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Note

Rapid and sensitive determination of ethotoin as well as carbamazepine, phenobarbital, phenytoin and primidone in human serum

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Ethotoin (ETT) [1], 3-ethyl-5-phenylhydantoin, has been reported to have lower anticonvulsant activity in epileptic patients than phenytoin (PHT). Thus, ETT is not considered to be a primary antiepileptic agent, although it lacks the typical side-effects of PHT, such as gingival hyperplasia, hirsutism and ataxia.

Recently, Carter et al. [2] reported that seizure was controlled, without side-effects, by ETT administration to sixteen out of seventeen patients who suffered from a variety of seizure disorders.

Little information on the disposition of ETT in patients has been reported because of the lack of an adequate quantitation method of ETT for the patients taking various other antiepileptic agents.

Naestoft and Larsen [3] reported a method for determining ETT using a selected-ion monitoring (SIM) technique. Bius et al. [4] developed a gas chromatographic (GC) method for the determination of ETT in human plasma to investigate the fate of ETT in humans. However, there have been no reports of

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the simultaneous quantitation of several antiepileptic agents including ETT. We have developed such a method for ETT, carbamazepine (CBZ), phenobarbital (PB), PHT and primidone (PMD) in serum using a SIM technique. Serum levels of these agents in paediatric patients were also determined by using this method.

EXPERIMENTAL

Materials

ETT and hexobarbital (HB) were purchased from Dainippon Pharmaceutical (Osaka, Japan) and Tokyo Chemical Industry (Tokyo, Japan), respectively. MethElute, a methylating agent for on-column derivatization of drugs in GC, was purchased from Pierce (Rockford, IL, U.S.A.). MethElute was labelled to contain a 0.2 *M* solution of trimethylanilinium hydroxide in anhydrous methanol.

All other reagents were of analytical grade and purchased from Wako (Osaka, Japan).

Extraction procedure

To a 100-μl portion of serum in a 10-ml glass test-tube, 1 ml of a saturated solution of sodium chloride and 1 ml of chloroform containing 2 μg of HB as internal standard were added. The sample was mixed for 10 s by a vortex-type mixer. After centrifugation, the upper aqueous layer was aspirated off. The remaining chloroform layer was then transferred to a 2-ml glass sample tube and evaporated to dryness in vacuo at 50°C. The residue was redissolved in 25 μl of MethElute, and a 1-μl aliquot of the resultant solution was injected into the gas chromatographic—mass spectrometric (GC—MS) system.

GC–MS conditions

A QP-1000 gas chromatograph—mass spectrometer (Shimadzu, Kyoto, Japan) was employed in this study. The GC part was equipped with a 1.2 m × 3 mm I.D. glass column of 3% OV-17 on Gas Chrom Q. The flow-rate of helium was 50 ml/min. The temperatures of the injection port, the transfer line to the mass spectrometer and the ion source of the mass spectrometer were 300, 250 and 250°C, respectively.

The following programme for the column temperature was employed: the initial temperature was 215°C with a hold-time of 1.5 min; the temperature was then raised at 10°C/min to a final temperature of 250°C.

Mass spectra were obtained in an electron-impact mode at an electron energy of 70 eV. SIM was performed at *m/z* 193, 218, 218, 232, 235 and 280 for CBZ, ETT, PMD, PB, HB and PHT, respectively. Peak-area ratios of the drug to the internal standard material were calculated to obtain the standard curves.

Standard curves

Known amounts of each drug corresponding to up to 100 μg/ml were added to a blank serum. The concentrations of each drug were estimated by the peak-area ratios of each drug to the internal standard. The standard curves were obtained for each set of the serum samples.

Patients

Ethical aspects of the present study were guided by the Declaration of Helsinki, since an institutional review board has not been established in our institution yet. An informed consent was obtained from each subject or his guardian.

A comparative study on the rate and extent of bioavailability of ETT powder and the tablet was performed in one patient who had been receiving PB, PHT and valproate.

RESULTS

Gas chromatography—mass spectrometry

A typical SIM chromatogram is shown in Fig. 1. Serum concentrations of five drugs could be determined within 5 min after injection. Retention times for CBZ, ETT, PB, PHT, PMD and HB were 2.4, 1.0, 1.3, 4.2, 2.8 and 1.1 min, respectively. Clear separation was observed between ETT and PMD, both of which were monitored at *m/z* 218. The whole procedure to quantitate anti-epileptic agents in a patient serum was completed within 1 h. Recovery values of five agents through the whole procedure are listed in Table I. No effort

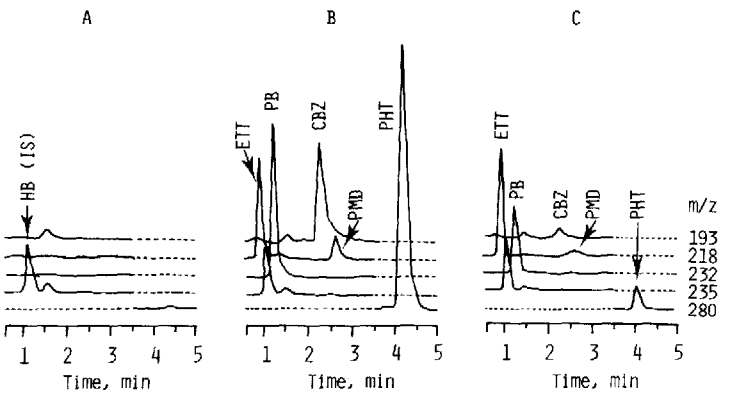

Fig. 1. Typical selected-ion monitoring chromatograms of: (A) a blank serum spiked with an internal standard; (B) a blank serum spiked with 100 μg/ml of each drug; (C) a patient sample containing 2.8, 60.6, 25.9, 6.4 and 5.0 μg/ml of CBZ, ETT, PB, PHT and PMD, respectively.

TABLE I

RECOVERY VALUES OF FIVE ANTIEPILEPTIC AGENTS THROUGH THE WHOLE EXTRACTION PROCEDURE (n = 9)

Compound	Concentration (μg/ml)	Recovery (%)
CBZ	7.0	88.5
ETT	50.0	95.8
PB	10.0	83.3
PHT	25.0	84.9
PMD	15.0	74.6

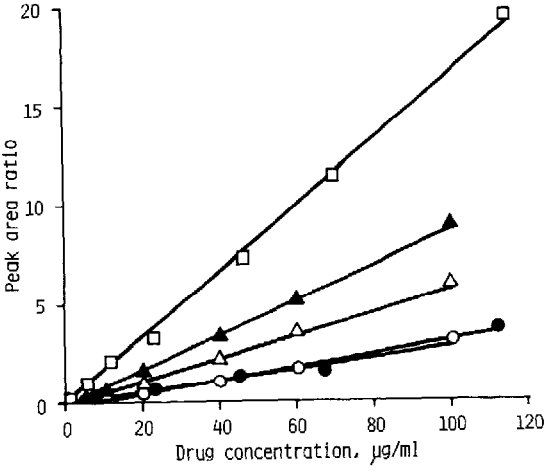

Fig. 2. Calibration curves of CBZ (▲), ETT (○), PB (△), PHT (□) and PMD (●) after extraction from serum.

TABLE II

INTER-DAY COEFFICIENTS OF VARIATION FOR QUANTITATION OF FIVE ANTIEPILEPTIC AGENTS (n = 8)

Concentration (μg/ml)	Coefficient of variation (%)				
	CBZ	ETT	PB	PHT	PMD
10	5.45	3.60	2.27	0.39	5.03
20	2.89	2.55	1.19	3.56	3.18
30	1.76	1.91	2.03	3.72	6.00
40	5.81	0.47	3.60	2.08	0.22
50	4.94	2.05	2.25	1.30	1.05
60	1.10	1.57	1.00	6.06	3.42
70	3.13	2.28	1.98	1.68	2.18
80	1.82	1.43	3.63	0.33	0.80
90	0.81	2.27	3.06	1.26	1.08
100	0.73	2.25	2.67	3.08	0.82

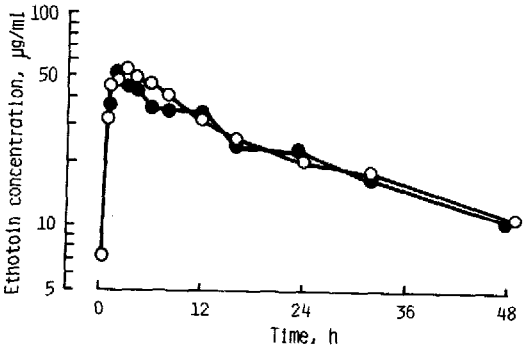

Fig. 3. Serum ethotoin concentration profiles of a patient who had received an oral dose of 2.0 g of ethotoin tablet (○) or powder (●) with a concurrent administration of PB, PHT and valproate.

was made to increase the recovery value of each agent, and 4 ng of each drug, corresponding to ca. 1 μg/ml, could be measured quantitatively. Linear relationships were obtained up to the serum level of 100 μg/ml for all five drugs, with correlation coefficients of 0.998–0.9999. Fig. 2 shows the typical standard curves of five agents. Inter-day coefficients of variation for quantitation are listed in Table II.

Patient samples

Fig. 3 shows the serum concentrations of ETT after administration of 2 g of ETT powder or the tablet. Pharmacokinetic parameters estimated by the non-linear regression analysis (Table III) indicate bioequivalency of ETT powder and the tablet after oral administration to one patient.

TABLE III

PHARMACOKINETIC PARAMETERS OF ETHOTOIN IN PATIENT RECEIVING 2.0 g OF ETHOTOIN IN TABLET OR POWDER

	$AUC_{0-\infty}$ (μg/ml) h	Mean residence $time_{0-\infty}$ (h)	$t_{1/2}$ (h)	C_{max} (μg/ml)	t_{max} (h)
Tablet	$1.61 \cdot 10^3$	38.5	11.6	53.3	3
Powder	$1.58 \cdot 10^3$	37.0	14.3	53.5	2

DISCUSSION

The use of GC–MS with a SIM technique for the determination of ETT has been reported previously [2, 3]. Our method differs from earlier reports principally with regard to the derivatization technique and can be applied to serum samples of patients who take various antiepileptic agents. Although this method can measure the serum level of ethosuximide, it could not be applied to the dosage regimen of ethosuximide because the concentration determined by this method includes ethosuximide and its major active metabolite, demethylated ethosuximide. Valproate can not be measured by the method, even when extraction to an organic solvent is included, because of its volatility. The presence of these drugs in the blood of patients who participated in this study did not interfere with the analysis of ETT, CBZ, PB, PHT and PMD.

The method described has the advantages of high specificity, sensitivity and rapidity and it is suitable for the routine monitoring of serum concentrations in epileptic patients receiving multi-drug therapy. Serum profiles of ETT after administration of ETT powder or the tablet fitted a one-compartment open model. Areas under the curve (AUC) of both formulations were quite similar to each other, indicating their bioequivalency. Elimination half-lives ($t_{1/2}$) calculated were 14.3 and 11.6 h after administration of powder and the tablet of ETT, respectively. These values were much longer than a reported value of 5.1 h [5]. Thus, administration of two or three doses per day is appropriate in ETT therapy without a large fluctuation of serum ETT levels.

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