Journal of Chromatography, 575 (1992) 306–310
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6232

Short Communication

Plasma determination of the novel anticonvulsant D,L-3-hydroxy-3-ethyl-3-phenylpropionamide and preliminary pharmacokinetic studies in the rat

Lisbeth E. Gomez[☆,*] and Pedro A. Lehmann F.

Departamento de Farmacología y Toxicología, Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Apartado Postal 14-740, Mexico City 07000 (Mexico)

(First received June 20th, 1991; revised manuscript received November 22nd, 1991)

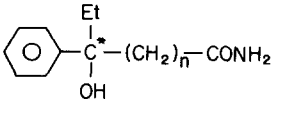
ABSTRACT

A sensitive gas chromatographic method with flame ionization detection was developed for the analysis in plasma of the novel anticonvulsant D,L-3-hydroxy-3-ethyl-3-phenylpropionamide (HEPP), using D,L-2-hydroxy-2-ethyl-2-phenylacetamide as the internal standard. HEPP was extracted from alkalinized plasma into dichloromethane and quantified after derivatization with bis(trimethylsilyl)-trifluoroacetamide. Standard curves were linear from 0.5 to 50 and from 2 to 100 μg/ml of plasma, using 1.5 and 5 μg of the internal standard, respectively. The lower limit of detection at a signal-to-noise ratio of 3 standard deviations was 0.33 μg/ml of sample. The sensitivity, accuracy and reproducibility of the method were shown to be satisfactory for pharmacokinetic studies of HEPP. After intraperitoneal administration of 50 mg/kg to Wistar rats, the principal kinetic parameters were: absorption half-life = 0.04 h; volume of distribution = 1.32 l/kg; clearance = 4.40 ml/min; peak concentration = 50 μg/ml; peak time = 0.25 h; mean residence time = 4.55 h.

INTRODUCTION

Despite substantial interest in developing safer and more efficacious antiepileptic drugs, at present six pharmacological classes are the source of all primary epilepsy treatment [1]. However, these drugs do not control the entire epileptic population, and all of them possess considerable toxicity [2]. The need for more selective and less toxic antiepileptic drugs is well recognized and their development is being actively pursued throughout the world [3].

A novel series of phenylalkylamide anticonvulsants (Fig. 1) have been synthesized [4] and were



n		Compound
0	—	HEPA
1	—	HEPP
2	—	HEPB

Fig. 1. Structures of the anticonvulsant homologous series.

☆ On leave from the Universidad Autónoma de Chihuahua, Chihuahua, Mexico.

0378-4347/92/$05.00 © 1992 Elsevier Science Publishers B.V. All rights reserved

found to possess a good anticonvulsant profile and relatively low toxicity. An overall comparative evaluation showed that D,L-3-hydroxy-3-ethyl-3-phenylpropionamide (HEPP) has a wide spectrum of activity and the lowest neurotoxicity of the three amides. These studies suggest that HEPP is a promising anticonvulsant, and it is currently undergoing preclinical development.

The aim of the present study was to develop a sensitive and simple gas chromatographic method with flame ionization detection (GC–FID) to measure HEPP in plasma for its preclinical pharmacokinetic evaluation. The applicability of the method to single-dose pharmacokinetic studies was demonstrated by the analysis of plasma samples following the intraperitoneal administration of HEPP (50 mg/kg) to Wistar rats.

EXPERIMENTAL

Chemicals and reagents

Racemic HEPP and D,L-2-hydroxy-2-ethyl-2-phenylacetamide (HEPA, internal standard) were donated by Dr. Guillermo Carvajal S. and his group at the Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional de México. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Aldrich (Milwaukee, WI, USA). All other reagents and solvents were analytical grade. Stock solutions of HEPP and HEPA (1 mg/ml) were prepared in methanol and stored at 4°C.

Chromatographic conditions

The GC system consisted of a Varian 3700 chromatograph with a flame ionization detector and a CDS 111 Varian integrator (Varian Instrument Group, Palo Alto, CA, USA). Separation was achieved on a 1.80 m × 2 mm I.D. glass column with 3% OV-17 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, USA). The GC conditions were: injection port temperature, 150°C; detector temperature, 260°C. The oven temperature was kept at 160°C for 1 min, followed by a 3°C/min programmed increase to the final temperature, 174°C, which was held for 2 min. The carrier gas (nitrogen) flow-rate was 35 ml/min. The hydrogen and air flow-rates were 35 and 300 ml/min, respectively.

Extraction procedure

Samples (0.5 ml) of heparinized rat plasma with 1.5 or 5 μg of HEPA were vortex-mixed with 0.2 ml of 0.5 *M* sodium hydroxide, 0.5 ml of 0.05 *M* phosphate buffer (pH 8), and 5 ml of dichloromethane in a 15-ml test-tube. The tube was shaken for 10 min and centrifuged for 15 min at 1650 *g*. The organic phase was transferred to concentration tubes (Laboratory Research, Los Angeles, CA, USA) and evaporated to dryness under a gentle stream of nitrogen. The residue was derivatized with 50 μl of BSTFA and allowed to stand at room temperature for at least 4 h before a 2–3 μl sample was injected into the gas chromatograph.

Quantitation

Owing to the wider range of concentrations seen in plasma in the pharmacokinetic studies, the assay linearity was checked by linear regression analyses in two separate sets of calibration curves. Seven standards of HEPP in the 0.25–25 μg (low) range and five standards in the 1–50 μg (high) range were assayed per 0.5 ml of blank sample ($n = 3$), and 1.5 or 5 μg of HEPA were added in the low and high range, respectively. The samples were processed according to the analytical procedure. The peak-area ratios of HEPP *versus* internal standard (HEPP/I.S.) were plotted against the concentration of HEPP. For each standard curve, the correlation coefficient, slope and *y*-axis intercept were obtained. The values of slopes and intercepts were used to calculate the unknown concentrations in controls and samples.

Recovery studies

Five replicate samples with different concentrations of HEPP and 5 μg of HEPA were prepared in blank plasma and processed as described above. The recovery was calculated by comparing peak-area ratios of HEPP to I.S. with and without extraction. Day-to-day precision and accuracy were calculated from the analysis of

three plasma control samples on five successive working days.

Pharmacokinetic studies

The pharmacokinetics of HEPP were determined by dosing seven male Wistar rats (200–250 g) per time-point with 50 mg/kg HEPP intraperitoneally. At specified times after dosing, the animals were exsanguinated by cardiac puncture and sacrificed by decapitation. The blood was immediately centrifuged at 1650 *g* for 15 min, and the plasma was stored at −20°C until analysis.

RESULTS AND DISCUSSION

Gas chromatography

Fig. 2 shows typical gas chromatograms for HEPA and HEPP. Their trimethylsilyl derivatives gave sharp and symmetrical peaks with retention times of 3.30 and 6.35 min, respectively. No interfering peaks of endogenous components were observed at these times (chromatogram A).

Linearity

Linearity of response was observed over the ranges of concentration studied. The mean calibration curves could be described by the following equations: $y = 0.163x + 0.039$, $r^2 = 0.99$, estimated standard deviation (S_y) = 0.09 in the low range; and $y = 0.264x - 0.204$, $r^2 = 0.99$, $S_y = 0.40$ in the high range.

The limit of detection, defined by the mass of analyte that produces a signal three times the standard deviation of the baseline noise [5] was 0.33 μg/ml.

Recovery

The mean extraction recovery of five different concentrations of HEPP was 96.1 ± 5.8%. The correlation between HEPP added and HEPP recovered is described by the equation $y = 0.99x - 0.74$, with $r^2 = 0.99$. The mean coefficient of variation (C.V.) was less than 10%. These results are shown in Table I. The precision and accuracy of the assay are reported in Table II. In general, the inter-day C.V. and error values ranged from 1.11 to 6.01% and from −10.2 to 4.4%, respectively.

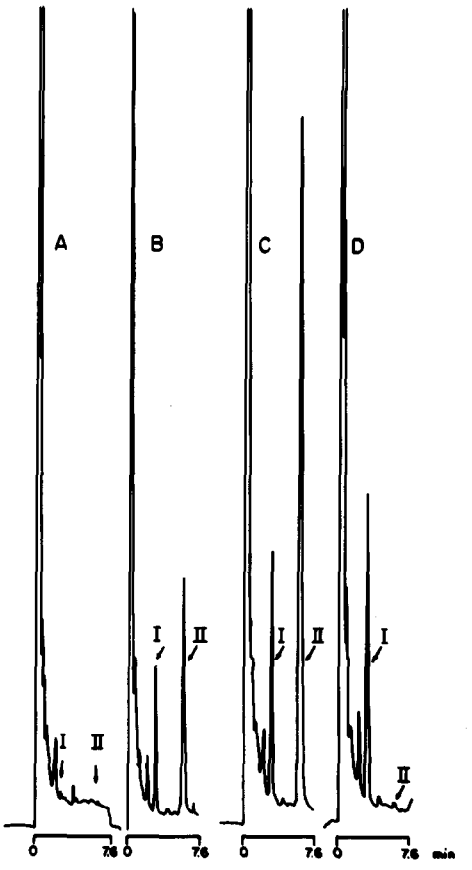


Fig. 2. Chromatograms of HEPP and HEPA from rat plasma after single i.p. administration of 50 mg/kg. (A) Blank plasma (pre-dose); the arrows show the absence of interference peaks at the retention times of HEPA (I) and HEPP (II), 3.30 and 6.35 min, respectively. (B) Plasma sample 1 min after dosage; calculated concentration 21 μg/ml. (C) Plasma sample 15 min after dosage; calculated concentration 54 μg/ml. (D) Plasma sample 16 h after dosage; caculated concentration 2 μg/ml.

Pharmacokinetic studies

Following the optimization of the chromatographic conditions and validation of the assay, it was used for preliminary pharmacokinetic studies in rats. Representative chromatograms obtained at different times after the intraperitoneal administration of 50 mg/kg HEPP are shown in Fig. 2. The concentration–time profile of HEPP in rat plasma is shown in Fig. 3. The data could be fitted to a two-compartment model, whose ad-

TABLE I

RECOVERY OF HEPP FROM RAT PLASMA

Mean recovery = 96.1%; S.D. = 5.8%; 95% confidence interval = 89–103%.

Amount added (μg)	Amount recovered (mean ± S.D., n = 5) (μg)	C.V. (%)	Recovery (mean ± S.D.) (%)
10	10.3 ± 0.5	4.86	102.9 ± 5.0
15	13.2 ± 1.0	7.74	87.8 ± 6.8
20	19.2 ± 1.0	5.40	96.3 ± 5.2
30	28.6 ± 1.9	6.68	95.2 ± 6.4
60	59.1 ± 3.4	5.80	98.5 ± 5.7

TABLE II

DAY-TO-DAY REPRODUCIBILITY AND ACCURACY OF THE PLASMA HEPP ASSAY OVER FIVE-DAY PERIOD

Amount added (μg)	Amount recovered (mean ± S.D., n = 5) (μg)	C.V.[a] (%)	Relative error (%)
5	4.49 ± 0.05	1.11	−10.2
25	26.10 ± 1.57	6.01	4.4
50	50.52 ± 2.59	5.13	1.0

[a] Mean C.V. = 4.33 ± 1.32%.

equacy was confirmed by examining the plot of residuals and by the PKCALC least-squares non-linear iterative computer program [6].

Kinetic parameters indicate that HEPP was rapidly absorbed (absorption constant, k_{abs} = 15.9 h^{-1}, absorption half-life, $t_{1/2}$ = 0.04 h), and extensively distributed into extravascular tissues. The values for the apparent volume of distribution (V_d) and clearance (Cl), calculated by the β method, were 1.32 l/kg and 4.40 ml/min/kg, respectively. The elimination process presents two exponential phases (α, β) with $t_{1/2}$ = 0.5 and 3.5 h, respectively. Peak concentration (C_{max}) was 50 μg/ml and peak time (T_{max}) was 0.25 h. The area under the plasma concentration *versus* time curve (AUC), calculated by the trapezoidal method from 0 to 16 h, was 182 (μg/ml) h; the area under the first statistical moment (AUMC), according to the polyexponential equation, was 897 (μg/ml) h^2 and the mean residence time (MRT) was 4.55 h.

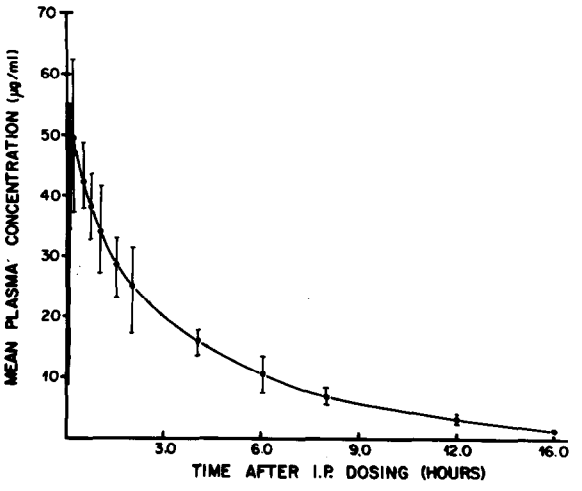


Fig. 3. Average plasma concentration–time profile of HEPP in rats after intraperitoneal administration of 50 mg/kg. Each point represents the mean ± S.D. of seven determinations.

CONCLUSION

This GC–FID method is sensitive and selective for monitoring HEPP concentrations in plasma and is applicable to pharmacokinetic studies.

ACKNOWLEDGEMENTS

The authors thank Dr. Guillermo Carvajal S. and Sergio Meza T. for supplying samples of the anticonvulsant compounds and Heinz Hemken P. for correcting the manuscript. This research was partially supported by CONACYT Grant No. P228CC0X891545. L.E.G. gratefully acknowledges financial support provided by the Universidad Autónoma de Chihuahua. Preliminary pharmacokinetic results of this work were presented at the *1991 Joint Meeting of the Western Pharmacological Society, Jan. 27–Feb. 1, 1991, Acapulco, Mexico.*

REFERENCES

1 R. L. Macdonald, *Epilepsia,* 30 (1989) S19.
2 R. L. Krall, J. K. Penry, H. J. Kupferberg and E. A. Swinyard, *Epilepsia,* 19 (1978) 393.
3 H. J. Kupferberg, *Epilepsia,* 30 (1989) S56.
4 S. E. Meza, M. T. Zenteno, E. Juárez, D. Martínez and G. Carvajal, *Arzneim.-Forsch.,* 40 (1990) 1289.
5 J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry,* Ellis Horwood, Chichester, 1984, p. 96.
6 R. C. Shumaker, *Drug Metab. Rev.,* 17 (1986) 331.