CORRELATION BETWEEN CONVULSIVE ACTION AND METABOLISM OF ISONIAZID

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It is well known that the animal, after a large dose of isoniazid, has a short series of clonic movements followed by a tonic convulsion with a sustained contraction of all muscles. The seizures are characterized by a relatively prolonged interval of time between the administration of the drug and the onset of epileptiform seizures (1, 2).

In our preceding paper (3), it has been described that this latent period differs with routes of administration and may be dependent on the isoniazid level in the central nervous system.

Assuming that there existed a critical concentration in regard to the brain level of isoniazid, it would reasonably be considered that the convulsion does not appear until the brain level exceeds this critical point, even if an animal received isoniazid sufficient to cause a maximal seizure.

Many reports, however, revealed that isoniazid undergoes a considerable metabolic change within a few hours after the administration to man and experimental animals (4, 5).

Therefore, it is felt that an investigation of the distribution and metabolic pattern of isoniazid in the animals under the drug-induced seizures may serve to determine if any correlation existed between the convulsive action and the metabolism of this compound.

The present paper describes distribution experiments in cats receiving a convulsive dose of isoniazid and also describes metabolic experiments carried out with mice receiving 14C-labelled isoniazid.

MATERIALS AND METHODS

1. Examination of brain for isoniazid

Cats, weighing 2 to 4 kg, were injected intraperitoneally with 150 or 250 mg/kg of isoniazid. In the first group, the animals were sacrificed 20 or 40 minutes after the injection. The central nervous tissues were removed and subjected to chemical analysis. In the second group, the animals were killed when the first seizure appeared. In the third group, the animals received 250 mg/kg. The tissues were removed immediately.
after the animals died from repeated convulsions.

Total isoniazid in tissues was determined by the method of Kelly and Poet (6).

2. Examination of radioactivity

White mice, weighing about 20 g, were used. Injection of isoniazid-carbonyl-\(^{14}\)C (71.5 \(\mu\)c/mg), diluted with non-labelled isoniazid, was made intramuscularly. The dosage was 0.5 mg/g corresponding to 0.5 \(\mu\)c/g. After observing onset of convulsion, mice were sacrificed. An average time to convulsion was 28 minutes.

Procedure for the isolation of metabolites in tissues: Isoniazid and its metabolites were separated by paper chromatography. The weighed tissue was homogenized in a glass homogenizer by adding ten volumes of 50% ethanol. Isoniazid and its metabolites in the homogenate were then extracted with boiling 80% ethanol (homogenate-ethanol, 1:10) for 20 minutes. After cooling, the filtrate was dried by evaporation. The residue was dissolved in 1 ml of 90% ethanol. This sample solution was then spotted directly on the paper.

A mixture of isopropanol-10% ammonia water (85:15) or isopropanol-water (85:15) was used as solvent. In order to obtain the Ferroat-positive spots, the chromatograms were sprayed with aqueous Ferroat reagent (7).

Autoradiography and paper-scanning: The autoradiograms were prepared by allowing the chromatograms to remain in contact with X-ray film (non-screen type) for more than three weeks. These chromatograms were also examined for radioactivity in a strip-counting autoscaner (Aloka FC-20).

Determination of total radioactivity: Sample solution in an appropriate dilution was placed on a stainless steel planchet and then dried under ordinary lamp. The radioactivity was measured in a windowless gas-flow counter (Aloka FC-1E). The usual corrections for background and self-absorption were made.

Whole body autoradiography: Thirty minutes after the injection, the mouse was sacrificed by immersing into a mixture of acetone and carbon dioxide ice. After allowing to stand for 24 hours in a cold room kept at -12°C, the whole body was cut longitudinally at median line (8). A thin polyvinyl membrane was applied to the cutting surface and exposure was made by apposition against X-ray film.

3. Materials

Isoniazid-carbonyl-\(^{14}\)C (Radiochemical Centre, England); l-isonicotinyl-2-acetylhydrazine hydrochloride, isonicotinyl hydrazones of pyruvic acid and \(\alpha\)-ketoglutaric acid were synthesized in our laboratory. Isonicotinuric acid was kindly supplied by Takeda Chemical Industries Co.

RESULTS

1. Relationship between concentration of isoniazid in cat's brain and onset of seizure

Concentrations of isoniazid in different areas of the brain and the upper portion of the spinal cord at various times following intraperitoneal injection of 150 or 250 mg/kg
of isoniazid were summarized in Table 1. The sites with the highest concentrations of the drug were cerebrum and thalamus. Moderate to high concentrations were noted in the cerebellum and medulla. Low concentration was found in the spinal cord.

As shown in Table 2, there seemed to exist a correlation between the onset of convulsion and isoniazid level in the cerebrum. In six cases where convulsive seizures were induced, the isoniazid level in the cerebrum was found to be more than 100 μg per gram of wet tissue, except for one case. In two cases where cats died from repeated clonic and tonic convulsions, there was a high concentration of the drug over the whole length of the central nervous tissues. These observations indicate that the brain level of isoniazid in concentrations as high as 100 μg/g might be critical for cats.

2. Tissue distribution experiments on mice

The overall distribution of radioactivity thirty minutes after the intramuscular administration of 14C-isoniazid was shown in Fig. 1. From this autoradiogram, it can be seen that isoniazid is easily absorbed from the injection site and is distributed throughout the whole body.

The stomach contained a high concentration of radioactivity. Moderate concentrations of the activity could be observed in the brain and liver. The similar results were also obtained from the determination experiment of total radioactivity found in different organs (Table 3).

3. Identification of the isoniazid metabolites in tissues and urine

Aliquots of the tissue- or urine-extract were placed on paper together with samples of the following authentic materials: isoniazid, 1-isonicotinyl-2-acetylhydrazine hydro-
chloride, isonicotinic acid, isonicotinuric acid, isonicotinamide, pyridoxalphosphate isonicotinylhydrazone, and isonicotinylhydrazone of pyruvic acid or alpha-ketoglutaric acid.

After the chromatogram was developed, the paper was sprayed with Ferroat reagent. The Rf values of the metabolites were compared with those of the standards. Radioactivity on the chromatogram was then scanned by plotting against distance from the origin.

**Brain:** The scanning pattern of the chromatogram of mouse brain, together with the autoradiogram, was illustrated in Fig. 2. The scan revealed five peaks. The major peak of radioactivity could be observed at Rf 0.62 which was the identical location of the coloured spot due to acetylisoniazid. The scan also revealed a rather broad band at Rf 0.54, indicating that this compound is identical with isonicotinic acid. A small peak with Rf 0.81 corresponded to unchanged isoniazid. The result indicates that the majority of isoniazid undergoes metabolic changes in less than thirty minutes after the administration. One of the remaining two peaks with Rf 0.92 was considered to be an unknown substance concomitant with the labelled isoniazid (Fig. 3).

According to Manthei (9) and Albert *et al.* (10), isoniazid can be slowly converted *in vitro* to diisonicotinylhydrazine which emits fluorescence under Wood's light. The unknown substance under consideration had a resemblance to such an artifact in respect
of solubility in organic solvents or of fluorescent character. However, no further examination was made.

The nature of the compound which remained at the origin on the chromatogram seemed to be of interest. As shown in Fig. 6, urine contained high amount of this compound. However, it did not represent any of the compounds listed above.

Liver: Chromatographic pattern of the liver-extract was qualitatively the same as the brain-extract but was characterized by a broad band of radioactivity at position of ifonicotinic acid (Fig. 4).

Kidney and urine: Fig. 5 shows the radioactive compounds in samples of kidney. It may be noted that the kidney seems to metabolize isoniazid to a variety of compounds.

Fig. 6 shows a chromatogram of urine collected during thirty minutes after the administration of $^{14}$C-isoniazid. The main radioactive spot corresponded to isonicotinic acid, indicating that the mouse excretes isoniazid mainly as forms of being hydrolyzed.
FIG. 4. Scan and autoradiogram of paper chromatogram of liver 30 min after injection of $^{14}$C-isoniazid.

FIG. 5. Scan and autoradiogram of paper chromatogram of the kidney extract.
The urine contained no detectable amount of isonicotinuric acid which could be detected in the kidney-extract. Failure to find the urinary output of this compound may probably be due to a time lag. It is noteworthy that the urine had a large amount of unidentified radioactive compound which remained at the origin on the chromatogram.

DISCUSSION

In research program designed to investigate the mode of convulsive action of hydrazines and hydrazides, attention has been focused mainly on the metabolism of gamma-aminobutyric acid (GABA). Evidence has been presented to suggest that the inhibition of glutamic acid decarboxylase and GABA-transaminase is the result of interaction between formyl group of pyridoxal phosphate and hydrazides (11, 12). Actually in vitro studies have demonstrated that isoniazid, one of the hydrazides, easily combines with pyridoxal phosphate to form the corresponding hydrazone (13). Our present experiments failed to present direct in vivo demonstration of the pyridoxal phosphate complexes. However, the existence of such a substance would be anticipated if so-called "GABA-depletion theory" supported by some investigators is followed for the mechanism of action of isoniazid on the central nervous system. But, no satisfying proof of such an idea is yet available.
Meanwhile, a rough estimate of pyridoxal phosphate of animal tissues is given at about 1 \( \mu g/g \) (14). Provided that the combination of isoniazid with the endogenous pyridoxal phosphate proceeds in an equimolar ratio, it may be said that the tissue isoniazid concentration necessary for the inactivation of the decarboxylase and/or transaminase need not be so high. However, the brain levels of isoniazid, determined at times of onset of convulsion in cats, were found to be more than 100 \( \mu g/g \). And there was no sign of seizure when the brain isoniazid was at the lower level. Supposing that the turnover of pyridoxine metabolism in the central nervous system is fairly well, the brain isoniazid in concentrations as high as 100 \( \mu g/g \) seems to be more than enough.

This discrepancy between in vitro and in vivo studies may partially be explained by the fact that, in the brain, a greater part of isoniazid underwent acetylation or hydrolytic cleavage in half an hour after the administration and an amount of unchanged isoniazid was surprisingly low. However, more reasonable explanation remains to be proved.

Medina (15) has recently reported that direct relationship can not be obtained between the metabolism of GABA and the convulsive action of hydrazines with or without vitamin B. Maynert et al. (16) also observed that hydrazine is capable of inducing seizures under the higher level of GABA in the brain.

According to Balzer et al. (17), isoniazid produces convulsive seizures which are not alleviated but potentiated by pyridoxal phosphate. Hado (18) also reported that the time to onset of convulsion induced by pyridoxal phosphate hydrazone of isoniazid was about one-half that by isoniazid itself.

The above-mentioned findings seem to be in conflict with the "GABA-depletion theory." Moreover, our present study gave no satisfying proof of the availability of this theory. Unlike the mechanisms of convulsive action of semicarbazides, those of hydrazines and hydrazides seem to be more complicated.

**SUMMARY**

The distribution of isoniazid in the central nervous system of the cat receiving a convulsive dose of the drug and the metabolic fates of \(^{14}\text{C}-\text{isoniazid} \) in the mouse were investigated.

In cats, high concentrations of isoniazid were observed in the cerebrum and thalamus. Low concentration was noted in the spinal cord. In six cases where convulsive seizures were induced by isoniazid, the level of total isoniazid in the cerebrum was found to be more than 100 \( \mu g \) per gram of wet tissue.

In mice, \(^{14}\text{C}-\text{isoniazid} \) was widely distributed throughout the whole body. The chromatographic patterns of the radioactive metabolic products differed markedly among the organs tested.

In the brain, a greater part of isoniazid was metabolized within half an hour by acetylation or hydrolytic process. However, the pyridoxal phosphate hydrazone complexes could not be detected.
Based on a certain assumption, possible relationship was discussed between metabolism and convulsive action of isoniazid.

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