Published in final edited form as:

Hepatology. 2014 March ; 59(3): 746–748. doi:10.1002/hep.26707.

Isoniazid Hepatotoxicity: Progress in Understanding the Immunologic Component

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The report by Metushi *et al.* "Detection of Anti-Isoniazid and Anti-CYP Antibodies in Patients with Isoniazid-Induced Liver Failure" addresses the role of the immune response in isoniazid (INH) mediated liver injury. Isoniazid is an essential drug in the treatment of tuberculosis. It is used as one component of combination therapy for the treatment of tuberculosis and as prophylaxis for patients deemed to have been exposed to tuberculosis.(1) While INH is generally considered to be safe and cost-effective, liver-related adverse drug reactions may occur and involve two forms of liver injury. Mild hepatitis is typically asymptomatic and self-limiting, occurs in the early stages of a course of treatment, and is thought to occur in approximately 10% of patients receiving monotherapy. Alanine aminotransferase (ALT) elevations are generally less than threefold the upper limit of normal. Overt hepatitis occurs in 0.5–1% of patients receiving INH as monotherapy, generally occurs early in the course of therapy, and is associated with gastrointestinal complaints, jaundice, and progression to liver failure.(1) Identified risk factors for INH acute liver failure (ALF) include female gender, age > 35 years, concomitant treatment with other anti-tuberculous drugs, slow acetylator NAT2 genotype (NAT2*7), cytochrome P450 2E1 (CYP 2E1 c1/c1) genotype, daily dose of INH > 50 mg/kg, concomitant treatment with acetaminophen, and daily ethanol use.(2)

While the metabolism of INH (ie., oxidation of INH to a reactive metabolite) is known to contribute to the development of toxicity,(3, 4) the potential role of the immune system in the development of liver injury is unclear. Previous clinical and laboratory data have been inconclusive in this area, because INH liver injury is not universally associated with fever or rash, does not necessarily recur with rechallenge, and anti-INH antibodies have not been detected in previous studies.(2) However, new data,(5) including Metushi's report in this issue of *Hepatology*, contribute to an evolving understanding regarding the contribution of the immune response to the etiology of INH hepatotoxicity.(5)

Utilizing various immunoassay approaches, Metushi and colleagues measured antibodies to INH and native proteins in clinical samples from two patient groups. Patient samples (n=19) obtained from the Acute Liver Failure Study Group (ALFSG) registry deemed to have at least probable or higher (>50%) likelihood of toxicity due to INH represented the first group. Comparison samples were obtained from patients receiving INH prophylaxis (n=20), of whom 15 had no liver injury response, while five developed mild hepatitis (ALT 47-147 IU/L). Baseline demographic characteristics differed between the two groups by gender (73.7% female, ALFSG group vs. 40% female, prophylaxis group), while mean age was

comparable between the groups. An antigen for detection of anti-INH antibody in patient's serum was prepared by modifying lysozyme with the N-hydroxysuccinimide ester of isonicotinic acid (INA).(2) To test the specificity of the patient's antibody to INH, free INH was mixed with patient serum.

Western blot assays showed inhibition of binding, confirming the assay's specificity for INH. Samples from the two patient groups were subsequently tested with the synthetic Lys-INH antigen. Eight of the 19 samples from patients with INH ALF were positive (42%), while the samples from the INH prophylaxis group were all negative for antibodies to LYS-INH.

Additional assays were performed in the ALFSG samples to examine the immune response to native proteins involved in the metabolism of INH. These assays showed that a significant fraction (14 of 19) of ALFSG samples contained antibodies that reacted with one or more of the cytochromes P450, specifically CYP2E1, CYP3A4, or CYP2C9. These CYP P450 isoforms activate INH, as confirmed in this report and previously supported by clinical reports showing that INH inhibits the metabolism of known pharmacologic substrates of CYP2E1, CYP3A4, and CYP2C9.(6) While some of the patient samples contained antibodies to INH and several of the CYP P450's, the pattern of detection of the various antigens was variable among the eight patients with positivity to INH. In addition, the ALFSG samples were tested with a composite antigen comprised of CYP-2E1 modified with INA to produce CYP-2E1-INH. Thus, the composite antigen contained antigenic determinants that would be recognized by the same antibodies that recognize LYS or LYS-INH and/or CYP-2E1 protein. Some patient samples (eg., ALF-3 and ALF-10) were negative for anti-CYP-2E1, but were positive for anti INH (LYS-INH) and CYP-2E1-INH. In this context, the positive reaction of select patients (ALF 1,6,13,15,16,17) to the CYP-2E1-INH composite antigen appears to be due to reactivity with the 2E1 protein antigenic determinants; the positive reaction of patient ALF 7 appears to be due to reactivity with the INH antigenic determinant; and the positive reaction of other patients (ALF-3,10,14,18, 19) could be due to either of both antigenic determinants. This concept could be ultimately confirmed in the future by competitive inhibition studies. For example, one could predict that the anti-CYP-2E1-INH reactivity of ALF 1,6,13,15,16,17 could be inhibited by incubating the antibody with free INH.

The overall conclusion of the Metushi study is consistent with the concept that in some INH hepatotoxicity patients, CYPs bioactivate INH to a reactive metabolite that covalently modifies the CYP and/or other native proteins, and that in some (as yet undefined) circumstances, this reaction constitutes an immunogenic experience, and that the resultant immune response may have specificity for the INH hapten, CYPs, or both. While the Metushi data adds support for an immune mechanism of injury, there is no proof that the detected anti-INH antibodies are associated with injury. The anti-INH antibodies and autoantibodies may be the result of a non-pathogenic immune response associated with INH-metabolism, but not associated with injury. Additional, larger studies are needed to more fully examine the relationship of detectable immune responses to LYS-INH and relevant CYP P450's in relationship to the onset and degree of liver injury, with appropriate comparison to non-INH exposed human samples and INH prophylaxis samples. In addition,

additional work is needed to examine whether or not the immune response to the CYP P450's examined in this report (CYP2E1, CYP3A4, CYP2C9) with activity to INH is limited to these particular CYP P450 isoforms. For example, INH also interacts with CYP2C19, a highly polymorphic enzyme involved in the metabolism of a large number of drugs used clinically such as phenytoin.(6) The drug-drug interaction of INH and phenytoin is well documented and represents a clinically important issue for patients receiving INH, due to the potential for phenytoin toxicity secondary to the inhibitory effects of INH on the metabolism of phenytoin. *In vitro* studies have shown that INH potently inhibits the catalytic activity of CYP2C19 and CYP3A in a concentration dependent manner, while it is a non-competitive inhibitor of CYP2E1.(6) Other drug interactions with potential relevance in the clinical setting of INH hepatotoxicity include the concurrent use of INH with CYP2C19 substrates (eg., omeprazole, diazepam, citalopram, nelfenavir), CYP3A4 substrates (eg., carbamazepine, ethosuximide, vincristine) and/or CYP2E1 substrates (acetaminophen, ethanol).(6, 7)

Clinical studies of drug induced liver injury are very challenging due to the rare and the multi-factorial nature of the condition and the lack of mechanism-based analytical assays. Future strategies involving the collaborative use of international clinical sample banks - representing broad genetic diversity - would broaden and facilitate ongoing research in this area. An analysis of INH antibody positivity by ethnicity, drug metabolism genotype status (eg., N-acetylation, CYP P450), concomitant use of other medications, and the inclusion of appropriate negative controls would help to advance our growing understanding of the role of the immune system and its interaction with drug metabolism in the pathogenesis of INH hepatotoxicity. Ultimately, a strategy that incorporates new analytical approaches - addressing both the immune response and pharmacogenetic vulnerability - can be envisioned.

Acknowledgments

NIH grant support: DK079387; DK081406

Drs. James and Roberts are part owners of Acetaminophen Toxicity Diagnostics, LLC and are developing a diagnostic test for acetaminophen toxicity.

References

1. Black M, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. *Gastroenterology*. 1975; 69:289–302. [PubMed: 1150039]
2. Metushi IG, Cai P, Zhu X, Nakagawa T, Uetrecht JP. A fresh look at the mechanism of isoniazid-induced hepatotoxicity. *Clin Pharmacol Ther*. 2011; 89:911–914. [PubMed: 21412230]
3. Mitchell JR, Thorgeirsson UP, Black M, Timbrell JA, Snodgrass WR, Potter WZ, Jollow HR, et al. Increased incidence of isoniazid hepatitis in rapid acetylators: possible relation to hydrazine metabolites. *Clin Pharmacol Ther*. 1975; 18:70–79. [PubMed: 1149365]
4. Huang YS, Chern HD, Su WJ, Wu JC, Chang SC, Chiang CH, Chang FY, et al. Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. *Hepatology*. 2003; 37:924–930. [PubMed: 12668988]
5. Metushi IG, Nakagawa T, Uetrecht J. Direct oxidation and covalent binding of isoniazid to rodent liver and human hepatic microsomes: humans are more like mice than rats. *Chem Res Toxicol*. 2012; 25:2567–2576. [PubMed: 23016703]

6. Desta Z, Soukhova NV, Flockhart DA. Inhibition of cytochrome P450 (CYP450) isoforms by isoniazid: potent inhibition of CYP2C19 and CYP3A. *Antimicrob Agents Chemother.* 2001; 45:382–392. [PubMed: 11158730]
7. Epstein MM, Nelson SD, Slattery JT, Kalhorn TF, Wall RA, Wright JM. Inhibition of the metabolism of paracetamol by isoniazid. *Br J Clin Pharmacol.* 1991; 31:139–142. [PubMed: 2049229]