[Chem. Pharm. Bull. 22(10)2372—2376(1974)]

UDC 547.91.09 : 615.22.033

Studies on Drug Metabolism by Use of Isotopes. XIII.[1] Isotope Effect on Metabolism of Deuterated *l*-Ephedrine

KUNIO KAWAI and SHIGEO BABA

Tokyo College of Pharmacy[2]

(Received April 16, 1974)

Isotope effect which is among problems on the applicability of a drug labeled with deuterium on the benzene ring to the metabolic studies in man was investigated. *l*-Ephedrine[α-^{14}C,arom.-$^{2}H_5$] or *l*-ephedrine[α-^{14}C] was subcutaneously injected to rats and rabbits, and the urine excreted during 24 hr after dosing was submitted to the inverse isotope dilution analysis. The urinary excretion of ^{14}C activity in rats or rabbits given the deuterio-compound was not significantly different from that given the protio-compound. No significant difference was observed in the amount of main urinary metabolites (in rabbits, 1-phenyl-1,2-propanediol and hippuric acid, and in rats, *p*-hydroxyephedrine) between the deuterio- and the (proti-odrug) except the amount of unchanged ephedrine which was excreted more by rats receiving deuterated ephedrine. The increased excretion of unchanged ephedrine after dosing of the deuterated compound did not seem to be due to deuterium-isotope effect by another experiment using a mixture of *l*-ephedrine-[α-^{14}C,arom.-$^{2}H_5$] and *l*-ephedrine[arom.U-$^{3}H_1$].

Metabolism of drugs in experimental animals has recently been under study using radioactive isotope (RI)-labeled compounds to produce many fruitful results, while administration of RI-labeled drugs to man *per se* has been largely limited by the regulations of the International Commission on Radiological Protection. Because of wide species differences in the metabolism of drugs, however, the selection of appropriate species as a substitute for man in pharmacological studies is difficult to rationalize.

In an attempt to develop a practical procedure which is applicable to metabolic studies in man using a drug labeled with deuterium on the benzene ring,[3] *l*-ephedrine was chosen as a model to be labeled for this purpose, since the metabolic patterns of the drug differ considerably among animal species.[4] First, the stability of hydrogen isotope labeled on the aromatic ring in biological systems was investigated by the double tracer technique using ^{3}H- and ^{14}C-labeled *l*-ephedrine.[5] Next, *l*-ephedrine[arom.-$^{2}H_5$] was synthesized from $C_6{}^{2}H_6$.[6] Mass spectral problems in the quantitative analysis of deuterated compounds were studied in detail.[1] The purpose of the present work was to investigate whether isotope effect is observed in the metabolism of deuterated *l*-ephedrine *in vivo*.

Experimental

Labeled Compounds——*l*-Ephedrine[α-^{14}C] hydrochloride (0.358 μCi/mg) (I-^{14}C)[7] and *l*-ephedrine-[arom.U-$^{3}H_1$] hydrochloride (3.80 μCi/mg) (I-^{3}H)[5] were samples previously prepared in this laboratory. *l*-Ephedrine[α-^{14}C,arom-$^{2}H_5$] hydrochloride (0.371 μCi/mg) (I-^{14}C-d_5) was prepared from $C_6{}^{2}H_6$ (99.5 atom%

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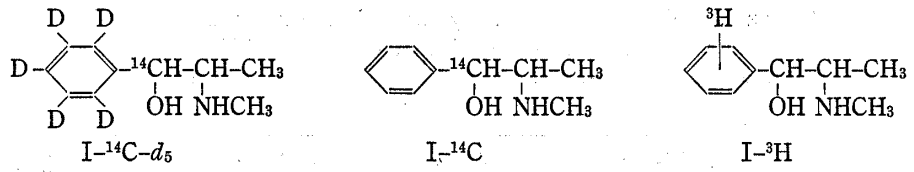

I-^{14}C-d_5 I-^{14}C I-^{3}H

Fig. 1. Structure of Isotopically Labeled *l*-Ephedrines

^{2}H, Merck, Darmstadt) and sodium propionate[1-^{14}C] (Daiichi Pure Chemicals Co., Tokyo) according to a method similar to that described by Baba, *et al.*[6]

Unlabeled Compounds——*l*-Ephedrine hydrochloride (JP grade) and hippuric acid (GR grade) were purchased from Sanko Seiyaku Kogyo Co., Tokyo and Tokyo Kasei Kogyo Co., Tokyo, respectively. *p*-Hydroxyephedrine hydrochloride was supplied from Hoechst Japan Co., Tokyo. 1-Phenyl-1,2-propanediol was synthesized.[8]

Animals and Dosing——Three male Wistar albino rats weighing 250±30 g and three male white rabbits weighing 3.0±0.2 kg were used in one experimental group. I-^{14}C-d_5, I-^{14}C, and I-^{14}C-d_5 (10 mg/kg) were subcutaneously injected successively to the same rats at intervals of 4 days or to the same rabbits at intervals of 3 days. An equimolar mixture (^{3}H/^{14}C=10.0) of I-^{14}C-d_5 and I-^{3}H (10 mg/kg) was subcutaneously injected to another group of rats.

Determination of ^{14}C Activity in 24 hr Urine——The urine of rats and rabbits was collected for 24 hr after dosing and then diluted with water to 100 ml and 500 ml, respectively. The ^{14}C activity in each diluted urine was determined conventionally with a liquid scintillation counter (Aloka Model 502, Japan Radiation & Medical Electronics, Inc., Tokyo).

Analysis of Main Urinary Metabolites——An aliquot (5 ml) of each urine sample from the rabbits was boiled in a water bath for 3 hr with 0.25 ml of concentrated hydrochloric acid. An aliquot (5 ml) of each urine sample from the rats was adjusted to pH 5.0 with 0.2M acetate buffer and incubated with β-glucuronidase (12000 Fishman units, Tokyo Zoki Co., Tokyo) for 24 hr at 37°. 1-Phenyl-1,2-propanediol and hippuric acid or ephedrine and *p*-hydroxyephedrine in the hydrolyzed urine of the rabbits or rats, respectively, were quantitatively determined by the inverse isotope dilution analysis as described in the previous paper.[4] The ^{3}H/^{14}C ratio in unchanged ephedrine excreted by the rats given the mixture of I-^{14}C-d_5 and I-^{3}H was determined according to the method of Baba, *et al.*[5]

Results and Discussion

The complete substitution of hydrogens on the benzene ring of drugs with deuterium may result in a primary isotope effect which should decrease the rate of aromatic hydroxylation when cleavage of a carbon-hydrogen bond is a rate-determining step. Any change of physicochemical properties with deuteration may also affect the absorption, metabolism, and excretion of the drugs; these are considered as "secondary" isotope effect. In order to evaluate correctly the metabolic data, obtained from a tracer experiment using drugs labeled with deuterium on the benzene ring, it is necessary to investigate isotope effect on the metabolism *in vivo*.

There are many evidences that cleavage of a carbon-hydrogen bond is a rate-determining step in the oxidation of alkyl side-chain *in vivo* and *in vitro*. For example, *N*-demethyldiazepam, deuterated at the C-3-position, was found to be hydroxylated at C-3 more slowly than the unlabeled compound.[9] Similar observations have been reported with morphine-[methyl-d_3],[10] *o*-nitroanisole[methyl-d_3],[11] and 3-dideuterio-pentobarbital.[12] On the other hand, no isotope effect was observed in the aromatic hydroxylation of acetanilide[13] and phenobarbital.[14]

In the previous paper,[4] it was shown that over 90% and about 70% of the ^{14}C activity administered was excreted in the urine of rabbit and rat, respectively, within 24 hr after

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Table I. Difference in Urinary Excretion between Deuterated and Protiated *l*-Ephedrine in Rabbits and Rats

	% of dose			
	I-^{14}C-d_5[a]	I-^{14}C[a]	I-^{14}C-d_5[a]	Mean[b] ± S.D.
No. of rabbits				
1	92.9	94.0	97.5	94.8±2.0
2	95.7	98.7	95.4	96.6±1.5
3	88.7	90.3	95.0	91.3±2.7
Mean[c] ± S.D.	92.4±2.9	94.3±3.4	96.0±1.1	
No. of rats				
1	77.4	77.1	71.5	75.3±2.7
2	71.5	65.4	69.1	68.7±2.5
3	61.2	67.3	71.6	66.7±4.3
Mean[c] ± S.D.	70.0±6.7	69.9±5.1	70.7±1.2	

a) I-^{14}C-d_5, I-^{14}C, and I-^{14}C-d_5 were administered successively at intervals of 3 days to the same rabbit and 4 days to the same rat, and 24 hr urine after dosing was collected.
b) Comparison of these mean values indicates the individual difference.
c) Comparison of these mean values may indicate the difference due to isotope effect and/or daily variation in the urinary excretion.

dosing of ^{14}C-*l*-ephedrine and there was no detectable ^{14}C activity in the urine on the fourth day in rabbit and on the fifth day in rat. Consequently, deuterio-(I-^{14}C-d_5) and protio-*l*-ephedrine (I-^{14}C) were administered successively at intervals of 3 days to rabbits and 4 days to rats. The urinary excretion of the ^{14}C activity in rabbits and rats is shown in Table I. There was no significant difference in the urinary excretion between I-^{14}C-d_5 and I-^{14}C.

In the rabbit, the main metabolic pathway of ephedrine is demethylation and subsequent deamination, followed by side-chain degradation. As the main urinary metabolites were 1-phenyl-1,2-propanediol and its conjugate, and hippuric acid,[4] isotope effect on the metabolism could be assessed from the amount of hippuric acid or the diol in the acid-hydrolyzed urine; under the hydrolysis conditions of this experiment, the conjugate of the diol was converted into the free form but not hippuric acid into benzoic acid. The amount of hippuric acid or the diol in the urine from two rabbits after dosing of I-^{14}C-d_5 was greater than that of I-^{14}C, but not in one rabbit (Table II). Because the output of metabolites of a drug may vary both individually and daily, the presence of deuterium-isotope effect on the metabolism could not be concluded from these data.

Table II. Difference in Metabolism between Deuterated and Protiated *l*-Ephedrine in Rabbits

No. of rabbits	% of dose			
	I-^{14}C-d_5[a]	I-^{14}C[a]	I-^{14}C-d_5[a]	Mean[b] ± S.D.
Hippuric acid				
1	31.4	31.5	20.4	27.8±5.2
2	41.9	32.6	34.3	36.3±4.0
3	32.8	29.0	31.7	31.2±1.6
Mean[c] ± S.D.	35.4±4.7	31.0±1.5	28.8±6.0	
1-Phenyl-1,2-propanediol (free+conjugated)				
1	15.0	13.9	15.0	14.6±0.5
2	15.4	11.2	16.3	14.3±2.2
3	10.8	12.4	12.4	11.9±0.8
Mean[c] ± S.D.	13.7±2.1	12.5±1.1	14.6±1.6	

a) and *b*) See the footnote of Table I.
c) Comparison of these mean values may indicate the difference due to isotope effect and/or daily variation in the metabolism.

In the rat, ephedrine is excreted mainly unchanged and partly as *p*-hydroxyephedrine and its glucuronide in the urine.[4] Any information for judging isotope effect on the metabolism could be obtained from the amount of ephedrine and/or *p*-hydroxyephedrine in the enzyme-hydrolyzed urine. If the rate-determining step in the *p*-hydroxylation of ephedrine involves cleavage of the carbon-hydrogen bond, then I-^{14}C-d_5 may be less *p*-hydroxylated and more excreted unchanged than I-^{14}C. As shown in Table III, two of the three rats administered I-^{14}C-d_5 excreted less *p*-hydroxyephedrine than those given I-^{14}C, but in one rat I-^{14}C-d_5 was, conversely, more *p*-hydroxylated than I-^{14}C, *i.e.*, a definite tendency suggesting a primary isotope effect in the aromatic hydroxylation was not observed. I-^{14}C was, however, 3 to 4% less excreted unchanged than I-^{14}C-d_5; the mean amount of the unchanged drug with the former was about 90% of those with the latter (32.5/36.5×100=89, 32.5/35.8×100=91). It was suggested that the increased excretion of unchanged ephedrine might be attributed to a secondary isotope effect, which is mainly dependent on factors such as pK_a and lipid solubility of the drug. In order to determine whether this tendency observed was due to a secondary isotope effect, an equimolar mixture of I-^{14}C-d_5 and I-^{3}H was administered to rats. Baba, *et al.* have found that the excretion of the unchanged drug with I-^{3}H was almost the same as that with I-^{14}C in the rat.[5] If I-^{14}C-d_5 is excreted more rapidly than I-^{3}H as unchanged, a lower ^{3}H/^{14}C ratio in ephedrine excreted than the ratio (^{3}H/^{14}C=10.0) administered must be observed; the ratio in the unchanged drug was expected to be 9.0 from values described above. Table IV shows that there was no significant difference in the excretion of unchanged ephedrine between I-^{14}C-d_5 and I-^{3}H within experimental error. From this fact, the difference in the excretion of unchanged ephedrine, as shown in Table III, is not possibly attributed to the secondary isotope effect, but daily variation in the excretion of the unchanged drug.

Table III. Difference in Metabolism between Deuterated and Protaited *l*-Ephedrine in Rats

No. of rats	% of dose			
	I-^{14}C-d_5[a]	I-^{14}C[a]	I-^{14}C-d_5[a]	Mean[b] ± S.D.
Ephedrine				
1	41.8	34.8	35.7	37.4±3.1
2	40.0	34.9	39.9	38.3±2.4
3	27.6	27.9	31.8	29.1±1.9
Mean[c] ± S.D.	36.5±6.3	32.5±3.3	35.8±3.3	
p-Hydroxyephedrine (free+conjugated)				
1	11.1	17.1	10.6	12.9±3.0
2	14.0	9.6	12.7	12.1±1.8
3	9.0	16.5	15.6	13.7±3.3
Mean[c] ± S.D.	11.4±2.0	14.4±3.4	13.0±2.0	

a) and *b*) See the footnote of Table I.
c) See the footnote of Table II.

Table IV. Ratio of ^{3}H/^{14}C in Ephedrine Excreted after Administration of an Equimolar Mixture of I-^{14}C-d_5 and I-^{3}H (^{3}H/^{14}C=10.0) in Rats

No. of rats	^{3}H/^{14}C in ephedrine excreted	No. of rats	^{3}H/^{14}C in ephedrine excreted
4	9.80±0.20	6	9.79±0.14
5	9.54±0.18	Mean	9.71

Each value represents the mean ±S.D. of 10 measurements.

Evaluation of the isotope effect which was based on the difference in the metabolism between I-^{14}C-d_5 and I-^{14}C was complicated by the daily variation in the output of metabolites. The difference between I-^{14}C-d_5 and I-^{14}C was, however, smaller than the individual difference in rats or rabbits (*cf.* Table II and Table III). From these facts, there was found to be no significant difference in the metabolism, at least *in vivo*, between I-^{14}C-d_5 and I-^{14}C.

When deuterium-labeled drugs are used for a tracer experiment, it is important to choose a position of labeling which must not result in isotope effect on the metabolism. From the present result, deuteration on the benzene ring would be suitable for the labeling of a durg. Therefore, compounds labeled with deuterium on the benzene ring would be useful for drug metabolism studies in man as a substitute for RI-labeled compounds.