BIOMEDICAL CHROMATOGRAPHY, VOL. 6, 12–15 (1992)

Quantitation of the Enantiomers of Rimantadine and its Hydroxylated Metabolites in Human Plasma by Gas Chromatography/Mass Spectrometry

Nadia Choma, Preston P. Davis, Richard W. Edom and Elaine K. Fukuda*
Department of Drug Metabolism, Hoffmann-LaRoche Inc., Nutley, NJ 07110, USA

A gas chromatographic/mass spectrometric procedure has been developed for the quantitation in human plasma of the enantiomers of rimantadine and its three hydroxylated metabolites. The assay utilized derivatization of all analytes with the optically active reagent *S*-α-methyl-α-methoxy(pentafluorophenyl)acetic acid, selective ion monitoring, methane negative ion chemical ionization mass spectrometry and stable isotope dilution techniques. This method has been used to measure plasma concentrations of the enantiomers of rimantadine, *m*-hydroxyrimantadine and *p*-hydroxyrimantadine (equatorial and axial epimers) in the ranges 2.5–250, 2.5–50, 1.25–62.5 and 1.25–62.5 ng/mL, respectively, in six subjects given a single 200 mg dose of racemic rimantadine. Although there are no significant differences in the concentration–time profiles of *R*- and *S*-rimantadine, large stereospecific differences in the disposition of their metabolites are observed.

INTRODUCTION

The importance of stereochemisty on the pharmacodynamics, pharmacokinetics and metabolism of drugs is well known and has been recently reviewed by Ariens (1986), Drayer (1986), Hubbard *et al.* (1986) and Williams and Lee (1985). In these reviews, numerous examples of enantiomers which have different pharmacodynamic properties are given. In spite of this, many drugs are still administered as racemates; for example, Ariens (1986) stated that up to 90% of β-adrenergic blockers, anti-epileptics and oral anticoagulants are administered as racemic mixtures. Rimantadine, an antiviral agent described by Galasso *et al.* (1984), is such a drug.

Rimantadine is an interesting racemate to study because of its extensive metabolism and relatively simple metabolic profile in man as reported by Rubio *et al.* (1988). It is metabolized to three hydroxylated species, *m*-hydroxyrimantadine and the two epimers of *p*-hydroxyrimantadine (equatorial and axial), whose structures are shown in Fig. 1.

Recently, Miwa *et al.* (1988) have reported a gas chromatographic/mass spectrometric (GC/MS) assay for the quantitation of the enantiomers of rimantadine in human plasma and urine. This paper reports an extension to that original assay which allows the quantitation of the enantiomers of rimantadine and its three hydroxylated metabolites in human plasma. The method involves the use of [$^2H_4$]-stable isotope analogues, extraction at alkaline pH, derivatization with *S*-α-methyl-α-methoxy(pentafluorophenyl)acetic acid (MMPA), methane negative ion chemical ionization (NICI) mass spectrometry and selective ion monitoring (SIM). The MMPA derivative of rimantadine is shown in Fig. 1; the hydroxy metabolites are similarly derivatized with MMPA to form the corresponding amide. The tetradeutero analogues of rimantadine, *m*-hydroxyrimantadine and *p*-hydroxyrimantadine contain deuteriums on the side-chain methyl and on the chiral carbon.

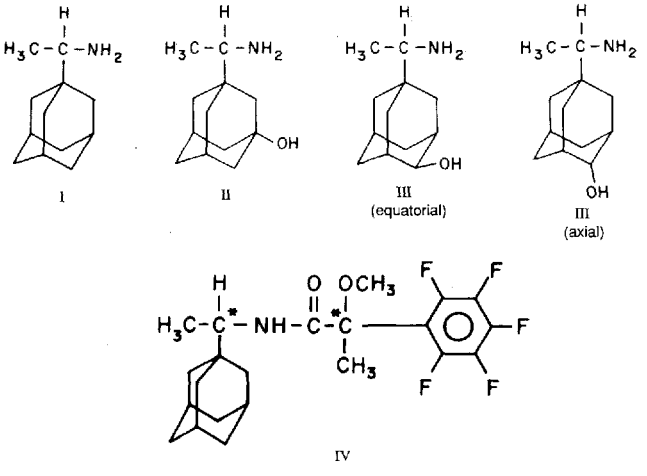

Figure 1. Structures of rimantadine (I), *m*-hydroxyrimantadine (II), the equatorial and axial epimers of *p*-hydroxyrimantadine (III) and the MMPA derivative of rimantadine (IV). The asterisks on structure IV denote the two chiral centres.

EXPERIMENTAL

The experimental details concerning the extraction method, calibration standards and (QA) sample preparation, and the derivatization method have been reported previously by Miwa *et al.* (1988) and Rubio *et al.* (1989).

Briefly, the plasma sample is fortified with the [$^2H_4$]-stable isotope analogues of rimantadine, *m*-hydroxyrimantadine and *p*-hydroxyrimantadine. After basifying the plasma sample, it is extracted with cyclohexane:chloroform (2:1). The extract is evaporated to dryness and derivatized with MMPA.

* Author to whom correspondence should be addressed.

0269–3879/92/010012–04 $05.00

Received 11 September 1991
Accepted 28 February 1991

Excess reagents are removed by adding 0.1 M NaOH and re-extracting the sample with dichloromethane. After drying the extract again, the residue is redissolved in 50 μL of ethyl acetate and analysed by GC/NICI MS.

Gas chromatography. A Hewlett-Packard Model 5889 gas chromatograph (Palo Alto, CA, USA) was interfaced to a Kratos MS890 tandem hybrid mass spectrometer (described below) and equipped with a DB-5 capillary column (15 m × 0.25 m i.d., film thickness 0.25 μm; J & W, Folsom, CA, USA). The GC oven was temperature programmed from 150 to 300°C at 20°C/min. Hydrogen was used as the carrier gas at a pressure of about 3 psi. A glass moving needle injector (Cat. No. 8992; Chrompack, Netherlands) was operated at 300°C and the GC/MS interface was maintained at 275°C.

GC/MS and data acquisition. A Kratos MS890 tandem hybrid mass spectrometer (Ramsey, NJ, USA) equipped with a System 80 Data General computer using DS90 software (Kratos Analytical, Manchester, UK) was used to acquire both full scan and SIM data. The mass spectrometer was tuned to give the optimal response at a resolution of about 1000. Methane was used as the NICI gas. The actual ions monitored were the $[M-HF-CH_3OH]^-$ ions from the derivatized analytes (*m/z* 379 and *m/z* 395) and their respective reference standards (*m/z* 383 and *m/z* 399). Peak heights were calculated using the DS90 software and QSIMPS (Rubio *et al.*, 1988) was used to calculate the analyte concentrations. Specifically, the ion ratios (*m/z* 379 : *m/z* 383 and *m/z* 395 : *m/z* 399) and the concentration value from the duplicate calibration standards were fitted, using weighted ($1/R^2$) non-linear regression, to the equation $R=(a+x)/(bx-c)$. In this equation, R is the ion ratio, x is the analyte concentration and a, b and c are parameters adjusted to give the best fit to the calibration data. For this study, the value of b was small, i.e. the calibration curve was virtually linear. Given an ion ratio from an experimental sample and the best-fit values for a, b and c, a value of x can be easily calculated.

RESULTS AND DISCUSSION

The methane NICI mass spectra of the diastereomers of derivatized rimantadine have been reported previously by Miwa *et al.* (1988). The base peaks for both derivatized *R*- and *S*-rimantadine are *m/z* 379, the $[M-HF-CH_3OH]^-$ ion. Similarly, a major ion at *m/z* 395 is observed for all derivatized *R*- and *S*-hydroxylated rimantadine species; this ion is also a $[M-HF-CH_3OH]^-$ ion. Because the hydroxylated diastereomers are chemically distinct despite their identical elemental composition, their mass spectra are not identical. In fact, the base peaks for the derivatized hydroxy metabolites are either *m/z* 395, 396 or 397, which differ only in the loss of one or two additional hydrogens. However, *m/z* 395 is nearly the base peak for all derivatized hydroxy metabolites, so this ion was chosen for quantitation. All deuterated derivatized internal standards each gave a $[M-HF-CH_3OH]^-$ ion four amu higher than their unlabelled analogue.

Figure 2 shows selected ion current profiles from a derivatized extract of a 48 h post-dose plasma sample from a healthy human subject given a single 200 mg dose of rimantadine. This sample was fortified with 100, 50 and 50 ng of $[^2H_4]$-rimantadine, $[^2H_4]$-*m*-hydroxyrimantadine and $[^2H_4]$-*p*-hydroxyrimantadine, respectively. The first eluting diastereomeric pair of ions at *m/z* 379 are from derivatized rimantadine. The later eluting diastereomeric pairs of ions at *m/z* 395 are from derivatized *m*-hydroxyrimantadine, *p*-hydroxyrimantadine (equatorial epimer) and *p*-hydroxyrimantadine (axial epimer), respectively. The concentrations of the *R* and *S* enantiomers of rimantadine, *m*-hydroxyrimantadine, and the equatorial and axial epimers of *p*-hydroxyrimantadine are 19, 22, 3.0, 6.9, 15, nonmeasureable, 4.4 and 2.7 ng/mL. Despite the fact that a peak is observed for *S*-*p*-hydroxyrimantadine (equatorial epimer), its calculated concentration was less than 1.25 ng/mL, the lowest calibration standard concentration (our definition of the lower limit of quantitation), and was therefore reported as nonmeasurable.

The absolute configuration of each enantiomer was assigned based on the analyses of model aromatic amines derivatized with MMPA by Pohl *et al.* (1973) and Valente *et al.* (1980). The absolute configuration of the first eluting diastereomer is assumed to have the *RS* configuration and the second eluting diastereomer is assumed to have the *SS* configuration. The same elution order is assumed for the hydroxy metabolites although they have different structures from the model

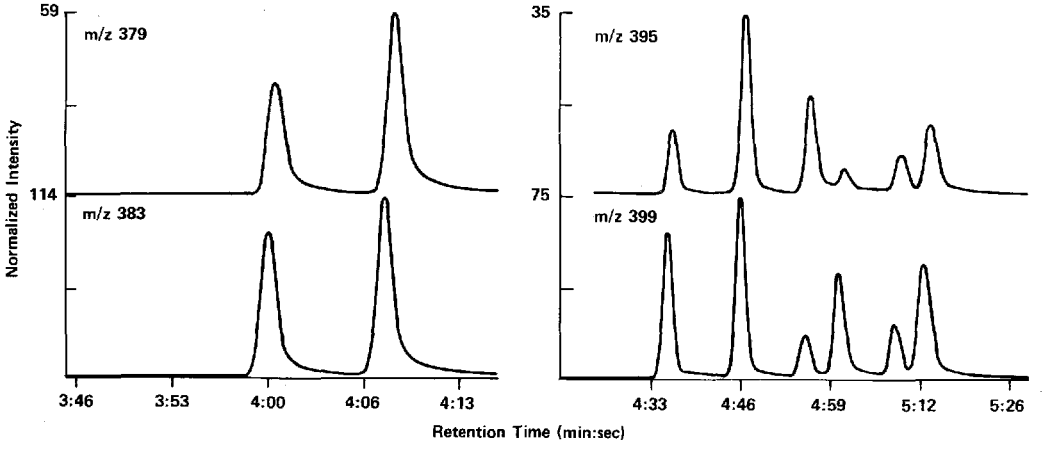

Figure 2. Selected ion current profiles from a derivatized extract of a 48 h post-dose plasma sample from a healthy human volunteer given a single 200 mg dose of rimantadine. This sample was fortified with 100, 50 and 50 ng of the appropriate $[^2H_4]$-reference standards.

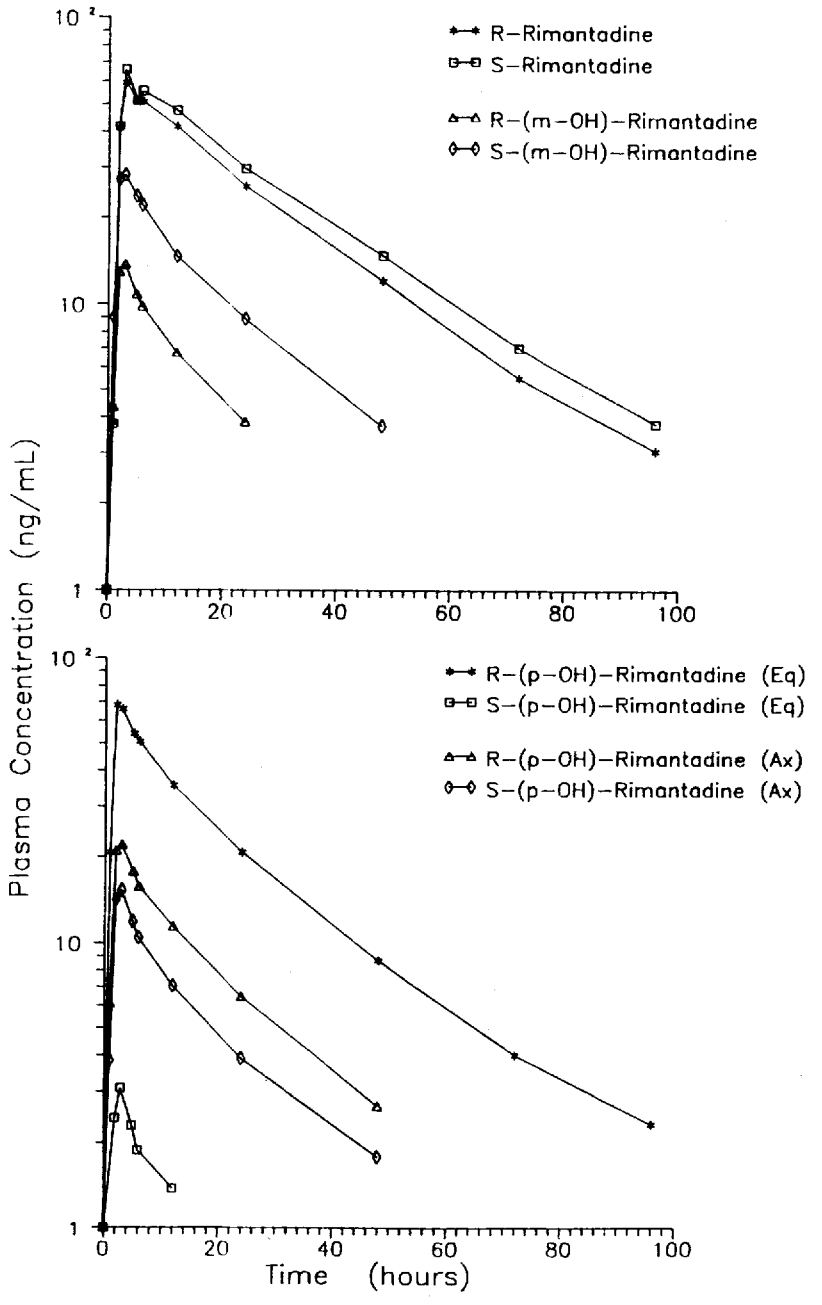

Figure 3. Plasma concentration vs. time profiles of the enantiomers of rimantadine and its hydroxylated metabolites in an individual given a single 200 mg dose of racemic rimantadine.

compounds referenced above. However, based on the above model systems, the *RS* diastereomer was assumed to elute first in all cases. In addition, this elution order is consistent with diastereomers prepared by reaction of (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride, a congener of MMPA, with several other aromatic amines, e.g. amphetamine (Gal, 1977), tocainide (Gal *et al.*, 1982) and *p*-chloroamphetamine (Ames and Frank, 1982).

The inter-assay precision of this assay for each enantiomer was estimated from the analysis of the precision of duplicate standards in each experiment. This was done by comparing the percent derivation of each found concentration to the back-calculated concentration obtained from the regression line. The inter-assay precision estimate was calculated by taking the mean percent deviation of all ten calibration standards. Based on the analysis of the eight calibration curves, the assay has an estimate overall inter-assay precision of 1.4 and 1.9%, 1.3 and 1.8%, 2.9 and 2.1%, and 2.3 and 1.6% for both diastereomers of rimantadine, *m*-hydroxyrimantadine, *p*-hydroxyrimantadine (equatorial epimer) and *p*-hydroxyrimantadine (axial epimer), respectively.

The intra-assay precision of the assay was estimated from the reproducibility of duplicate analyses of calibration standards and the QA sample from day to day. Specifically, a ratio of each duplicate standard and QA sample concentration was calculated; the percent deviation from a ratio of 1.0 was calculated for each pair. The intra-assay precision was estimated from the mean percent deviation of all duplicates. Based on the analysis of the same eight calibration curves and QA samples, the overall intra-assay precision was calculated to be 6.2 and 5.9%, 5.5 and 3.2%, 8.8 and 7.4%, and 8.5 and 6.0%, respectively.

Figure 3 shows typical plasma concentration vs. time profiles for the enantiomers of rimantadine and its hydroxylated metabolites of a helthy human subject given a single 200 mg oral dose of rimantadine. No significant differences in the profiles of *R*- and *S*-rimantadine are observed; Miwa *et al.* (1988) obtained similar results. However, the metabolites of rimantadine show significant stereospecific differences. Although the difference between *R*- and *S*-*m*-hydroxyrimantadine is small, the profiles of the four *R* and *S* epimers of *p*-hydroxyrimantadine are very different.

The observation of stereospecific differences in the metabolism of rimantadine is not surprising, given the fact that any enzymatic process is expected to be somewhat stereospecific, even for relatively non-specific P-450 enzymes. Also, because the elimination rate constant of rimantadine is smaller than that of its metabolites, the time–concentration profiles of all analytes decline in parallel (Rowland and Tozer, 1989).

Similar time–concentration profiles were obtained from the analysis of plasma samples from an additional five subjects. The pharmacokinetic analysis of the *R* and *S* enantiomers of rimantadine has been previously reported by Miwa *et al.* (1988); no pharmacokinetic analysis of the *R* and *S* enantiomers of the hydroxy metabolites is possible with the data reported herein.

In summary, a GC/NICI MS assay has been developed for the quantitation of the enantiomers of rimantadine and its hydroxylated metabolites in human plasma. This assay is being applied to study the stereospecific oxidative metabolism of rimantadine in man in healthy and diseased states, and also to study the stereospecific conjugation of rimantadine and its metabolites. Additionally, studies using resolved *R*- and *S*-rimantadine, as well as the individual racemic metabolites of rimantadine, are planned. Experiments with resolved *R*- and *S*-rimantadine will make it possible to determine if there are any stereochemical interactions between the two enantiomers which affect the pharmacokinetics of each enantiomer in the presence of the other enantiomer.

Acknowledgements

The authors wish to thank Dr. R. Wills for designing the protocol and Dr. R. Limjuco for carrying out the clinical work.

REFERENCES

Ames, M. M. and Frank, S. K. (1982). *Biochem. Pharmacol.* **31**, 5.

Ariens, E. J. (1986). *Med. Res. Rev.* **6**, 451.

Drayer, D. E. (1986). *Clin. Pharmacol. Ther.* **40**, 125.

Gal, J. (1977). *J. Pharm. Sci.* **66**, 169.

Gal, J., French, T. A., Zysset, T. and Haroldsen, P. E. (1982). *Drug. Metab. Dispos.* **10**, 399.

Galasso, G. J., Merigan, T. C., Merigan, T. C. and Buchanan, R. A. (1984). *Animal Agents and Viral Diseases of Man*, 2nd ed., Chap. 8. Raven Press, New York.

Hubbard, J. W., Ganes, D., Lim, H. K. and Midha, K. K. (1986). *Clin. Biochem.* **19**, 107.

Miwa, B. J., Choma, N., Brown, S. Y., Keigher, N., Garland, W. A. and Fukuda, E. K. (1988). *J. Chromatogr. Biomed. Applic.* **431**, 343.

Pohl, L. R. and Trager, W. F. (1973). *J. Med. Chem.* **16**, 475.

Rowland, M. and Tozer, T. N. (1989). *Clinical Pharmacokinetics, Concepts and Applications*, 2nd ed., pp. 350–352. Lea & Febiger, Philadelphia, PA.

Rubio, F. R., Fukuda, E. K. and Garland, W. A. (1988). *Drug Metab. Dispos.* **16**, 773.

Rubio, F., Choma, N. and Fukuda, E. K. (1989). *J. Chromatog. Biomed. Applic.* **497**, 147.

Valente, E. J., Pohl, L. R. and Trager, W. F. (1980). *J. Org. Chem.* **45**, 543.

Williams, K. and Lee, E. (1985). *Drugs* **30**, 333.