Pharmacological Properties of Galenical Preparation. XVI.¹⁾ Pharmacokinetics of Evodiamine and the Metabolite in Rats

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In an attempt to evaluate its pharmacokinetics, [³H]evodiamine, which is one of the characteristic alkaloids of *Evodia* fruit was synthesized. The pharmacokinetics of [³H]evodiamine were investigated in rats.

In plasma, the main source of radioactivity was a metabolite of *d*-evodiamine (EM). One hour after oral administration of 200 µg/kg of [³H]evodiamine, the radioactivity level in the plasma was maximal. The radioactivity declined in a biphasic manner with half-life times of 1.6 and 78.4 h. The distribution volume was 560 ml/kg.

Radioactivity in tissues was higher in the liver, kidney, heart, lung, and adipose tissue than in plasma, but radioactivity in other tissues was lower than that in plasma. In all tissues the radioactivity proportionally decreased to the level of that in plasma.

At 24 h after administration, 19% and 63% of orally administered radioactivity was excreted in urine and bile, respectively.

Keywords [³H]evodiamine; pharmacokinetics; *Evodia* fruit; metabolite; *Evodia officinalis*; rat

Evodia fruit²⁾ is the dried unripe fruit of *Evodia officinalis* DODE (Rutaceae), and is used in combination with Chinese medicinal prescription, "Goshuyu-to (呉茱萸湯)." Goshuyu-to is a drug to maintain body temperature and eliminate chills. It is used for treating cold in the extremities which accompanying lowered metabolism, damage to digestive tissues, and hemicrania. We demonstrated the effectiveness of Goshuyu-to in maintaining body temperature, and also showed that this effect was due to *d*-evodiamine which is the main alkaloid in *Evodia* fruit.³⁾

In a biological pharmaceutical study of *Evodia* fruit components, we earlier reported on evocarpine as a quinoline alkaloid,⁴⁾ however, there has been no biological pharmaceutical study on evodiamine. In this paper, the pharmacokinetics of [³H]*d*-evodiamine was investigated after oral and bolus intravenous administration to rats. Distribution of [³H]*d*-evodiamine in tissues was also analyzed.

Materials and Methods

Crude Drugs *Evodia* fruit was purchased from a market in Osaka in 1987, and fit the regulation of Japanese pharmacopeia XII.

[³H]*d*-Evodiamine The chemical synthesis of evodiamine from rutaecarpine by Nakazato *et al.*⁵⁾ was modified to obtain [³H]*d*-evodiamine as follows.

Thirty mg of rutaecarpine (100 µmol) was dissolved in 20 ml of benzene. Then, 185 MBq of dimethyl sulfate (1 µmol) was added, and the mixture was stirred at 80°C for 24 h. Fifty ml of 0.01 N HCl was added to the reaction mixture, and nonreacted matter was removed by ethereal extraction (50 ml, 3 times). After adding ammonia reagent to the water layer, extraction was performed 3 times with 50 ml of ether. This ether layer was washed once with 50 ml of 0.01 N NH₄OH and then was concentrated to obtain 36.37 MBq of [³H]hydroxyevodiamine. The radiochemical yield was 19.7%.

[³H]Hydroxyevodiamine (29.6 MBq) was dissolved in 5 ml of methanol, and 10 mg of NaBH₄ was added. The solution was kept at room temperature for 30 min. Following removal of the solvent by distillation *in vacuo*, the residue was diluted with 50 ml of water, and extraction was performed 3 times with 50 ml of ether. After washing with 0.01 N HCl once, the organic solvent layer was evaporated. The residue was further refined by preparative HPLC, and 28.01 MBq of [³H]*d*,*l*-evodiamine was obtained. The preparative HPLC conditions for optical resolution were as follows: the column used was C18—20 (22 i.d. × 300 mm; Kusano Kagakukikai Co., Ltd.), methanol/water=80/20 was used for the mobile layer, and the flow rate was 10 ml/min.

This racemic mixture was separated by preparative HPLC for optical isomer resolution, and we obtained 10.80 MBq (36.5%) of *d*-evodiamine and 8.81 MBq (29.8%) of *l*-evodiamine. The preparative HPLC conditions for optical resolution were as follows: the column used was Chiralcel-OC (10 i.d. × 250 mm; Daicel Chemical Industries, Ltd.), hexane/ethanol=70/30 was used for the mobile layer, and the flow rate was 5 ml/min (Chart 1).

Animals Wistar male rats (6 weeks old, 150–180 g body weight) were purchased from Shizuoka Laboratory Animal Center. The animals were bred in a breeding room with a temperature of 24 ± 1°C, humidity of 50 ± 5%, and 12 h dark-light cycle. They were given tap water and fed normal foods *ad libitum*, then were fasted for about 24 h before the experiment.

Oral Administration: The administration liquid was prepared by mixing an aqueous solution of 2% Tween 80 and 12 µl of a [³H]*d*-evodiamine ethanol solution. The administration volume was 10 ml/kg. The dose was 200 µg/kg, and the radioactivity dose was 1.85 MBq/kg.

Intravenous Administration: The administration liquid was prepared by adding 60 µl of a [³H]*d*-evodiamine ethanol solution to 900 µl of a 5% albumin aqueous solution, then adding a further 5% albumin aqueous solution to obtain 1 ml. The administration volume was set at 2 ml/kg. The dose was 200 µg/kg, and the radioactivity dose was 1.85 MBq/kg.

Plasma Disposition [³H]*d*-Evodiamine was orally administered to one group of 5 rats. After a designated time period, blood was collected from the carotid artery under ether anesthesia, centrifuged at 3000 rpm for 10 min and plasma was obtained. One hundred µl of plasma was dissolved in 10 ml of Clear-sol, and radioactivity was measured by a liquid

Chart 1. Chemical Synthesis and Optical Resolution of *d*- and *l*-Evodiamine

scintillation counter (LSC-900, Aloka).

Urinary and Biliary Excretion Rats were put in a metabolism cage and fasted for one day, and were then given [^3H]*d*-evodiamine. Urine was collected at specified intervals thereafter.

A polyethylene tube (fr. No. 3, Hibiki) was inserted into the rat bile duct under amobarbital anesthesia. After administration of [^3H]*d*-evodiamine to operated rats, bile was sampled at the designated interval. One hundred μl of the urine or bile was dissolved in 10 ml of Clear-sol and used as a specimen for radioactivity analysis.

Metabolite of *d*-Evodiamine [^3H]*d*-Evodiamine was intravenously administered to rats. After 30 min, blood was collected from the carotid artery under ether anesthesia. Plasma was analyzed by preparative HPLC (column used was C18—20 (22 i.d. $\times$ 300 mm; Kusano Kagakukikai Co., Ltd.), methanol/water = 80/20 was used for the mobile layer, and flow rate was 5 ml/min. Fractions were collected every minute. Each fraction (5 ml) was added to 10 ml of Clear-sol, and radioactivity was measured by a liquid scintillation counter. Rat bile was treated the same as plasma.

Tissue Distribution [^3H]*d*-Evodiamine was intravenously administered to one group of 5 rats. After a designated time period, the rats were anesthetized by ether, and their blood entirely substituted by normal saline. Each tissue was then excised quickly, the wet weight of each tissue was determined, and 10% KOH in an amount equal to ten times the tissue weight was added. The tissues were then homogenized by homogenizer (HG30, Hitachi) and dissolved by ultrasonic waves (47 kHz, Branson, Yamato). Five hundred μl of this mixture was dissolved in 10 ml of Clear-sol and used as a specimen for radioactive analysis.

Data Analysis The radioactivity-time curve was plotted semi-logarithmically. The half-life ($T_{1/2}$) was calculated from the linear region by linear regression analysis. The maximum plasma concentration (C_{max}) and the time of its occurrence (T_{max}) were obtained from the observed values. The area under the drug plasma concentration-time curve (AUC) was calculated by the trapezoidal method from a graph for up to 24 h.

Results

^3H Labeled Compound [^3H]*d*,*l*-Evodiamine was synthesized from rutaecarpine (Chart 1). Using HPLC, *d*- and *l*-evodiamine were separated. The *d*-evodiamine agreed in IR and UV spectral data with *d*-evodiamine obtained from nature. The radiochemical purity of the *d*-evodiamine and the *l*-evodiamine was 99% or higher according to HPLC, and their chemical purity was 99% or higher by this means.

Ratio of Intact *d*-Evodiamine and Its Metabolite The results of HPLC analysis showed three radioactive compounds. The compounds of the retention times 2, 20, 25 min were called EM-1, EM-2 and EM-3, respectively. From the radioactivity HPLC chart (Fig. 1), it was shown that the main compound in plasma was EM-2 and amounts of the other compounds were very small. EM-3 was identified as *d*-evodiamine by HPLC analysis. The chemical structure of EM-1 and EM-2 have not been confirmed because the amounts were too small to analyze. The result

showed that *d*-evodiamine changed quickly to EM-2 in blood.

Plasma Disposition Figure 2 shows the transition of [^3H]EM-2 concentration in plasma with the administration of 200 $\mu\text{g/kg}$. T_{max} was 1 h after oral administration. By calculating radioactivity at that time, the evodiamine concentration in plasma showed C_{max} 232 ng/ml. The elimination pattern was biphasic: the $T_{1/2}$ of the first log-linear phase was 1.6 h, and that of the terminal log-linear phase was 78.4 h (Fig. 2). The elimination pattern by [^3H]*d*-evodiamine intravenous administration was also biphasic, and the compound was eliminated at the same ratio when administered orally (Fig. 2). The ratio of AUC of oral administration to intravenous administration ($AUC_{\text{p.o.}}/AUC_{\text{i.v.}}$) was 0.95.

Urinary and Biliary Excretion It was confirmed that 62.7% of the orally administered [^3H]*d*-evodiamine was excreted to bile and 18.7% to urine within 24 h of administration (Fig. 3). It was also confirmed that 73.2% of the intravenously administered [^3H]*d*-evodiamine was excreted to bile and 19.8% to urine within 24 h (Fig. 3).

Tissue Distribution Table I shows tissue distribution of [^3H]*d*-evodiamine intravenously administered to rats. The concentration in the plasma was 273 ng/ml at 30 min after administration, while its concentration in the liver, kidney, heart, lung, and adipose tissue was higher. Concentration in the adrenal gland was almost equal to that in plasma, but in other tissues the concentration was lower than that

Fig. 1. Elution Profile of Plasma (A) and Bile (B) in Rat

HPLC conditions: a Shimadzu LC-6A gradient system equipped with Shimadzu SPD-6A UV detector and data processor. Column: C18—20, 22 i.d. $\times$ 300 mm, Kusano Kagakukikai Co., Ltd.; mobile phase; methanol/water = 80/20; flow rate, 5 ml/min. Fractions were collected every minute. Each fraction was added to 10 ml of Clear-sol, and radioactivity was measured by a liquid scintillation counter.

Fig. 2. Time Course of Radioactivity in Plasma after Intravenous (A) and Oral (B) Administration of 200 $\mu\text{g/kg}$ of [^3H]Evodiamine to Rats ($n=5$)
Each point and vertical bar represents the mean and S.D.

Fig. 3. Time Course of Radioactivity in Bile after Intravenous (A) and Oral (B) Administration of 200 µg/kg of [³H]Evodiamine to Rats (*n* = 5). Each point and vertical bar represents the mean and S.D.

TABLE I. Tissue Distribution of Radioactivity after Intravenous Administration of [³H]*d*-Evodiamine (200 µg/kg) in Rats

Tissue	Concentration (ng eq of [³ H]evodiamine/g or ml)				
	30 min	1 h	2 h	4 h	24 h
Plasma	272.8 ± 21.6	229.4 ± 23.9	149.1 ± 18.8	61.1 ± 7.4	21.1 ± 1.3
Brain	79.9 ± 5.8	69.1 ± 2.0	43.9 ± 2.5	17.8 ± 2.5	6.3 ± 1.9
Submaxillary gland	110.2 ± 15.8	81.5 ± 14.8	50.7 ± 6.1	19.3 ± 2.4	—
Thymus	122.5 ± 16.7	103.0 ± 10.2	70.6 ± 6.4	30.1 ± 4.1	8.3 ± 1.6
Heart	986.8 ± 84.2	832.5 ± 75.7	586.6 ± 47.2	292.3 ± 35.0	49.8 ± 4.7
Lung	918.4 ± 86.9	734.1 ± 62.1	461.8 ± 39.4	189.4 ± 27.2	137.3 ± 14.2
Liver	1108.0 ± 95.7	993.4 ± 85.3	693.7 ± 50.3	326.9 ± 20.4	95.3 ± 9.0
Kidney	1039.9 ± 74.0	807.0 ± 36.6	495.7 ± 34.6	181.4 ± 15.2	87.8 ± 6.6
Adrenal gland	299.3 ± 22.9	206.2 ± 20.6	135.0 ± 12.7	50.1 ± 8.1	—
Testis	147.7 ± 12.5	126.1 ± 12.4	84.3 ± 8.5	31.0 ± 4.1	11.4 ± 1.3
Adipose tissue	531.1 ± 48.6	486.8 ± 54.2	319.6 ± 19.83	81.1 ± 7.6	25.7 ± 2.3

The data are expressed as the mean ± S.D. of five rats.

in plasma. Its elimination from tissues occurred in proportion to its concentration in plasma.

Discussion

We previously confirmed the effect of Goshuyu-to on maintaining body temperature, owing to *d*-evodiamine.³⁾ However, this effect is not found in rutaecarpine or hydroxyevodiamine,³⁾ which have a similar structures. These compounds differ in that evodiamine is a N(14)-CH₃ form, and rutaecarpine has no methyl group in position 14.⁶⁾ While hydroxyevodiamine has a methyl group in position 14, it has a hydroxyl group in position 13b.^{6,7)} Accordingly, from the structure-activity relationship, the presence of N-CH₃ in position 14 and the absence of the hydroxyl group in position 13b cause this effect. Thus, we believe it is very useful to label the methyl group in position 14 with ³H in studying the kinetics of *d*-evodiamine. We obtained *d,l*-evodiamine from a satisfactory radiochemical yield. By optical resolution by HPLC with a Chiralcel-OC column, we obtained each [³H]*l*- and [³H]*d*-evodiamine.

In blood, *d*-evodiamine changed quickly to EM-2. It was the main radioactive compound in plasma and the amounts of the others were very small. In fact the amounts of all compounds other than EM-2 were negligible, for all practical purposes.

The elimination of [³H]EM-2 from rat plasma showed

TABLE II. Pharmacokinetic Parameters of [³H]Evodiamine

Dose ^{a)} (µg/kg)	<i>V_d</i> ^{a)} (ml/kg)	<i>T</i> _{1/2} ^{a)} (h)	<i>AUC</i> ^{a)} (ng · h/ml)	Absorption ^{a)} ratio (%)	Excretion (24 h) ^{b)}	
					Bile (%)	Urine (%)
i.v.						
200	563	1.53	608	—	73.2 ± 3.7	19.8 ± 2.2
p.o.						
200	—	1.61	569	95.3	62.7 ± 4.1	18.7 ± 2.0

a) See the text for details. b) Mean ± S.D. (*n* = 5).

a biphasic pattern. It seemed that the terminal log-linear phase was based on excretion of ³H₂O due to [³H]*d*-evodiamine metabolism from its half-life. The half-life of EM-2 was thought to be 1.6 h obtained from the straight-line section in the first phase.

The absorption rate was 95% as estimated by the *AUC* ratio (*AUC*_{p.o.}/*AUC*_{i.v.}) up to 0–72 h (Table II). The total amount excreted in urine and bile within 24 h of oral administration accounted for 81.4% of the dosage, and that within 24 h of intravenous administration for 93.0%. From these results, the absorption rate of 88% was obtained. This almost agreed with the value calculated from the *AUC* ratio, suggesting that [³H]*d*-evodiamine has excellent absorption characteristics. However, *d*-evodiamine changed quickly to EM-2 in blood.

It was thought that the main excretion route in rats was

in feces through in bile. The concentration of [^3H]EM-2 in the liver, kidney, heart, lung and fat tissues was higher than that in plasma, while its concentration in the adrenal gland was almost equal to that in plasma, and in other tissues was lower. Its elimination from tissues proceeded in proportion to its concentration in plasma. These results suggested that EM-2 does not accumulate in tissues. It seems that EM-2 is the active compound, but at present, the chemical structure of EM-2 has not been confirmed. We will continue study of this subject.

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