Chem. Pharm. Bull. 17(4) 841—843 (1969)

UDC 615.281.033.034

Distribution and Excretion of ^{3}H-Amantadine HCl

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(Received July 8, 1968)

Amantadine hydrochloride (1-aminotricyclo [3,3,1,1,^{3,7}]decane) has been known as an antiviral agent against various strains of influenza virus.[2,3]

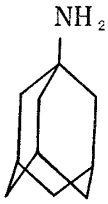

Fig. 1. Amantadine Hydrocholoride

Recently Bleidner, *et al.*[4] reported the distribution and excretion of Amantadine administered to various experimental animals. Since their studies were based on the extraction of administered amantadine from organs followed by quantitative analysis by gaschromatography and since the sensitivity of gas chromatogaphy requires more than a certain amount of amantadine, a dose far exceeding the doses used in humans had to be employed.

It is generally recognized that depending upon the variation of dose a large difference is to appear in the rate of distribution *in vivo* and in the rate of absorption and excretion. Therefore, a study using the dose silmilar to clinical use has become desirable. Among the methods of analysis, the most sensitive tracer method was selected.

Although it is difficult to label on a specific position of Amantadine, non-specific labelling with ^{3}H is not particularly difficult. In the present experiment, ^{3}H labelled Amantadine was used at the dose level of 1.6 mg/kg.

Experimental

Preparation of ^{3}H-Amantadine HCl——Acoording to the method of Wilzbach, irradiation with 140 curies–days of ^{3}H gas was applied to 194 mg of Amantadine HCl to obtain crude ^{3}H-Amantadine HCl. This was dissolved in water, made alkaline with 5N NaOH, and extracted with benzene. An adequate amount of 1N HCl solution was added to the benzene solution for extraction. This extraction procedure was repeated three times and the third benzene extract was sufficiently washed to remove excess alkali. Final extraction with 1N HCl was carried out, the pH of the sample was then adjusted to nearly neutral and the total volume was made up to 25 ml (Solution I).

Chromatography——1) Thin–Layer Chromatography: As the developing solvent, a mixture of butanol–acetic acid–water (4:1:5) was used. The temperature and time for development was 16—20° and 90 minutes. As the staining reagent, a mixture of pyridine-1%–ninhydrin-0.5%–ascorbic acid (2:1:1) was used.

2) Gaschromatography and radio-gaschromatography: Shimadzu gaschromatograph (GC–2C) and radio isotope detector (RID–2A) were employed. The detecors were hydrogen flame type for mass detection and solid scintillator–flow type for ^{3}H–counting after conversion of gas chromatographic effluent into ^{3}H–gas by a combustion furnace. A column packed with HMDS–treated chromosorb-W coated with 20% carbowax was used in gaschromatographic and radio-gaschromatographic studies. The conditions were as follows: column temperature, 98°; He–gas pressure, 0.6 kg/cm^2; range 100 cps; and time constant 5 sec.

Animal Experiment——1) Method of Administration and Maintenance of Animals: 0.1 ml of solution I diluted 10 times was orally administered in male mice of dd-strain weighing 23—25 g (^{3}H-Amantadine HCl 1.6 mg/kg, 0.312 mc/kg). The animals were individually kept in small metabolic cage to collect feces and urine.

2) Extraction of ^{3}H-Amantadine from Tissues: Mice were decapitated and lungs, heart, liver, kindeys and spleen were removed. After weighing, lung, heart, and spleen were homogenized with each 50 volumes

1) Location: *Kita-4-bacnho, Sendai.*
2) H.A. Wendel, *Fed. Proc.*, 23, 387 (1964).
3) R.R. Grunert, J,W. McGahen and W.L. Davies, *Virology*, **26**, 262 (1965).
4) W.E. Bleidner, J.B. Harmon, W.E. Hewes, T.E. Lynes and E.C. Hermann, *J. Pharmacol. Exptl. Therap.*, **150**, 484 (1965).

of 5N NaOH, while the liver and kidneys were homogenized with 10 and 20 volumes of 5N NaOH, respectively. Ten ml of benzene was added to the homogenate and the mixture was shaken vigorously, centrifuged at 3500 rpm for 5 minutes to separate the water and benzene layer. One ml of benzene layer was placed in a counting vial together with toluene scintillator fluid to measure the radioactivity.

For the estimation of recovery from tissue homogenate, the second and the third extraction were carried out by the analogous procedure with that mentioned above.

3) Extraction of ^{3}H-Amantadine from Blood: Blood was obtained from the mice sacrified by decapitation in a beaker made humid with double oxalate solution (1.25 g of ammonium oxalate and 0.75 g of potassium oxalate dissolved in water to make up 100 ml). To 1 ml of blood, 15 ml of 5N NaOH and 10 ml of benzene were added for extraction. The subsequent procedure is described in 2).

4) Extraction of ^{3}H-Amantadine from Urine: The food on the filter paper and the filter paper infiltrated by urine were chopped into small pieces and kept for half a day after the addition of 30 ml of 5N NaOH. Extraction was then carried out with 70 ml of benzene. The subsequent procedure is described in 2).

5) Extraction of ^{3}H-Amantadine from Feces: After weighing the feces collected on filter paper, 20 volumes of 5N NaOH was added and the mixture was kept for 24 hr. After sufficient fragmentation, 10 ml of benzene (30 ml if more than 12 hr has elapsed) was added for extraction. The subsequent procedure is described in 2).

6) Confirmation of ^{3}H-Amantadine and Its Metabolite in Benzene Extract: Each benzene extract was concentrated and developed on TLC and analyzed by radio-gaschromatography by the method described in chromatography to study the substances contained.

Results and Discussion

Examination of ^{3}H-Amantadine Prepared——^{3}H-Amantadine prepared by ^{3}H-gas irradiation and subsequent processing was chromatographed on thin-layer plate. After development the thin layer was divided into 18 portions, scraped off and placed in a vial to determine radioactivity using 10 ml of toluene scintillator fluid with Cab-O-sil gel powder. Fig. 2 showed the satisfactory distribution of radioactivity. The shaded spot represents the location of Amantadine confirmed by staining reagent following the development of Amantadine under the same conditions.

Distribution and Excretion of ^{3}H-Amantadine——The recovery of Amantadine by the first extraction was about 80% from the homogenate of each organ and 60% from urine. The values shown in Table I—III represent those obtained upon raising the recovery to about 100% after 3 extractions.

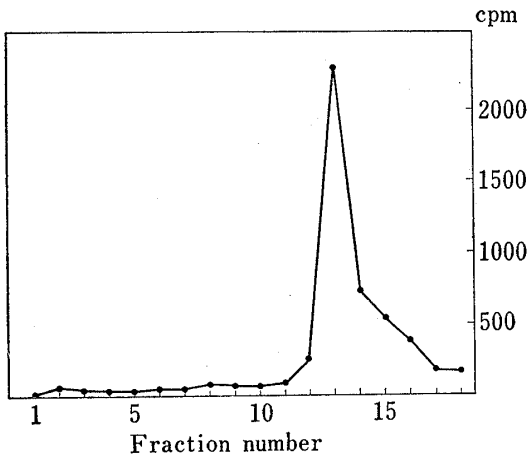

Fig. 2. ^{3}H-Distribution in the Fraction Scraped off Thin-Layer Plate

n-BuOH:AcOH:H_2O=4:1:5

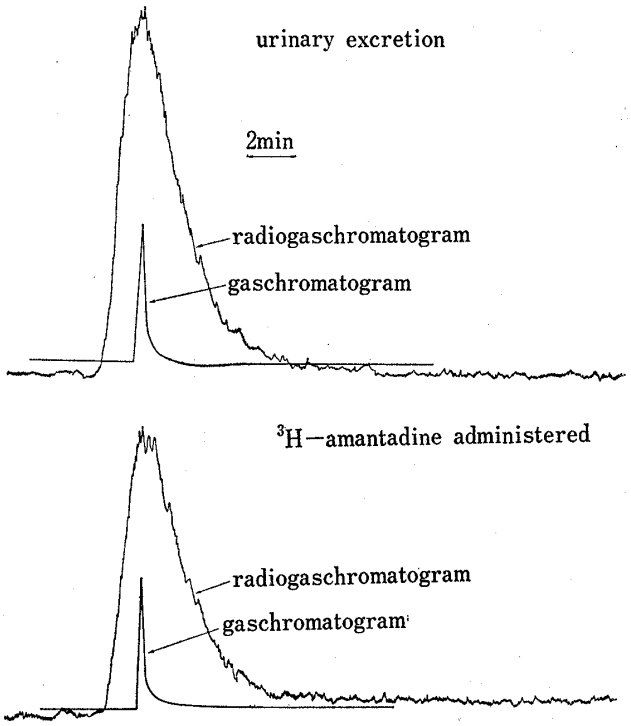

Fig. 3. Gaschromatograms and Radiogaschromatograms of Amantadine Administered and Recovered

Table I. The Distribution of Amantadine in Mouse Tissues Following Signle Oral Dose of 1.6 mg/kg

Tissue / Hour	Heart mμg/tissue	%	Lung mμg/tissue	%	Liver mμg/tissue	%	Kidney mμg/tissue	%	Spleen mμg/tissue	%
0.25	93	0.2	295	0.7	4776	11.9	1707	4.3	189	0.5
0.5	133	0.3	810	2.0	4967	12.4	2063	5.2	275	0.7
1	63	0.2	384	1.0	2254	5.6	1363	3.4	120	0.3
2	28	0.1	208	0.5	965	2.4	1139	2.8	50	0.1
6	18	—a)	100	0.3	449	1.1	237	0.6	18	—
12	18	—	34	0.1	174	0.4	91	0.2	14	—
48	6	—	13	—	43	0.1	14	—	7	—
100	4	—	9	—	18	—	4	—	1	—

a) indicates below 0.1%

Table II. Urinary or Fecal Excretion of Amantadine in Mouse Following Single Oral Dose of 1.6 mg/kg

Hour	Feces μg/total feces	%	Urine μg/total urine	%
0.25	—	—	0.24	0.6
0.5	—	—	0.06	0.2
1	0.12	0.3	4.10	10.2
2	0.23	0.6	5.15	12.9
4	0.16	0.4	13.53	33.8
6	0.43	1.1	15.98	40.0
12	0.71	1.8	24.73	61.8
24	1.25	3.1	27.31	68.3
48	1.58	4.0	29.68	74.2
100	2.98	7.5	34.64	86.7

Values indicate a cummulative amount of Amantadine in excretion obtained from individual animals for each analysis.

Table III. Blood Levels of Amantadine in Mouse Subjects Following Single Oral Dose of 1.6 mg/kg

Hour after dose	mμg/ml	% of dose	Hour after dose	mμg/ml	% of dose
0.25	176	0.4	6.0	54	0.1
0.5	186	0.5	12.0	32	0.1
1.0	87	0.2	48.0	14	—
2.0	45	0.1	100.0	—a)	—

a) not detectable

The distribution of Amantadine is maximum in liver among organs. The changes in the amount of Amantadine were approximately same in all organs tested, namely the peak was appeared at 30 minutes after administration. Even at 100 hours after administration, trace retention of Amantadine was detected.

Comparing with the other drugs selected arbitrary, the distribution of Amantadine into lungs was seemed to be significant, which may be the preferable tendency as anti-influenza drug. The present results are likely to reflect the fate of Amantadine of clinical use.

The results of analysis of gaschromatography and radiogaschromatography were shown in Fig. 3. Single peak was found both in gaschromatography and radiogaschromatography. The presence of ^{3}H-labeled metabolites was not demonstrated.