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Note

Simultaneous, rapid high-performance liquid chromatographic microanalysis of plasma carbamazepine and its 10,11-epoxide metabolite

Applications to pharmacokinetic studies in humans

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Carbamazepine is an anticonvulsant used in the treatment of grand mal, psychomotor epilepsy and also in trigeminal neuralgia. Side-effects are fairly common and therapeutic drug monitoring should be carried out during treatment.

Plasma concentrations of carbamazepine may be closely related to its therapeutic as well as toxic effects [1]. Carbamazepine exerts a useful therapeutic effect at plasma concentrations of 2—5 μg/ml [2, 3]. Plasma levels higher than 12 μg/ml are often associated with adverse effects. Carbamazepine 10,11-epoxide, the major metabolite in plasma [4], is as potent as the parent compound in preventing electroshock-induced seizures in the rat [5]. It is necessary, therefore, to measure the parent drug and its active metabolite, as both may contribute to the therapeutic effect of the drug [6].

Gas—liquid chromatographic (GLC) [7, 8], thin-layer chromatographic [9], spectrophotometric [10], fluorescence [11], and high-performance liquid chromatographic (HPLC) [12, 13] methods have been used to determine carbamazepine alone or in combination with other agents.

All proposed methods suffer from one more of the following requirements: an excessive amount of manipulation time; extraction (either single or multiple steps) to clean the sample; sample dilution; a fairly large volume of plasma; chemical derivatization (in GLC). Furthermore, the poor stability of carbamazepine and its 10,11-epoxide at high temperatures (GLC) may cause errors in the determination of true plasma levels.

The method developed has none of these drawbacks. Quantitation can be achieved within 15–20 min after obtaining the plasma sample. No derivatives are made, and no extraction used. This method is ideally suited for the simultaneous determination of the two compounds in plasma using as little as 50 μl of plasma, and was used to investigate the kinetics of carbamazepine and its metabolite in humans.

EXPERIMENTAL

Reagents

Acetonitrile (HPLC grade) was supplied by Merck (Darmstadt, G.F.R.). Carbamazepine and its 10,11-epoxide were obtained from Ciba-Geigy (Basel, Switzerland) by generous gift. Nitrazepam was supplied by Roche (Basel, Switzerland).

Apparatus and chromatographic conditions

An LC3 liquid chromatograph (Pye Unicam, Cambridge, Great Britain) equipped with a variable-wavelength detector (Pye Unicam) was used in a reversed-phase system with Partisil ODS-2 as the stationary phase (250 mm × 4.6 mm I.D.; particle size 10 μm; Whatman), and acetonitrile—water (50:50, v/v) as the mobile phase. Assays were performed using column thermoregulation (25°). The flow-rate of the mobile phase was maintained at 0.9 ml/min (±0.02). The effluent stream was monitored at 288 nm. The volume of sample injected was 20 μl (Rheodyne injector).

Standard curve and sample preparation

A standard curve was prepared for carbamazepine and its 10,11-epoxide in the following manner. Aliquots of 200 μl of pooled human plasma were spiked with various quantities of methanolic stock solution (1 g/l) of each compound. The stock solutions were kept refrigerated and sealed until use (stable for 2 months at +4°). A plasma that was known to be free of the compounds to be analysed and five plasma samples, with concentrations ranging from 1 to 20 μg/ml of carbamazepine and its epoxide, were Vortex mixed for about 20 sec. These samples were deproteinized by the addition of 200 μl of a freshly prepared (daily) acetonitrile solution of internal standard, nitrazepam, at 4 μg/ml (methanolic stock solution of 1 g/l, stored at +4° in the dark up to one month), Vortex mixed 10 sec and centrifuged for 5 min at 650 *g*. Aliquots of 20 μl of the clear supernatant were chromatographed. Peak heights were used for quantitation.

Drug recovery study

Five samples each of 200 μl of water and pooled human plasma were spiked

with a stock solution of carbamazepine, its 10,11-epoxide and nitrazepam to give a final concentration of 5 μg/ml and analysed as described above.

Drug interference study

Many compounds were tested for possible interference. A reference toxicological plasma was used (Hyland, Costa Mesa, Calif., U.S.A.).

In vivo study

Six in-patients participated voluntarily in the study. Carbamazepine, 3 mg/kg or 6 mg/kg of body weight in capsule form, was administered as a single oral dose at 8.00 a.m. No food was allowed for 12 h before dosing. Venous blood samples were collected in heparinized vials at various times and were centrifuged within 10 min at 650 *g* (+4°) to obtain plasma fractions (stored at -20° until analysis).

RESULTS AND DISCUSSION

Method

The standard curves of carbamazepine and its 10,11-epoxide were linear in the concentration ranges studied. For example, the carbamazepine plot linear regression line was $y = 0.322x - 0.037$ ($r = 0.9999$), and that for the epoxide $y = 0.179x - 0.019$ ($r = 0.9977$), where y is the ratio of the peak height (in centimeters) of carbamazepine or the epoxide to that of nitrazepam, and x is the concentration in μg/ml.

The peak height measurements were found to be superior to peak area estimation. For example, the reproducibility, estimated by the ratio (S.D.) (peak of carbamazepine)/(mean)(peak height or area), was 0.016 for peak height and 0.0304 for peak area measurements.

Reproducibility was calculated by 30 replicate analyses for each concentration on the standard curve (Table I) and was satisfactory.

Recovery was essentially complete: 99.8% ± 0.2 (mean ± S.D., $n = 30$). Under the conditions of this analysis, the minimum concentration that can be

TABLE I

REPRODUCIBILITY OF CARBAMAZEPINE (I) AND ITS 10,11-EPOXIDE METABOLITE (II) FROM PLASMA

Each value represents the mean of 30 experiments (± S.D.).

	Amount added to plasma (μg/ml)	(peak height I or II)/(peak height IS) ± S.D.	Amount found (μg/ml) ± S.D.
I	2.0	0.474 ± 0.009	1.90 ± 0.40
	5.0	1.446 ± 0.027	5.15 ± 0.12
	10.0	2.644 ± 0.019	9.76 ± 0.08
	20.0	6.043 ± 0.067	20.06 ± 0.74
II	2.0	0.350 ± 0.008	1.98 ± 0.30
	5.0	0.915 ± 0.020	5.05 ± 0.15
	10.0	2.066 ± 0.024	9.80 ± 0.10
	20.0	4.087 ± 0.059	20.30 ± 0.62

measured accurately is about 200 ng/ml of carbamazepine and of the epoxide. This value for carbamazepine is well below the therapeutic level.

Lower concentrations give poor accuracy because of error in peak height measurement. Carbamazepine exhibits maximal absorption at around 285 nm and its epoxide at 288 nm. Detection at 288 nm was adequately sensitive and endogenous and exogenous interferences were minimized.

Column regulation permits no variation in retention time and accurate reproducibility of the peak height ratio of drug to internal standard (IS). Retention times for carbamazepine and the 10,11-epoxide were 7.4 min and 8.4 min, respectively (IS = 6.6 min). The flow-rate of 0.9 ml/min was optimal. The efficiencies (plates per metre) were determined using various flow-rates. At 0.9 ml/min, the number of plates per metre was 4772 for carbamazepine and 5088 for the 10,11-epoxide with resolution factor $R = 1.04$. A typical chromatogram is shown in Fig. 1.

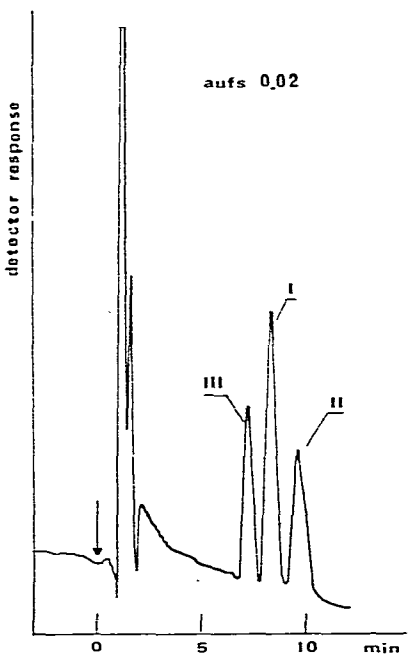

Fig. 1. Chromatogram (HPLC) of carbamazepine (I) and its 10,11-epoxide metabolite (II) (5 μg/ml of each), and nitrazepam (III), (internal standard, 4 μg/ml), using pooled, spiked human plasma.

The drug interference study showed that only phenytoin interfered with carbamazepine but at a concentration much higher than would usually be encountered clinically. Pooled human plasma spiked with 5 μg/ml of carbamazepine and 30 μg/ml of phenytoin gives 5.27 μg/ml for carbamazepine (recovery 105.2%, error 5.2%).

After centrifugation the clear supernatant may be stored in a sealed glass culture tube for 2—4 days without significant change.

In vivo study

The data for humans are graphically presented in Fig. 2.

The various pharmacokinetic parameters (and their mean values) calculated are set out in Table II.

The mean values of K and $T_{1/2}$ obtained in the present study are reasonably similar to these reported by others [7, 13—15]. Dose dependence of K_{el} and V_d was studied in our laboratory and the results [16] suggest that the elimination rate constant tends to increase with increasing drug dose.

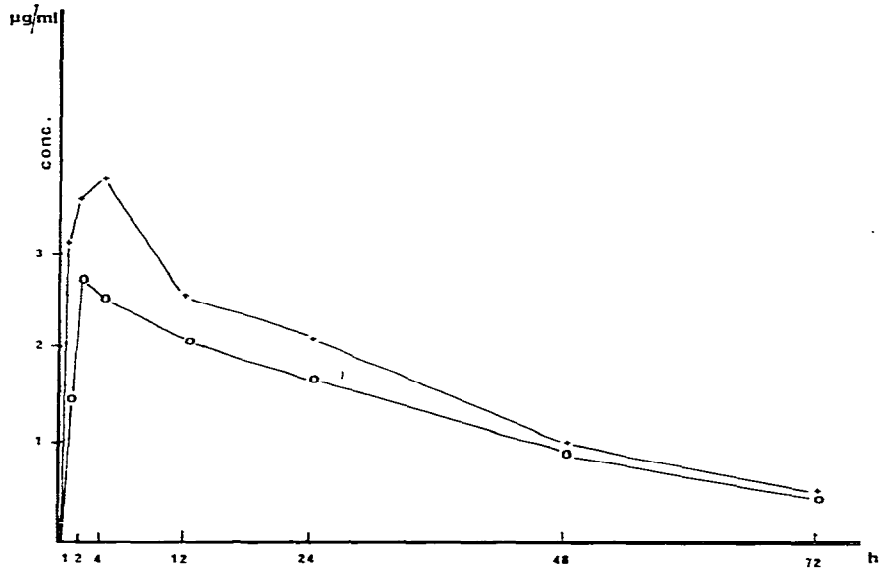

Fig. 2. Plasma levels of carbamazepine after a single oral dose (○, 3 mg/kg; +, 6 mg/kg) of carbamazepine to human volunteers.

TABLE II

PHARMACOKINETICS PARAMETERS* AND THEIR VALUES FROM SIX VOLUNTEERS

Dose	Patient	$T_{1/2}$ (h^{-1})	K_{el} (h^{-1})	AUC_0^∞ ($mg\,l^{-1}\,h$)	V_d ($l\,kg^{-1}$)
3 mg/kg	Del.	50.4	0.0134	273	0.78
	Ric.	35.0	0.0198	190	0.85
	Bon.	30.5	0.0227	130	0.99
Mean		38.6	0.0186	197	0.87
S.D.		± 10.4	± 0.0047	± 71	± 0.11
6 mg/kg	Lab.	25.9	0.0267	151	1.46
	Zel.	43.3	0.0160	225	1.65
	Mar.	22.9	0.0302	98	1.10
Mean		30.7	0.0243	158	1.40
S.D.		± 11.0	± 0.0074	± 63	± 0.28

*$T_{1/2}$, elimination half-life; K_{el}, first-order elimination rate constant; AUC_0^∞, area under the plasma concentration curves; V_d, apparent volume of distribution.

REFERENCES

1 E. Martindale, The Extra Pharmacopeia, 27th ed., The Pharmaceutical Press, London, 1977, p. 1237.
2 E. Singlas, La Revue de Médecine, No. 20—21 (1978) 15—22 mai.
3 A. Astier, in Bulletin de la Commission des Médicaments, Assistance Publique, Paris, 1979, No. 6.
4 A. Frigerio, R. Farrelli, P. Biandrate, S. Passerini, P.L. Moreselli and S. Garattini, J. Pharm. Sci., 61 (1972) 1144.
5 P.L. Moreselli, P. Biandrate, A. Frigerio, M. Gerna and C. Tognoni, in J.W.A. Meyer, H. Meinardi, C. Gardner-Thorpe and E. van der Klein (Editors), Methods of Analysis of Antiepileptic Drugs, Excerpta Medica, Amsterdam, 1973, p. 169.
6 M. Eichelbaum, K. Ekbom, L. Bertilsson, V.A. Ringberger and A. Rane, Eur. J. Clin. Pharmacol., 8 (1975) 337.
7 A. Gérardin, F. Abadie and J. Laffont, J. Pharm. Sci., 64 (1975) 1940.
8 M.A. Schwertner, H.E. Marulein and J.E. Wallace, Clin. Chem., 24 (1978) 895.
9 D.B. Faber and W.A. Man in 't Veld, J. Chromatogr., 93 (1974) 238.
10 J. Furh, Arzneim.-Forsch., 43 (1964) 69.
11 S. Lauffer, E. Schmidt and F. Weist, Arzneim.-Forsch., 19 (1969) 1965.
12 M. Eichelbaum and L. Bertilsson, J. Chromatogr., 103 (1975) 135.
13 A. Gérardin and J. Hirtz, in W. Birmayer (Editor), Int. Symp., Epileptic Seizure Behaviour — Pain, St. Moritz, Huber, Berne, Stuttgart, Vienna, 1976, p. 151.
14 L.M. Cotter, M.J. Eadie, W.D. Hopper, C.M. Lauder, G.A. Smith and J.H. Tyrer, Eur. J. Clin. Pharmacol., 12 (1977) 451.
15 A.P. Gérardin, F.V. Abadie, J.A. Campestrini and W. Theobald, J. Pharmacokin., Biopharm., 4 (1976) 521.
16 A. Astier and M.M. Plat, Colloque Chimiothérapie Antiépileptique, Lyon, France, 20 Avril 1979, in press.