TISSUE DISTRIBUTION OF HYDRAZINE AND ITS METABOLITES IN RATS

YOSHIHARU KANEKO,* SADAO IGUCHI,** HIROAKI KUBO, NAOMI IWAGIRI AND KENJI MATSUYAMA***

Faculty of Pharmacy & Pharmaceutical Sciences, Fukuyama University,* Sanzo, Higashimura-cho, Fukuyama and Faculty of Pharmaceutical Sciences, Kyushu University,*** Maidashi, Higashi-ku, Fukuoka

(Received January 17, 1984)

The tissue distribution and the urinary excretion of hydrazines, hydrazine, acetylhydrazine and 1,2-diacyethylhydrazine, were determined by mass fragmentography using a gas chromatography-mass spectrometer equipped with a multiple ion detector-peak matcher. Using the compounds labeled with a stable isotope as an internal standard, namely the isotope dilution method, made it possible to estimate trace amounts of hydrazine and its metabolites in the tissues. Significantly high levels of all hydrazines were detected in the kidney. Especially, acetylhydrazine, a metabolite of hydrazine, accumulated to a great extent in the kidney. Free hydrazine which was liberated from acetylhydrazine was detected both in the tissues and in the urine after the administration of acetylhydrazine. This demonstrates clearly that the metabolic pathway between hydrazine and acetylhydrazine is reversible.

Keywords—hydrazine; acetylhydrazine; 1,2-diacyethylhydrazine; tissue distribution; urinary excretion; gas chromatography-mass spectrometry

INTRODUCTION

In recent studies we have found that free hydrazine (HZ) is detectable in the urine of patients on isoniazid (INH)-treatment. In order to elucidate the mechanism of HZ formation, the metabolism of INH including the acetylation process was then examined using isolated hepatocytes of rats. The results clearly demonstrated that the greater part of HZ is formed through the direct hydrolysis of INH. Furthermore, HZ could be successfully detected in the tissues of rats following the administration of INH.

Pharmacological and biochemical effects of HZ have been described, such as hypoglycemia, increased ammonia levels in the blood and spinal fluid, decreased hepatic lipid mobilization, blood dyscrasias and convulsions. Biancifiori et al. reported that hydrazine sulfate induces liver and lung tumors in mice. They suggested that the tumors observed after the administration of INH in the mice are mainly due to the liberation of HZ. This idea was supported by the work of Braun et al. on the mutagenicity of INH, suggesting that HZ represents the active principal in the mutagenesis in mice. More recently, the possibility that HZ may induce a syndrome similar to systemic lupus erythematosus was pointed out by Durant and Harris.

In contrast to extensive studies of the biological effects of HZ, the metabolic disposition of the compound is still not completely elucidated. We developed a highly sensitive and specific method for determining the hydrazines, i.e., HZ, acetylhydrazine (AchZ) and 1,2-diacyethylhydrazine (DiAchZ) as well as INH and acetylsioniazid by gas chromatography-mass spectrometry (GC-MS). Using this method, tissue distribution and urinary excretion of HZ and its acetylated metabolites were examined in detail.

** To whom communications should be directed.
MATERIALS AND METHODS

Chemicals — The specific reagent grade of hydrazine sulfate and of AcHZ were purchased from Wako Pure Chemicals Ind. Ltd. and Tokyo Chemical Ind. Co., Ltd., respectively. Hydrazine sulfate-\textsuperscript{15}N\textsubscript{2} (99 atom\%\textsuperscript{15}N) and acetic anhydride-\textsuperscript{18}O (99 atom\%\textsuperscript{18}O) were obtained from the British Oxygen Co., Ltd. and Merck Sharp & Dohme Canada Ltd., respectively. DiAcHZ was prepared by the known method.\textsuperscript{13} Trideuterobenzylacetates of this compound, d\textsubscript{3}-DiAcHZ, was synthesized by the same method using the acetic anhydride-\textsuperscript{18}O instead of acetic anhydride. Trideuterobenzylacetate (d\textsubscript{3}-AcHZ) was prepared as the hydrochloride salt by the method of Nelson et al.\textsuperscript{14}

Animal Experiment — Male, Wistar rats weighing 200—250 g who had been fasted for 12 h, were given a subcutaneous injection of 0.31 mmol/kg of hydrazine sulfate and of AcHZ dissolved in 1 ml of physiological saline. Liver, kidney, lung and plasma samples were taken at 0.5, 1, 2, 4 and 8 h after the drug administration. For the investigation of the metabolism, urine samples were collected at 12-h or 24-h intervals to 48 h after dosing and were stored at −20°C until analyzed.

Assay Procedure — One to two g of the tissue samples which were obtained from the animal experiment, 5 ml of saline and 1 ml of mixed solution containing \textsuperscript{15}N\textsubscript{2}-HZ, d\textsubscript{3}-AcHZ and d\textsubscript{3}-DiAcHZ as an internal standard, were homogenized. The enzymatic reaction was stopped by the addition of 4 g of (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. The homogenate was centrifuged at 6000 \times g for 10 min, and the supernatant was further filtered to remove protein using the membrane cone. The filtrate or the suitably diluted urine sample spiked with the labeled compounds as an internal standard was placed in a centrifuge tube containing 5 g of (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} to salt out the water-soluble metabolites. Half a ml of 5 (v/v)\% benzaldehyde ethanol solution was then added to the sample solution. The mixture was shaken for 30 min at room temperature, then extracted twice with 15 ml of ethyl acetate by shaking for 20 min. The organic layer was filtered through anhydrous sodium sulfate to remove water, then combined and evaporated to dryness. The residue was trimethylsilylated with N, O-bistrimethylsilylethynylacetamide (BSA) in pyridine. One μl of this solution was injected into a gas chromatography-mass spectrometer.

Gas Chromatography-Mass Spectrometry (GC-MS) — The instrument used was a Shimadzu GCMS-7000 equipped with a multiple ion detector-peak matcher. A glass column, 1.0 m in length and 3 mm i.d., containing 1.5% OV-17 coated onto 80/100 mesh Shimalite W, was used. The temperature of injection port for the DiAcHZ measurement was 150°C and that for the others was 250°C. The oven temperatures for the measurement of DiAcHZ, AcHZ and HZ were 100, 160 and 220°C, respectively. Mass spectrometer conditions were as follows: accelerating voltage, 3 kV; ionizing current, 300 μA; ionizing energy, 23 eV; separator temperature, 260°C.

HZ, its internal standard \textsuperscript{15}N\textsubscript{2}-HZ, AcHZ and d\textsubscript{3}-AcHZ were mixed in aqueous solution with benzaldehyde to form benzalazine, \textsuperscript{15}N\textsubscript{2}-benzalazine, 1-acetyl-2-benzylidene-hydrazine (ABH) and d\textsubscript{3}-ABH, respectively. Furthermore, these compounds except benzalazine each were mono- or dtrimethylsilylated (TMS or di-TMS) by the addition of BSA. Mass fragmentography was employed in the analysis of these derivatives. To determine the amount of HZ, the relative height of the molecular ion peak of benzalazine at m/z 208(M\textsuperscript{+}) to that of \textsuperscript{15}N\textsubscript{2}-benzalazine at m/z 210(M\textsuperscript{+}) was measured. In the case of AcHZ, the relative peak height of ABH-TMS at m/z 219 (M\textsuperscript{+}−15) to d\textsubscript{3}-ABH-TMS at m/z 222 (M\textsuperscript{+}−15) was determined. DiAcHZ was measured by the ratio of DiAcHZ-di-TMS at m/z 245(M\textsuperscript{+}−15) to d\textsubscript{3}-DiAcHZ-di-TMS at m/z 248 (M\textsuperscript{+}−15). More detailed information has been previously described.\textsuperscript{1,3,12}

RESULTS

Fig. 1 shows the tissue levels of HZ after administration of hydrazine sulfate (0.31
mmol/kg) in rats. The hypodermic injection resulted in rapid absorption and distribution of the compound to the tissues in which the maximum levels were attained within 0.5 h. The elimination half-lives in liver, kidney, lung and plasma were 3.3, 2.7, 3.0 and 2.3 h respectively, and the greater part of the drug disappeared within 8 h. As can be seen in Fig. 1, the HZ concentrations in the kidney were significantly higher than those in the other tissues except 8 h after administration.

The tissue levels of the monoacetylated metabolite, AcHZ, are shown in Fig. 2. The relatively low concentrations in liver, lung and plasma were almost the same, peaked in 1–4 h and decreased gradually with the elapse of time. Time lag in the appearance of the metabolite is attributed to the rate-determining step of acetylation. The AcHZ concentrations in the kidney were also markedly higher than those in the other tissues (Fig. 2). The tissue levels of DiAcHZ, further acetylated metabolite of HZ, were extremely low, and only trace amounts were detected in each tissue.

Three urinary products, HZ, AcHZ and DiAcHZ, were quantitated. As shown in Table I, about 30% of a 0.31 mmol/kg dose was recovered in 48 h after injection. Unchanged HZ comprised 82% of the hydrazines recovered in urine; the proportion of the acetylated metabolites was small. The amounts of AcHZ (2.9±0.7%) and DiAcHZ (2.5±0.4%) excreted in the urine were almost equal, however, evidently the excretion of DiAcHZ was slow compared to that of AcHZ. This is attributed to the rate-determining step of acetylation, and may cause the extremely low levels of DiAcHZ in the tissue.

Fig. 3 shows that AcHZ was absorbed rapidly from the injection site and distributed quickly to each organ. The levels were relatively higher than those of HZ in each tissue at the same dose (Figs. 1 and 3). The elimination half-lives were longer

---

**FIG. 1. Tissue Concentrations of Hydrazine (HZ) after Subcutaneous Administration of Hydrazine Sulfate (0.31 mmol/kg) in the Rat**

- : liver, ○: kidney, △: lung and ×: plasma. Each point indicates the mean value for 3–9 rats. The vertical bar represents standard deviation of the mean.

a) p < 0.01, when compared with the other tissue levels.

**FIG. 2. Tissue Concentrations of Acetylhydrazine (AcHZ) after Subcutaneous Administration of Hydrazine Sulfate (0.31 mmol/kg) in the Rat**

- : liver, ○: kidney, △: lung and ×: plasma. Each point indicates the mean value for 3–9 rats. The vertical bar represents standard deviation of the mean.

a) p < 0.01, when compared with the other tissue levels.
Distribution of Hydrazine and Its Metabolites

TABLE I. Urinary Excretion of Hydrazine and Acetylated Metabolites after Subcutaneous Administration of Hydrazine Sulfate (0.31 mmol/kg) in the Rat

<table>
<thead>
<tr>
<th></th>
<th>% to dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0—12 h</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>18.6±5.2</td>
</tr>
<tr>
<td>Acetylhydrazine</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>Diacetylhydrazine</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>Total</td>
<td>21.2±5.3</td>
</tr>
</tbody>
</table>

Results are the means ± S.D. for 3—9 rats.

than those of HZ, being 4.8, 4.7, 4.9 and 4.2 h in the liver, kidney, lung and plasma, respectively. Also in Fig. 3, the levels of AchHZ in the kidney are significantly higher than those in the other tissues at 2, 4 and 8 h after administration.

Hydrazine which was liberated from AchHZ was detected in the tissues (Fig. 4). Markedly high level of HZ in the kidney was noted. This phenomenon is very similar to that of AchHZ shown in Fig. 2.

DiAchHZ could be detected in the tissues when AchHZ was administered. As shown in Fig. 5, the levels of DiAchHZ were low and the elimination was slow. The levels of DiAchHZ in the kidney were also higher than those in the other tissues.

Table II shows the percentage of urinary excreted metabolites after the administration of AchHZ. Around 40% of a 0.31 mmol/kg dose was recovered in 48 h. This is 10% higher than that of HZ. The proportion of the acetylated metabolite, DiAchHZ was relatively high, sharing 14% of the dose. Free HZ of 6.2% was recovered in the urine.

DISCUSSION

Dambrauskas and Cornish examined the HZ tissue distribution at 2 and 20 h after its subcutaneous injection (1.88 mmol/kg) in rats by the spectrophotometric method with p-dimethylaminobenzaldehyde. They reported that although a rather uniform distribution throughout most tissues, the kidney concentration was considerably higher than other tissues. As shown in Fig. 1, this was confirmed by following the time-course of the HZ level in the tissues.

They measured only HZ by the colorimetric method which suffers poor specificity and sensitivity. In this study, using the compounds labeled with a stable isotope as an internal standard, namely the isotope dilution method, we made it possible to determine trace amounts of
not only HZ but also AcHz and DiAcHz in the tissues quantitatively. By this method, it was found that either AcHz and DiAcHz are also distributed highly in the kidney (Figs. 3 and 5).

Investigation of the fate of HZ in the animals was undertaken by Mckennis et al.\textsuperscript{16-18} They reported that dogs excrete approximately 50\% of 0.47 mmol/kg dose as unchanged HZ within the first two days after injection.\textsuperscript{17} They also identified DiAcHz as a metabolite in the urine following the administration of HZ and AcHz.\textsuperscript{17} Dambrauskas and Cornish\textsuperscript{15} showed that approximately 50\% of 1.25–3.13 mmol/kg doses of HZ injected subcutaneously into mice is

---

**FIG. 4. Tissue Concentrations of Hydrazine (HZ) after Subcutaneous Administration of Acetylhydrazine (0.31 mmol/kg) in the Rat**
Each point indicates the mean value for 6–9 rats.
The vertical bar represents standard deviation of the mean.
a) \( p < 0.01 \), when compared with the other tissue levels.

**FIG. 5. Tissue Concentrations of Diacetylhydrazine (DiAcHz) after Subcutaneous Administration of Acetylhydrazine (0.31 mmol/kg) in the Rat**
Each point indicates the mean value for 6–9 rats.
The vertical bar represents standard deviation of the mean.
a) \( p < 0.05 \), when compared with the other tissue levels.

**TABLE II. Urinary Excretion of Acetylhydrazine and Its Metabolites after Subcutaneous Administration of Acetylhydrazine (0.31 mmol/kg) in the Rat**

<table>
<thead>
<tr>
<th></th>
<th>% to dose</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–24 h</td>
<td>24–48 h</td>
<td>Total</td>
</tr>
<tr>
<td>Acetylhydrazine</td>
<td>18.1±1.5</td>
<td>0.7±0.2</td>
<td>18.8±1.5</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>5.4±1.3</td>
<td>0.8±0.4</td>
<td>6.2±1.4</td>
</tr>
<tr>
<td>Diacetylhydrazine</td>
<td>13.4±0.9</td>
<td>0.6±0.2</td>
<td>14.0±1.0</td>
</tr>
<tr>
<td>Total</td>
<td>36.9±2.2</td>
<td>2.1±0.5</td>
<td>39.0±2.3</td>
</tr>
</tbody>
</table>

*Results are the means ± S.D. for 7 rats.*
excreted unchanged in the urine within 48 h. Recently, Wright and Timbrell\textsuperscript{190} reported that when hydrazine hydrate (0.16 mmol/kg, i.p.) was given to rats, 10.3% of the dose was recovered as HZ in the 0 to 24-h urine, and 2.2 and 1.2% as AcHZ and DiAcHZ, respectively. The recovery of unchanged HZ, which depends on the dose, was less than that of ours. However, their results were similar to those obtained in our study (Table I).

More recently, Springer et al.\textsuperscript{200} recovered about 75% of the single dose of HZ (1 mmol/kg, i.p.) from rats utilizing both $^{15}$N$_2$-labeled HZ and conventional method. In 48 h about 30% appeared in the urine as HZ and about 20% emerged as derivatives that were acid hydrolyzable to HZ. About 25% was converted to nitrogen gas, most of which appeared in less 30 min after the administration.

Since N-acetylation and N-deacetylation enzymes occur as independent metabolic systems and their actions in vivo oppose each other, the possibility of deacetylation must also be dealt with in considering the extent to which a compound is acetylated in vivo. The fate of AcHZ was then examined. Fig. 4 and Table II show that the N-deacetylation activity is significant. This demonstrates that the relative quantitative importance between N-acetylation and N-deacetylation activities is a factor which determines the extent of acetylation of HZ.

In the previous paper,\textsuperscript{4} we reported that isoniazid is distributed highly in the kidney. In this study, it was found that not only HZ but also AcHZ and DiAcHZ are distributed highly in the kidney. Figs. 2 and 4 show that AcHZ which is the acetylated metabolite of HZ and HZ which is liberated from AcHZ accumulate in the kidney tissue markedly higher than other cases.

On the other hand, the elimination half-lives of HZ and AcHZ in the kidney tissue are almost equal to those in the other tissues (Figs. 1 and 3). A similar tendency can be seen in Figs. 2 and 4. Furthermore, we have experimental results which indicate that transport of HZ into rat kidney slices is mediated by an energy-dependent pro-

These findings strongly suggest that tubular secretion might exist at least in the excretion of HZ. Uptake and biotransformation of the hydrazines by the kidney tissue are now being studied and will be reported elsewhere.

REFERENCES


21) Y. Kaneo: Unpublished data.