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Short communication

Determination of gabapentin in plasma by high-performance liquid chromatography

Gerard Forrest, Graeme J. Sills*, John Paul Leach, Martin J. Brodie

Epilepsy Research Unit, University Department of Medicine and Therapeutics, Western Infirmary, Glasgow G11 6NT, UK

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Abstract

A rapid and simple method for determination of the novel antiepileptic compound gabapentin [1-(aminomethyl)cyclohexaneacetic acid] in plasma is described. Blank human plasma was spiked with gabapentin (1.0–10.0 μg/ml) and internal standard [1-(aminomethyl)-cycloheptaneacetic acid; 5.0 μg/ml]. Individual samples were treated with 2 *M* perchloric acid, centrifuged and then derivatised with *o*-phthalaldehyde-3-mercaptopropionic acid. Separation was achieved on a Beckman Ultrasphere 5 μm reversed-phase column with mobile phase consisting of 0.33 *M* acetate buffer (pH 3.7; containing 100 mg/l EDTA)–methanol–acetonitrile (40:30:30, v/v). Eluents were monitored by fluorescence spectroscopy with excitation and emission wavelengths of 330 and 440 nm, respectively. The calibration curve for gabapentin in plasma was linear (r=0.9997) over the concentration range 1.0–10.0 μg/ml. Recovery was seen to be ≥90%. The inter- and intra-assay variations for three different gabapentin concentrations were ≤10% throughout. The lower limit of quantitation was found to be 0.5 μg/ml. Chromatography was unaffected by a range of commonly employed antiepileptic drugs or selected amino acids.

Keywords: Gabapentin

1. Introduction

Gabapentin (GBP) is a novel antiepileptic drug (AED) that has recently been approved in the UK and USA for the treatment of partial seizures. It is a hydrophilic analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), and was designed to act as a $GABA_A$ receptor agonist that could freely cross the blood–brain barrier [1].

Despite its structural similarity to GABA (Fig. 1), GBP has demonstrated only limited effects on the GABAergic system. It is devoid of effect on GABA-mediated ion conductances, GABA receptor binding and GABA metabolism [2,3]. Although the mechanism of GBP action remains to be fully characterised, substantial evidence now suggests that it may interact specifically with a plasma membrane site proposed to be the system L-amino acid transporter [4,5].

GBP has an experimental anticonvulsant profile similar to that of valproic acid [6]. It is effective against tonic seizures induced by a variety of chemoconvulsants [6] and is also active in the maximal electroshock test [6] and in several rodent models of genetic reflex epilepsy [7]. Clinically, the drug has demonstrated efficacy against both partial and generalised tonic–clonic seizures [8].

GBP is rapidly absorbed and exhibits a dose-

*Corresponding author.

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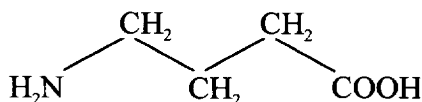

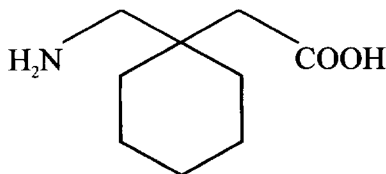

Fig. 1. Comparison of the chemical structures of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the novel antiepileptic compound gabapentin (GBP).

dependent bioavailability as a result of a saturable uptake process [9]. Maximum concentrations occur 2–3 h after administration and the elimination half-life is approximately 5–7 h [10]. There is no significant binding to plasma proteins, and the drug is excreted unchanged in the urine with a clearance rate equivalent to that for creatinine [11]. Despite extensive pharmacokinetic investigations and the report that GBP is free of important drug interactions [9], the requirement for a reliable, routine laboratory assay of GBP concentration remains, particularly for those who advocate therapeutic monitoring of this drug in the treatment of epilepsy [12].

Of the three published methods for the laboratory measurement of GBP in plasma, all have significant drawbacks. The gas chromatographic (GC) and the capillary electrophoretic (CE) assays [13,14] are likely to be limited by the routine availability of these techniques. The only reported high-performance liquid chromatographic (HPLC) method [15] would appear to be hampered by a multistep derivatisation involving 2,4,6-trinitrobenzenesulphonic acid (TNBS) followed by an extraction into toluene, and a methodology incompatible with the use of modern automated HPLC systems.

We have developed an automatable, one-step derivatisation method for the determination of GBP in plasma by HPLC with fluorimetric detection. This rapid and reliable assay, which obviates the requirement for hazardous chemicals such as TNBS and toluene, is a modification of the method of Durkin et al. [16] for the analysis of neurotransmitter amino acids in brain.

2. Experimental

2.1. Reagents

GBP [1-(aminomethyl)cyclohexaneacetic acid] and the internal standard [1-(aminomethyl)cycloheptaneacetic acid] were supplied by Parke-Davis (Ann Arbor, MI, USA). Methanol and acetonitrile (HPLC grade) were from Rathburn (Walkerburn, UK). All other chemicals (reagent grade) were obtained from Sigma (Poole, UK).

2.2. Standards

Stock solutions of GBP (1 mg/ml) and internal standard (1 mg/ml) were prepared in de-ionised water and stored at −20°C for up to seven days. Working standard solutions of GBP (10–100 μg/ml) and internal standard (50 μg/ml) were prepared daily in de-ionised water. The derivatisation reagent, *o*-phthalaldehyde-3-mercaptopropionic acid (OPA-MPA), was prepared weekly by dissolving 50 mg of OPA in 4.5 ml of methanol and adding 0.5 ml of borate buffer and 50 μl of 3-MPA. The borate buffer was prepared on a weekly basis by adjusting 0.5 *M* boric acid to pH 9.5 with 1 *M* NaOH.

2.3. Sample preparation

GBP standards were prepared by the addition of 50 μl of the appropriate working standard (10–100 μg/ml) and 50 μl of working internal standard to 0.4 ml of blank human plasma. Samples for analysis were prepared by adding 50 μl of working internal standard to 0.45 ml of unknown plasma. Pooled plasma, spiked at high (5.0 μg/ml), medium (2.5 μg/ml) and low (0.5 μg/ml) GBP concentrations, was used to determine intra- and inter-assay variations.

2.4. Derivatisation

A 100-μl volume of 2 *M* perchloric acid was added to each standard and sample before vortex-mixing for 10 s and centrifuging for 3 min at 15 000 g at room temperature. A 50-μl aliquot of the resulting supernatant was reacted with 200 μl of methanol, 200 μl of 0.5 *M* borate buffer (pH 9.5) and 50 μl of OPA-MPA solution. The reaction mixture was allowed to stand at room temperature for 5 min to allow full derivatisation prior to injection of 20 μl onto the column. Fully derivatised GBP and internal standard were found to be stable for a further 25 min.

2.5. High-performance liquid chromatography

Chromatography was carried out at room temperature on a Beckman Ultrasphere octadecyl silane (ODS) 5 μm reversed-phase column (250×4.6 mm I.D.; 80 Å pore size; Beckman Instruments, Fullerton, CA, USA). The chromatographic system consisted of a Waters 6000A pump (Waters-Millipore, Harrow, UK), a Shimadzu SIL-9A autoinjector (Dyson Instruments, Houghton-le-Spring, UK) and a Perkin-Elmer LS5 fluorescence spectrophotometer (Perkin-Elmer, Beaconsfield, UK). The excitation and emission wavelengths were 330 and 440 nm, respectively, with slit widths set at 15 and 20 nm, respectively. The mobile phase consisted of 0.33 *M* acetate buffer (containing 100 mg/l EDTA)–methanol–acetonitrile (40:30:30, v/v). The acetate buffer was prepared by diluting 7.5 ml of glacial acetic acid (approximately 17.4 *M*) to 400 ml with water, adding 40 mg EDTA and adjusting the pH to 3.7 with 3 *M* NaOH. Flow-rates were 1.5 ml/min throughout.

2.6. Calculations

Chromatograms were recorded and integrated on a Jones Chromatography JCL6000 chromatography data system (Crawford Scientific, Strathaven, UK). GBP concentrations were determined by comparison of peak-height ratios of analyte to internal standard, quantified in relation to volume, and expressed as μg/ml. Pearson's product moment correlation coefficient is quoted.

3. Results

GBP and the internal standard were well resolved from one another and from the solvent front (Fig. 2). The calibration line was shown to be linear from 1.0–10.0 μg/ml (n=6; r=0.9997). The slope (±S.E.M.) was found to be 296.76 (±3.61) and the y-intercept (±S.E.M.) was calculated at 39.44 (±18.6). The intra-assay (n=6) variations at 0.5, 2.5 and 5.0 μg/ml were 4.1, 2.2 and 3.8%, respectively. The inter-assay (n=6) variations for the same samples were 10.0, 2.0 and 2.6%, respectively. Recoveries were shown to be ≥90% throughout. The lower limit of quantitation, defined as the lowest quantifiable concentration with an associated variation of ≤10%, was found to be 0.5 μg/ml.

There were no interfering peaks from any of the

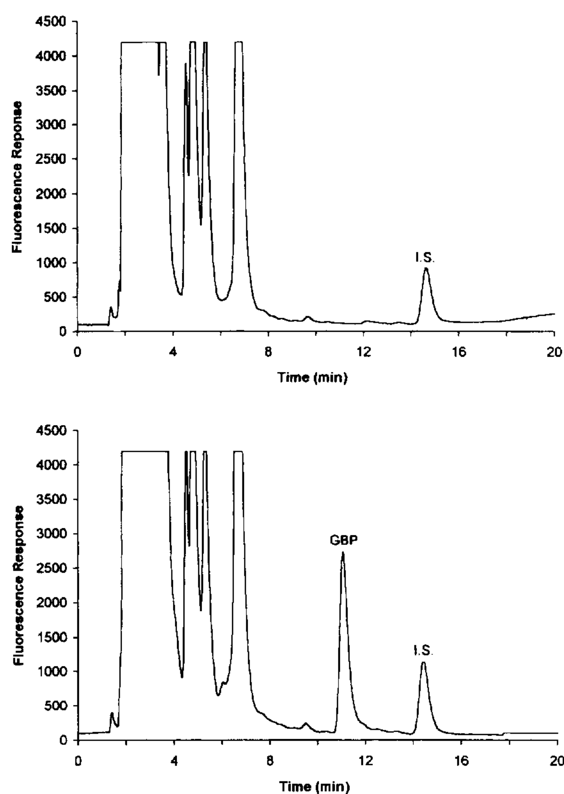

Fig. 2. Typical chromatograms highlighting gabapentin (GBP) and internal standard (I.S.) peaks in one patient's sample from each phase of a double blind crossover trial of GBP and placebo. Upper chromatogram represents the placebo phase and the lower chromatogram the active phase. Daily GBP dose was 2400 mg.

following AEDs: phenytoin, carbamazepine, sodium valproate, phenobarbital, primidone, clobazam, clonazepam, lamotrigine, vigabatrin, oxcarbazepine, felbamate, tiagabine or remacemide.

Similarly, there were no interfering peaks from any of the following amino acids: L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine or L-valine.

Typical GBP chromatograms are illustrated in Fig. 2. Samples were taken from a patient currently undergoing a double blind, crossover trial with GBP and placebo. One sample was taken from each treatment arm of this study, where the daily GBP dose was 2400 mg.

4. Discussion

This method employed pre-column derivatisation and fluorimetric detection for the quantitation of GBP concentrations in plasma by HPLC. It facilitated clear detection and resolution of the drug and its appropriate internal standard, with intra- and inter-assay variations of an acceptable degree. Chromatography was unaffected by other commonly employed AEDs or by a variety of endogenous amino acids.

When compared to previously published methods for the measurement of GBP in plasma, this assay applied a more readily available laboratory technique than that afforded by either GC [13] or CE [14] methods. In contrast to the only available HPLC assay for GBP analysis [15], the methodology proved to be both more rapid and simple, and obviated several complicated steps, including the requirement for pH adjustment of relatively small volumes. Other important advantages of this method over that previously reported for HPLC [15] included a reduced requirement for the use of hazardous chemicals, such as toluene and TNBS, and a methodology compatible with modern HPLC systems which facilitate automated pre-column derivatisation.

Although this new method, with a limit of quantitation of 0.5 μg/ml, compared favourably in terms of both sensitivity and resolution, with the recently reported CE assay of Garcia et al. [14], it appeared less favourable when compared with the HPLC assay of Hengy and Kölle [15] and the GC assay of Hooper and colleagues [13]. In our clinical practise, however, the expected plasma concentration on the least effective GBP dose (1200 mg daily) exceeds 2 μg/ml. While saturable absorption must be taken into account, this daily dose of GBP is relatively low when compared to those of up to 6400 mg now being administered to patients with refractory epilepsy, Thus, it would appear that, with the exception of the most sensitive pharmacokinetic requirements, the new assay is more than adequate for the routine analysis of plasma GBP concentrations in the epilepsy clinic.

In conclusion, the method reported above represents a significant advance in the laboratory analysis of the novel antiepileptic drug GBP. In comparison to previously published methods [13–15], this HPLC assay is rapid, simple, safe and readily available and automatable. In addition, it appears to possess a sensitivity more than adequate for the routine monitoring of GBP concentrations in patients with intractable epilepsy.

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References

[1] M. Foot and J. Wallace, in F. Pisani, E. Perucca, G. Avanzini and A. Richens (Editors), New Antiepileptic Drugs, Elsevier, Amsterdam, 1991, p. 109.
[2] K.L. Goa and E.M. Sorkin, Drugs, 46 (1993) 409.
[3] D.M. Rock, K.M. Kelly and R.L. Macdonald, Epilepsy Res., 16 (1993) 89.
[4] B.H. Stewart, A.R. Kugler, P.R. Thompson and H.N. Bockbrader, Pharmacol. Res., 10 (1993) 276.
[5] C.P. Taylor, M.G. Vartanian, P.W. Yuen and C. Bigge, Epilepsy Res., 14 (1993) 11.
[6] M.A. Rogawski and R.J. Porter, Pharmacol. Rev., 42 (1990) 223.
[7] G.D. Bartoszyk and M. Hamer, Pharmacol. Res. Commun., 19 (1987) 429.
[8] D. Chadwick, Lancet, 343 (1994) 89.

[9] M.A. Dichter and M.J. Brodie, New Eng. J. Med., in press.
[10] M.J. McLean, Neurology, 44 (1994) S17.
[11] J.P. Leach and M.J. Brodie, Seizure, 4 (1995) 5.
[12] P.J.W. McKee and M.J. Brodie, in J. Engel and T.A. Pedley (Editors), Epilepsy: A Comprehensive Textbook, Raven Press, New York, in press.
[13] W.D. Hooper, M.C. Kavanagh and R.G. Dickinson, J. Chromatogr., 529 (1990) 167.
[14] L.L. Garcia, Z.K. Shihabi and K. Oles, J. Chromatogr. B., 669 (1995) 157.
[15] H. Hengy and E.U. Kölle, J. Chromatogr., 341 (1985) 473.
[16] T.A. Durkin, G.M. Anderson and D.J. Cohen, J. Chromatogr., 428 (1988) 9.