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Letter to the Editor

Gas chromatographic–mass spectrometric analysis of *threo*-methylphenidate enantiomers in plasma

Sir,

Methylphenidate (MPD), which is used in the treatment of attention deficit disorder and narcolepsy, is usually administered as a racemic mixture of the *threo* pair of enantiomers It is well known that the plasma concentration of racemic MPD after oral administration to humans is quite low A number of analytical methods have been developed for the determination of racemic MPD in biological fluids [1–8] On the other hand, it has been shown that (+)-MPD is more effective pharmacologically than the (−)-enantiomer [9] Recently, gas chromatographic (GC) methods were developed for the stereoselective determination of MPD in biological fluids [10, 11], and used to investigate the stereoselective disposition of MPD in humans [11] However, there is no information on the disposition of MPD enantiomers in experimental animals In the present study, a GC–chemical ionization mass spectrometric (GC–CI-MS) method was developed for the determination of MPD enantiomers, for application in the analysis of small plasma samples obtained from a disposition study in rats

EXPERIMENTAL

Enantiomers of MPD·HCl were separated from racemic MPD·HCl [Ciba-Geigy (Japan)] according to the method of Patrick et al [9] The optical rotation of (+)-MPD·HCl was $[88°]_D^{22}$, 0 8% in methanol (lit $[+89°]_D^{22}$), and that of (−)-MPD·HCl was $[-81°]_D^{22}$, 0 8% in methanol (lit $[-89°]_D^{22}$) [12] Racemic *threo*-form ethylphenidate·HCl as an internal standard (I S) was synthesized by esterification of racemic ritalinic acid (Ciba-Geigy) according to the method of Gal et al [5] N-Heptafluorobutyryl-L-prolyl chlo-

0378-4347/89/$03 50

ride (HFBPC) was synthesized according to the method of Lim et al [10] The GC–MS system included a JMA DX-300 mass spectrometer (JEOL, Japan) and a MS-GCG05 gas chromatograph (JEOL) equipped with a glass column (1 m×2 6 mm I D) packed with 1 5% OV-7/1 5% OV-210 on Chromosorb W AW DMCS, 80–100 mesh (Gasukuro Kogyo, Japan) The GC–MS analysis was performed in isobutane CI mode. The column, injection-port, separator and ion-source temperatures were 255, 265, 285 and 200 °C, respectively The flow-rate of the helium carrier gas was 37 ml/min The electron current was 300 μA and the electron energy was 200 eV

The extraction procedure was a slightly modified version of the methods of Lim et al [10] and Nakajima et al [8] To 0 2 ml of plasma were added 100 μl of methanol containing the I S , and 1 ml of a saturated solution of sodium tetraborate (pH 9 2–9 5) These procedures were performed on ice to prevent the degradation of MPD in the sample This finding was based on our results mentioned below for the stability of MPD enantiomers in plasma MPD and the I S in the mixture were then extracted into 4 ml of cyclohexane by shaking for 5 min After the two layers had been separated by centrifugation, the organic phase was transferred to a tube containing 0 3 ml of 1 *M* HCl in methanol and evaporated to dryness with nitrogen To the residue was added 1 ml of 0 3 *M* carbonate buffer (pH 9). The mixture was vortexed and allowed to stand for 5 min in an ice-bath Then 20 μl of 0 02 *M* HFBPC in dichloromethane were added to the mixture After this acylation had proceeded for 20 min in an ice-bath, 4 ml of cyclohexane were added and the mixture was shaken and centrifuged The organic phase was transferred to a tube and evaporated to dryness with nitrogen The residue was redissolved in 30 μl of methanol An aliquot (1–4 μl) was injected into the column

RESULTS AND DISCUSSION

Quasi-molecular ions at m/z 527 $[M+1]^+$ for the HFBP derivative of (+)- or (−)-MPD and m/z 541 $[M+1]^+$ for that of the I S observed as the base peak in the CI mass spectra were used as the appropriate ions for the selected-ion monitoring (SIM) analysis Peaks of the HFBP derivatives of MPD and the I S enantiomers were clearly separated on the SIM chromatogram (Fig 1) The peak-area ratios of m/z 527 to m/z 541 were calculated The first eluting peak of racemic ethylphenidate (I S) on the chromatogram, which may correspond to the (+)-enantiomer, was used for the quantification (Fig 1) The calibration graphs for plasma assay were linear in the range 0.04–2 ng per 0 2 ml of MPD enantiomers when 4 ng of the I S were used (regression equations $y=0\ 655x-0\ 010$ for (+)-MPD and $y=0\ 605x-0\ 015$ for (−)-MPD) Similarly, the calibration graphs were linear in the range 2–80 ng per 0 2 ml of MPD enantiomers when 30 ng of the internal standard was used (regression equations $y=0\ 088x-0\ 003$ for (+)-MPD and $y=0\ 084x-0\ 005$ for (−)-

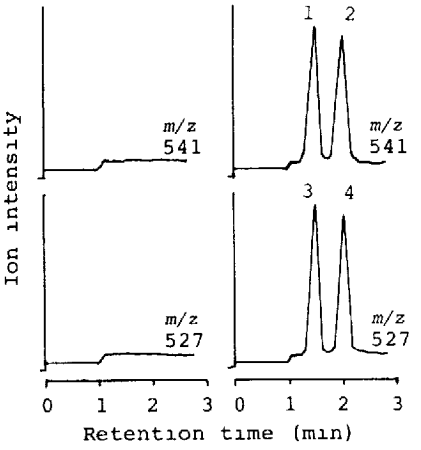

Fig 1 SIM chromatograms of a rat plasma sample containing 15 ng of (+)-MPD, 15 ng of (−)-MPD and 30 ng of racemic ethylphenidate (right panel), and blank rat plasma (left panel) Peaks 1 = the first eluting peak of HFBP derivative of ethylphenidate (I S), 2 = the second eluting peak of HFBP derivative, 3 = HFBP derivative of (+)-MPD, 4 = HFBP derivative of (−)-MPD

MPD) The reproducibility of the assay was examined for plasma sample at 30 and 180 min after intravenous (i v) administration of a 1 mg/kg dose of racemic MPD to a rat The coefficients of variation in the assay ($n=4$) were less than 4% for (+)-MPD and 5% for (−)-MPD

It was reported that racemic MPD was hydrolysed in the plasma of various species [8], so the stability of the MPD enantiomers in rat plasma was examined at 0°C and 25°C When the plasma containing 100 ng/ml (+)- or (−)-MPD was incubated at 25°C, both enantiomers were gradually degraded The residual percentages were 90% for (+)-MPD and 77% for (−)-MPD at 120 min This may suggest that (−)-MPD was more labile in plasma than (+)-MPD On the other hand, at 0°C, no degradation of either enantiomer was observed at least up to 90 min Therefore the degradation of each enantiomer in plasma after sampling could be prevented when the sample was rapidly treated at ca 0°C

The method was applied to the determination of the plasma concentration of MPD enantiomers after i v administration of racemic MPD to rats (270–280 g) As shown in Fig 2, the plasma concentrations of MPD enantiomers declined rapidly after administration The difference in the concentrations between (+)- and (−)-MPD after 2 h should be noted

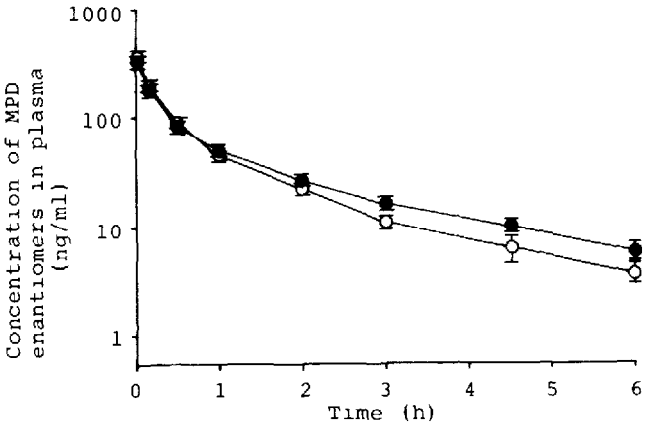

Fig 2 Time courses of plasma concentration of MPD enantiomers after i v administration of 1 mg/kg dose of racemic MPD to rats Data points (●)(+)-MPD, (○) (−)-MPD Data represent mean ± standard deviation of three rats

In conclusion, MPD enantiomers in plasma could be precisely determined in amounts as low as 0 04 ng by the present method using a 0 2-ml sample The method should be useful for disposition studies of MPD enantiomers in small animals, such as the rat

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