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DETERMINATION OF CARBAMAZEPINE AND ITS EPOXIDE METABOLITE IN PLASMA BY HIGH-SPEED LIQUID CHROMATOGRAPHY

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SUMMARY

A liquid-chromatographic method for the simultaneous determination of carbamazepine and its active metabolite (carbamazepine 10,11-epoxide) in plasma has been developed. The two compounds were identified in plasma by mass spectrometry. The lower limit of sensitivity is about 4 and 40 ng for the drug and its metabolite, respectively. 10,11-Dihydrocarbamazepine is used as internal standard for the determinations, which have precisions of 2.2 and 4.2%, respectively. No derivatization is needed. The specificity of the method for carbamazepine is shown by the significant correlation ($r = 0.99$) between the results obtained by this method and by mass fragmentography for the drug in plasma of patients.

INTRODUCTION

The determination of plasma concentrations of several drugs has been useful in therapeutic studies[1]. Carbamazepine (Tegretol®, Ciba-Geigy, Basel, Switserland) is increasingly used in the treatment of convulsive disorders and is the drug of choice in trigeminal neuralgia. Gaschromatographic, spectrophotometric and thin-layer chromatographic methods for determining carbamazepine have been available for some years[2–11], and a sensitive method for its determination by high-speed liquid chromatography has been described[12]; in addition, a mass-fragmentographic method has recently been reported[13]. As this last-named method is highly specific even at low concentrations of the drug in plasma, it has been possible to perform detailed pharmacokinetic analysis after single oral doses of the drug[14]. The levels observed during chronic treatment are much lower than the predicted levels calculated from single-dose pharmacokinetic parameters, implying that carbamazepine may induce its own metabolism[14].

Carbamazepine is partly metabolized to its 10,11-epoxide, which has been identified in the urine of man and rat[15]. In the rat, this metabolite has been shown to exert anticonvulsant activity of the same order of magnitude as the parent drug[16,17]. It is reasonable to assume that this metabolic pathway may be induced during

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chronic treatment with the drug. The formation of this active metabolite may explain the difficulties in establishing a relationship between plasma level and therapeutic effect of the parent drug[18–20]. It is therefore essential to measure the epoxide metabolite as well as the carbamazepine itself, particularly in order to evaluate the relationship between the plasma levels of the drug and its effect.

When the epoxide metabolite is injected into a gas chromatograph, it rearranges to 9-acridinecarboxaldehyde[21]. This product may also be formed from other metabolites, *e.g.*, 10,11-dihydro-10,11-dihydroxycarbamazepine[22]. Attempts in this laboratory to transform the epoxide into a stable derivative for mass-fragmentographic analysis have been unsuccessful. This paper describes the use of liquid chromatography for the concurrent determination of carbamazepine and its epoxide metabolite in plasma.

MATERIALS AND METHODS

Chemicals

The synthesis of the internal standard, 10,11-dihydrocarbamazepine, has been described[13]. Carbamazepine and carbamazepine 10,11-epoxide were obtained from Ciba–Geigy AG, Basel, Switzerland. Solvents and other chemicals used were of analytical grade.

Liquid chromatography

A Chromatronix Cheminert metering pump was used, with air as the pressurizing gas. The detector was a UV photometer (Chromatronix model 200), operated at 254 nm. The glass column (1100 mm × 3 mm I.D.) was packed with Durapak Carbowax 400–Corasil (Waters Ass., Framingham, Mass., U.S.A.) by the modified tap-and-fill method of Kirkland[23]. The mobile phase was *n*-hexane–dichloromethane–dimethyl sulphoxide (76:22.8:1.2); the flow-rate was 1 ml per min.

Gas chromatography–mass spectrometry

An LKB 9000 gas chromatograph–mass spectrometer was used to record the mass spectra of reference compounds and of compounds eluted from the liquid chromatograph. The compounds were introduced into the direct-inlet system. Conditions for the mass-fragmentographic determination of carbamazepine have been described[13].

Procedure

Venous blood (10 ml) from patients chronically treated with carbamazepine was withdrawn into heparinized tubes and the plasma was separated by centrifugation and stored at −20° until analyzed. To 1.0 ml of plasma in a 10-ml glass tube were added 20 μg of the internal standard, 10,11-dihydrocarbamazepine (in 20 μl of ethanol) and 3 ml of dichloromethane. The tube was shaken for 3 min and centrifuged at 4000 × *g* for 5 min; the aqueous phase was then aspirated off and discarded. The organic phase was decanted into another tube, and 2 ml of 0.1 *M* aqueous sodium hydroxide were added. The tube was then shaken and centrifuged as before, the aqueous phase was discarded, and the organic phase was transferred to another tube and evaporated to dryness in a stream of nitrogen at 40°. The residue was dissolved

in 100 μl of the solvent used for the chromatographic separation, and 30–40 μl of this solution were injected into the liquid chromatograph.

Standard curves for the determination of carbamazepine and its 10,11-epoxide were prepared by treating a series of blank plasmas, containing known amounts of the two compounds, in the same way as described above. The ratios between the peak heights of the compounds being determined and the internal standard were calculated and plotted against the known concentration.

RESULTS AND DISCUSSION

As previously described[12], small amounts of carbamazepine can be determined by using a UV detector (254 nm) on the effluent of a liquid chromatograph (see Fig. 1B). As carbamazepine 10,11-epoxide has no double bond in the 10,11-position, the sensitivity for this compound is 1/15 of that for carbamazepine. However, about 40 ng (corresponding to 0.1 μg per ml of plasma) can be detected.

For the determination of carbamazepine in plasma by mass fragmentography, 10,11-dihydrocarbamazepine was used as internal standard[13]. This compound is

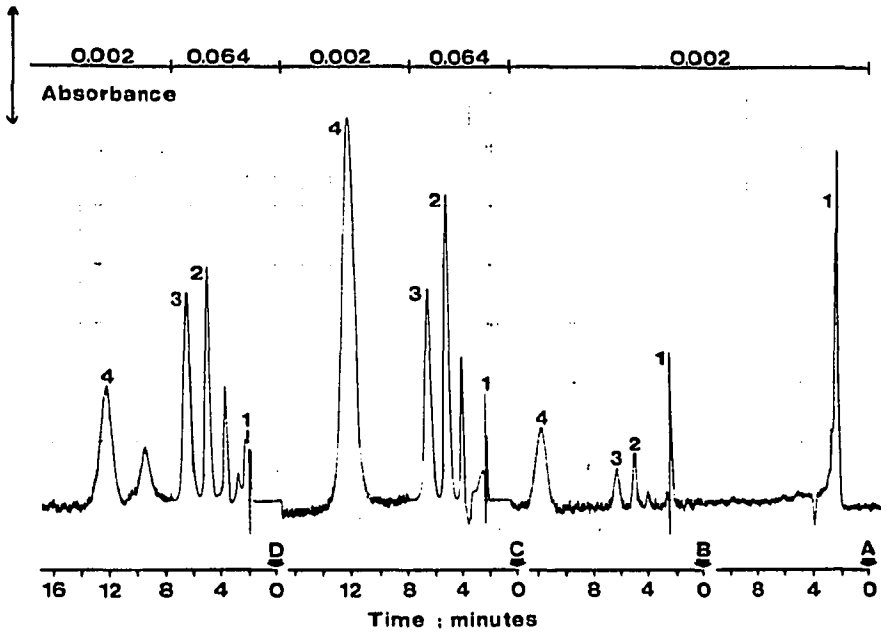

Fig. 1. Liquid chromatograms for: (A) material extracted from plasma obtained from a patient receiving phenytoin, chlorpromazine and digoxin, but not carbamazepine; (B) reference compounds, 100 ng of 10,11-dihydrocarbamazepine, 8 ng of carbamazepine and 190 ng of carbamazepine 10,11-epoxide; (C) material extracted from blank plasma (1.0 ml) to which carbamazepine (2.6 μg), carbamazepine 10,11-epoxide (2.4 μg) and the internal standard (20 μg) had been added; (D) material extracted from plasma obtained from a patient chronically treated with carbamazepine; the internal standard (20 μg) was added to the plasma. Analysis showed that the concentration of carbamazepine and the epoxide were 3.3 and 1.0 μg per ml of plasma, respectively. The peaks indicated are: (1) solvent front; (2) the internal standard (10,11-dihydrocarbamazepine); (3) carbamazepine; (4) carbamazepine 10,11-epoxide.

separated from carbamazepine and its epoxide in the liquid-chromatographic system described here (see Fig. 1), and can therefore serve as internal standard for the determination of both compounds. Standard curves were rectilinear in the range tested (0.1–15 μg/ml) for both compounds. Owing to the low UV-absorbing properties of the epoxide compared with carbamazepine itself, the sensitivity of the detector had to be changed during the analysis (Fig. 1C and D). When samples containing the same amounts of carbamazepine and the epoxide were analyzed on different days over one month, the daily variation of the peak-height ratio was less than 4% of the mean.

Carbamazepine and its epoxide metabolite could be almost quantitatively extracted from plasma by dichloromethane (recovery 87–93%). Epileptic patients often receive carbamazepine concomitantly with other drugs, *e.g.*, phenytoin, phenobarbitone and primidone. It was thus necessary to extract the organic phase with aqueous sodium hydroxide to remove these drugs and their metabolites, as these compounds have retention volumes similar to those of the internal standard and the epoxide.

Chromatogram A in Fig. 1 was obtained from a patient on some common drugs. Interfering peaks could not be detected in analyses of plasma samples from 20 epileptic patients taking the following drugs: phenytoin, phenobarbitone, primidone, ethosuximide, maliasin, diazepam, nitrazepam, amitriptyline, nortriptyline, digoxin, acetyldigoxin, digitoxin, caffeine, furosemide, chlorthalidone, prometazine, chlorpromazine, mephenetoin, penicillin, sulfonamides or vitamins A, B, C or D.

Both the parent drug and the epoxide metabolite are present in plasma from patients receiving carbamazepine. Fig. 1D shows the liquid-chromatographic effluent of an extract of plasma obtained from a patient treated with carbamazepine. The mass spectra (obtained with a direct-inlet system) of compounds 3 and 4 were identical with those obtained from reference carbamazepine and the 10,11-epoxide, respectively[15]. The two compounds are eluted unchanged from the column, which is an advantage compared with gas chromatography, in which technique carbamazepine is partly decomposed to iminostilbene[4,13] and the epoxide is rearranged to 9-acridinecarboxaldehyde[21].

Iminostilbene, which has been shown to be present in the urine of rats treated with carbamazepine[24], was not detected in plasma from patients. This compound has a retention time of 3.2 min and a few nanograms may be detected by using system the described here.

The peak preceding that for the internal standard in Fig. 1C and D is an unknown endogenous compound. The compound with a retention time of 9.5 min (Fig. 1D) was only detected in plasma from patients on carbamazepine and might be a metabolite of this drug.

The precision of the liquid-chromatographic method for the determination of the two compounds in plasma was obtained by analyzing duplicates. The standard deviation for carbamazepine was 2.2% ($n = 21$; concentration range, 1.2–16.5 μg/ml; mean 6.2 μg/ml) and for the epoxide it was 4.2% ($n = 18$; concentration range, 0.6–2.3 μg/ml; mean 1.2 μg/ml). Analysis for carbamazepine in the same plasma sample by the present method and by a mass-fragmentographic method[13] gave almost identical results (see Fig. 2), indicating the high specificity of the proposed method.

As pointed out by Gauchel *et al.*[12], carbamazepine can be determined in capillary-blood plasma by liquid chromatography. If only 2 μg of the internal stan-

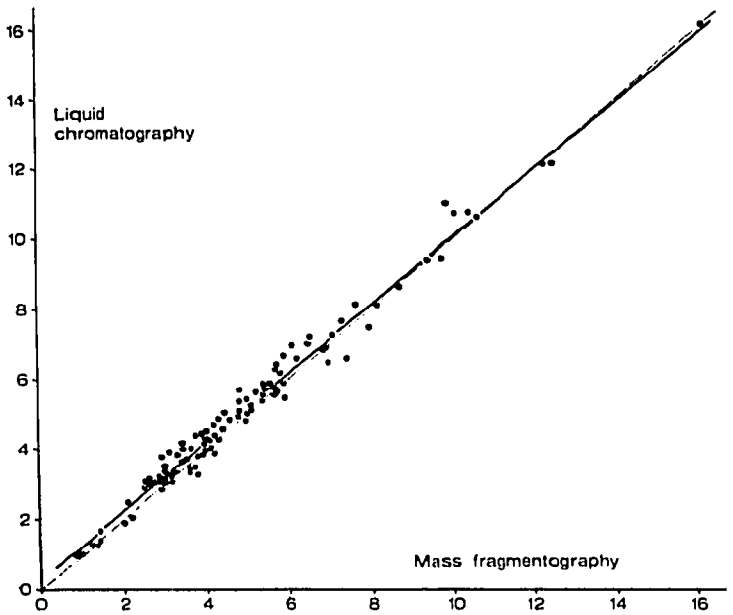

Fig. 2. Comparison of results for carbamazepine in plasma from patients chronically treated with the drug. There is a significant regression of the concentration as determined by liquid chromatography (y) on the concentration determined by mass fragmentography (x) ($n = 108$; $r = 0.99$; $p < 0.001$). The regression line $y = 0.98x + 0.28$ is indicated by a solid line; the dashed line is $y = x$.

dard is added and the sensitivity of the detector is increased, 100 μl of plasma may be used for the determination of carbamazepine by the present method. However, if carbamazepine 10,11-epoxide is to be concurrently measured, 1 ml of plasma is needed.

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