Journal of Chromatography, 183 (1980) 141—148
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 587

CHEMICAL IONISATION MASS FRAGMENTOGRAPHIC MEASUREMENT OF DOTHIEPIN PLASMA CONCENTRATIONS FOLLOWING A SINGLE ORAL DOSE IN MAN

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(First received Janaury 3rd, 1980; revised manuscript received March 10th, 1980)

SUMMARY

A method is described for the measurement of the antidepressant drug dothiepin in human plasma.

The procedure involves the addition of deuterodothiepin as an internal standard, extraction and measurement by chemical ionisation mass fragmentography. The minimum measurable concentration of 0.5 ng/ml enabled the pharmacokinetics of dothiepin in man to be studied after a single oral dose of 75 mg of dothiepin hydrochloride.

Unlike other tricyclic antidepressants the apparent half-life of elimination (approximately 24 h) showed very little intersubject variation. However, the apparent volume of distribution was much more variable and could account for the wide range of steady state concentrations which have been found in patients taking dothiepin.

INTRODUCTION

Although the tricyclic antidepressant, dothiepin hydrochloride or dosulepin hydrochloride, Prothiaden [11-(3-dimethylaminopropylidene)-6,11-dihydrodibenz[*b*,*e*]thiepin hydrochloride], has been in clinical use for a number of years, the pharmacokinetics after a single dose in man have not been studied, as a method with suitable sensitivity has not been available. This is because dothiepin, like other tricyclic antidepressants, is extensively metabolised in man resulting in very low plasma concentrations of unchanged drug.

We have predicted from studies in laboratory animals, that in order to study the single dose pharmacokinetics in man, an analytical method capable of measuring subnanogram amounts of dothiepin is required.

Whilst techniques using gas—liquid chromatography [1,2] or high-performance liquid chromatography [3] are suitable for the measurement of steady state levels of dothiepin (range 20—420 ng/ml) they are not sufficiently sensitive to enable a complete plasma profile to be obtained after a single dose of 25—75 mg of the drug.

0378-4347/80/0000-0000/$02.25

For the measurement of tertiary tricyclic amines, mass fragmentographic methods have been developed. Electron impact (EI) mass fragmentographic methods have been published for many tricyclic antidepressants [4] and more recently chemical ionisation (CI) methods have been reported [5]. However, there is no published mass fragmentographic method for dothiepin.

EXPERIMENTAL

Six healthy male volunteers within ± 10% of the Metropolitan Life Insurance tables of desirable weights for males and females (age 22—36 years, mean 26; weight 65—76 kg, mean 71.0; height 178.5—185.5 cm, mean 181.0) gave written consent to participate in the study. All volunteers undertook a screening procedure which included a full physical examination, electrocardiogram, urinalysis, haemoglobin, white blood cell and differential count, platelets, serum glutamic—oxaloacetic transaminase (SGOT), serum glutamic—pyruvic transaminase (SGPT), bilirubin and sodium and potassium levels.

Each volunteer received 75 mg (3 25-mg capsules) of dothiepin hydrochloride with 100 ml of water after an overnight fast. Breakfast was allowed 3 h after taking the dose. Blood samples were taken immediately prior to drug ingestion and after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 12, 24, 36, 48, 72 and 96 h. Samples were taken into heparinised containers, the first ten by indwelling cannula and the remaining samples by repeated venipuncture. For the first 8 h of the test the volunteers were under constant medical supervision. Plasma was separated by centrifugation at 850 *g* for 20 min and stored at −20°C until assayed.

Gas chromatography—mass spectrometry

All analyses were carried out on a Hewlett-Packard Model 5982A gas chromatograph—mass spectrometer fitted with a dual EI/CI source and coupled to a Hewlett-Packard Model 5947A multiple ion detector.

The gas chromatographic separation was carried out on silanised glass columns (1 m × 2 mm I.D.) packed with 3% OV 17 on 100—120 mesh Gas-Chrom Q (Chromatography Services, Merseyside, Great Britain).

The injection port, column oven and transfer lines were maintained at 250, 220 and 250°C respectively. Research grade methane (B.O.C., Derby, Great Britain) was used both as carrier gas and as CI reactant gas. The flow-rate of methane was adjusted to optimise the source pressure (80—93 Pa), which corresponded to a flow-rate of 8—10 ml/min through the column and gave satisfactory chromatography. The source temperature was 150°C, the electron beam energy was 250 eV and the filament current 200 μA. Other parameters were adjusted to optimise the performance of the instrument.

Reagents and standards

Internal standard. Deuterodothiepin HCl (2 mg) dissolved in distilled water (10 ml) diluted 1 to 100 with distilled water to give a solution containing 20 ng/10 μl.

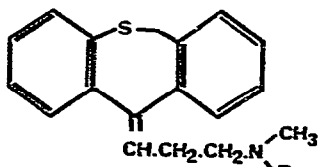
Carrier. Imipramine HCl (Courtine and Warner, Lewes, Great Britain) (25 mg) dissolved in distilled water (10 ml) diluted 1 to 10 with distilled water to give a solution containing 250 ng/10 μl.

Standard. Dothiepin HCl (The Boots Company, Nottingham, Great Britain) (11.2 mg) dissolved in distilled water (10 ml) diluted 1 to 1000 with distilled water to give a solution containing 1 ng/μl of dothiepin free base.

Reagents and solvents used were 1 *N* sodium hydroxide solution prepared from sodium hydroxide AR (B.D.H., Poole, Great Britain), Nanograde hexane (Camlab, Cambridge, Great Britain) and methyl acetate (B.D.H.).

Synthesis of internal standard [11-(3-N-trideuteromethyl-N-methylamino-propylidene)-6,11-dihydrodibenz[b,e] thiepin]

The N-methyl-N-ethoxycarbonyl derivative (I) (Fig. 1) of dothiepin was prepared by reaction of dothiepin (II) with ethyl chloroformate [6]. The carbamate was reduced to deuterodothiepin (III) with lithium aluminium deuteride and isolated as the hydrochloride.



I. R=CO_2.Et
II. R=CH_3
III. R=$C[^2H]_3$

Fig. 1. Structural formulae of deuterodothiepin and intermediates. I = N-methyl-N-ethoxycarbonyl derivative; II = dothiepin; III = deuterodothiepin.

Extraction and measurement

To 1 ml plasma in a 15-ml glass stoppered centrifuge tube were added 10 μl internal standard solution, 10 μl carrier solution, 200 μl 1 *N* sodium hydroxide solution and 10 ml hexane. Extraction was effected by rotation on an extraction wheel (Scientific Industries International, Loughborough, Great Britain) at 45 rpm for 30 min. After centrifugation (1500 *g* for 10 min), the hexane phase was transferred to a 10-ml pointed glass tube and evaporated to dryness at 45°C under a stream of nitrogen to concentrate the extract into the point of the tube. The residue was finally redissolved in 5 μl of methyl acetate and injected into the gas chromatograph.

To minimise adsorption losses all glassware was soaked overnight in 0.1 *N* sodium hydroxide solution, rinsed with distilled water and dried in an oven at 110°C.

The multiple ion detector was set to monitor approximately *m/e* 296 (M + 1 ion of dothiepin) and approximately *m/e* 299 (M + 1 ion of internal standard). Fractional mass settings required to give maximum response were dependent on the mass marker offset at the time of operation. Optimum mass settings and gain settings were therefore determined for each batch of samples by examining standard solutions of dothiepin and deuterated dothiepin.

The peak height for dothiepin (*m/e* 296) and deuterodothiepin (*m/e* 299) were measured and the ratio *m/e* 296/*m/e* 299 used to calculate the concentration of dothiepin relative to a standard calibration curve.

RESULTS AND DISCUSSION

Methodology

Electron impact ionisation results in the complete fragmentation of the dothiepin molecule such that no fragment ion greater than 2% of the base peak (*m/e* 58) is found (Fig. 2). Monitoring of the base peak was found to be insufficiently selective to allow the reliable measurement of subnanogram amounts of dothiepin in plasma or serum extracts. Chemical ionisation on the other hand gives rise to an abundant quasimolecular ion at *m/e* 296 (Fig. 3) and monitoring of this ion results in the specific detection of dothiepin with the minimum of sample preparation.

A calibration curve constructed from 40 measurements, over a period of 14 days, on control plasma to which had been added known amounts of dothiepin in the range 0—60 ng/ml is shown in Fig. 4. Regression analysis of these data gave a linear relationship between peak height ratio and dothiepin concentration, viz., dothiepin concentration (ng/ml) = (peak height ratio) × 2.095 − 2.225, with a relative standard deviation of 10.1%.

The known metabolites of dothiepin, which are present in blood at concentrations greater than the parent drug [7] and which interfere with the gas—liquid chromatographic assay, do not interfere with the mass fragmentographic assay of dothiepin when present at concentrations of 100 ng/ml.

The limit of detection of the method as reported is 0.5 ng/ml.

The main limitation on the sensitivity of the method described is the contribution at *m/e* 296 from the deuterated internal standard which is responsible for the positive intercept of the least squares plot. This ion at *m/e* 296 does not reflect the isotopic purity of the internal standard. One possible explanation is the exchange of the $-C[^2H]_3$ label with methane reactant gas in the mass

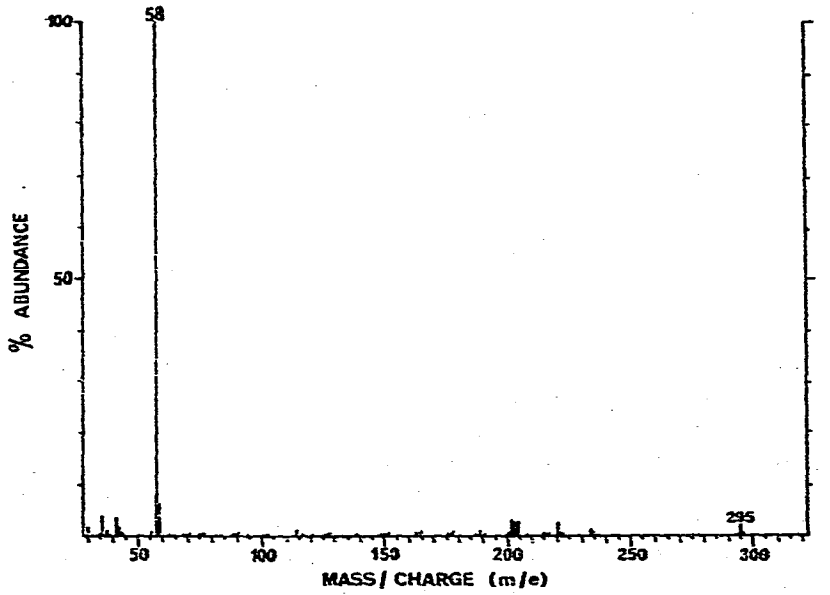


Fig. 2. Electron-impact mass spectrum of dothiepin hydrochloride.

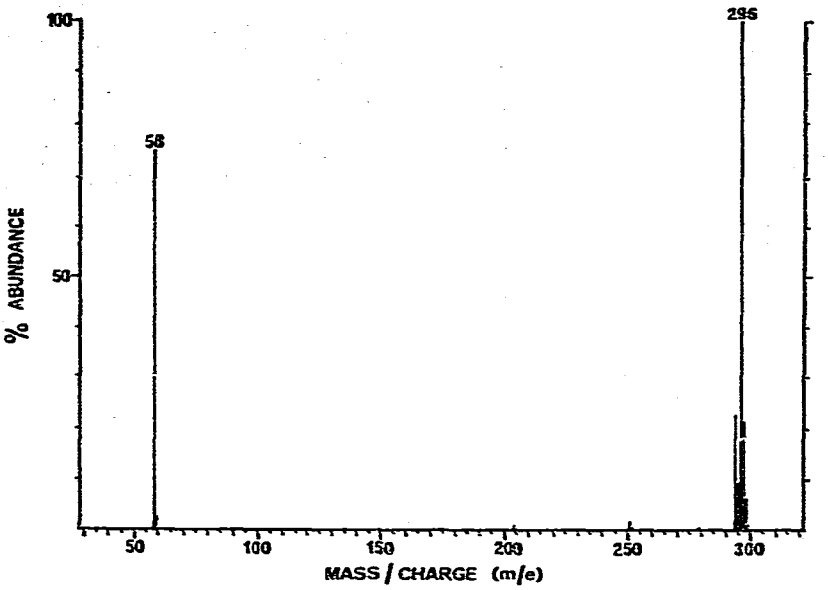


Fig. 3. Chemical ionisation (methane) mass spectrum of dothiepin hydrochloride.

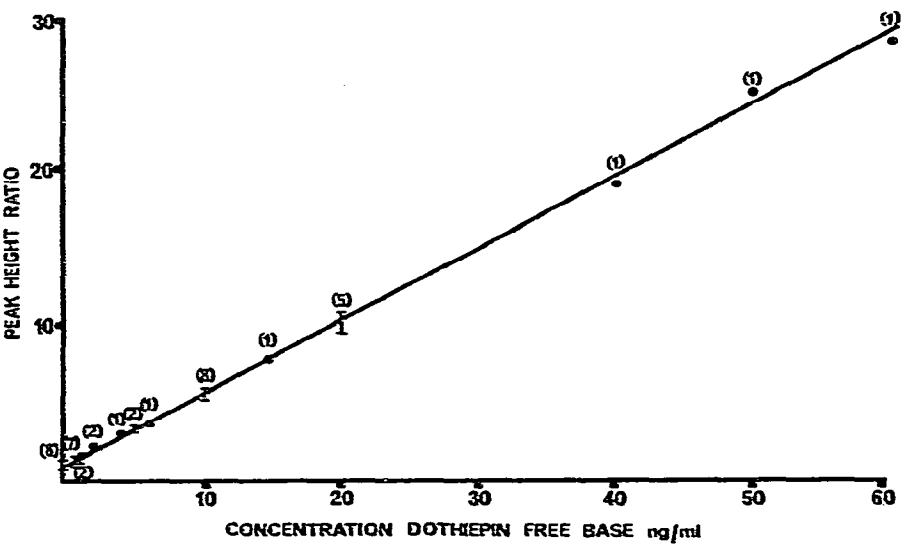


Fig. 4. Calibration curve for dothiepin in plasma. Numbers in parentheses indicate number of measurements. Vertical bars indicate the range of replicate determinations — single or identical values. The full line gives the least squares best fit.

spectrometer source, a phenomenon first reported by Ford et al. [8]. This effect remained constant at a fixed source temperature and was not affected by small changes in source pressure.

The use of isobutane, or preferably ammonia, as reactant gas eliminates the interference at *m/e* 296 and could form the basis of a much more sensitive assay if this was required.

TABLE I

PLASMA CONCENTRATIONS (ng/ml) OF DOTHIEPIN IN VOLUNTEERS AFTER A SINGLE 75-mg ORAL DOSE OF DOTHIEPIN HYDROCHLORIDE

Dothiepin hydrochloride in 3 25-mg Prothiaden capsules. Peak values are underlined. S.E.M. = standard error of the mean; N.D. = not detected (< 0.5 ng/ml) taken for calculation purposes as zero.

Volunteer	Time (h)															
	0	0.5	1	1.5	2	2.5	3	3.5	4	6	12	24	36	48	72	96
1	N.D.	1.4	103.6	69.1	87.5	81.3	95.1	81.5	76.3	49.5	58.0	36.5	31.3	20.1	13.3	7.7
2	N.D.	N.D.	1.2	5.0	7.1	9.8	25.2	29.6	22.3	20.0	19.1	10.4	5.8	3.3	1.8	1.2
3	0.9	1.5	39.1	92.8	91.1	80.4	78.4	85.7	73.2	49.5	37.8	28.6	13.0	9.3	4.6	2.6
4	N.D.	3.6	69.9	92.4	68.5	70.7	61.9	56.5	48.7	37.0	31.8	16.2	7.2	5.2	1.6	0.7
5	N.D.	N.D.	1.7	16.1	25.6	27.1	38.2	35.4	31.4	18.5	17.3	8.7	7.4	4.2	1.9	1.4
6	N.D.	1.5	33.3	53.9	70.9	42.2	70.6	58.3	54.3	41.3	36.7	26.8	19.0	12.6	7.4	2.5
Mean	0.2	1.3	41.5	54.8	58.5	51.9	61.6	57.8	51.0	36.0	33.5	21.2	14.0	9.1	5.1	2.7
± S.E.M.	0.2	0.5	16.3	15.3	14.0	12.3	10.6	9.4	8.9	5.6	6.1	4.5	4.0	2.6	1.9	1.0

TABLE II

PHARMACOKINETIC PARAMETERS OF SIX VOLUNTEERS AFTER A SINGLE 75-mg ORAL DOSE OF DOTHIEPIN HCl (EQUIVALENT TO 66.7 mg FREE BASE)

$$V_d = \frac{\text{dose}}{\text{body weight}} \times \frac{1}{A+B}\,;\; t_{\frac{1}{2}} = \frac{\ln 2}{\beta}\,;\; Cl = \frac{\text{dose}}{\text{A.U.C.}_{\infty}}\,;\; \text{A.U.C.}_{\infty} = \frac{A}{\alpha} + \frac{B}{\beta};\; \text{A.U.C.}_{96\,h} \text{ measured using trapezoidal rule.}$$

Volunteer	Body weight (kg)	Apparent volume of distribution of central compartment (l/kg) V_d	Terminal elimination half-life $t_{\frac{1}{2}}$ (h)	Clearance (l/h) Cl	Area under curve to ∞ (calculated) (h·ng/ml) A.U.C.$_{\infty}$	Area under curve to 96 h (measured) (h·ng/ml) A.U.C.$_{96\,h}$	Fraction of dose not metabolised at first-pass
1	76	2.9	29.6	21.8	3065	2662	0.81
2	73.5	24.5	23.1	90.4	738	630	0.50
3	73	6.4	21.3	36.2	1842	1672	0.71
4	65	11.8	23.1	61.7	1082	1128	0.59
5	69.3	12.2	23.5	87.4	764	672	0.51
6	69.3	9.2	23.1	35.8	1865	1713	0.72
Mean	71.0	11.2	23.9	55.6	1560	1413	0.64
± S.E.M.	1.6	3.0	1.2	11.8	363	314	0.05

Pharmacokinetics

No serious adverse effects were observed in any of the volunteers after administration of 75 mg dothiepin hydrochloride. Side effects were limited to slight drowsiness in all subjects and a dry mouth in one individual. Pulse and blood pressure recordings taken throughout the study did not change to any clinically significant extent.

The plasma concentrations in six healthy male volunteers who had each received 75 mg of dothiepin hydrochloride are given in Table I and mean results shown graphically in Fig. 5. The results show that there was a large intersubject variation in peak plasma concentrations of dothiepin which occurred from 1 to 3.5 h after the dose. In four of the six subjects secondary peaks occurred up to 4 h after administration of the dose. We have also observed this effect, which is attributed to biliary recycling, in baboons. One consequence of this observation was that absorption parameters could not be accurately calculated.

Non-linear regression analysis of the elimination phase showed this to be biphasic, and the data could be fitted to a bi-exponential equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ where C_t (ng/ml) is the concentration at time t (h) and $A = 210$, $\alpha = 0.71$, $B = 42$ and $\beta = 0.029$ for the composite curve. The closeness of fit between experimental data and the computer generated curve ($r = 0.999$) strongly supports the applicability of a two-compartment model to describe the elimination pharmacokinetics of dothiepin in man. The composite apparent distribution and elimination half-lives were calculated as 2.9 and 23.7 h respectively.

Using equations derived for a two-compartment open model [9] individual data were used to predict the predose steady state levels of dothiepin which would be expected during a 50-mg three times a day dose regimen. The pre-

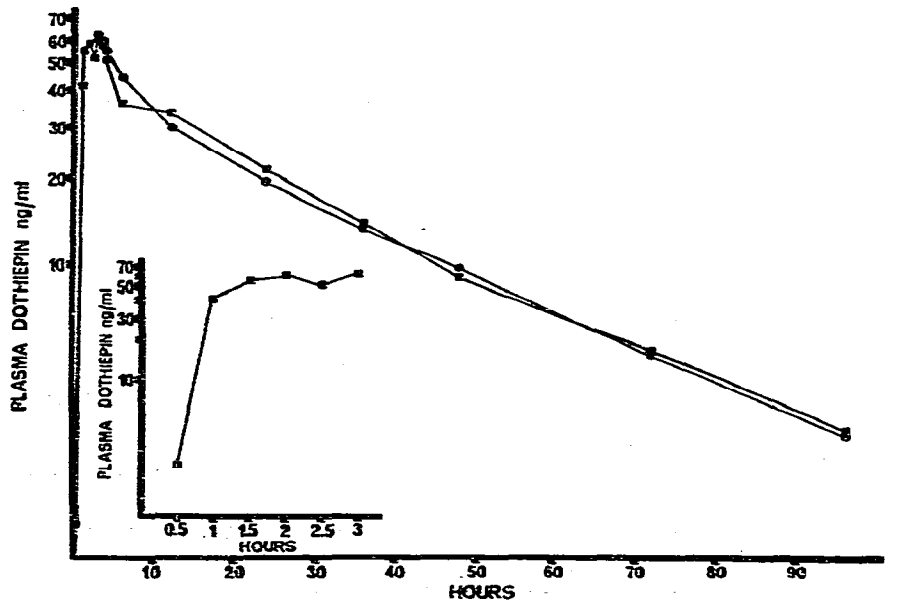


Fig. 5. Mean plasma concentrations of dothiepin after a single oral dose of 75 mg dothiepin hydrochloride.

dicted value of 107 ± 28 (S.E.M.) ng/ml is in good agreement with the mean value of 106 ± 12 (S.E.M.) ng/ml found in 16 patients receiving dothiepin hydrochloride 50 mg three times a day [10].

Volumes of distribution, areas under plasma concentration versus time curves (0—∞) and clearances were calculated from the bi-exponential constants of the individual profiles (Table II). There were large intersubject variations in these parameters consistent with a drug that is extensively metabolised [11]. However, there was very little intersubject variation in elimination half-lives of unchanged dothiepin in contrast to reports published for other tricyclic antidepressants [12—14].

The variations in steady state concentrations of tricyclic antidepressants have been attributed to variations in half-life of elimination and theoretical volume of distribution [15, 16]. If the elimination kinetics of dothiepin in patients are similar to those in normal subjects, then the range of steady state concentrations achieved clinically (20—420 ng/ml) is more likely to be a consequence of variations in apparent volume of distribution. Since there is a linear correlation ($r = 0.84$) between apparent volume of distribution and the calculated [17] fraction of drug metabolised at first-pass, it is suggested that steady state levels of dothiepin are controlled primarily by the extent of first-pass metabolism.

ACKNOWLEDGEMENT

The authors wish to thank Dr. K.J. Nichol, Research Department, The Boots Company for the synthesis of deuterodothiepin.

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