Direct Oxidation and Covalent Binding of Isoniazid to Rodent Liver and Human Hepatic Microsomes: Humans Are More Like Mice than Rats

Supplementary Materials

Table S1. Percentage of BSA and Blue Carrier protein lysine residues modified by INA-NHS

Protein	pH	Buffer	% modification of Lysine residues	# of Lysine residues coupled
BSA	7.0	H_2O	19	11
BSA	4.7	0.9% NaCl	10	6
BSA	8.6	0.1M $NaHCO_3$	16	9
BSA	8.6	0.1 M Na_2HPO_4 + 0.15 M NaCl	23	14
Protein Blue	8.6	0.1 M Na_2HPO_4 + 0.15 M NaCl	25	75 - 150

BSA or Blue Carrier protein was reacted with INA-NHS in the presence of different buffers to evaluate the optimal conditions for INA coupling to protein. BSA was used as the model protein and the optimal conditions were also used for Blue Carrier protein. The % of modified Lysine proteins were calculated by monitoring absorbance at 335 nm and using the formula: {1 – [OD of modified protein (INA-BSA)/OD of native protein (BSA)] × 100} = Modification %. Calculations were based on a total number of 58 lysine residues for BSA and 300 – 600 lysine residues for Blue Carrier protein.

Table S2. GLDH and ALT activities after 5 weeks of treatment of BN rats with INH.

	GLDH	ALT
Control	2.2 ± 0.1	23.8 ± 0.6
INH (gavage)	2.0 ± 0.2	7.4 ± 0.3 *
INH (food)	2.3 ± 0.2	1.1 ± 0.2 *

INH was given by gavage at 150 mg/kg/day or in food at 0.2% of INH by weight in food. Values represented as mean ± S.E. of 4 animals per group. Analysed for statistical significance by Mann-Whitney U test. Significantly different from control group (*$p < 0.05$).

Table S3. SDH and ALT activities in Balb C and C57BL/6 mice.

SDH (U/L)

Male Balb C	Control INH	23 ± 1 27 ± 2
Female Balb C	Control INH	23 ± 2 39 ± 4 *
Male C57BL/6	Control INH	22 ± 1 25 ± 1
Female C57BL/6	Control INH	22 ± 2 24 ± 1

ALT (U/L)

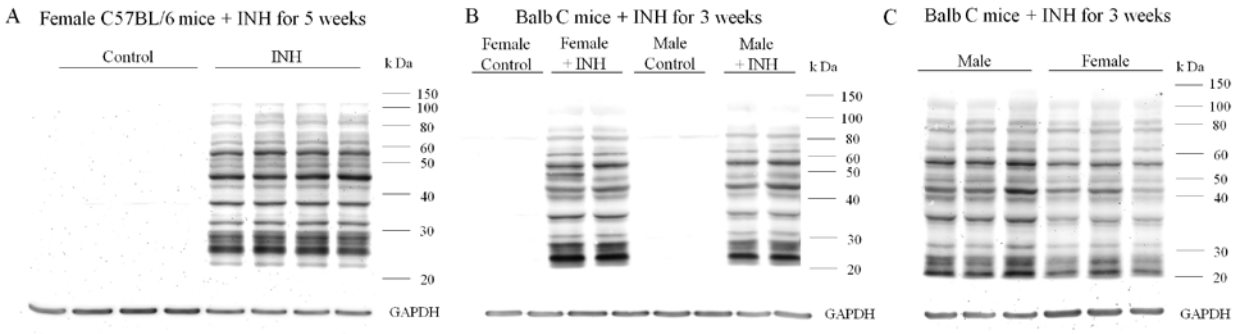
Male Balb C	Control INH	19 ± 5 0 ± 3 *
Female Balb C	Control INH	16 ± 3 2 ± 2 *
Male C57BL/6	Control INH	25 ± 2 -2 ± 9 *
Female C57BL/6	Control INH	21 ± 6 9 ± 2

Balb C mice were treated at 0.2% of INH by weight in food for 3 weeks and C57BL/6 mice were treated at 0.2% of INH by weight in food for 5 weeks. Values represented as mean ± S.E. of 4 animals per group. Analysed for statistical significance by Mann-Whitney U test. Significantly different from the control group (*$p < 0.05$).

Supplemental Figure Legends

Figure S1. Covalent binding of INH to hepatic proteins in mice. A) Female C57BL/6 (n = 4) untreated controls or treated with INH (0.2% of INH by weight in food) for 5 weeks. B) Female or male Balb C mice (n = 2) untreated controls or treated with INH (0.2% of INH by weight in food) for 3 weeks. C) Male vs. female Balb C mice (n = 3) treated with INH (0.2% of INH by weight in food) for 3 weeks.

Figure S2. Liver histology of mice and rats treated with INH. A-B) Wistar rats, control or treated with INH at a dose of 150 mg/kg/day for up to 4 weeks. C-D) BN rats, control or treated with INH by gavage at 400 mg/kg/day for 7 days. D-F) Female C57BL/6 mice, control or treated at 0.2 % of INH by weight in food. H&E stain, 100X magnification.



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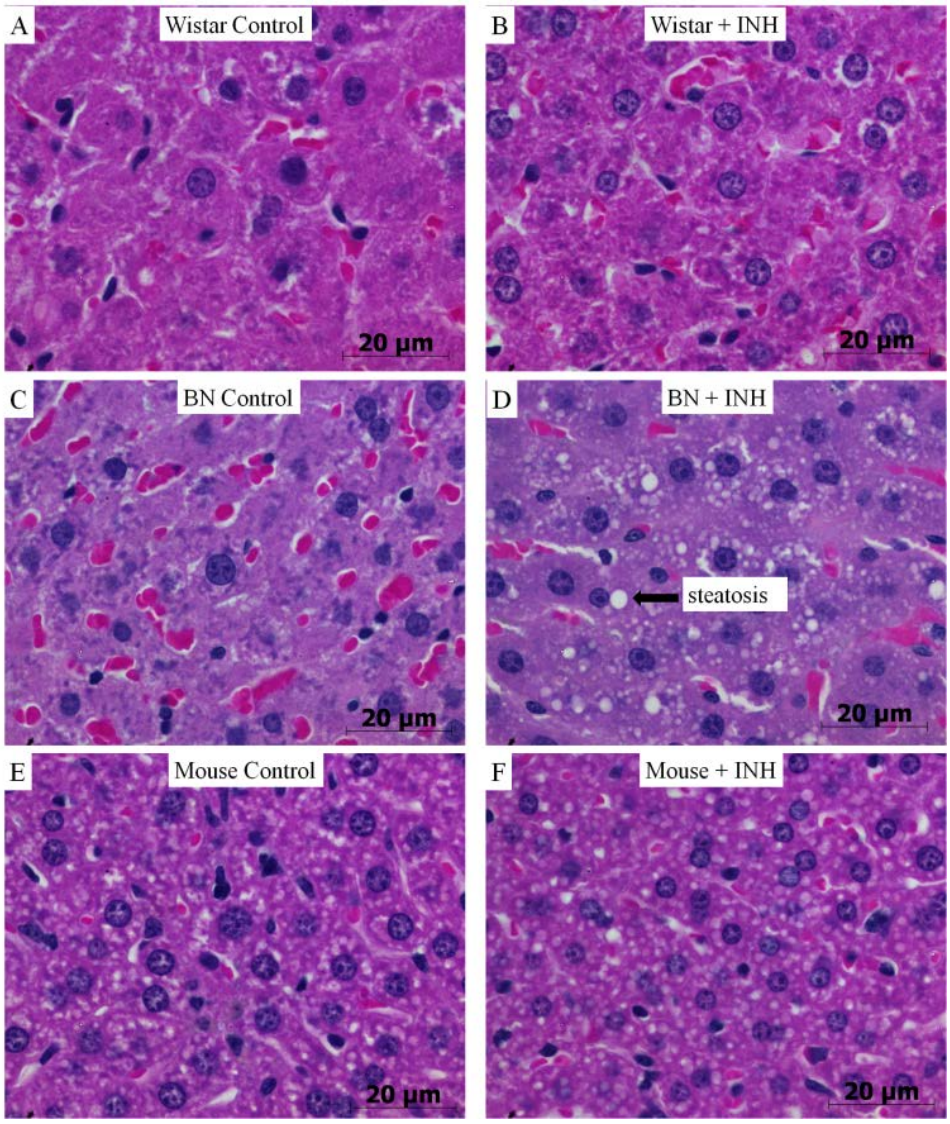

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