proteins than those in rat while treating them with the same dose of AcINH.

Even though amidases are responsible for the hydrolysis of INH, AcINH, and AcHz, the specific form of amidases that mediate these hydrolysis reactions is elusive. Besides, all previous studies were performed in microsomes, primary hepatocytes, or in animal models rather than in pure enzymatic systems. In addition, BNPP is a non-specific amidase inhibitor and it inhibits both amidases and esterases, thus the involvement of esterase in INH metabolism cannot be excluded. A recent study suggests that genetic variations in a carboxylesterase gene (CES1) was possibly associated with INH hepatotoxicity; however, the authors also realized that the results are not conclusive and replication in a larger size of population needs to be performed to confirm these correlations.

5. Role of P450s in INH metabolism and hepatotoxicity

Cytochrome P450 (P450) isoenzymes are a group of heme-containing membrane proteins that are majorly expressed in the endoplasmic reticulum. In animal cells, P450s are also expressed in the inner membrane of mitochondria. P450s are major drug metabolizing enzymes. In addition, they also metabolize endogenous molecules and play important roles in hormone homeostasis (estrogen and testosterone), cholesterol synthesis, and vitamin D metabolism. CYP3A4, 1A2, 2C9, 2C19, 2D6, 2E1 are the major P450s that are involved in drug metabolism and catalyze the oxidation of almost 90% of human drugs. P450s were proposed to be involved in the oxidation of Hz and AcHz to reactive metabolites. Pretreating rats with phenobarbital increased the covalent binding of AcHz to liver proteins, whereas pretreatment with cobalt chloride plus phenobarbital decreased the formation of covalently bound proteins. Besides, P450s are also involved in the oxidation of Hz, as well as its toxic effects. The cytotoxic effect of Hz is limited by 1-aminobenzotriazole, a non-selective P450 inhibitor in rat hepatocyte. Pretreating rats with phenobarbital increases the extent of hepatic necrosis caused by INH, whereas pretreatment with cimetidine decreases the toxic effects of INH. In addition, pretreatment with other P450 inducers, such as 3-methylcholanthrene and rifampicin, also increased the production of reactive metabolites and hepatic necrosis in rats. However, it is still unclear which P450 is responsible for these reactions since rifampicin and phenobarbital are non-specific P450 inducers. Besides, cimetidine and 1-aminobenzotriazole are non-specific P450 inhibitors.

CYP2E1 is well-known to be involved in the formation of reactive oxidative species and other reactive metabolites of hepatotoxins, like acetaminophen and carbon tetrachloride. Based on the important roles in the formation of reactive metabolites, CYP2E1 was proposed to play important roles in INH hepatotoxicity. CYP2E1 is highly polymorphic, in which the CYP2E1 c1/c1 genotype had a higher CYP2E1 activity. Lots of studies investigated the roles of the CYP2E1 polymorphism in INH hepatotoxicity, but the results were inconsistent in different populations. Some studies suggest a higher CYP2E1 activity is associated with an increasing risk of INH hepatotoxicity. Patients with homozygous wild genotype CYP2E1 c1/c1 have a higher risk of INH hepatotoxicity (20.0%; odds ratio [OR], 2.52) than those with mutant allele c2 (CYP2E1 c1/c2 or c2/c2, 9.0%, P = 0.009) in a Chinese population, suggesting the CYP2E1 c1/c1 genotype is an independent risk factor for INH hepatotoxicity after adjustment for acetylator status and age. Another study showed that the CYP2E1 polymorphism is a useful tool to predict INH hepatotoxicity. However, there are some reports that showed different results. CYP2E1 c1/c2 polymorphism did not show a significant association with hepatotoxicity in a study with 175 TB patients who were treated with anti-TB drugs in Argentina. Another study performed in Chinese patients in the Xinjiang Uyghur autonomous region showed no correlation between the CYP2E1 genotypes with anti-TB drug-induced liver injury. Involvement of CYP2E1 in INH hepatotoxicity was proposed on the basis of the roles of CYP2E1 in the formation of reactive metabolites, but there was no direct evidence to support it. Structures of the reactive metabolites are also unclear. However, in animal studies using Cyp2e1 knockout mice to study the roles of CYP2E1 in INH hepatotoxicity, the authors point out that CYP2E1 might not be involved in INH-induced liver injury. Another study showed that the CYP2E1 inhibitor, diallylsulfide, can potentiate INH-induced oxidative stress rather than decrease the toxic effects in rat primary hepatocytes. Overall, roles of CYP2E1 in INH metabolism and hepatotoxicity remain controversial and require further studies.

Besides the oxidation of Hz and AcHz, P450s are also involved in the activation of INH itself. INH can bind to microsomal
proteins in an NADPH-dependent manner, suggesting the key roles of P450s in INH bioactivation, but which P450 is responsible for that remains unclear. Another study carried out by the same group showed the presence of anti-P450 antibodies (anti-CYP2C19, 2E1, and 3A4) in the serum of patients with severe liver injury caused by INH\(^6\), suggesting the interaction between CYP2C19, 2E1, and 3A4 with INH. INH is also a mechanism-based inhibitor of CYP1A2, 2A6, 2C19, and 3A4 in human liver microsomes, which suggests that INH interacts with these P450s.\(^{102,110-118}\) Furthermore, cimetidine administration in man did not decrease the oxidation of AChz, suggesting that the role of P450s in INH hepatotoxicity in man is different from that in rats.\(^{105}\)

6. Role of glutathione S-transferases (GSTs) in INH metabolism and hepatotoxicity

Glutathione S-transferases (GSTs, E.C. 2.5.1.18) comprise a multi-gene family of phase II metabolizing isozymes that are involved in the detoxification of chemicals.\(^{119}\) Most GSTs are soluble enzymes and are located in cytosol; a small family of GSTs has been reported to associate with INH hepatotoxicity in clinics.\(^{123,124}\) although the detoxification of the reactive metabolites produced by oxidization of Hz and AcHz\(^6\), INH can condense with pyridoxal to form pyridoxal isonicotinoyl hydrazine in INH hepatotoxicity still needs further investigation. INH was also shown to decrease the oxidation of AcHz, suggesting that the role of P450s in INH hepatotoxicity in man is different from that in rats.\(^{105}\)

8. Hydrazones and other adducts of INH

INH is known to interact with ketone acids, which leads to the formation of hydrazones.\(^{125,126}\) INH can condense with pyruvic and \(\alpha\)-ketoglutaric acid to form the corresponding hydrazones, isonicotinoyl glycine, and \(1\)-isonicotinoyl-2-acetylhydrazine, respectively, in rat urine.\(^{124}\) A recent study identified a series of INH-hydrazones in human urine using a LC–MS-based metabolomic approach.\(^{127}\) Five novel hydrazones were identified as the condensation of isoniazid with keto acids that are intermediates in the metabolism of leucine and/or isoleucine, lysine, tyrosine, tryptophan, and phenylalanine. The formation of these INH hydrazones is totally a chemical reaction, without the requirement of any enzyme. The formation of these INH hydrazones might affect the metabolism of amino acids, but their role in INH hepatotoxicity still needs further investigation. INH was also found to condense with pyridoxal to form pyridoxal isonicotinoyl hydrazine in human urine.\(^{129}\) This is also an enzyme-independent reaction. Since pyridoxal isonicotinoyl hydrazine is a strong iron-chelator,\(^{130}\) it might affect iron homeostasis.

9. INH and endobiotic homeostasis

INH has been found to affect the metabolism of bile acids, cholesterol, triglycerides, and free fatty acids in a chronic treatment of INH.\(^{127}\) Cheng et al.\(^{127}\) treated WT and Cyp2e1-null mice with INH for 1 month. They found that serum fatty acids were significantly decreased in WT mice, but not in Cyp2e1-null mice. Besides, serum cholesterol and triglycerides, and hepatic bile acids were increased in WT mice after INH treatment. These results suggested INH can also interrupt homeostasis of cholesterol, fatty acids, and bile acids in liver. Besides, conjugation of INH with vitamin B6 (pyridoxal and pyridoxyl 5-phosphate) leads to the depletion of pyridoxal 5-phosphate in both humans and lab animals.\(^{51,130}\) INH has also been found to reduce the plasma concentrations of calcium and phosphate ions. These reductions are due to its inhibitory action on the active form of vitamin D.\(^{37}\)

10. Conclusions

INH metabolism and its role in INH-induced liver injury have been extensively studied. However, the available data are inconsistent and even controversial. We summarized and updated the pathways of INH metabolism and discussed the association of INH metabolism with INH-induced liver injury. NAT2 is the primary enzyme that contributes to INH metabolism. NAT2 deficiency increases the risk of INH-induced liver injury. However, the
detailed mechanism, by which NAT2 deficiency leads to INH hepatotoxicity, remains unknown. Amidases hydrolyze INH and AcINH to produce Hz and AcHz, but the specific isoform of amidases that is involved in this metabolic pathway remains unclear. CYP2E1 and other P450s have been proposed to be associated with INH and AcHz bioactivation. Nevertheless, the products of P450-mediated bioactivation of INH and AcHz have not been identified. GSTs have been proposed to detoxify products of AcHz bioactivation, but the detoxified metabolites have not been determined. In addition to these classical pathways of INH metabolism, INH can form adducts with multiple endogenous metabolites. The significance of the interactions between INH and endobiotics in INH-induced liver injury is understudied. In summary, further studies are needed to explore the field of INH metabolism and hepatotoxicity.

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