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Acetylation of Acetylhydrazine, the Toxic Metabolite of Isoniazid, in Humans. Inhibition by Concomitant Administration of Isoniazid[1]

ELMAR PERETTI, GEORG KARLAGANIS and BERNHARD H. LAUTERBURG
Department of Clinical Pharmacology, University of Berne, Switzerland
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ABSTRACT

The effect of isoniazid and its metabolites on the disposition of acetylhydrazine, a toxic metabolite formed from isoniazid, was studied in humans. Acetylhydrazine was administered i.v. with and without prior ingestion of 300 mg of isoniazid. In the studies with isoniazid, $^{15}N_2$-acetylhydrazine was administered in order to distinguish exogenous acetylhydrazine from the unlabeled acetylhydrazine formed from isoniazid. In each subject (two fast and three slow acetylators) the rate of elimination of acetylhydrazine was similar to the rate of elimination of isoniazid suggesting that the two compounds are subject to the same acetylation polymorphism. In the presence of isoniazid the rate of elimination of acetylhydrazine was consistently lower than in the absence of isoniazid, and the urinary excretion of diacetylhydrazine and the ratio of diacetyl/acetylhydrazine in urine decreased. The data indicate that therapeutic concentrations of isoniazid and its metabolites inhibit the acetylation of acetylhydrazine in humans. The inhibition of this detoxification pathway could contribute to the hepatotoxicity of isoniazid.

The hepatotoxicity of isoniazid is well recognized (Mitchell *et al.*, 1975; Black *et al.*, 1975; Musch *et al.*, 1982; Dickinson *et al.*, 1981). Studies in experimental models of isoniazid hepatotoxicity have shown that the metabolic activation to a reactive intermediate of acetylhydrazine, a metabolite of isoniazid, is responsible for the hepatocellular injury (Nelson *et al.*, 1976; Mitchell *et al.*, 1976; Timbrell *et al.*, 1980). To protect hepatocytes from damage detoxifying metabolic steps compete with the toxifying step for the available acetylhydrazine. The most important detoxifying step is the further acetylation of acetylhydrazine to the nontoxic diacetylhydrazine (Wright and Timbrell, 1978). Besides the amount of acetylhydrazine generated from isoniazid the balance between metabolic activation and acetylation may, therefore, be a key determinant of the toxicity of isoniazid (Lauterburg *et al.*, 1985a).

The factors determining the acetylation of acetylhydrazine in humans are poorly understood. Circumstantial evidence indicates that the acetylation may be subject to the same acetylation polymorphism as isoniazid (Lauterburg *et al.*, 1985b; Ellard and Gammon, 1976), the pharmacokinetics of acetylhydrazine in humans, however, have not been formally studied. Moreover, it is not known whether relevant interactions between the acetylation of isoniazid and acetylhydrazine might occur with therapeutic doses of isoniazid in humans. In rats, for example, isoniazid inhibits the acetylation of acetylhydrazine and increases the toxicity of acetylhydrazine (Wright and Timbrell, 1978; Timbrell and Wright, 1979).

To address these questions we studied the pharmacokinetics of acetylhydrazine in the absence and presence of isoniazid. In order to follow the kinetics of acetylhydrazine in the presence of acetylhydrazine formed from isoniazid, $^{15}N_2$-acetylhydrazine was administered and the two species of acetylhydrazine and their metabolites were followed by capillary GC-MS.

Materials and Methods

Protocol of the study. Five healthy male volunteers, age 29 to 42, gave informed consent to participate in the study which had been approved by the local ethics committee. They did not smoke, drank less than 10 g of ethanol per day and were not taking any medication. The kinetic studies were done after an overnight fast starting at 8:00 A.M. The volunteers were allowed to eat and to pursue their regular daily activities 1 hr after administration of acetylhydrazine. They all had been phenotyped regarding their acetylator status with sulfadimidine (Evans, 1969).

On day 1, 7.4 mg (100 μmol) of acetylhydrazine fumarate dissolved in 0.9% NaCl were injected i.v. and blood samples were obtained at intervals for 9 hr. Urine was collected for 9 hr. Serum and urine samples were frozen and stored under argon at −70°C until analysis.

One week later the study was repeated. This time the subjects ingested 300 mg (2.19 mmol) of isoniazid dissolved in water, and 100 μmol of $^{15}N_2$-acetylhydrazine were injected i.v. 90 min later.

Synthesis of $^{15}N_2$-acetylhydrazine. $^{15}N_2$-hydrazine was synthesized from $^{15}N_2$-hydrazine sulfate (95% ^{15}N, Cambridge Isotope Laboratories, Woburn, MA) and methyl acetate as described previously

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ABBREVIATION: GC-MS, gas liquid chromatography-mass spectrometry.

(Karlaganis *et al.*, 1987). A ^{1}H-NMR spectrum in chloroform showed proton resonances at 2.0 ppm (singlet, 3H from CH_3CO), 3.9 ppm (singlet, 2H from NH_2) and 7.0 ppm (multiplet, 1H from −NH).

Synthesis of acetylhydrazine fumarate. Five millimoles of fumaric acid were added to 10 mmol of acetylhydrazine (Aldrich Chemie, Steinheim, FRG) in 80 ml of hot ethanol. The fumarate salt of acetylhydrazine precipitated when the stirred solution was cooled. The salt was recrystallized from hot ethanol: yield, 31%; melting point, 110°C under decomposition. A ^{1}H-NMR spectrum in D_2O showed peaks at 1.7 ppm (singlet, 6H from CH_3CO) and 6.4 ppm (singlet, 2H from fumarate), indicating an acetylhydrazine/fumarate ratio of 2:1.

Synthesis of internal standards. Isoniazid, acetylhydrazine and hydrazine hydrate (between 1–5 mmol/reaction) were added to d_6-benzaldehyde (98% d, Merck Sharp and Dohme, Ltd., Montreal, Canada) in 20 ml of methanol in a 1:1, respectively, 1:2 in the case of hydrazine, molar ratio. The solutions were stirred for 1 hr at 60°C. After evaporation of the methanol *in vacuo* the deuterated hydrazones were recrystallized two times from ethyl acetate. Acetylisoniazid was synthesized according to Mitchell *et al.* (1975).

Analysis of serum samples. To 2.5 ml of serum were added 0.1 ml of 12.5% hydrochloric acid and 1 g of ammonium sulfate. The mixture was vortexed, centrifuged at 2500 × *g* for 20 min and 1.5 ml of the supernatant were transferred to 1 ml of 0.5 M sodium citrate, pH 6, and 5 ml of dichloromethane to extract lipids interfering with the GC-MS analysis (Karlaganis *et al.*, 1987). After centrifugation 2 ml of the acqueous phase were derivatized with 0.1 ml of methanolic benzaldehyde (0.1 ml/ml) while shaking for 1 hr at room temperature. After the addition of the three internal standards the sample was extracted twice with 6 ml of dichloromethane. The dichloromethane phases were combined, dried with sodium sulfate and evaporated at 40°C under a stream of nitrogen. The residue was taken up in 0.1 ml of ethyl acetate and 1 μl was injected into the GC-MS system.

Analysis of urine samples. Urine samples were adjusted to pH 6 with 0.5 M sodium citrate and extracted with dichloromethane. The urine was then derivatized with benzaldehyde and extracted with dichloromethane after addition of internal standards as described for serum samples. For the analysis of diacetylhydrazine an aliquot of the extracted urine was acidified to pH 1 with 25% HCl and hydrolyzed at 45°C for 20 hr (Timbrell *et al.*, 1977). The hydrolyzed urine was then adjusted to pH 6, derivatized and extracted as described above.

GC-MS. Capillary GC-MS was performed on a Finnigan 1020 instrument (Karlaganis *et al.*, 1987). Selected ion monitoring was carried out at the following masses corresponding to M + 1: *m/z* 163 (benzaldehyde acetylhydrazone); *m/z* 165 (benzaldehyde $^{15}N_2$-acetylhydrazone); *m/z* 169 (benzaldehyde-d_6 acetylhydrazone, internal standard); *m/z* 209 (benzaldehyde azine); *m/z* 211 (benzaldehyde $^{15}N_2$-azine); *m/z* 221 (benzaldehyde-d12 azine, internal standard); *m/z* 226 (benzaldehyde isonicotinoylhydrazone); and *m/z* 232 (benzaldehyde-d6 isonicotinoylhydrazone, internal standard).

Statistics. Statistical differences between different treatments were assessed by Student's *t* test for paired samples.

Results

Representative ion chromatograms of monitored ions in serum of a subject who had received isoniazid and $^{15}N_2$-acetylhydrazine are shown in figures 1 and 2. The individual serum concentration-time curves of isoniazid, acetylhydrazine and $^{15}N_2$-acetylhydrazine are shown in figure 3. As expected, the plasma half-life of isoniazid was longer than 2 hr in the three slow acetylators and less than 2 hr in the two rapid acetylators. Acetylhydrazine disappeared monoexponentially from the circulation in all volunteers, but the rate of elimination was markedly lower in the slow acetylators compared to the rapid acetylators (fig. 3; table 1). There was a close correlation between the rate of disappearance of isoniazid and the rate of disappearance of acetylhydrazine ($r^2 = 0.72$), suggesting that the same enzyme may be responsible for the acetylation of isoniazid and acetylhydrazine. The estimated volume of distribution of acetylhydrazine averaged 1.5 liters/kg and thus exceeds reported values of the volume of distribution of isoniazid which averages 0.7 liters/kg (Kergueris *et al.*, 1986).

After the administration of isoniazid the rate of disappearance of exogenous acetylhydrazine, *i.e.* $^{15}N_2$-acetylhydrazine, was lower ($P < .05$) in each subject (fig. 3; table 1). The apparent volume of distribution was similar with the labeled and the unlabeled acetylhydrazine. In the one subject (E. R.) who received $^{15}N_2$-acetylhydrazine on both occasions the elimination rate constant also was lower after isoniazid indicating that the difference in the kinetics cannot be attributed to different handling of the labeled compound. For the duration of the study the serum concentration of isoniazid exceeded the concentration of exogenous acetylhydrazine by at least one order of magnitude. The concentrations of acetylhydrazine formed from isoniazid and of exogenous acetylhydrazine were similar initially. Later, however, the concentrations of exogenous acetylhydrazine was substantially lower than the concentrations of endogenous acetylhydrazine because the apparent half-life of acetylhydrazine formed from isoniazid is about 5 times longer than the half-life of isoniazid (Lauterburg *et al.*, 1985a). This divergent time course is due to the continuous formation of the metabolite from the parent compound.

The decreased rate of elimination of acetylhydrazine in the presence of isoniazid and its metabolites suggests that the acetylation of acetylhydrazine is inhibited. This conclusion is supported by the urinary data shown in figure 4. The excretion of diacetylhydrazine formed from the exogenous acetylhydrazine decreased in each subject whereas the excretion of exogenous acetylhydrazine increased in most after administration of isoniazid. Consequently, the ratio of diacetylhydrazine to acetylhydrazine in urine decreased significantly ($P < .05$). The urinary excretion of hydrazine after the administration of acetylhydrazine demonstrates that hydrazine is a metabolite not only of isoniazid but also of acetylhydrazine.

Discussion

The present data demonstrate that the rate of disappearance of acetylhydrazine from the circulation is similar to the rate of disappearance of isoniazid. Inasmuch as the clearance of acetylhydrazine is mainly accounted for by the acetylation of the compound to diacetylhydrazine (Wright and Timbrell, 1978), the data suggest that acetylhydrazine is subject to the same acetylation polymorphism as isoniazid. Earlier data from our laboratory also have shown a close correlation between the rate of elimination of isoniazid and the apparent rate of elimination of acetylhydrazine formed from isoniazid (Lauterburg *et al.*, 1985a). However, the apparent half-life of acetylhydrazine formed from isoniazid is in the average 5 times longer than the half-life of isoniazid because the serum concentration-time curve reflects not only the elimination but also the formation of the metabolite. The only other reported administration of acetylhydrazine to two subjects also suggested that the acetylation of acetylhydrazine is genetically controlled but proceeds at a lower rate than the acetylation of isoniazid (Ellard and Gammon, 1976). The p.o. rather than i.v. administration of acetylhydrazine in that study and the kinetic analysis of its excretion in urine rather than of serum concentrations may

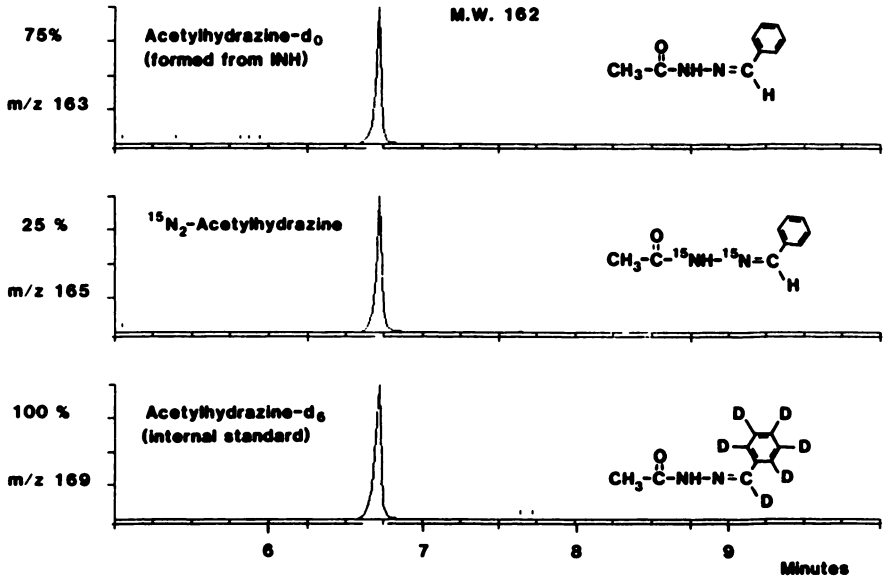

Fig. 1. Mass chromatograms of acetylhydrazine derivatives in a serum sample of a healthy volunteer who had received isoniazid and $^{15}N_2$-acetylhydrazine.

have led to an underestimation of the actual rate of elimination of acetylhydrazine.

After the administration of isoniazid the systemic clearance of exogenous acetylhydrazine and the clearance of the compound to its acetylated metabolite decreased in each subject. A qualitatively similar effect of isoniazid on the acetylation of acetylhydrazine has been described in experimental animals (Wright and Timbrell, 1978). For the duration of the kinetic study the serum concentrations of isoniazid and of acetylhydrazine formed from isoniazid were both substantially higher than the concentration of exogenous acetylhydrazine. Thus, both compounds could be responsible for the inhibition of the acetylation step. The initially low concentrations of acetylhydrazine and the roughly 10 times higher concentrations of isoniazid suggest that isoniazid may be mainly responsible for the inhibitory effect.

In contrast to acetylhydrazine, the excretion of hydrazine formed from acetylhydrazine decreased in the presence of isoniazid (fig. 4). Hydrolysis of both, isoniazid and acetylhydrazine, will generate hydrazine. Thus, the decreased formation of hydrazine from ^{15}N-acetylhydrazine could possibly be due to competition of isoniazid and acetylhydrazine for hydrolysis.

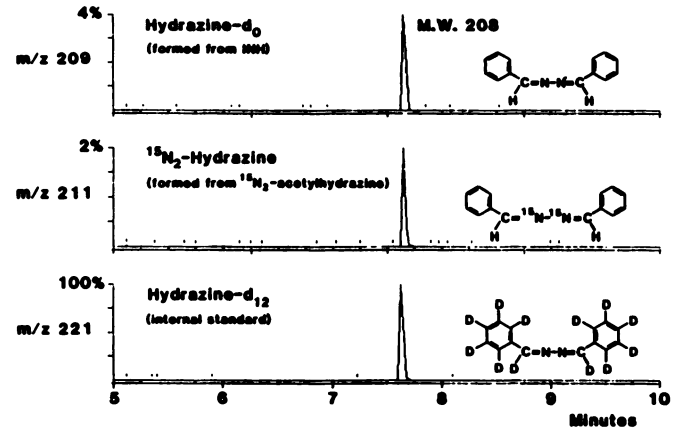

Fig. 2. Mass chromatograms of hydrazine derivatives in a urine sample of a healthy volunteer who had received isoniazid and $^{15}N_2$-acetylhydrazine. Hydrazine may be formed from isoniazid and acetylhydrazine, respectively.

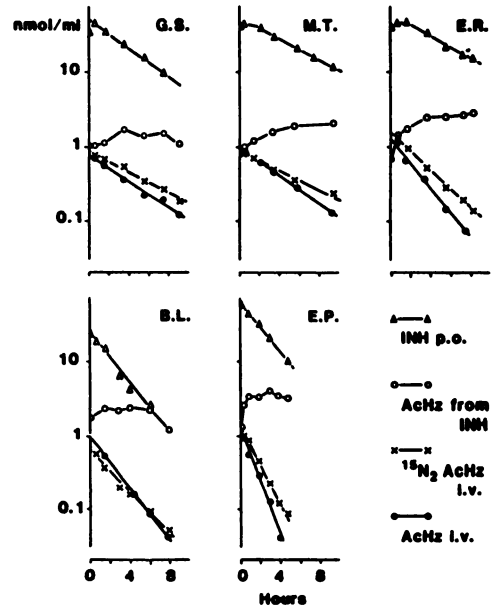

Fig. 3. Serum concentrations of acetylhydrazine (AcHz i.v.) after i.v. administration of AcHz and of $^{15}N_2$AcHz ($^{15}N_2$AcHz i.v.), AcHz (AcHz from INH), and isoniazid (INH p.o.) after the ingestion of 300 mg of INH p.o. and the i.v. administration of $^{15}N_2$-AcHz to three slow acetylators (upper panel) and two rapid acetylators (lower panel). In the presence of INH the rate of elimination of exogenous AcHz is consistently lower, indicating an inhibition of the rate of acetylation of AcHz by INH and its metabolites. AcHz from INH is AcHz in serum formed from INH, *i.e.* unlabeled AcHz. In contrast to $^{15}N_2$-AcHz the time course of the serum concentration of AcHz from INH depends on the rate of formation from INH *via* acetylisoniazid and the rate of elimination of AcHz.

In experimental animals the inhibition of the acetylation of acetylhydrazine by isoniazid increases the toxicity of acetylhydrazine (Timbrell and Wright, 1979). Based on the present data it is conceivable that the same mechanism could contribute to the hepatotoxicity of isoniazid in humans. If acetylhydrazine is responsible for isoniazid hepatitis a slow-release form of isoniazid resulting in lower serum concentrations of isoniazid and thus a lesser inhibition of the acetylation of acetylhydrazine might decrease the hepatotoxicity of isoniazid.

TABLE 1

Pharmacokinetics of acetylhydrazine in the presence ($^{15}N_2$-acetylhydrazine) and absence (acetylhydrazine) of isoniazid

Subject	Body wt.	Acetylhydrazine			$^{15}N_2$-Acetylhydrazine			Isoniazid
		Vd*	kel	Cl	Vd	kel	Cl	kel
	kg	l	hr^{-1}	$ml \cdot min^{-1} kg^{-1}$	l	hr^{-1}	$ml \cdot min^{-1} kg^{-1}$	hr^{-1}
B. H. L.	69	100.92	0.41	10.00	102.69	0.34	8.43	0.35
E. P.	64	81.69	0.83	17.65	76.84	0.59	11.80	0.40
M. T.	67	100.52	0.21	5.25	113.31	0.15	4.23	0.17
C. S.	66	120.54	0.20	6.08	114.63	0.16	4.63	0.19
E. R.	60	79.91	0.38	8.44	65.30	0.29	5.26	0.18

* Vd, volume of distribution; kel, elimination rate constant; Cl, systemic clearance.

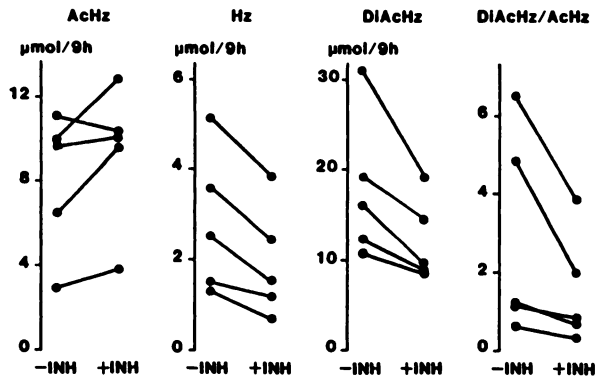

Fig. 4. Urinary excretion of i.v. administered acetylhydrazine (AcHz) and $^{15}N_2$-AcHz and their metabolites in 9 hr. $^{15}N_2$-AcHz was administered i.v. 90 min after ingestion of 300 mg of isoniazid (+INH), AcHz was administered i.v. without INH (−INH). The decreased excretion of diacetylhydrazine (DiAcHz) and the decreased Di/AcHz ratio in the presence of INH indicates an inhibition of the acetylation of AcHz by INH and its metabolites.

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Send reprint requests to: Dr. Bernhard H. Lauterburg, Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne, Switzerland.