Published in final edited form as:

Toxicol Appl Pharmacol. 2013 January 15; 266(2): 245–253. doi:10.1016/j.taap.2012.10.024.

CYP2E1-dependent elevation of serum cholesterol, triglycerides, and hepatic bile acids by isoniazid

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Abstract

Isoniazid is the first-line medication in the prevention and treatment of tuberculosis. Isoniazid is known to have a biphasic effect on the inhibition–induction of CYP2E1 and is also considered to be involved in isoniazid-induced hepatotoxicity. However, the full extent and mechanism of involvement of CYP2E1 in isoniazid-induced hepatotoxicity remain to be thoroughly investigated. In the current study, isoniazid was administered to wild-type and *Cyp2e1*-null mice to investigate the potential toxicity of isoniazid in vivo. The results revealed that isoniazid caused no hepatotoxicity in wild-type and *Cyp2e1*-null mice, but produced elevated serum cholesterol and triglycerides, and hepatic bile acids in wild-type mice, as well as decreased abundance of free fatty acids in wild-type mice and not in *Cyp2e1*-null mice. Metabolomic analysis demonstrated that production of isoniazid metabolites was elevated in wild-type mice along with a higher abundance of bile acids, bile acid metabolites, carnitine and carnitine derivatives; these were not observed in *Cyp2e1*-null mice. In addition, the enzymes responsible for bile acid synthesis were decreased and proteins involved in bile acid transport were significantly increased in wild-type mice. Lastly, treatment of targeted isoniazid metabolites to wild-type mice led to similar changes in cholesterol, triglycerides and free fatty acids. These findings suggest that while CYP2E1 is not involved in isoniazid-induced hepatotoxicity, while an isoniazid metabolite might play a role in isoniazid-induced cholestasis through enhancement of bile acid accumulation and mitochondria β -oxidation.

Keywords

Isoniazid; CYP2E1; Metabolomics; Drug-induced hepatotoxicity

Introduction

Isoniazid (INH) is a first-line medication used in the treatment of tuberculosis. However, INH therapy is associated with serious hepatotoxicity and potentially fatal liver injury. Hepatotoxicity manifests with nausea and right upper-quadrant abdominal pain, and can also be asymptomatic; diagnosis depends on measuring serum levels of bilirubin and the liver enzyme alanine aminotransferase (Black et al., 1975; Centers for Disease and Prevention,

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Conflict of interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.taap.2012.10.024>.

2010). While the detailed mechanism of INH toxicity remains underdetermined, hepatotoxicity of INH is considered to be due to metabolism of the nitrogen-containing group in its chemical structure resulting in reactive metabolites that lead to hepatitis (Woo et al., 1992).

Isoniazid-induced hepatotoxicity was thought to be due in part to the activity of CYP2E1 (Hussain et al., 2003; Yue and Peng, 2009; Yue et al., 2004). CYP2E1 is involved in the oxidative metabolism of numerous therapeutic and environmentally important chemicals such as alcohol, aliphatic and aromatic hydrocarbons, solvents and industrial monomers (Gonzalez, 2007). However, there is no direct evidence indicating a role for CYP2E1 in INH-induced hepatotoxicity. In the liver, INH is metabolized to acetylisoniazid via *N*-acetyltransferase 2 (NAT2), followed by hydrolysis to acetylhydrazine (Possuelo et al., 2008). Acetylhydrazine might be oxidized by CYPs to form hepatotoxic intermediates.

It is now possible to investigate the full extent and mechanism of isoniazid-induced liver injury in vivo by use of *Cyp2e1*-null mice (Lee et al., 1996). *Cyp2e1*-null mice have an absence of CYP2E1 protein expression, while CYPs in subfamilies, 1A, 2A, 2B, 2C, and 3A were expressed at normal levels (Ghanayem and Hoffler, 2007). In addition, *Cyp2e1*-null mice did not exhibit any phenotypic or pathological abnormalities. Wild-type (WT) and *Cyp2e1*-null mice share an identical 129/SV genetic background and are housed in the same environment, and thus are ideal tools for the screening and characterization of endogenous biomarkers of CYP2E1.

Metabolomics, as a high-throughput technology platform to measure metabolic fluctuations in biological samples, possesses the analytical power to comprehensively examine the metabolic pathways of both exogenous and endogenous substances, and to determine the metabolizing properties of enzymes and their physiological functions in biological systems. In this study, a mass spectrometry-based metabolomics approach was applied to examine the role of CYP2E1 in isoniazid metabolism and its relationship to hepatotoxicity between WT and *Cyp2e1*-null mice. The current findings suggest that CYP2E1 might not be involved in isoniazid-induced hepatotoxicity; however, an isoniazid metabolite derived from CYP2E1 appears to play a role in isoniazid-induced hepatotoxicity through enhancement of bile acid accumulation and mitochondria β -oxidation.

Method and materials

Animals and chemicals

WT and *Cyp2e1*-null male mice were housed in temperature- and light-controlled rooms and given water and pelleted chow ad libitum. All animal experiments were carried out in accordance with the Institute of Laboratory Animal Resources Guidelines and approved by the National Cancer Institute Animal Care and Use Committee. Isoniazid was obtained from Sigma-Aldrich (St. Louis, MO). Isoniazid metabolites I (isoniazid conjugated with alpha-ketoglutaric acid), II (isoniazid conjugated with 2-oxo-3-phenylpropanoic acid), III (isoniazid conjugated with 3-(4-hydroxyphenyl)-2-oxopropanoic acid), and IV (isonicotinic acid) were obtained as previously described (Li et al., 2011).

Experimental design

Two- to three-month-old WT and *Cyp2e1*-null male mice were fed AIN-93G purified diet for 1 month, concomitant with 50 mg/kg/day isoniazid in the water. Control groups of WT and *Cyp2e1*-null male mice were fed with AIN-93G diet and water. Urine samples from mice housed individually in Nalgene metabolic cages (Tecniplast USA, Inc., Exton, PA) were collected over continuous 24-h periods with alternate 24-h rest intervals when mice were treated INH for 15 days and 1 month respectively. Urine was collected over 24 h to

avoid the effects of diurnal variation on urine metabolite profiles. All urine samples were stored at -80°C until analyzed. For INH metabolite treatment, WT and *Cyp2e1*-null male mice were fed with the AIN-93G purified diet for 15 days, concomitant with 50 mg/kg/day INH metabolites orally administered by gavage. Control groups of WT and *Cyp2e1*-null male mice were fed with AIN-93G diet and gavaged orally with saline. Serum was collected by retro-orbital bleeding and all mice were killed by CO_2 asphyxiation after INH or INH metabolite administration, respectively. Tissue samples were harvested and stored at -80°C before analysis.

Assessment of liver injury

For assessment of macroscopic liver damage, liver tissue was flushed with PBS and fixed in 10% buffered formalin. Liver injury was scored by double-blinded analysis on a routine hematoxylin and eosin-stained section and red oil staining according to the morphological criteria previously described (Schulte, 1991). Drug-induced liver injury was further evaluated by measuring alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum. Briefly, 1 μl of serum was mixed with 200 μl of ALP, AST or ALT assay buffer (Catachem, Bridgeport, CT) in a 96-well microplate, and the oxidation of NADH to NAD^+ was monitored at 340 nm for 5 min. Blood urea nitrogen (BUN) was used to determine INH-induced kidney injury. Plasma levels of non-esterified free fatty acid, triglycerides and total cholesterol in serum and liver homogenate were measured in overnight-fasted mice using assay kits from Wako Diagnostics (Wako Diagnostics, Richmond, VA). In addition, plasma bilirubin and albumin analyses were performed using the VetScan VS2 comprehensive and liver profiles (Abaxis, Union City, CA). For determination of liver bile acid pool size, liver, gallbladder, and small intestine with its contents were removed from 4 h-fasted mice, weighed and freeze-dried overnight. To measure the hepatic bile acid levels, 20 mg of frozen liver was homogenized in 400 μl of 75% ethanol, incubated at 50°C for 2 h, and then centrifuged. The supernatant (aqueous fraction) was retained, evaporated, and resuspended in 200 μl of 0.9% saline. Twenty microliters was used for bile acid quantification using the VetSpec Bile Acids kit (Catachem, Oxford, CT).

RNA analysis

Hepatic RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and qPCR was performed using cDNA generated from 1 μg of total RNA with SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). Primers for qPCR were designed using the Primer Express software (Applied Biosystems, Foster City, CA); sequences are available in the Supplemental Table 1. qPCR reactions were carried out using SYBR Green PCR master mix (SuperArray, Frederick, MD) by using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Values were quantitated using the comparative cycle threshold (CT) method, and results were normalized to mouse β -actin.

LC-MS analyses of urinary metabolomics

Samples for LC-MS were prepared by mixing 40 μl of urine with 160 μl of 50% aqueous acetonitrile and centrifugation at $18,000\times g$ for 10 min to remove protein and particulates. Supernatants were injected into a UPLC (Waters Corporation, Milford, MA) and C18 column (Waters) was used to separate chemical components at 35°C . The mobile phase flow rate was 0.5 ml/min with an aqueous acetonitrile gradient containing 0.1% formic acid over a 10-min run (0% acetonitrile for 0.5 min to 20% acetonitrile by 5 min to 95% acetonitrile by 9 min, then equilibration at 100% water for 1 min before the next injection). The QTOF PremierTM mass spectrometer was operated in the positive electrospray ionization mode. Capillary voltage and cone voltage were maintained at 3 kV and 20 V, respectively. Source temperature and desolvation temperature were set at 120°C and 350°C .

°C, respectively. Nitrogen was used as both cone gas (50 l/h) and desolvation gas (600 l/h), and argon was used as collision gas. For accurate mass measurements, the time-of-flight mass spectrometry (TOFMS) was calibrated with sodium formate solution (range m/z 100–1000) and monitored by the intermittent injection of the lock mass sulfadimethoxine ($[M+H]^+=311.0814$ m/z) in real-time. Mass chromatograms and mass spectral data were acquired and processed by MassLynx software (Waters) in centroid format. Metabolites were quantitated via peak areas that were normalized using an internal standard.

Principal components analysis (PCA) of urinary metabolomic data

Chromatographic and spectral data were deconvoluted by MarkerLynx (Waters, Inc.) software. A multivariate data matrix containing information on sample identity, ion identity (retention time and m/z) and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The intensity of each ion was calculated by normalizing the single ion counts versus the total ion counts in the whole chromatogram. The data matrix was further exported into SIMCA-P™ software (Umetrics, Kinnelon, NJ) and transformed by mean-centering and Pareto scaling, a technique that increases the importance of low abundance ions without significant amplification of noise. Principal components of urine were generated by PCA analysis, to represent the major latent variables in the data matrix, which were depicted in a scores scatter plot.

Statistics

Experimental values are expressed as mean±standard deviation (SD). Statistical analysis was performed with two-tailed Student's *t* tests with a *p* value of <0.05 considered statistically significant.

Results

Administration of INH led to slight cholestasis in WT mice

Histological analysis revealed that INH administration had no clear effect on normal liver histology in *Cyp2e1*-null mice (Supplemental Fig. 1A), while a slight cholestasis was observed in WT mice although without significant difference of the histological score. There was no influence on the ratio of liver versus body weight or kidney versus body weight in the two mouse lines (Supplemental Fig. 1B). In addition, serum biochemistry including ALP, ALT, bile acid pool size and serum bilirubin, showed no significant difference between WT and *Cyp2e1*-null mice. However, INH significantly increased the serum cholesterol, serum triglycerides, and hepatic bile acids in WT mice compared to no change observed in *Cyp2e1*-null mice, in contrast to suppression the abundance of serum free fatty acids and BUN in WT mice compared to no changes in *Cyp2e1*-null mice (Fig. 1).

INH metabolism in WT and *Cyp2e1*-null mice

PCA analysis of the urinary metabolome revealed a clear separation between control and INH-treated mice (WT and *Cyp2e1*-null) (Fig. 2A). Loading plots from PCA analysis of the urinary metabolomes revealed that the isoniazid metabolites I (250.11⁺), II (284.10⁺), III (300.09⁺), and IV (124.04⁺) are the major ions contributing to the distinctive clustering of control and INH-treated mice (Fig. 2B). The elemental composition and chemical structure of these metabolites were proposed based on accurate mass measurements and MS/MS fragmentography. The metabolites were defined as I: isoniazid conjugated with α -ketoglutaric acid, II: isoniazid conjugated with 2-oxo-3-phenylpropanoic acid, III: isoniazid conjugated with 3-(4-hydroxyphenyl)-2-oxopropanoic acid, and IV: isonicotinic acid by comparison with the authentic standards (Li et al., 2011) (Fig. 2C).

Quantitation of INH metabolites I–IV revealed that metabolite I is significantly higher in WT mice compared to *Cyp2e1*-null mice, while metabolite II was slightly decreased in WT mice compared to *Cyp2e1*-null mice. These results indicate that the high abundance of metabolite I might be correlated with change in levels of endogenous compounds such as cholesterol, fatty acids, and bile acids, in WT mice.

Expression of enzymes responsible for INH metabolism to isoniazid and conjugation with alpha-ketoglutaric acid

Conjugation of INH with α -ketoglutaric acid (INH metabolite I) requires the addition of phenylpyruvate (Li et al., 2011). Phenylpyruvate is directly produced from phenylalanine (Mitchell et al., 2011), and multiple enzymes are involved in this metabolism including L-amino acid oxidase, tyrosine transaminase, aspartate transaminase, phenylpyruvate tautomerase, N-acetyltransferase type 2, and D-alanine transaminase. L-Amino acid oxidase was significantly higher in WT mice compared to *Cyp2e1*-null mice, which might contribute to the higher production of isoniazid α -ketoglutaric acid in WT mice (Fig. 3). However, *Cyp2e1* is not significantly increased in WT mice treated with INH (Supplemental Fig. 2).

Endogenous metabolomics profile after INH administration in WT and *Cyp2e1*-null mice

PCA analysis of the urinary metabolome revealed a clear separation between untreated control WT, *Cyp2e1*-null mice from WT and *Cyp2e1*-null mice treated with INH (Fig. 4A) after exclusion of INH and its metabolites. Loading plots from the PCA analysis of the urinary metabolomes showed p-cresol glucuronide, p-cresol sulfate, carnitine, and palmitoylcarnitine as major ions contributing to the distinctive clustering of control and INH-treated mice (Fig. 4B). The elemental composition and chemical structure of the metabolites were proposed based on the accurate mass measurements and MS/MS fragmentography. Quantitation of p-cresol glucuronide, p-cresol sulfate, carnitine, and palmitoylcarnitine revealed that these four endogenous substances are significantly higher in WT mice compared to *Cyp2e1*-null mice (Fig. 4C), in contrast to no significant difference of diuresis after treatment of INH either for 15 days or 1 month (Fig. 4D). Although urinary levels of metabolites in INH-treated WT mice at 15 day are less than WT controls, the variations between individual mice led to no statistical difference between these two groups. p-Cresol glucuronide and p-cresol sulfate were reported to be metabolites of gut microflora and to be correlated with bile acid metabolism (Cho et al., 2009). Carnitine and palmitoylcarnitine are the two major carnitines responsible for energy metabolism and are reflective of mitochondrial function (Reuter and Evans, in press). These results indicate that INH administration might contribute to increased bile acid metabolism and mitochondrial function in WT mice.

PCA analysis revealed a clear separation of bile acid components between WT and *Cyp2e1*-null mice treated with INH, while there was no wide separation between untreated WT and *Cyp2e1*-null mice (Fig. 5A). The loading plots showed taurocholic acid (TCA) and tauro- β -muricholic acid (T- β -MCA) as the major ions contributing to the distinctive clustering of control and INH-treated mice, which was confirmed by quantitation of TCA and T- β -MCA (Figs. 5B and C). TCA and T- β -MCA are the two major bile acids responsible for emulsification of fats and regulation of energy metabolism (Trauner et al., 2010). These results also indicate that INH administration might be correlated with increased bile acid metabolism in WT mice.

The expression of genes encoding enzymes involved in carnitine metabolism revealed no significant difference between WT and *Cyp2e1*-null mice (Supplemental Fig. 3). However, mRNAs encoded by genes related to bile acid synthesis (*Cyp7a1*) and bile acid transport, organic anion transporting polypeptide 2 (*Oatp2*) or bile salt export pump (*Bsep*), showed

significant changes in expression in WT mice compared to other genes encoding proteins responsible for bile acid metabolism and transport (Fig. 5D, Supplemental Fig. 4). Bsep and Oatp2 both control the export of bile acids out of hepatocytes (Kubitz et al., in press), while Cyp7a1 is involved in bile acid synthesis (Chen et al., 2012). Suppression of Bsep and Oatp2, and up-regulation of Cyp7a1 in hepatocytes of WT mice might contribute to the higher accumulation of bile acids in liver (Figs. 1 and 5).

INH metabolite administration directly induced liver toxicity

To further confirm the potential function of INH metabolite I in WT mice, this compound was administered to WT mice for 15 days. The results revealed that isoniazid α -ketoglutaric acid significantly increased the body weight of *Cyp2e1*-null mice, and also increased the body weight of WT mice despite of no significant effect (Fig. 6A), but had no effect on the ratios of liver versus body weight and kidney versus body weight in WT and *Cyp2e1*-null mice (Fig. 6B). However, it was noted that INH metabolite I induced hepatic cholesterol, triglyceride (Fig. 6C) and bile acid abundance (Fig. 6D) in WT mice after a 15 day-administration, while no sign of liver toxicity was found as revealed by AST activity (Fig. 6E).

Discussion

Isoniazid, one of the most important drugs used in the treatment tuberculosis, causes hepatotoxicity in a subset of patients. It would be of great value to be able to predict those patients that are susceptible to INH toxicity. Differences in INH-induced toxicity have been attributed to genetic variability at several loci encoding the drug-metabolizing enzymes NAT2, CYP2E1, glutathione s-transferase mu 1 (GSTM1) and glutathione S-transferase theta 1 (GSTT1) (An et al., 2012). Some studies suggest that the activity of CYP2E1 is also modulated by polymorphisms at several sites in the gene and higher activity of this enzyme may increase the production of hepatotoxic metabolites (Teixeira et al., 2011). Two polymorphisms upstream of the *CYP2E1* transcriptional start site appear to be in complete linkage disequilibrium (Teixeira et al., 2011). The presence of *CYP2E1* *1A/*1A as a genetic marker of anti-TB drug-induced liver injury has been evaluated in a few studies with discordant results. Due to differences in *NAT2*, *CYP2E1*, *GSTM1* and *GSTT1* genotype frequencies among ethnic groups, evaluation of these genetic markers in the predisposition to drug-induced hepatitis during TB treatment may be of value in prediction of clinical therapy and drug toxicity (Sotsuka et al., 2011).

Compared to multiple reports on the *CYP2E1* polymorphism correlation with isoniazid-induced liver toxicity, there are no studies to confirm with certainty that CYP2E1 directly causes toxicity as a result of INH metabolism. The global INH metabolites in human urine were investigated revealing that multiple INH metabolites are produced by condensation of INH with α -keto acids that are intermediates in the metabolism of essential amino acids, including leucine and/or isoleucine, lysine, tyrosine, tryptophan and phenylalanine (Li et al., 2011). One of the INH metabolites, isoniazid conjugated with α -ketoglutaric acid, was determined in the present study to correlate with INH-induced hepatotoxicity. Isoniazid metabolite I alone causes elevated cholesterol, triglycerides and hepatic bile acids in WT mice, similar to the INH effect on WT mice, and thus it could be assumed that it is the main metabolite of INH that leads to the slight cholestasis in WT mice. Phenylalanine metabolism is essential for production of isoniazid α -ketoglutaric acid. A 3-fold higher expression of L-amino acid oxidase was found in WT mice that contribute to the increased formation of isoniazid metabolite I due to the over-supply of phenylpyruvate. While the higher expression of L-amino acid oxidase in WT mice needs to be further investigated at the mechanistic level, the basal expression of L-amino acid oxidase in liver of WT mice is not significantly different from *Cyp2e1*-null mice (data not shown). The up-regulation of L-amino acid

oxidase might be due to INH administration-mediated differences in the endogenous metabolism in WT and *Cyp2e1*-null mice.

Owing to the potential effect from INH metabolites, carnitine and palmitoylcarnitine were up-regulated in urine of WT mice. Carnitine (levocarnitine) is a naturally occurring compound found in all mammalian species (Reuter and Evans, in press). The most important biological function of L-carnitine is in the transport of fatty acids into the mitochondria for subsequent β -oxidation, a process that results in the esterification of L-carnitine to form acylcarnitine derivatives. As such, the endogenous carnitine pool is composed of L-carnitine and various short-, medium- and long-chain acylcarnitines. The homeostasis of carnitine is multifaceted with concentrations achieved and maintained by a combination of oral absorption, de novo biosynthesis, carrier-mediated distribution into tissues and extensive, but saturable, renal tubular reabsorption. It was reported that up-regulation of carnitine can lead to reduced free fatty acids, which is reflected in the present data in which serum free fatty acids are decreased in WT mice coupled with an increase of carnitine upon INH administration (McCarty and Gustin, 1999; Stefanovic-Racic et al., 2008).

Moreover, total hepatic bile acids and individual primary bile acids including TCA and T- β -MCA are elevated in WT mice treated with INH. Some of the bile acids, especially hydrophobic bile acids like TCA, can cause cell injury in cultured hepatocytes (Hofmann, 2004). Therefore, the predominant hypothesis of the mechanism of hepato-cellular injury during cholestasis assumes that toxic bile acids accumulating in hepatocytes are the main cause of cell death. Although bile acids such as TCA can cause necrosis through the promotion of mitochondrial oxidative stress, the more recent focus was on apoptotic cell death by some bile acids like T- β -MCA (Denk et al., 2012; Perez et al., 2005). The present data revealed that TCA and T- β -MCA are increased in WT mice, which might be attributed to increased production of an INH metabolite. Oatp2 was shown to facilitate the sodium-independent uptake of taurocholic acid and Bsep augments the export bile acids out of hepatocytes, while Cyp7a1 is involved in bile acid synthesis. Suppression of Bsep and Oatp2, and up-regulation of Cyp7a1 in hepatocytes of WT mice might contribute to the higher accumulation of bile acids in the liver of INH-treated WT mice. Furthermore, increased bile acid levels were also related to elevated triglyceride levels, which might reflect the influence of triglycerides on bile acid synthesis, transport and excretion in the enterohepatic circuit (Liu et al., 2012).

In the clinic, INH generally is dual-administrated to tuberculosis patients with rifampicin. Co-administration of INH and rifampicin would be another case to induce liver toxicity. A recent study provided evidence that rifampicin exacerbated isoniazid toxicity in human hepatocytes but not in rat hepatocytes (Yue et al., 2009). The main conclusion was that the difference in CYP2E1 induction by rifampicin between rat and human hepatocytes accounted for the difference in exacerbation of isoniazid hepatotoxicity by rifampicin. However, another group (Shen et al., 2008) suggests that CYP3A is the key factor regulating this toxic reaction, since rifampicin is a strong inducer of human CYP3A4 (Cheng et al., 2009), and activated CYP3A led to increased metabolism of rifampicin. Toxic intermediates of rifampicin aggravate rifampicin and isoniazid-induced hepatotoxicity (Shen et al., 2008). Additionally, more experiments are needed to support the conclusion that rifampicin increased CYP2E1 mRNA expression in human hepatocytes. The present study suggests that in addition to the known enzymes that might be related to INH-induced toxicity, including NAT2, CYP3A, and CYP2E1, amino acid oxidase might contribute to toxicity through production of specific INH metabolites.

In summary, this is the first study to investigate the role of Cyp2e1 in INH-induced hepatotoxicity by use of metabolomics. This targeted metabolomic analysis identified a

novel correlation between an INH metabolite and INH-induced toxicity, and provides an opportunity to establish a biomarker that can be used to monitor INH induced toxicity activity through noninvasive measurements. Accordingly, metabolomics, can be used as a tool for measuring small-molecule metabolite profiles and fluxes in biological matrices, and thus monitor endogenous and exogenous variation of drug metabolism in animal models and in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Serum biochemistry of WT and *Cyp2e1*-null mice treated with INH compared to control. ALP, ALT, bile acid pool size, bilirubin, serum cholesterol, serum triglycerides, serum free fatty acids, BUN and hepatic bile acids in WT and *Cyp2e1*-null mice. Cont: control group; INH: INH treatment. * p < 0.05 compared to control in same group, ** p < 0.01 compared to control in same group.

Fig. 2.

Metabolomics analysis of urine in positive mode from WT, *Cyp2e1*-null control mice and INH treated WT and *Cyp2e1*-null mice. A. PCA analysis of the urinary metabolome revealed a clear separation of WT (□) and *Cyp2e1*-null (○) control from WT (■) and *Cyp2e1*-null (●) mice treated with INH. B. Loading plots from the PCA analysis of urinary metabolomes showing isoniazid metabolites: I (250.11⁺), II (284.10⁺), III (300.09⁺), and IV (124.04⁺) contributing to the distinctive clustering of control and INH-treated mice. C. INH metabolites are identified as I: isoniazid conjugated with α-ketoglutaric acid, II: isoniazid conjugated with 2-oxo-3-phenylpropanoic acid, III: isoniazid conjugated with 3-(4-hydroxyphenyl)-2-oxopropanoic acid, IV: isonicotinic acid and V: isoniazid by comparison

with the authentic standard. Quantitation of INH metabolites I–IV in WT and *Cyp2e1*-null mice. Structures of each metabolite are listed to the left of each panel. Cont: control group; INH: INH treatment. * $p < 0.05$ compared to control.

Fig. 3.

Expression of mRNAs encoding enzymes responsible for production of isoniazid conjugated with α -ketoglutaric acid. L-Amino acid oxidase, tyrosine transaminase, aspartate transaminase, phenylpyruvate tautomerase, N-acetyltransferase type 2, and D-alanine transaminase in WT and Cyp2e1-null mice. * $p < 0.05$ compared to control mice in the same group.

Fig. 4.

Metabolomics analysis of urine from WT, *Cyp2e1*-null control mice and WT, *Cyp2e1*-null INH treated mice with exclusion of INH and INH metabolites. A. PCA analysis of the urinary metabolome in negative WT ($\square$) and *Cyp2e1*-null ($\circ$) control from WT ($\blacksquare$) and *Cyp2e1*-null ($\bullet$) mice treated with INH after exclude of INH metabolites and INH. B. The loading plots from the PCA analysis of urinary metabolomes showing *p*-cresol glucuronide, *p*-cresol sulfate, carnitine, and palmitoyl carnitine as major ions contributing to the distinctive clustering of control and INH-treated mice. C. Quantitation of *p*-cresol glucuronide, *p*-cresol sulfate, carnitine, and palmitoylcarnitine in WT mice and *Cyp2e1*-null mice. D. Diuresis after treatment of INH for 15 days or 1 month. Cont: control group; INH: INH treatment. ** $p < 0.01$ compared to control in same group.

Fig. 5. Metabolomics analysis of bile in negative mode from WT and *Cyp2e1*-null control mice and WT and *Cyp2e1*-null INH treated mice. A. PCA analysis separation of bile acid components between WT (■) and *Cyp2e1*-null (●) mice treated with INH, and WT (□) and *Cyp2e1*-null (○) mice treated with control. B. Loading plots showing taurocholic acid and tauro-β-muricholic acid as ions contributing to the distinctive clustering of control and INH-treated mice. C. Quantitation of taurocholic acid and tauro-β-muricholic acid revealed that taurocholic acid and tauro-β-muricholic in WT and *Cyp2e1*-null mice. D. Expression of mRNA proteins involved in bile acid synthesis and transport in WT mice: *Oatp2*, *Bsep* and *Cyp7a1* mRNAs. Cont: control group; INH: INH treatment. * $p < 0.05$ compared to control in same group.

Fig. 6. Characterization of the function of isoniazid metabolite I in WT mice. The compound was administered at 50 mg/kg/day for 15 consecutive days to WT mice. **A.** Body weight of WT and *Cyp2e1*-null mice. **B.** Effect of metabolite I on the ratios of liver vs. body weight and kidney vs. body weight in WT and *Cyp2e1*-null mice. **C.** Effect of metabolite I on hepatic cholesterol, triglycerides and hepatic free fatty acids. **D.** Effect of metabolite I on bile acid abundance in WT mice. **E.** Effect of metabolite I on AST activity. Cont: control group; INH: INH treatment. * $p < 0.05$ compared to control. ** $p < 0.01$ compared to control. NEFA: non-esterified fatty acid.