

Disposition of *N*-methyl- $[\text{ring-3,5-}^3\text{H}]$ tyramine in rabbits and miceHAI Hua, GUO Zhao-Gui², WANG Ji-Ming¹

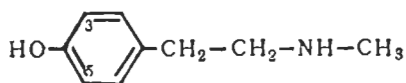
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ABSTRACT After iv bolus injection of *N*-methyl- $[\text{ring-3,5-}^3\text{H}]$ tyramine ($[\text{ring-3,5-}^3\text{H}]$ MT) 14.8 MBq/kg in rabbits, the plasma concentration-time data was found to be in accordance with the 2-compartment model. The pharmacokinetic parameters were: $T_{1/2\alpha} = 0.3$ min, $T_{1/2\beta} = 5.6$ min, $K_{12} = 0.69/\text{min}$, $K_{21} = 0.21/\text{min}$, $K_{10} = 1.6/\text{min}$, $V_C = 0.4$ L/kg, $Cl = 0.62$ L/kg \cdot min⁻¹. $[\text{ring-3,5-}^3\text{H}]$ MT was taken up by organs rapidly and extensively. Two min after administration, a large amount of radioactivity was detected in every organ sampled. The highest amounts were in the kidney and liver, followed by lung, small intestine, heart, skeletal muscle, spleen, brain and fat. The drug was metabolized extremely fast *in vivo*. The metabolites were found in the plasma chromatogram just 0.5 min after dosing, while over 80% were found in the urine within 1 h. After a 1 h collecting period, the radioactivity recovered in the urine amounted to 79% of the injected dose. By the end of a 6 h collection, almost no drug was detected in the body.

KEY WORDS *p*-hydroxy- $[\text{ring-3,5-}^3\text{H}]$ amphetamine; pharmacokinetics; two-compartment open models; tissue distribution; metabolism; excretion

N-methyltyramine is one of the active principles of Zhishi (枳实, *Ructus aurantii immaturus*). It possesses not only a marked pressor effect, but also increases cardiac contractility and urinary flow. Therefore, it is useful for the treatment of shock.⁽¹⁾ Studies of its effect on the cardiovascular system have been carried out since the 1970's, however, no report has been published on its pharmacokinetics so far. This experiment employed the technique of tracer radioisotope combined with thin-

layer chromatography to investigate the disposition of the ^3H -labeled drug ($[\text{ring-3,5-}^3\text{H}]$ MT) in animals.



N-Methyl- $[\text{ring-3,5-}^3\text{H}]$ tyramine ($[\text{ring-3,5-}^3\text{H}]$ MT)

Materials and methods

N-Methyltyramine acetate was provided by the Hunan Medical and Pharmaceutical Institution. It was labeled by the Measurement and Analysis Institution of Guangdong Province using halogen-tritium substitution. $[\text{ring-3,5-}^3\text{H}]$ MT was 96.6% pure as measured by thin-layer chromatography. Its specific radioactivity was 1028.6 GBq/mmol.

Animals, dosage and administration
New Zealand rabbits weighing $2.5 \pm \text{SD}$ 0.5 kg and NIH mice weighing $20 \pm \text{SD}$ 2 g (both sexes) were used. The $[\text{ring-3,5-}^3\text{H}]$ MT ethanol solution was dried. An adequate amount of nonlabeled MT was added as carrier. It was diluted with distilled water to the final concentration of 7.4 MBq/ml for rabbits and 3.7 MBq/ml for mice. For studying the plasma concentration changes with time, rabbits were given an iv bolus injection of $[\text{ring-3,5-}^3\text{H}]$ MT 14.8 MBq (2 mg)/kg within 15 s, while to study the distribution, metabolism and excretion, $[\text{ring-3,5-}^3\text{H}]$ MT 37 MBq (5 mg)/kg was injected via the mouse tail vein.

Determination of drug plasma concentration and metabolites The rabbit femoral artery was cannulated under sodium pentobarbital anaesthesia. 0.5, 1, 2, 5, 10, 15, 30 min after iv $[\text{ring-3,5-}^3\text{H}]$ MT, 2.5 ml blood was drawn separately into heparin saline

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tubes. 1.0 ml plasma was obtained after centrifugation and alkalinized with 25% aqueous ammonia to make its pH over 11. Then it was extracted 5 times with an equivalent volume of ethyl acetate (recovery rate of [^3H]MT was 96.5%). The acetate was completely volatilized by nitrogen current and the residuals were dissolved with 50 μl methanol. 20 μl of the solution were taken for chromatography by applying it onto thin-layer plates made of silica gel G (size: 20 \times 10 cm). 10 μg of MT were simultaneously spotted at each of the origins as a standard agent or carrier. The layer was developed in an ascending system of *n*-butyl alcohol: 12.5% aqueous ammonia: 95% ethanol (13:3:3) for 15 cm and dried in an oven at 60–70°C for 45 min to volatilize the remaining ammonia. Ninhydrin 0.2% was sprayed for the visualization of MT. The chromatograms were cut into strips 1.0 cm in length and the radioactivity of the strips counted directly. To observe metabolic changes, 2 ml of blood were drawn prior to the drug injection. An *in vitro* mixture of 1 ml plasma, 37 MBq [^3H]MT and 1 mg MT was made and kept in a bath at 37°C for 30 min. Subsequent procedures were the same as those described above.

Distribution Mice were divided into groups of 4. At intervals of 2, 5, 10, 15 and 30 min and 1, 2, 4 and 6 h after *iv* the drug, one group of animals was killed. About 70 mg each of heart, lung, liver, small intestine, kidney, skeletal muscle, brain, spleen and fat tissue were removed. The tissues were separately treated by means of perchloric acid digestion. The digested preparation was diluted to 1 ml with distilled water and 0.1 ml for counting.

Excretion Four mice were given *iv* [^3H]MT and raised in metabolic cages. Urine was collected 1, 2, 4 and 6 h after injection and diluted to 1 ml. 10 μl of

diluted urine were added directly to scintillation liquid for radioactivity determination. Urine was chromatographed in the same solvent as described before. Feces was collected for 24 h, baked at 60–70°C and then ground into powder. About 10 mg of powder was digested and decolorized. A 10 μl digested sample was used for measurement.

Radioactivity was measured by LKB-1215 RackBeta II liquid scintillation counter. The scintillation mixture was 0.6% PPO xylene. When the diluted urine, digested tissues and feces were counted, the cocktail had to be replaced by 0.6% PPO xylene: ethanol (7:3) in order to allow these aqueous samples to be incorporated into the scintillant. Each sample was solubilized in 5 ml scintillation liquid prior to counting and then counted for 1 min. The correction for quenching of all samples was checked by internal standardization.

Results

Plasma concentration of [^3H]MT and pharmacokinetic analysis Fig 1 is the plot of the semilogarithmic mean plasma concentration *vs* the time curve. It shows that 1 min after injection, the plasma radioactivity dropped to half the value from the peak. Five to 10 min later, the rate of declination decreased. After 30 min, plasma radioactivity could not be detected anymore.

Each pair of concentration–time data were inputted into an IBM microcomputer. The MCPKP-automatic pharmacokinetic program (worked out by the Veterinary Institution of the Chinese Academy of Agricultural Sciences) was employed for judgement of the drug kinetic pattern in the body, fitting of the concentration–time curve, selection of the optimal compartment model and calculation of the pharmacokinetic parameters. Kinetic analysis demonstrated that the elimination of [^3H]MT in 6 rabbits followed first-order kinetics. The concen-

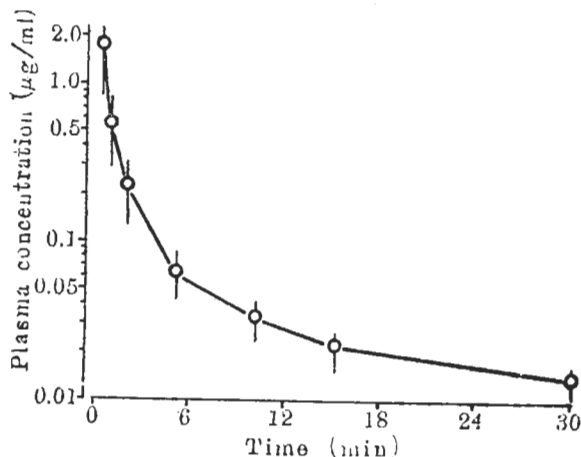


Fig 1. Plasma *N*-methyl-[ring-3,5- ^3H]-tyramine (^3H]MT) concentration after iv 2 mg/kg in 6 rabbits. $\bar{x} \pm \text{SD}$.

tration-time curve was fitted to one, two and three compartment models, respectively.

The weighting factor was $\frac{1}{c} / \frac{1}{n} \Sigma \left(\frac{1}{c} \right)$.

By comparing the values of r^2 , Re (residual sum of squares), AIC and the F test,⁽²⁾ the 2-compartment open model was chosen as the optimal one to describe the disposition of ^3H]MT in the body. Using the mathematical formulas⁽³⁾ of this model, the pharmacokinetic parameters were generated. Because the $T_{1/2\alpha}$ of ^3H]MT was very short (less than 20 s, calculated by iv bolus or by intravenous infusion), the duration of iv injection (15 s) must be taken into account as an important influential factor. For this reason, we considered that the parameters generated by intravenous infusion were more rational in reflecting the kinetic course of ^3H]MT. Pharmacokinetic parameters were first generated from the concentration-time data of each animal after iv administration of ^3H]MT 2 mg/kg. Parameters obtained from 6 rabbits were expressed as $\bar{x} \pm \text{SD}$ as follows: $K_{12} = 0.69 \pm 0.20/\text{min}$, $K_{21} = 0.21 \pm 0.09/\text{min}$, $K_{10} = 1.6 \pm 0.3/\text{min}$, $\alpha = 2.3 \pm 0.4/\text{min}$, $\beta = 0.14 \pm 0.06/\text{min}$, $T_{1/2\alpha} = 0.30 \pm 0.05/\text{min}$, $T_{1/2\beta} = 5.6 \pm 2.2/\text{min}$, $\text{AUC} = 2.9 \pm 1.0 \text{ mg/L} \cdot \text{min}$, $V_c = 0.4 \pm 0.15 \text{ L/kg}$, $\text{Cl} = 0.62 \pm 0.20$

$\text{L/kg} \cdot \text{min}^{-1}$.

Tissue distribution Distribution of radioactivity of ^3H]MT in the tissues at different times was illustrated in Fig 2.

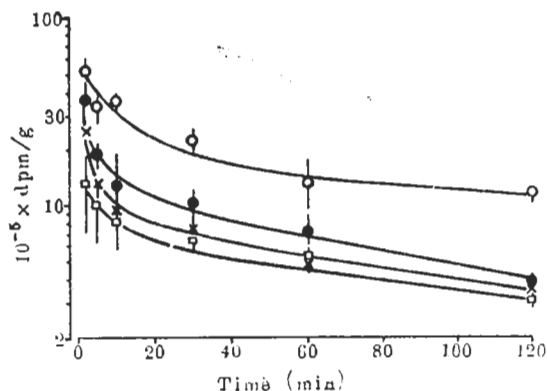


Fig 2. Distribution of radioactivity in various tissues after iv ^3H]MT 37 MBq/kg in mice. $n = 4$. $\bar{x} \pm \text{SD}$. (\circ) liver, (\bullet) lung, (\times) heart, (\square) brain.

The results showed that soon after dosing a large amount of radioactivity was taken up by each tissue sampled. The highest radioactive level was at 2 min after injection of the drug, except for that of the spleen and fat. The radioactivity tended to decrease gradually, and 30 min later it reached 50% of the peak level. About 2 h after administration, the radioactivity remained at low levels. However, in the liver, intestine and kidney, the decrease was relatively slow. It took 4 or 6 h for the disappearance of radioactivity. At peak concentration (2 min after iv), the degree of intensity of specific radioactivity in the tissues was: kidney > liver > lung > small intestine > heart > skeletal muscle > brain > spleen > fat.

Primary investigation in metabolism

The radiochromatography of rabbit plasma both *in vitro* and *in vivo*, as well as that of mouse urine are illustrated in Fig 3.

When ^3H]MT was mixed with plasma *in vitro*, the thin-layer chromatogram exhibited only a single peak with identical mobility to the parent drug (the R_F value

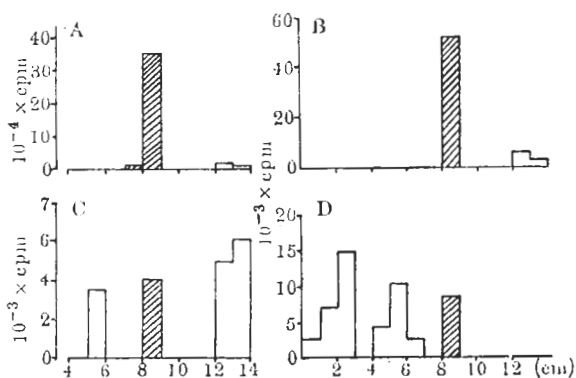


Fig 3. Thin-layer chromatograms of rabbit plasma and mouse urine in butanol: 12.5% aqueous ammonia: 95% ethanol (13 : 3 : 3). Rabbit plasma, [^3H]MT 37 MBq/1 mg being added *in vitro* and kept at 37°C for 30 min (A); rabbit plasma, 0.5 min (B) and 15 min (C) after iv [^3H]MT 14.8 MBq/kg; mouse urine, 1 h after iv [^3H]MT 37 MBq/kg (D). Hatched column: standard, blank column: metabolites.

was 0.54) (Fig 3 A). The small peak at the front containing only 5% of the total radioactivity was considered to be caused by impurities. This indicated that the blood itself did not have an obvious effect on the drug metabolism. Once the drug entered the body, metabolic changes occurred immediately. This change was reflected by the quickly reduced percentage of the radioactive parent drug, which decreased from between 70 to 80% initially to less than 15% at the 30th min. Even 0.5 min after administration, a radioactive peak appeared at the front of the standard MT. Its content was over 15% of the total radioactivity (Fig 3 B). Moreover, 15 min later, another smaller peak was found at the back of the parent drug (with a similar R_F value of 0.86 to the former metabolite). Meanwhile, the front peak became much higher (Fig 3 C). The chromatogram of 1 h urine revealed that the radioactivity gathered largely at the end close to the origin. The unchanged drug consisted of only 17%, whereas the percentages of the two metabolites reached 50 and 33%, respectively (Fig 3 D).

This case meant that 1 h after dosing, most of the [^3H]MT had been broken down and excreted into the urine in the form of metabolites. The same occurred in the other urine chromatograms of subsequent times. By the end of 6 h collection, there was only a peak less than 10% of the total radioactivity remaining at the position corresponding to the parent drug.

Excretion The excretion rates of radioactivity in the urine and feces over the 6 or 24 h collecting period are shown in Tab 1. It can be seen that [^3H]MT and its metabolites were excreted mainly from the urine and feces. 89% was excreted through the urinary tract, while less than 5% was excreted via the feces in 24 h. The total radioactivity recovered in urine and feces within 24 h amounted to 93% of the dose administered. Owing to the radioactive contamination on the wall of the metabolic cages and the collecting containers, the actual total excretion rate of radioactivity should be still higher. [^3H]MT excretion via the urine was very rapid. 79% of the tritium was excreted within the first hour, and in 6 h almost all of the drug had passed out of the body.

Tab 1. Excretion rate of radioactivity in urine and feces after iv [^3H]MT 37 MBq/kg in mice. $n = 4$. $\bar{x} \pm \text{SD}$

Time (h)	Dose excreted in urine (%)	Time (h)	Dose excreted in feces (%)
0-1	79.0 ± 5.0	0-1	0.23 ± 0.05
1-2	6.1 ± 0.5	1-2	0.23 ± 0.18
2-4	3.6 ± 2.0	2-4	0.5 ± 0.4
4-6	0.8 ± 0.3	4-8	0.8 ± 0.7
		8-12	0.7 ± 0.6
		12-24	0.8 ± 0.6
Total	89.0 ± 5.0	Total	2.3 ± 1.3

Discussion

The results have revealed that [^3H]MT is distributed throughout the body very rapidly. Its plasma half-life is only 5.6 min. The extremely short T_{+a} (0.3 min)

indicates a rapid drug distribution from blood to tissues. Two min after injection, a great deal of radioactivity was detected in all tissues sampled. A certain degree of [^3H]MT content found in mice brains early in administration implies that small amounts of the highly concentrated drug can penetrate the blood brain barrier. The large distribution to the kidney correlates with the drug's effects on renal vessels.⁽¹⁾ The radioactivity in the tissues decreased rapidly with time, and the fact that nearly 80% of the dose was recovered in the urine within the first hour demonstrates that the drug does not accumulate in the body. The radioactive chromatograms of plasma and urine showed that at least three metabolites were produced. Further investigations concerning their structures should be carried out. The facts that the plasma and urine samples were chromatographed in the same solvent system and that the metabolites occurred successively anterior and posterior to the standard MT suggest that a less polar intermediate may be derived first from metabolism, following which it is transformed into the two terminal products having higher polarity which are excreted in urine.

Due to the very short half-life of

[^3H]MT and its high speed of distribution; metabolism and excretion, it is more appropriate to give the drug by intravenous infusion in order to maintain its therapeutic level in blood. In the 1970's, clinicians recognized by repeated practice that MT had little effect as a shock remedy if it was given iv at intervals of 15 to 30 min because blood pressure would drop quickly during these intervals. Only when the drug was given first by iv injection as a loading dose followed by immediate intravenous infusion could a satisfactory antishock effect be achieved⁽⁴⁾. Our study provides the experimental basis for this rational clinical dosage regimen.

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N-甲基[环-3,5- ^3H]酪胺在兔和小鼠的体内过程

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提要 N-甲基酪胺是从中药枳实提取的升压抗休克有效成分。兔 iv [^3H]MT 14.8 MBq/kg 后, 血浓度-时间数据表明, 该药体内药动学过程符合二室开放模型。药动学参数如下: $T_{1/2\alpha} = 0.3 \text{ min}$, $T_{1/2\beta} = 5.6 \text{ min}$, $K_{12} = 0.69/\text{min}$, $K_{21} = 0.21/\text{min}$, $K_{10} = 1.6/\text{min}$, $V_C = 0.4 \text{ L/kg}$, $\text{Cl} = 0.62 \text{ L/kg}\cdot\text{min}^{-1}$ 。小鼠组织分布实验证明, [^3H]MT 体内分布迅速而广泛, iv 后 2 min 器官即可测到大量放射性, 以肾、肝含量最高, 肺、小肠、心次之。该药进入体内后很快代谢, 给药

后 0.5 min 血中即出现代谢物, 1 h 内排出的尿液中, 代谢物占 80% 以上。 [^3H]MT 主要自尿中排泄, 1 h 排出放射性占总放射性的 79%, 6 h 内基本排完。

关键词 p-羟基[环-3,5- ^3H]苯异丙胺; 药物动力学; 二室开放模型; 组织分布; 代谢; 排泄

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