METABOLISM OF ISONIAZID AND FREE HYDRAZINE FORMATION IN ISOLATED RAT HEPATOCYTES*

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Isoniazid (INH) was incubated with isolated rat hepatocytes, and the amounts of acetyl-INH, monoacetylhydrazine, diacetylhydrazine and hydrazine formed were determined by gas chromatography-mass spectrometry (GC-MS). Subsequent analyses of kinetic parameters of metabolic pathways and enzymological study of rat liver homogenate revealed that hydrazine was formed preferentially from INH, catalized by soluble hydrolytic enzymes.

Keywords—isoniazid; hydrazines; isolated rat hepatocytes; K_m and V_{max} ; mass fragmentography

As described in a preceding report,¹⁾ hydrazine (Hz), a hazardous metabolite of isoniazid (INH), was commonly detected by GC-MS in the urine of tuberculous patients following the oral administration of 600 mg of INH, amounting to, in an extreme case, as much as 1.2 mg during 8 hr of pursuit. Though a large portion of INH was metabolized in the liver of experimental animals or human subjects^{2,3)} to acetyl-INH (Ac-INH), other minor metabolites such as hydrazines have attracted much attention because of their possible carcinogenic activities.^{4,5)} Isolated hepatocyte system is reported to catalyze sequential drug-metabolizing reactions including phases I and II under the conditions similar to those *in vivo*.⁶⁾ Therefore, the system could serve as a suitable model in investigating INH metabolism as mentioned above.

Isolated hepatocytes were prepared from male Wister rats by collagenase perfusion methods as described by Moldéus *et al.*⁷⁾ The incubation of cell suspensions (6–20 ml/flask) with various drugs was performed in rotary round-bottom

flasks at 37° under an atmosphere of 95% O₂–5% CO₂ gas. The concentration of drugs in the mixture ranged from 5×10^{-5} to 10^{-3} M, and the number of liver cells was 4×10^6 cells/ml. After incubation for a certain time period, a portion of the mixture (4 ml) was combined with an equal volume of aqueous internal standard solution and was boiled for 2 min to terminate the reaction, followed by centrifugation for 20 min at 2500 rpm. The supernatant thus obtained was filtered with Centriflo (Amicon Co., CF 25) and subjected to analyses performed by mass fragmentography. As internal standards, benzoic acid hydrazide (BAH), *d*₃-AcINH, *d*₃-monoacetylhydrazine (*d*₃-AcHz), *d*₃-diacetylhydrazine (*d*₃-DAcHz) and ¹⁵N-Hz were used for the determination of INH, AcINH, AcHz, DAcHz and Hz, respectively. The viability of isolated hepatocytes was checked periodically by LDH (lactic dehydrogenase) latency test⁸⁾ and was found to be 98–99% throughout the experiment.

To check the outline of INH metabolism in isolated rat hepatocytes, 10^{-4} M INH was incu-

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FIG. 1. Metabolic Pathways of INH and Ratios of Metabolites found 15 min after Incubation with Isolated Rat Hepatocytes

Initial concentration of INH: 10^{-3} M, Number of liver cells: 4×10^6 cells/ml

*The amounts of the compounds were not determined.

bated with liver cells and the amounts of AcINH, AcHz, DAcHz and Hz formed were determined. As the rates of formation of the products were found to be linear for at least 10 min, the percent ratio of the metabolites 15 min after incubation were determined and are shown in Fig. 1. About 20% of the INH was consumed during the incubation period. Consistent with the previous findings,⁹⁾ the major metabolite of INH was AcINH, and relatively large amounts of hydrazones or isonicotinate derivatives (INA) should have been formed concomitantly. Further metabolism of hydrazines was also probable, for example, approximately 10% of Hz was metabolized when 10^{-4} M Hz sulfate was incubated with the liver cells for 30 min. The amount of DAcHz formed from INH was too small to be identified by the method employed, while DAcHz was detectable when AcHz (10^{-3} to 5×10^{-4} M) was incubated with the cells as the substrate. Acetylation of AcHz could be competitively inhibited by INH, in the presence of excess INH.¹⁰⁾

The apparent K_m and V_{max} values of INH metabolism routes are summarized in Table I. Clearly, N-acetylation of INH functions most actively at low substrate concentrations which is reasonable under the physiological conditions. As shown above (Fig. 1), an appreciable amount of Hz was formed by the incubation of INH, and this might be formed by a simple hydrolysis of INH. Comparison of the K_m and V_{max} values of the two possible metabolic routes, $\text{INH} \rightarrow \text{Hz}$

TABLE I. Kinetic Parameters of Metabolic Pathways of INH

Metabolic pathways	K_m ^{a)}	V_{max} ^{b)}
$\text{INH} \rightarrow \text{Hz}$	0.19	1.8
$\text{AcINH} \rightarrow \text{AcHz}$	0.38	1.1
$\text{AcHz} \rightarrow \text{Hz}$	0.88	11.8
$\text{INH} \rightarrow \text{AcINH}$	0.03	1.5
$\text{Hz} \rightarrow \text{AcHz}$	0.16	2.4
$\text{AcHz} \rightarrow \text{DAcHz}$	0.28	6.0

a) mM.

b) nmol/min/ 4×10^6 cells.

and $\text{AcHz} \rightarrow \text{Hz}$, shows that the former is definitely probable.

Additional experiments revealed that the activity which catalyzed formation of Hz from INH existed exclusively in the soluble fraction of liver homogenate (specific activity: 5.0 pmol/min/mg, in 0.1M phosphate buffer, pH 7.4, at 37°), and trypsin (10 units/ml) also liberated Hz from INH. In contrast to our findings, isocarboxazid was hydrolyzed not by a soluble enzyme but by microsomal arylamidase.¹¹⁾ The details will be reported in the nearest future.

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