Supporting Information

Chronic treatment with isoniazid causes protoporphyrin IX accumulation in mouse liver

Madhav Sachar†, Feng Li‡, Ke Liu†, Pengcheng Wang†, Jie Lu†, Xiaochao Ma†

Table of Contents

Table S1. Water consumption and dosage of isoniazid (INH).

Figure S1. Validation of methods for western blotting of FECH and ALAS1.

Figure S2. Evaluation of liver damage in mice treated with INH.

Figure S3. Hemoglobin in the liver of mice treated with INH.

Figure S4. Effect of INH and its metabolites on FECH activity.

Figure S5. Effect of INH on FECH mRNA expression in mouse liver.

Table S1. Water consumption and dosage of isoniazid (INH). Mice were treated with 0, 100, 200 and 400 mg/L INH in drinking water for 14 days. All data are expressed as mean $\pm$ SD (n = 4).

INH treatment (mg/L water)	Daily water intake (ml)	Body weight (g)	Daily water consumption vs body weight (ml/g)	Daily INH dosage (mg/Kg)
0	4.3 $\pm$ 0.3	27.7 $\pm$ 0.9	0.16 $\pm$ 0.01	0
100	4.4 $\pm$ 0.5	24.7 $\pm$ 1.4	0.18 $\pm$ 0.02	18.8 $\pm$ 2.0
200	3.3 $\pm$ 0.1	26.2 $\pm$ 0.9	0.13 $\pm$ 0.004	25.2 $\pm$ 0.8
400	3.5 $\pm$ 0.1	24.7 $\pm$ 0.5	0.14 $\pm$ 0.004	56.7 $\pm$ 1.6

Figure S1. Validation of methods for western blotting of FECH and ALAS1. Ten μg to 250 μg of liver proteins were loaded for analysis of FECH (A) and ALAS1 (B). Protein was visualized using the Odyssey infrared imaging system (LICOR, Lincoln, NE) and was quantified using the corresponding software.

Figure S2. Evaluation of liver damage in mice treated with INH. (A-E) Histological analysis of the liver in mice treated with 400 mg/L INH in drinking water for 0 (A) 3 (B), 7 (C), 14 (D) and 28 (E) days. H&E staining, 200 X. (F) Serum activity of ALT in mice treated with INH for 0-28 days. All data were expressed as means $\pm$ SD (n = 3 or 4). No statistical difference was observed from day 0 to day 28.

Figure S3. Hemoglobin in the liver of mice treated with INH. Mice were treated with 400 mg/L INH in drinking water for 0-14 days. Hemoglobin was analyzed using western blotting. GAPDH was used as loading control.

Figure S4. Effect of INH and its metabolites on FECH activity. Liver mitochondria of WT mice were used for analysis of FECH activity. All data were expressed as means $\pm$ SD (n=3). The data in control group was set as 100%. $**P < 0.01$ vs control. N-methyl PPIX was used as a positive control. AcINH, acetyl isoniazid; Hz, hydrazine; AcHz, acetyl hydrazine.

Figure S5. Effect of INH on FECH mRNA expression in mouse liver. The liver samples were analyzed by qPCR. (A) FECH mRNA expression in the liver of mice treated with 400 mg/L INH in drinking water for 0-14 days. (B) FECH mRNA expression in the liver of mice treated with 0, 100, 200 and 400 mg/L INH in drinking water for 14 days. All data were expressed as mean $\pm$ SD (n = 4). The data in the control group were set as 1.