Communications to the Editor

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Mass Fragmentographic Determination of *d*- and *l*-Chlorpheniramine with Aid of the Stable Isotope Technique

To investigate the pharmacokinetics of *d*-chlorpheniramine (*d*-CPA) and its *l*-isomer, *d*-CPA-d_6 and *l*-CPA-d_0 were administered orally and simultaneously to healthy adult volunteers. Both isomers in the serum were measured by chemical ionization-mass fragmentography using *dl*-CPA-d_{13} as an internal standard.

The concentration of *d*-isomer was higher than that of *l*-isomer and the ratio (*d*/*l*) of both isomers was 1.9—3.1, and the biological half-lives of *d*- and *l*-isomer were 24 hr and 15 hr, respectively.

Chlorpheniramine (CPA) maleate is used as an antihistaminics and it is well known that the pharmacological action of its *d*-isomer in the clinic is more effective than that of its *l*-isomer.[1] The investigations on the metabolism of enantiomers of some drugs provided that the metabolic pathways or the amounts of the metabolites of them were different each other after administration to mammalians, and so the plasma clearance and the rate of urinary excretion of the *d*-isomers were distinct from those of the *l*-isomers.[2]

Therefore, gas chromatography, especially gas chromatography–mass spectrometry has been used for investigation of the pharmacokinetics of these enantiomers because this technique has an advantage that this is not only very sensitive but also highly specific for qualitative and quantitative analyses of the drugs in biological fluids.

There are two ways to separate the enantiomers by gas chromatography: One of them is a derivatization technique which converts them to their diasteroisomers with chiral reagents such as N-trifluoroacetyl-(*S*)-(−)-prolyl chloride,[3] chrysanthemoyl chloride[4] and *l*-teresantalinyl chloride,[5] and another is a direct separation technique by the use of chiral stationary phases.

In these techniques, however, there are the following disadvantages: the former requires a functional group with active hydrogen in the molecule, and in the latter it is difficult to obtain the stationary phase which is stable at high temperature.

Mass fragmentography makes it possible to discriminate the enantiomorphic mixture, when one enantiomer is labelled previously with stable isotope even if they are not separable gas chromatographically.

The present paper deals with mass fragmentographic determinations of non-labelled *l*-CPA (*l*-CPA-d_0) and its *d*-isomer labelled with deuterium in human serum after the simultaneous administration of these isomers to the volunteers.

Hexadeuterium labelled *d*-CPA (*d*-CPA-d_6) was synthesized by the method of Sunagawa, *et al.*[6] using pentadeuteropyridine and monodeuteroformic acid (HCOOD) and was isolated with *d*-phenylsuccinic acid[7] and then *d*-CPA-d_6 was converted to its maleate [$[\alpha]_D^{26.8}$ +44.23° (*c*=1, DMF)].

On the other hand, *dl*-CPA-d_{13} was synthesized by the same method described in *d*-CPA-d_6 using heptadeuterodimethylformamide and dideuteroformic acid, and it was used as an

1) G. Babcok and L.A. Packard, *Clin. Med.*, **6**, 985 (1969).
2) L.M. Gune, *Biochem. Pharmacol.*, **16**, 863 (1967).
3) A.H. Beckett and B. Testa, *J. Pharm. Pharmacol.*, **25**, 382 (1973).
4) C.J.W. Brooks, M.T. Gilbert, and J.D. Gilbert, *Anal. Chem.*, **45**, 896 (1973).
5) T. Nambara, J. Goto, T. Taguchi, and T. Iwata, *J. Chromatogr.*, **100**, 180 (1974).
6) G. Sunagawa, K. Murayama, and J. Nakazawa, *Yakugaku Zasshi*, **74**, 1177 (1954).
7) L.A. Walter (to Schering Corp.), U.S. Patent 3061517 (1962) [*C.A.*, **58**, 6744 (1963)].

internal standard for compensating the losses in the processes of extraction, purification and measurement.

No quasi-molecular ion (QM^+) of CPA appeared, when a small amount of CPA (10 ng) was measured by gas chromatography-mass spectrometry in chemical ionization mode using methane, isobutane and ammonia as reagent gases. While the QM^+ without fragment ions was obtained by using trimethylamine as a reagent gas.[8]

The detection and determination limits of CPA were 1 pg and 10 pg, respectively and the calibration curve was linear in the range of 1 to 50 ng/ml serum of CPA and *d*-CPA-d_6.

Prior to the measurement of this pharmacokinetics using CPA-d_6 it was examined by utilizing 1:1 mixture technique[9] whether this d_6-variant exhibits the biological isotope effect or not.

Each 3 mg of non-labelled *d*-CPA-d_0 maleate and its d_6-variant was administered orally and simultaneously to three healthy adult volunteers who were under the medical supervision of Associate Professor B. Fukushima, Medical Faculty, Teikyo University, and then the concentrations of these drugs in the serum at each definite time were determined by chemical ionization-mass fragmentography.

As shown in Fig. 1A, the doublet at *m/e* 275 ($[M+H]^+$ of *d*-CPA-d_0) and 281 ($[M+H]^+$ of d_6-variant) has been kept at nearly equal intensities. Fig. 2A shows the time course of *d*-CPA-d_0 and its d_6-variant in the serum.

The above results revealed that the *d*-CPA-d_6 has no biological isotope effects.

Then, each 3 mg of *d*-CPA-d_6 maleate and *l*-CPA-d_0 maleate was administered simultaneously to other three healthy adult volunteers.

Fig. 1B and 2B show the mass fragmentogram and the time course of these compounds.

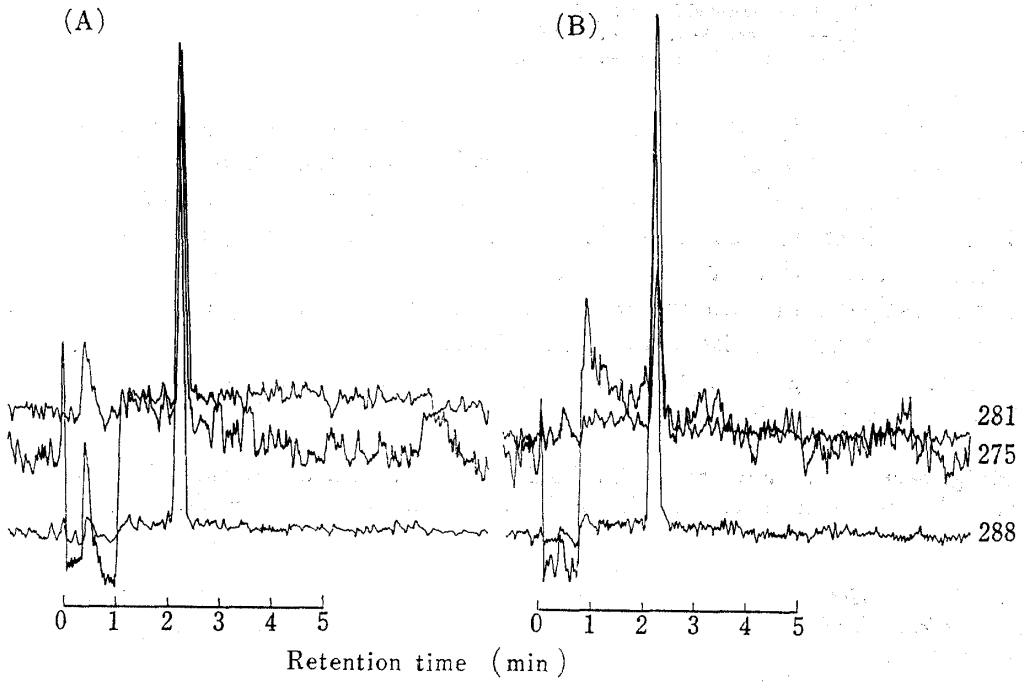

Fig. 1. Chemical Ionization Mass Fragmentograms of Chlorpheniramines in Serum Extract

A Shimadzu LKB-9000 GC-MS system equipped with MID was employed, column: Ucon Oil 50-HB-5100 and 0.05% KOH 2m on GLC110, column temp.: 185° ionization volt.: 500eV, accelerating volt.: 3.5kV, ion source temp.: 250°, reagent gas: trimethylamine 1 torr, carrier gas (He): 20ml/min.

8) M. Miyazaki and H. Abuki, *Biomed. Mass Spectrom*, "submitted".
9) D.R. Knapp, T.E. Gaffney, R.E. McMahon, and G. Kiplinger, *J. Pharmacol. Exp. Ther.*, **180**, 784 (1972).

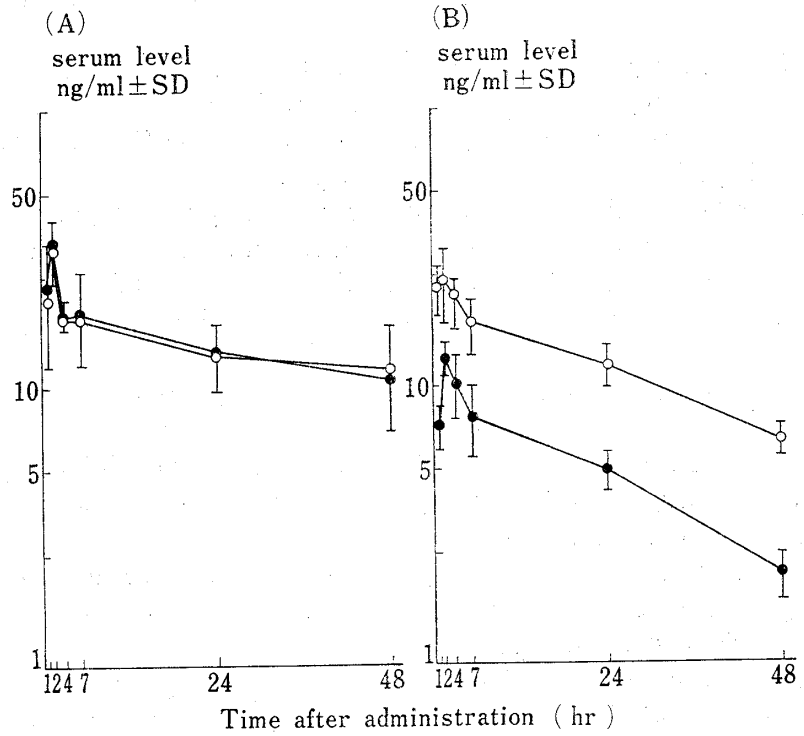

Fig. 2. Mean Time Course of Serum Level of Chlorpheniramines after Oral Administration to Three Healthy Adult Volunteers

The optical rotations of the chlorpheniramine maleates in this work were $[\alpha]_D^{26.8}$ + 44.23° (*d*-CPA-d_6), $[\alpha]_D^{25}$ + 44.3° (*d*-CPA-d_0) and $[\alpha]_D^{25}$ − 43.15° (*l*-CPA-d_0). They were measured in their DMF solutions c=1).
(A) —○—: *d*-CPA-d_6, —●—: *d*-CPA-d_0
(B) —○—: *d*-CPA-d_6, —●—: *l*-CPA-d_0

As shown in Fig. 2B, the serum concentrations between *d*-CPA-d_6 and *l*-CPA-d_0 were significantly different at each time after the oral administration of these drugs.

The concentration of *d*-isomer in the serum was higher than that of *l*-isomer and the ratio (*d*/*l*) of both isomers was 1.9—3.1. Furthermore, the biological half-lives of *d*- and *l*-isomers were 24 hr and 15 hr, respectively.

Further investigation on the metabolism and the rate of urinary excretion of both enantiomers by this technique will be reported elsewhere.

This technique may generally enable to discriminate the enantiomers which can not be separated gas chromatographically.

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