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A High-performance Liquid-chromatographic Microanalytical Procedure for the Rapid Estimation of Piracetam in Plasma or Cerebrospinal Fluid

M. H. DOHENY, M. T. O'CONNELL AND P. N. PATSALOS

Pharmacology and Therapeutics Unit, Epilepsy Research Group, University Department of Clinical Neurology, Institute of Neurology, London, UK

Abstract

Presently available GC and HPLC methods for analysis of piracetam, require large samples and suffer from interference.

A micro scale, isocratic high-performance liquid-chromatographic method is described for the determination of piracetam in plasma (25 μL) or cerebrospinal fluid (10 μL) using ultraviolet absorbance at 215 nm. The limit of quantitation is 4 μg mL^{-1} and the within-batch and between-batch coefficients of variation are less than 10%. No interference from other commonly prescribed antimyoclonic or antiepileptic drugs was observed and thus the method can be used to monitor piracetam in patients on polytherapy antimyoclonic or antiepileptic drug regimens.

Because of the sensitivity and rapidity of the method it is suitable for pharmacokinetic and mechanistic studies and for analysis of paediatric samples.

Piracetam, 2-oxo-1-pyrrolidine acetamide, the prototype nootropic drug has recently been licensed in the UK for the treatment of myoclonus. Although piracetam was developed as a cyclic analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and has been shown to have beneficial effects in patients with numerous disorders such as memory impairment, epilepsy, alcoholism, senile dementia and Alzheimer's disease, its mechanism of action is unknown (Richardson & Bereen 1977; Kunneke & Malan 1979; Chouinard et al 1983; Tallal et al 1986; Obeso et al 1989; Barnas et al 1990).

Piracetam is rapidly and almost completely absorbed into the blood after oral administration, peak plasma levels being reached within 1·5 h (Gobert 1977). It crosses the placental and blood-brain barriers and is almost exclusively excreted by the kidneys, urinary excretion being dose-dependent. No metabolites of piracetam have yet been found.

Various analytical approaches based on GC (Gobert & Baltes 1977; Hesse & Schulz 1979; Alebic-Kolbah & Hirsl-Starcevic 1990) and HPLC (Rieck & Platt 1982; Nalbandian et al 1983; Kumar & Stadler 1986; Mascher & Kikuta 1989; Louchahi et al 1995) have been described for the analysis of piracetam in plasma. These methods require large sample volumes (300 to 1000 μL) and suffer from severe interferences from the biological matrix and, consequently, require extensive extraction procedures. The use of perchloric acid, a very corrosive and hazardous chemical, as the mobile phase can, furthermore, be considered a major disadvantage (Mascher & Kikuta 1989). We have developed a rapid, sensitive and reproducible microanalytical HPLC procedure for the measurement of piracetam using ultraviolet (UV) detection. The extraction procedure is simple and requires small sample volumes (25 μL plasma, 10 μL CSF) and is thus highly suited for studies of pharmacokinetics and mechanisms of action of piracetam in small rodents. The method is also suitable for routine therapeutic drug monitoring in patients.

Correspondence: P. N. Patsalos, Pharmacology and Therapeutics Unit, University Department of Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

Experimental

Materials

Piracetam and α-ethyl-2-oxo-1-pyrrolidine acetamide, the internal standard, were kindly provided by UCB s.a., Pharmaceutical Sector (Belgium). Acetonitrile (BDH, Poole, UK) and water (Alpha-Q System, Millipore, Brussels, Belgium) were of HPLC grade; all other chemicals were of analytical grade.

The stock solution of piracetam (10 mg mL^{-1}) was prepared in 20% methanol. Working standards were made by diluting the stock solution with drug-free serum or simulated cerebrospinal fluid; they were stored at $-$ 70°C until required for analysis. The stock solution of internal standard (10 mg mL^{-1}) was prepared in acetone; the working internal standard (80 μg mL^{-1}) was prepared by dilution with acetone. These solutions were observed to be stable for at least ten months at 4°C.

Instrumentation

Isocratic HPLC was performed with a Gilson 305 solvent delivery system, a Rheodyne model 7125 injection valve, a Hewlett-Packard HP 3396A integrator, and a Spectra Physics 8440 UV-visible detector fitted with a 20-μL loop (Spectra-Physics, Maidenhead, UK). The analytical column was 25 cm × 4.9 mm i.d. stainless steel packed with 5 μm Spherisorb S5CN (Hichrom, Reading, UK).

Operating conditions

HPLC analysis was performed at ambient temperature using acetonitrile-water, 98 : 2 (v/v) as mobile phase; before use the

mobile phase was filtered through a 0·22-μm Millipore filter and degassed with helium. The flow-rate was 2·0 mL min^{-1} and the column eluate was monitored at an absorption wavelength of 215 nm with a sensitivity range of 0.005 aufs; the chart speed was 0·5 cm min^{-1}.

Plasma and CSF extraction procedure

To construct the calibration curve and to establish the recovery, linearity, reproducibility and accuracy of the extraction procedure, human plasma or simulated CSF containing known concentrations of piracetam (4, 8, 16, 32, 64, 128 and 256 μg mL^{-1}) were extracted and analysed. Plasma (25 μL) or cerebrospinal fluid (10 μL), working internal standard solution (10 μL) and acetone (750 μL) were dispensed into 1·5-mL polypropylene microcentrifuge tubes which were then capped and sonicated for 5 min in a general purpose ultrasonic bath. After shaking for 5 min, the tubes were centrifuged for 5 min at 10 500 *g* (Abbott Microcentrifuge, Chicago, USA) and the upper supernatant liquid was transferred into a clean 1·5-mL polypropylene tube and evaporated to dryness at 60°C in a Gyro-Vap evaporator (V. A. Howe, Oxfordshire, UK) (50 min). The dried extract was then reconstituted with acetone (150 μL) vortex mixed, centrifuged and finally evaporated to dryness as previously described. The dry samples were stored in the refrigerator. Before analysis the extracts were reconstituted with 30 μL mobile phase and 20 μL was injected into the HPLC system.

Results

A linear relationship between the ratio of the peak area of the drug to that of the internal standard was confirmed for piracetam concentrations in the range 4–256 μg mL^{-1} by adding known amounts of piracetam to blank plasma and simulated cerebrospinal fluid and subjecting them to the extraction procedure and chromatography. Quantification of piracetam was determined from the ratio of the peak area of piracetam to that of the internal standard. Calibration data from five plasma standard curves for piracetam fit linear equations with correlation coefficients ranging from 0·9983 to 0·9997. The relative standard deviation of the slopes of the five calibration curves ranged from 1·55 to 1·62%. The limit of quantitation for piracetam was 4 μg mL^{-1} as determined by precision and accuracy and where CVs of < 10% were targeted. Similar values were achieved for cerebrospinal fluid. On the basis of a signal-to-noise ratio of 3 : 1, the limit of detection was observed to be 1 μg mL^{-1}.

The recovery (extractability) of different concentrations of piracetam from plasma (after deproteinization with acetone) and cerebrospinal fluid was determined over the concentration range 4–256 μg mL^{-1}. Recoveries were calculated by comparing the chromatographic peaks obtained by extraction of plasma or cerebrospinal fluid to which piracetam had been added with those obtained from pure solutions of piracetam (constituted in acetonitrile); values ranged from 69 to 79%.

Within-batch precision was determined by analysis of plasma samples to which piracetam had been added at seven different concentrations ranging from 4 to 256 μg mL^{-1} (Table 1). The coefficients of variation ranged from 1·2 to 8·6%. Between-batch precision was similarly determined over a period of several days and coefficients of variation ranged from 1·8 to 10·0%. Typical chromatograms obtained from blank plasma containing known amounts of piracetam and internal standard, blank plasma (containing neither piracetam nor internal standard), a blank plasma containing internal standard only, and a plasma sample from a patient taking piracetam are shown in Fig. 1. The analysis of the sample from the patient was undertaken as part of her routine clinical management.

Table 1. Within-batch and between-batch precision for the determination of piracetam in plasma.

Piracetam concn (μg mL^{-1})	Within-batch concn measured (μg mL^{-1})	CV (%)	Between-batch concn measured (μg mL^{-1})	CV (%)
4	5·2 ± 0·4	7·7	4·9 ± 0·5	10·0
8	9·3 ± 0·8	8·6	9·0 ± 0·8	8·9
16	16·3 ± 1·0	6·1	16·1 ± 0·3	1·9
32	31·1 ± 1·0	3·2	31·9 ± 1·0	3·1
64	63·8 ± 1·4	2·2	63·8 ± 1·2	1·9
128	124·9 ± 3·3	2·6	126·0 ± 2·9	2·3
256	258·1 ± 3·1	1·2	254·7 ± 4·7	1·8

$n = 5–11$, CV = coefficient of variation, values are means ± s.d.

Chromatograms (not shown) of blank CSF and blank CSF to which piracetam and internal standard had been added were similar to those shown (Fig.1).

Fig. 2 shows typical plasma and CSF pharmacokinetic profiles of piracetam after intraperitoneal administration of 150 mg kg^{-1} piracetam to a single male Sprague-Dawley rat (320 g). The rat was housed in a perspex cage and fed freely on Scientific Dietary Services (Witham, Essex, UK) normal laboratory diets R and R number 1 expanded and on water. A 12-h light-dark cycle (lights on at 0600 h) and an ambient temperature of 25°C were maintained. Twenty four hours before the pharmacokinetic study the rat was anaesthetized with pentobarbitone (60 mg kg^{-1}) and catheters were implanted in the cisterna magna for sampling of cerebrospinal fluid, and the right jugular vein for sampling blood, as previously described by Patsalos et al (1992). Sampling of blood (200 μL) and CSF (30 μL) was undertaken at time intervals shown in Fig. 2.

As piracetam is commonly prescribed as add-on therapy in the management of myoclonus, the possibility of chromato-

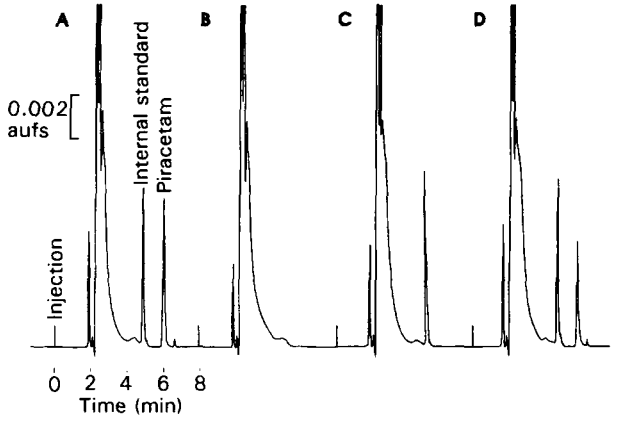

FIG. 1. Chromatograms obtained from (A) blank plasma to which 50 μg mL^{-1} piracetam and 1 μg mL^{-1} internal standard have been added, (B) blank plasma, (C) blank plasma to which 50 μg mL^{-1} piracetam only has been added, and (D) a sample from a patient, containing 32 μg mL^{-1} piracetam. The patient (a female aged 36 years and weighing 53 kg) was taking 21·6 g piracetam, 3·0 g sodium valproate and 4 mg clonazepam daily.

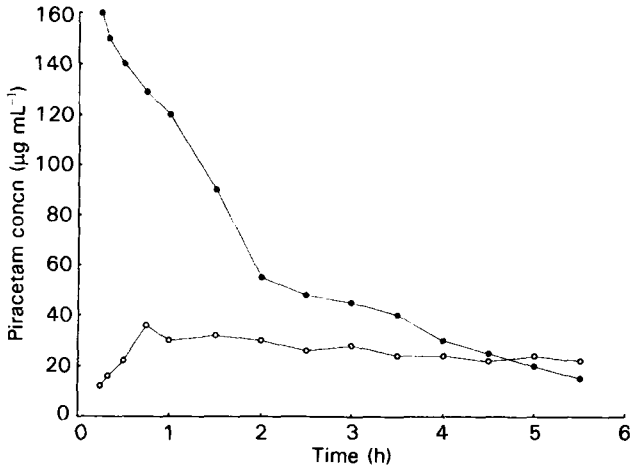

FIG. 2. Typical concurrent pharmacokinetic profiles showing relationship between piracetam concentration and time for plasma (○) and cerebrospinal fluid (●) from a rat administered 150 mg kg^{-1} piracetam intraperitoneally.

graphic interference by other antimyoclonic and antiepileptic drugs was investigated. Carbamazepine, its primary metabolite carbamazepine-10,11-epoxide, lamotrigine, phenobarbitone, phenytoin, primidone and valproic acid were added to blank plasma which was then analysed. These drugs did not interfere with the analysis of piracetam.

Discussion

A simple, rapid and specific HPLC procedure has been developed for the quantitation of piracetam. Because only 25 μL plasma or 10 μL CSF is required, the method is ideally suited for paediatric samples and for pharmacokinetic studies in both man and animal models.

Although only one carbamazepine metabolite, carbamazepine-10,11-epoxide, was specifically tested for interference, our extensive experience with this method for the determination of piracetam in patients taking polytherapy antimyoclonic and antiepileptic drug regimens suggests that other drug metabolites do not interfere.

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