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PHYSIOLOGICAL DISPOSITION OF IPRONIAZID IN MICE*

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ABSTRACT The time-course of [^{14}C]iproniazid was followed for 96 h after administration of a single dose (ip) to mice on either control or pyridoxine-deficient diets. Pharmacokinetic data showed an α-decay phase in plasma and tissues of 0.5-1.0 h and β-decay phases ranging from 13.5 to 47.5 h. The β-phase half-lives in mice on the pyridoxine-deficient diets were decreased in plasma and kidneys and increased in liver, as compared to control diet animals. Covalent binding of iproniazid to liver proteins was increased in mice on the pyridoxine-deficient diet.

KEY WORDS iproniazid; pyridoxine deficiency; pharmacokinetics; covalent binding; tissue disposition

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(MAOI)[2]. In patients treated with iproniazid, a number of instances of toxicity were reported[3]. Several unique strain differences in toxicity were reported[4], as well as differences in the comparative effects of MAOI's on brain biogenic amines[5].

A previous report from this laboratory[6] showed that single doses of iproniazid to Swiss-Webster mice caused a prolonged hypoglycemic reaction and transient biphasic changes in liver triglycerides. In contrast, the same dose of iproniazid in mice on a pyridoxine-deficient diet elevated plasma glucose, plasma fatty acids, and liver fatty acids and triglycerides. This differential effect in animals with a pyridoxine-deficient diet prompted an examination of the pharmacokinetics of iproniazid in mice fed normal or pyridoxine-deficient diets.

Adult, male Swiss-Webster mice (22-30 g) were obtained from Murphy Breeding Laboratories, Plainfield, IN and maintained on Purina lab chow and tap water *ad lib* until readied for experimental use. For 21 d prior to test, mice were divided into 2 groups and fed either complete or pyridoxine-deficient diets (ICN Nutritional Biochemicals, Cleveland, OH). Drug dosage was given i p at a level of 0.77mg/kg,using ^{14}C-side chain labeled iproniazid. Dosage volumes were 20 ml/kg and administering radioactivity at 200 μCi/kg.

Mice were killed by decapitation,blood was collected into heparinized beakers, and tissues removed, frozen, and stored at -40℃ until analysis. Levels of iproniazid were determined by adding 1.0 ml of plasma or 1.0 ml of tissue homogenate (1+3 in 0.01 N HCl) to a g.s. centrifuge tube containing 1.0 ml of 0.2 M phosphate buffer (pH=8.2), 6.0 ml of n-butanol, and 500 mg NaCl. After shaking and centrifugation, 5.0 ml of the organic phase was transferred to a clean g.s. shaking tube containing 5.0 ml of n-heptane and 1.0 ml of 0.1 N HCl. The tubes were shaken, centrifuged, and 0.5 ml of the aqueous phase was removed for liquid scintillation counting of ^{14}C-material. The method was specific for iproniazid, as determined by thin-layer chromatography of selected samples.

Table 1. Levels of [^{14}C]iproniazid in(N)mice at 30 min after ip 0.77 mg/kg.
* P>0.05, ** P<0.05

TISSUE	N	[^{14}C]Iproniazid (nmol/g±S D) Control	Pyridoxine-Deficient
Plasma	6	2.71±.07	3.27±.16**
Brain	6	3.51±.61	4.10±.43*
Heart	6	3.25±.84	3.93±.44*
Kidney	5	2.53±.79	3.36±.46*
Liver	6	5.79±.97	5.48±.46*
Lung	6	1.47±.52	1.93±.47*
Spleen	5	1.39±.21	2.10±.31**

Covalent binding was determined by adding 2.0 ml of 0.9 M trichloracetic acid to 1.0 ml of liver homogenate, centrifuging at 1,000×*g* for 15 min, and discarding the supernatant fluid. The pellet was then resuspended in 3.0 ml of 0.6 M trichloracetic acid, centrifuged at 1,000×*g* for 3 min, and the supernatant fluid discarded; this process was repeated 3 times. The pellet was then resuspended in 2.0 ml of 80% methanol, centrifuged at 1,000×*g* for 3 min, and the supernatant tested for ^{14}C. This procedure was repeated until no ^{14}C was detectable in the supernatant fluid (usually 2-3 times). The pellet was then

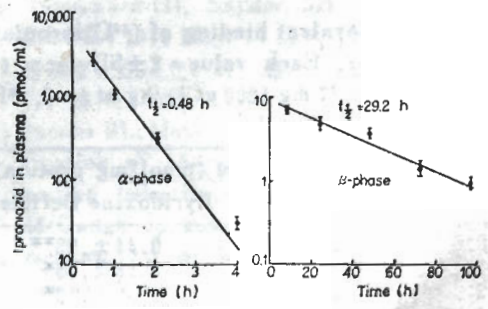

Fig 1. Iproniazid decay curve in plasma of mice fed a control diet

Table 2. Pharmacokinetic characteristics of [^{14}C] iproniazid in mice. Each value = $\bar{x} \pm$SD from 4-6 mice given ip 0.77 (200 μCi)/kg at t = 0. * P>0.05, ** P<0.05

Tissue	Diet*	Half-Life of [^{14}C]Iproniazid (h) α-Phase	β-Phase
Plasma	C	0.48±.05	29.2±3.1
	PD	0.46±.08	10.4±1.9**
Brain	C	0.73±.07	13.5±3.6
	PD	0.71±.06	18.6±3.9*
Heart	C	0.68±.04	20.3±1.7
	PD	0.66±.09	20.4±2.9*
Kidney	C	0.69±.10	47.5±5.3
	PD	0.66±.08	25.6±3.8**
Liver	C	0.48±.07	38.5±5.9
	PD	0.47±.09	86.0±9.3**
Lung	C	0.37±.05	18.5±1.9
	PD	0.38±.07	16.0±3.3*
Spleen	C	0.59±.07	25.7±4.6
	PD	0.56±.09	21.9±4.7*

* C = Control; PD = pyridoxine deficient

dissolved in 1.0 ml of 1.0 M NaOH, and protein and ^{14}C content determined on separate aliquots.

Data were analyzed by linear regression analysis and by ANOVA. Results are reported for groups of 4-6 mice in terms of mean ± S D.

Maximum levels of iproniazid were seen in all tissues at 30 min after dosage, as shown in Table 1. The only significant differences between control and pyridoxine-deficient animals were seen in plasma and spleen, where the levels in pyridoxine-deficient mice were higher. Tissue:plasma ratios ranged from 2.14 (liver) to 0.51 (lung) in control mice, and from 1.68 (liver) to 0.59 (lung) in pyridoxine-deficient mice.

Table 3. Covalent binding of [^{14}C]iproniazid to mouse liver. Each value = $\bar{x} \pm$SD from (N) mice given ip 0.77 mg (200 μCi)/kg at t = 0. *P> 0.05, **P<0.05

Time(h)	N	Iproniazid (pmol/mg protein) Control	Pyridoxine Deficient
0.5	6	0.33±.12	0.44±.05**
1	4	0.39±.12	0.38±.08*
2	4	0.40±.12	0.39±.10*
4	4	0.35±.08	0.56±.06**
8	4	0.42±.08	0.51±.08**

Groups of 6 mice on each diet were killed at various times (0.5 to 96 h) after a single dose of [^{14}C]iproniazid and tissues were assayed as described. Pharmacokinetic analysis of the data indicated biphasic decay curves; the data for plasma decay are shown in Fig 1. As can be seen, under these conditions, the half-life for the α phase was 0.48 h and for the β phase was 29.2 h. Table 2 presents the values for the half-life of [^{14}C]iproniazid in tissues of control and pyridoxine-deficient mice. The α phase values were not altered by the dietary manipulations. In contrast, the β phases in plasma and kidney were markedly reduced while that of liver was more than doubled.

This persistence in liver suggested that perhaps a significant degree of covalent binding of iproniazid to liver proteins was occurring. Accordingly, a study of such binding was performed. The results, presented in Table 3, show that this was indeed the case. The extent of covalent binding of iproniazid to liver proteins of mice on the control diet was relatively constant over the 8 h after administration of a single dose. In mice on a pyridoxine-deficient diet, however, the extent of covalent binding to liver proteins was significantly greater at 30 min as well as at the 4 and 8 h time points.

These results demonstrate that iproniazid has an extensive second (β) phase half-life in mouse tissues following a single ip dose. Clinical reports have shown that human hepatotoxicity is manifested by deranged liver function tests, and liver biopsy results biochemically and morphologically similar to viral hepatitis[7]. A previous report has shown that a single dose (50 mg/kg, p o) of iproniazid produces an hypoglycemia lasting more than 24 h in mice fed a control diet, but has little effect on blood glucose levels in mice on a pyridoxine-deficient diet[8]. In

contrast, liver fatty acids and triglycerides are elevated by iproniazid in the pyridoxine-deficient mice with variable effects in control mice.

The present results show that iproniazid persists in mice tissues after a single ip dose. In control mice, the half-life of the β-phase decay ranges from 13.5 h in brain to 47.5 h in kidney, while in mice on a pyridoxine-deficient diet, the values range from 10.4 h in plasma to 86.0 h in liver. Direct tissue comparisons show that the $t_{1/2}$ values for plasma and kidney were reduced (by 64% and 46%, respectively) while that for liver was increased (by 123%) in mice on a pyridoxine-deficient diet, as compared to control mice. This longer retention in the pyridoxine-deficient mice is supported by an increased level of covalent binding of iproniazid to liver proteins.

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