Metabolomic Analysis Reveals Novel Isoniazid Metabolites and Hydrazones in Human Urine

Feng Li1,†, Yan Miao2,†, Lirong Zhang3, Sarah Ann Neuenswander4, Justin T. Douglas4 and Xiaochao Ma1,*

1Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas, USA
2Department of Rheumatism and Nephrology, People’s Hospital of Henan Province, Zhengzhou, China
3Department of Pharmacology, School of Medicine, Zhengzhou University, Zhengzhou, China
4Nuclear Magnetic Resonance Laboratory, University of Kansas, Kansas, USA

†These authors contributed equally to this work.

Summary: Isoniazid (INH) is a first-line drug for tuberculosis control; the side effects of INH are thought to be associated with its metabolism, and this study was designed to globally characterize isoniazid metabolism. Metabolomic strategies were used to profile isoniazid metabolism in humans. Eight known and seven novel INH metabolites and hydrazones were identified in human urine. The novel products included two hydroxylated INH metabolites and five hydrazones. The two novel metabolites were determined as 2-oxo-1,2-dihydro-pyridine-4-carbohydrazide and isoniazid N-oxide. Five novel hydrazones were produced by condensation of isoniazid with keto acids that are intermediates in the metabolism of essential amino acids, namely, leucine and/or isoleucine, lysine, tyrosine, tryptophan, and phenylalanine. This study enhances our knowledge of isoniazid metabolism and disposition and may offer new avenues for investigating INH-induced toxicity.

Keywords: isoniazid; metabolism; toxicity; metabolomics; mass spectrometry; nuclear magnetic resonance (NMR) spectrometry

Introduction

Isoniazid (INH) is a highly effective anti-tuberculosis drug; however, INH frequently causes toxicity that includes liver injury, peripheral neuropathy, mild central nervous system effects, and sideroblastic anemia. The detailed mechanism of INH toxicity remains unknown. Hepatotoxicity of INH is thought to be mediated by the nitrogen-containing group in its chemical structure, as it is metabolized in the liver and converted into a reactive metabolite that causes hepatitis. INH-induced peripheral neuropathy, central nervous system effects, and sideroblastic anemia are thought to be associated with the effect of INH on pyridoxine (vitamin B6) homeostasis because INH may condense with pyridoxine to form hydrazone.

Because of the potential role of metabolism in INH toxicity, INH metabolism has been studied extensively. In the early 1950s, three metabolites of INH were identified: isonicotinic acid (INA), hydrazine (HZ), and ammonia (NH₃). Soon after, isonicotinoyl glycine and acetylisoniazid (AcINH) were also discovered. The advent of chromatographic techniques, radiochemical methods, and mass spectrometry (MS) enabled researchers to identify additional INH metabolites, such as acetylhydrazine (AcHZ) and N,N'-diacetylhydrazine (DiAcHZ), and hydrazone derivatives of pyruvic and α-ketoglutaric acids.

To achieve a global picture of INH metabolism and disposition, we revisited INH metabolism in humans using a metabolomic approach. Metabolomics combines high-resolution molecular spectroscopy, mainly MS or nuclear magnetic resonance (NMR) spectrometry, with multivariate data analysis for the rapid and systematic profiling of small molecular metabolites found in an organism. We took advantage of the speed and resolving power of ultra-performance liquid chromatography (UPLC) and the accurate mass determination of time-of-flight mass spec-
trometry (TOFMS) to monitor the metabolism of INH. Seven novel and eight known INH metabolites and hydrazones were identified in human urine.

Methods

Chemicals and reagents: INH, m-chloroperbenzoic acid, keto acids, and DMSO-d6 were purchased from Sigma-Aldrich (St. Louis, MO). 2-Oxo-1,2-dihydro-pyridine-4-carbohydrazide (metabolite X) was purchased from Shanghai Yuanding Chem. Sci. & Tech. Co. Ltd. (Shanghai, China). All solvents for liquid chromatography and MS were of the highest grade commercially available. Novel hydrazones were prepared by condensation of keto acids with INH.

Human subjects, treatment, and sample preparation: Six healthy subjects (Chinese, 3 men and 3 women), aged between 30 and 45 years, were recruited for this study. The research followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the Ethics Committee of Zhengzhou University. All participants provided written informed consent. None of the subjects had a history of significant medical illness and none had taken any drug for at least two weeks prior to and during the study. Their health status was judged on the basis of a physical examination and a blood screen, including a complete blood count, liver function test, urine analysis, and electrocardiogram performed prior to the study. Two urine samples per subject were collected. A control urine sample was collected at ~8:00 A.M. on the day of the study. At ~8:00 P.M., each subject received a single oral dose of 300 mg INH with 200 ml water. At ~8:00 A.M. the next day, a second urine sample was collected from each subject. Urine samples were prepared by mixing 80 µL of urine with 320 µL of 50% acetonitrile and centrifuging at 20,000 g for 10 min. Next, 100 µL of each supernatant was transferred to an autosampler vial for analysis. Because some INH metabolites, such as HZ and AcHZ, are undetectable by UPLC-TOFMS, we derivatized the sample using m-anisaldehyde (MA). MA can readily react with the hydrazone to form the hydrazone, which will be detected by UPLC-TOFMS. A total of 200 µL of each supernatant was transferred into a new Eppendorf vial, followed by addition of MA (final concentration of 3 mM) and formic acid (final concentration of 1.0 mM). Following incubation at 37°C for 40 min, 100 µL of each sample was transferred into an autosampler vial. Five microliters was injected into a system combining UPLC and TOFMS for metabolic analysis.

INH metabolism in vitro: Incubations were conducted in 1× phosphate-buffered saline (PBS, pH 7.4), containing 50 µM INH, 0.1 mg human liver microsomes or 2 pmol of each cDNA-expressed human CYP450 enzyme (control, CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) in a final volume of 190 µL. CYP450 activity was verified using relatively specific substrates and inhibitors (data not shown). After 5 min of pre-incubation at 37°C, the reaction was initiated by the addition of 10 µL of 20 mM NADPH (final concentration 1.0 mM) and was allowed to continue for 30 min with gentle shaking. Incubations in the absence of NADPH were used as controls. Incubations were terminated by adding 200 µL of acetonitrile and vortexing for 1 min and centrifuging at 20,000 g for 10 min. Each supernatant was transferred to an autosampler vial, and 5.0 µL was injected into the UPLC-TOFMS system for metabolite analysis. All the incubations were performed in duplicate.

Synthesis of isoniazid N-oxide (metabolite XI) and its hydrazone derivative: A solution of m-chloroperbenzoic acid (293 mg, 1.2 mmol, 70% purity) in CHCl3 (2 ml) was added gradually to an ice-cooled, stirred solution of isoniazid (137 mg, 1.0 mmol) in CHCl3 (3.0 ml). The resulting mixture was stirred for 2 h at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in CHCN2/\textsubscript{2}H\textsubscript{2}O (1:1, 5.0 ml), followed by addition of m-anisaldehyde (272 mg, 2.0 mmol) and formic acid (10 µL). The reaction mixture was incubated at 37°C for 30 min, diluted, and injected into the UPLC-TOFMS system to confirm the structure of metabolite XI.

Synthesis of novel hydrazones (XII–XVI): INH (274 mg, 2 mmol), the appropriate keto acid (2 mmol), and ethanol (4.0 ml) were added to a 50-ml round flask equipped with a cooler condenser. Then, 20 µL of formic acid was added to the reaction system and the resulting mixture was stirred and heated to reflux on a water bath. After 2 h, the mixture was cooled to room temperature, and the resulting solid product was separated from the solution by suction filtration. The solid was washed twice with water (15 ml) and twice with ethanol (10 ml). The products were dried under high vacuum and used for structural confirmation. 1H and 13C NMR spectra were recorded on a 500-MHz Bruker AVIII equipped with a cryoprobe. Chemical shifts are reported in ppm and coupling constants (J) are reported in Hz. Geminal protons are denoted arbitrarily with “a” and “b” subscripts. 1H NMR spectra of the synthesized hydrazones are provided in Supplemental Figure 1 in Supplemental materials.

Reaction with 4-methyl-2-oxopentanoic acid: XIIa was obtained as a white powder in 66% yield (328 mg); 1H NMR (500 MHz, DMSO-d6): δ 12.87 (s, 1H, COOH), 11.40 (s, 1H, CONH), 8.76 (s, 2H, pyridinyl-H), 7.68 (d, J = 5.8 Hz, 2H, pyridinyl-H), 2.63 (s, 2H, CH\textsubscript{2}), 1.96 (m, 1H, CH\textsubscript{2}), 0.90 (s, 6H, 2CH\textsubscript{3}); 13C NMR (125 MHz, DMSO-d6): δ 167 (COOH), 164 (CONH), 151 (C=N), 150 (pyridinyl-C), 141 (pyridinyl-C), 122 (pyridinyl-C), 34 (CH\textsubscript{2}), 26 (CH\textsubscript{2}), 22 (2CH\textsubscript{2}); HRMS (ESI, pos.): m/z [M + H]\textsuperscript{+} calcd for C\textsubscript{12}H\textsubscript{16}N\textsubscript{2}O\textsubscript{4}: 250.1192; found: 250.1198.

Reaction with 3-methyl-2-oxopentanoic acid: XIIb was obtained as a light yellow powder in 48% yield (238 mg); 1H NMR (500 MHz, DMSO-d6): δ 16.30 (s, 1H, COOH), 10.13 (s, 1H, CONH), 8.76 (s, 2H, pyridinyl-H), 7.67
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(d, J = 4.6 Hz, 2H, pyridinyl-H), 2.88 (m, 1H, CH), 1.69 (m, 1H, CH$_2$H$_3$), 1.34 (m, 1H, CH$_2$H$_3$), 1.01 (d, J = 7.0 Hz, 3H, CH$_3$CH$_3$), 0.84 (t, J = 7.4 Hz, 3H, CH$_3$CH$_2$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165 (COOH), 163 (CONH), 159 (C=N), 150 (pyridinyl-C), 140 (pyridinyl-C), 122 (pyridinyl-C), 38 (CH$_3$CH$_3$), 27 (CH$_3$CH$_3$), 12 (CH$_2$N$_2$); HRMS (ESI, pos.): m/z [M + H]$^+$ for C$_{12}$H$_8$N$_2$O$_5$: 250.1192; found: 250.1196.

Reaction with 2-oxohexanedioic acid: XII was obtained as a white powder in 60% yield (336 mg); $^1$H NMR (500 MHz, DMSO-$d_6$): δ 13.70 (s, 1H, COOH), 12.50 (s, 1H, COOH), 11.16 (s, 1H, CONH), 8.76 (s, 2H, pyridinyl-H), 7.74 (s, 2H, phenyl-H), 2.65 (s, 2H, CH$_2$), 2.28 (t, J = 5.0 Hz, 2H, phenyl-H), 1.65 (m, 2H, CH$_2$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 175 (COOH), 165 (COOH), 164 (CONH), 150 (pyridinyl-C), 149 (C=N), 140 (s, 1C, pyridinyl-C), 122 (d, 2C, pyridinyl-C), 33 (CH$_3$), 25 (CH$_2$), 21 (CH$_3$); HRMS (ESI, pos.): m/z [M + H]$^+$ for C$_{12}$H$_8$N$_2$O$_5$: 280.0933; found: 280.0942.

Reaction with 3-(4-hydroxyphenyl)-2-oxopropanoic acid: XIV was obtained as a white powder in 72% yield (396 mg); $^1$H NMR (500 MHz, DMSO-$d_6$): δ 13.87 (s, 1H, COOH), 11.45 (s, 1H, CONH), 8.76 (d, J = 3.6 Hz, 2H, pyridinyl-H), 7.67 (s, 2H, pyridinyl-H), 7.33 (d, J = 7.6 Hz, 2H, phenyl-H), 7.26 (m, 1H, phenyl-H), 7.23 (d, J = 7.3 Hz, 2H, phenyl-H), 4.15 (s, 2H, CH$_2$CH$_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 166 (COOH), 161 (CONH), 156 (phenyl-C), 150 (pyridinyl-C), 149 (C=N), 140 (pyridinyl-C), 130 (phenyl-C), 121 (pyridinyl-C), 115 (phenyl-C), 126 (phenyl-C), 30 (4-OH-C$_6$H$_5$CH$_2$); HRMS (ESI, pos.): m/z [M + H]$^+$ for C$_{15}$H$_{14}$N$_3$O$_6$: 300.0984; found: 300.0993.

Results and Discussion

**Metabolic profiles of INH in human urine:** The results of metabolomic analysis are shown in Figure 1 and Table 1. The OPLS-DA score plot (Fig. 1A) revealed two clusters corresponding to the control and INH-treated groups. The corresponding S-plot (Fig. 1B) generated from OPLS-DA displays the ion contribution to this group separation. For the MA-derivatized urine samples, the score plot and S-plot are presented in Figures 1C and 1D, respectively. The ions that highly contributed to group separation are INH and its metabolites and hydrazones (Figs. 1B and 1D). Overall, metabolomic analysis revealed seven novel and eight known metabolites and hydrazones (Table 1). Among the novel metabolites and hydrazones, two hydroxylated INH metabolites (X and XI) and five hydrazones (XII, XIII, XIV, XV, and XVI) were identified. The six healthy subjects exhibited similar INH metabolic profiles, but there were variations in the abundance of each
Identiﬁcation of hydroxylated INH metabolites: Two hydroxylated INH metabolites (X and XI) were identiﬁed from MA-derivatized human urine samples. The MA-conjugated product of metabolite X eluted at 4.24 min, having a mass of 272.1038, higher than that of the MA conjugated product of INH. X-MA has a similar pattern of MS/MS fragmentation to that of INH-MA. Compared with mass fragments of INH-MA at m/z 123 and 80, X-MA had fragments at m/z 139 and 96, indicating that oxidation occurred on the pyridinyl ring. The metabolite XI-MA was detected at 4.14 min and had a mass of 272.1035 m/z. The fragments at m/z 139 and 96 suggest that the oxidation took place on the pyridinyl ring. In addition, the structures of metabolite X and XI were conﬁrmed by comparing retention times and mass fragments with the authentic standards.

Role of CYP450 in INH metabolism: In the current study, we examined the role of CYP450s in INH metabolism. No P450-dependent INH metabolite was detected. As for the novel hydroxylated metabolites X and XI, we found that (1) the generation of metabolite X was NADPH-independent, suggesting that this metabolic pathway is not mediated by CYP450s, and (2) the generation of metabolite XI was NADPH-dependent (data not shown), but it was not mediated by CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4. Further studies are required to determine the enzyme(s) that contribute to the formation of the two hydroxylated INH metabolites X and XI. INH hepatotoxicity is thought to be dependent on metabolic activation by arylamine N-acetyltransferase and CYP2E1. In addition, INH toxicity is enhanced during combinational treatment with rifampicin. Rifampicin is a strong microsomal enzyme inducer; thus, it was thought that rifampicin might enhance INH hepatotoxicity via enzymatic induction. However, rifampicin does not regulate arylamine N-acetyltransferase or CYP2E1 expression. CYP3A is upregulated by rifampicin via the pregnane X receptor. Recently, multiple studies have suggested that CYP3A is involved in INH metabolism and toxicity; however, CYP3A4-dependent metabolic pathways of INH were not found in the current study.

Identification of INH hydrazones: Several INH hydrazones have been reported; these have been found conjugated with pyruvic acid, α-ketoglutaric acids, or glucose. Beyond these reported hydrazones, five novel metabolite and hydrazone (Supplemental Fig. 2 in Supplemental materials).

Fig. 1. Metabolomic analysis of urine samples from control and INH-administered healthy volunteers (A) Separation of control and INH-treated human urine samples in an OPLS-DA score plot. The t[1] and t[2] values represent the scores of each sample in principal component 1 and 2, respectively. (B) Loading S-plot generated by OPLS-DA. (C) Separation of control and INH-treated human urine samples derivatized with m-anisaldehyde (MA) in an OPLS-DA score plot. (D) Loading S-plot generated by OPLS-DA of MA-derivatized urine samples. The x-axis is a measure of the relative abundance of ions, and the y-axis is a measure of the correlation of each ion to the model. These loading plots represent the relationship between variables (ions) in relation to the first and second components present in A and C. Top ranking ions are marked in the S-plots (1B and 1D). The number of ions (metabolite identiﬁcation) was accordant with that in Table 1.
Table 1. Top ranking ions in the metabolomic analysis of human urine. Each subject received a single oral dose of 300 mg INH, and 12-h urine samples were collected for metabolite analysis

<table>
<thead>
<tr>
<th>Found mass (min)</th>
<th>RT (min)</th>
<th>Formula [M + H]</th>
<th>Mass error* (ppm)</th>
<th>i-Fit¹</th>
<th>Identification</th>
<th>Symbol</th>
<th>Significance score¹</th>
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<td>138.0674</td>
<td>1.01</td>
<td>C₆H₈N₃O</td>
<td>5.1</td>
<td>0.8</td>
<td>Isoniazid (INH)</td>
<td>INH</td>
<td>10.2</td>
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<td>256.1086</td>
<td>4.30</td>
<td>C₁₀H₁₀N₅O₂</td>
<td>3.5</td>
<td>−0.4</td>
<td>Isoniazid conjugated with MA</td>
<td>INH-MA</td>
<td>37.5</td>
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<tr>
<td>180.0782</td>
<td>1.02</td>
<td>C₆H₈N₃O</td>
<td>5.0</td>
<td>0.6</td>
<td>Acetylisoniazid (AcINH)</td>
<td>I</td>
<td>24.2; 17.6 (MA)</td>
</tr>
<tr>
<td>124.0406</td>
<td>0.95</td>
<td>C₆H₆NO₂</td>
<td>5.6</td>
<td>0.0</td>
<td>Isonicotinic acid (IANA)</td>
<td>II</td>
<td>23.5; 17.2 (MA)</td>
</tr>
<tr>
<td>269.1298</td>
<td>7.58</td>
<td>C₁₂H₁₁N₅O₂</td>
<td>3.0</td>
<td>0.4</td>
<td>NH₂NH₂ conjugated with MA</td>
<td>III-MA</td>
<td>36.0</td>
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<tr>
<td>181.0621</td>
<td>1.41</td>
<td>C₆H₈N₃O</td>
<td>4.4</td>
<td>0.6</td>
<td>Isonicotinoyl glyline</td>
<td>IV</td>
<td>9.7; 7.2 (MA)</td>
</tr>
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<td>4.62</td>
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<td>−0.5</td>
<td>1.3</td>
<td>AcNH₂NH₂ conjugated with MA</td>
<td>V-MA</td>
<td>4.4</td>
</tr>
<tr>
<td>300.1198</td>
<td>0.99</td>
<td>C₁₂H₁₂N₅O₁</td>
<td>0.7</td>
<td>0.6</td>
<td>INH conjugated with glucose</td>
<td>VII</td>
<td>5.4; 3.9 (MA)</td>
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<td>266.0778</td>
<td>2.57</td>
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<td>0.4</td>
<td>0.5</td>
<td>INH conjugated with α-ketoglutaric acid</td>
<td>VIII</td>
<td>11.8; 9.1 (MA)</td>
</tr>
<tr>
<td>208.0726</td>
<td>2.08</td>
<td>C₁₀H₈N₃O₀</td>
<td>1.9</td>
<td>0.6</td>
<td>INH conjugated with pyruvic acid</td>
<td>IX</td>
<td>6.2; 4.5 (MA)</td>
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<tr>
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<td>Hydroxylated INH conjugated with MA</td>
<td>X-MA</td>
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<td>272.1044</td>
<td>4.14</td>
<td>C₁₀H₉N₃O₂</td>
<td>3.3</td>
<td>0.6</td>
<td>N-oxide INH conjugated with MA</td>
<td>XI-MA</td>
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<td>3.50</td>
<td>C₁₀H₉N₃O₂</td>
<td>2.8</td>
<td>0.7</td>
<td>INH conjugated with 4-methyl-2-oxopentanoic acid and/or 3-methyl-2-oxopentanoic acid</td>
<td>XII</td>
<td>9.2; 7.2 (MA)</td>
</tr>
<tr>
<td>280.0945</td>
<td>2.81</td>
<td>C₁₀H₉N₃O₂</td>
<td>4.3</td>
<td>2.3</td>
<td>INH conjugated with 2-oxooxanediolic acid</td>
<td>XIII</td>
<td>2.4</td>
</tr>
<tr>
<td>300.0996</td>
<td>3.09</td>
<td>C₁₀H₉N₃O₂</td>
<td>4.0</td>
<td>2.5</td>
<td>INH conjugated with 3-(4-hydroxyphenyl)-2-oxo-3-phenylpropanoic acid</td>
<td>XIV</td>
<td>3.0; 2.2 (MA)</td>
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<td>284.1063</td>
<td>3.86</td>
<td>C₁₀H₉N₃O₂</td>
<td>9.9</td>
<td>2.4</td>
<td>INH conjugated with 2-oxo-3-phenylpropanoic acid</td>
<td>XV</td>
<td>1.5; 1.0 (MA)</td>
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<tr>
<td>323.1147</td>
<td>3.90</td>
<td>C₁₀H₉N₃O₂</td>
<td>0.9</td>
<td>1.8</td>
<td>INH conjugated with 3-(1H-indol-3-yl)-2-oxo-3-phenylpropanoic acid</td>
<td>XVI</td>
<td>1.6; 1.2 (MA)</td>
</tr>
</tbody>
</table>

*Mass error: the difference between the calculated and found mass. ¹-FIT: a method of isotope prediction applied to elemental composition analysis. The smaller the i-Fit value, the higher the accuracy. ¹Significance score: the contribution of each ion to group separation in the metabolomic analysis. The higher the significance score, the higher the abundance.

RT, retention time; MA, m-anisaldehyde.

Fig. 2. Representative extracted ion chromatograms of hydroxylated metabolites (X and XI) and their MS/MS structural elucidation

Urine samples were derivatized with MA and analyzed by UPLC-TOFMS. MS/MS fragmentation was conducted with collision energy ramping from 10 to 35 eV. (A) The extracted ion chromatograms of X and XI conjugated with MA. (B) MS/MS of INH conjugated with MA. (C) MS/MS of metabolite X conjugated with MA. (D) MS/MS of metabolite XI conjugated with MA.

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hydrazones were characterized in this study. The structures of these five novel hydrazones were confirmed by the use of synthetic standards. The extracted ion chromatograms of hydrazones (XII-XVI) and their MS/MS structural elucidations are shown in Figure 3.

XII (retention time 3.50 min, Fig. 3A) corresponded to a protonated molecule at m/z 250.1195 (C12H16N3O3). MS/MS of XII produced product ions at m/z 204 (loss of HCOOH), 123 (loss of C6H10NO2), and 80 (pyridinyl group) (Fig. 3B). The structure of XII was determined as the hydrazone formed from the conjugation of INH with 4-methyl-2-oxopentanoic acid (XIIa) and/or 3-methyl-2-oxopentanoic acid (XIIb). XIII was eluted at 2.81 min (Fig. 3A) and had a [M + H]+ = 280.0945 m/z.
(C₁₂H₁₄N₃O₅). MS/MS analysis showed product ions at $m/z$ 234 (loss of HCOOH), 216 (loss of HCOOH and H₂O), 123 (loss of C₆H₈NO₄), and 80 (pyridinyl group) (Fig. 3C). The structure of XIII was determined as the hydrazone formed from the conjugation of INH with 2-oxohexanedioic acid. XIV was detected at 3.09 min (Fig. 3A) and had a $[M+H]^+ = 300.0996$ $m/z$ (C₁₅H₁₄N₃O₄). MS/MS of XIV produced product ions at $m/z$ 254 (loss of HCOOH), 123 (loss of C₉H₈NO₂), and 80 (pyridinyl group) (Fig. 3D). The structure of XIV was determined as the hydrazone formed from the conjugation of INH with 3-(4-hydroxyphenyl)-2-oxopropanoic acid. XV was eluted at 3.86 min (Fig. 3A) and had a $[M+H]^+ = 284.1063$ $m/z$ (C₁₅H₁₄N₃O₃). The product ions of XV at $m/z$ 238 (loss of HCOOH), 123 (loss of C₉H₈NO₂), and 80 (pyridinyl group) are interpreted in Figure 3E. The structure of XV was determined as the hydrazone formed from the conjugation of INH with 2-oxo-3-phenylpropanoic acid. XVI eluted at 3.90 min, (Fig. 3A) had a $[M+H]^+ = 323.1147$ $m/z$ (C₁₇H₁₅N₄O₃). MS/MS analysis of XVI showed major product ions at $m/z$ 277 (loss of HCOOH), 201 (loss of C₆H₆N₂O₂), 123 (loss of C₁₁H₈N₂O₃), and 80 (pyridinyl group) (Fig. 3F). The structure of XVI was determined as the hydrazone formed from the conjugation of INH with 3-(1H-indol-3-yl)-2-oxopropanoic acid.

4-Methyl-2-oxopentanoic acid and/or 3-methyl-2-oxopentanoic acid, 2-oxohexanedioic acid, 3-(4-hydroxyphenyl)-2-oxopropanoic acid, 2-oxo-3-phenylpropanoic acid, and 3-(1H-indol-3-yl)-2-oxopropanoic acid are intermediates in the metabolism of essential amino acids, namely, leucine and/or isoleucine, lysine, tyrosine, tryptophan, and phenylalanine. The results of the current study suggest that INH might affect the metabolism of amino acids. In addition, some INH hydrazones have been identified as potent iron chelators that may have an effect on iron metabolism and homeostasis. Further studies are needed to investigate the implications of these novel INH hydrazones in INH-induced toxicity.

In summary, INH metabolism was re-investigated in humans using a metabolomic approach, and the map of INH metabolism and disposition was extended with seven novel and eight known pathways by using a metabolomic approach. Eight known INH metabolites and hydrazones were identified as AcINH (I), INA (II), HZ (III), AcHZ (V), isonicotinoyl glycine (IV), and hydrazone derivatives (VII–IX) of glucose, pyruvic acid, and α-ketoglutaric acid. Among the novel metabolites and hydrazones, two hydroxylated INH metabolites (X and XI) and five hydrazones (XII, XIII, XIV, XV, and XVI) were identified. Solid arrows: INH metabolites and hydrazones detected by UPLC-TOFMS; Dashed arrows: previously reported or proposed INH metabolites and hydrazones not detected in the current study.

Acknowledgment: We thank Dr. Martha Montello for editing the manuscript.

Novel Isoniazid Metabolites and Hydrazones

Fig. 4. INH metabolites and hydrazones in human urine
The map of INH metabolism and disposition was extended with seven novel and eight known pathways by using a metabolomic approach. Eight known INH metabolites and hydrazones were identified as AcINH (I), INA (II), HZ (III), AcHZ (V), isonicotinoyl glycine (IV), and hydrazone derivatives (VII–IX) of glucose, pyruvic acid, and α-ketoglutaric acid. Among the novel metabolites and hydrazones, two hydroxylated INH metabolites (X and XI) and five hydrazones (XII, XIII, XIV, XV, and XVI) were identified. Solid arrows: INH metabolites and hydrazones detected by UPLC-TOFMS; Dashed arrows: previously reported or proposed INH metabolites and hydrazones not detected in the current study.
References


