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Note

Analysis of plasma trifluoperazine by gas chromatography and selected ion monitoring

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The relatively high potencies of the piperazine side chain phenothiazines, coupled with extensive metabolism, lead to low plasma concentrations, making accurate determinations difficult. Radioimmunoassay [1, 2] and radioreceptor [3] methods offer high sensitivities, but doubtful selectivities render these methods unsuitable for pharmacokinetic work. Gas chromatography (GC) with electron-capture detection has been applied to 2-chlorophenothiazine derivatives [4] but is unsuitable for those with 2-trifluoromethyl substituents. Liquid chromatography (LC) using nitrile-bonded phases has been used to determine phenothiazine plasma concentrations down to approximately 1 ng ml^{-1} [5—7], Nitrogen—phosphorus detectors (NPD) have been used to determine fluphenazine and perhenazine [8, 9] by GC. Our experience with a GC—NPD assay of trifluoperazine led us to an alternative approach. Potential difficulties arise from the low concentrations involved: adsorptive losses onto glassware, oxidation and contamination from other drugs, etc. Consequently, GC with selective ion monitoring using stable isotope internal standard was chosen.

MATERIALS

Chemicals and glassware

Reagents were of analytical purity when available. Trifluoperazine dihydrochloride, [^{3}H]trifluoperazine (34.9 μCi mol^{-1}) and an authentic sample of desmethyltrifluoperazine dimaleate were provided by Smith, Kline and French Laboratories (Welwyn Garden City, Great Britain). Reagents for the synthesis

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of deuterium-labelled trifluoperazine were purchased from Aldrich (Gillingham, Great Britain). Iodomethane-d_3 had an isotopic purity of > 99%. Screw-capped glass tubes and disposable glass pipettes were purchased from Labco (Marlow, Great Britain) and Corning (Stone, Great Britain).

Biological material

Plasma for calibration and recovery studies was kindly provided by The London Hospital Blood Bank. Rat plasma samples were provided by Dr. P. Jenner, The Institute of Psychiatry, Denmark Hill, London, Great Britain. Two doses of trifluoperazine were administered chronically via drinking water as described previously [10]. The average doses were calculated as 0.7—0.9 mg kg^{-1} day^{-1} and 2.5—3.5 mg kg^{-1} day^{-1}. Human plasma was from a male patient (age 37) under the care of Dr. D. Lewis, St. Bernard's Hospital, Southall, Middlesex, Great Britain. The patient had been receiving oral trifluoperazine (15 mg, b.i.d.) for a number of years prior to this study. Concurrent drugs were: nitrazepam (10 mg, nocte) orphenadine (50 mg, t.d.s.) and lorazepam (2.5 mg, t.d.s.). Blood was drawn into heparinised tubes just before a usual morning dose of three 5-mg trifluoperazine tablets and at 0.5, 1.0, 1.5, 2.0, 4.0, 6.0 h afterwards. Plasma was separated and stored at —18°C until assayed.

EXPERIMENTAL

Synthesis of 3-(2-trifluoromethylphenothiazinyl-10)-propyl chloride (I)

2-Trifluoromethylphenothiazine (0.02 mol) in dimethylformamide (20 ml) was added to 1,3-bromochloroproprane (0.2 mol). Sodium hydride (50% dispersion in oil) was added over approximately 2 h, until the colour of the phenothiazinate ion was no longer visible. The mixture was poured into ice-water (50 ml) and extracted with diethyl ether (2 × 50 ml). The ether extract was washed with water (50 ml), dried (magnesium sulphate) and evaporated under reduced pressure, leaving a light brown oil which solidified on standing. Crystallization from methanol gave a pale yellow solid (84%), m.p. 68—72°C. This was used for the next step without further treatment. Recrystallization [light petroleum b.p. 40—60°C)] of an aliquot gave an analytical sample, m.p. 74—75°C. Analysis: calculated for $C_{16}H_{13}F_3C_1NS$: C, 55.90; H, 3.81; N, 4.07; found C, 55.45; H, 3.73; N, 3.94.

Synthesis of N-(3-(2-trifluoromethylphenothiazinyl-10)-propyl)-piperazine dimaleate (II)

Compound I (0.01 mol) and piperazine (0.1 mol) were refluxed in ethanol (10 ml) for 90 min. The reaction mixture was added to water (50 ml) and the product extracted and concentrated as described for I. The pale oil residue was dissolved in diethyl ether (20 ml) and added, with stirring, to maleic acid (2.4 g) in diethyl ether. The solid was filtered and dried, yield 68%. Crystallization from ethanol gave a white solid, m.p. 150—151°C. The melting point of an authentic sample was 151—152°C. Analysis: calculated for $C_{28}H_{30}F_3O_8S$: C, 53.76; H, 4.83; N, 6.71; found: C, 53.41; H, 4.87; N, 6.53.

Synthesis of trifluoperazine-d_3 dimaleate

Compound II (0.001 mol) was added to 1 *N* sodium hydroxide (1 ml) and the base was extracted into diethyl ether. The organic phase was transferred to a clean tube and evaporated. The dried residue was dissolved in benzene (1 ml) containing iodomethane-d_3 (0.0011 mol) and heated in a sealed tube at 60°C for 1 h. Diethyl ether (5 ml) was added and the organic solvent washed with water (2 × 5 ml), dried (magnesium sulphate) and evaporated. The residue was dissolved in diethyl ether (2 ml) and added to a solution of maleic acid in diethyl ether (2 g per 100 ml). The solid was filtered and crystallized from ethanol twice.

Plasma extraction

Plasma (1 ml for rat or 2—4 ml for human plasma) was pipetted into screw-cap tubes and trifluoperazine-d_3 dimaleate solution (100 ng in 0.1 ml ethanol) added as internal standard. Sodium hydroxide (2 *N*, 1 ml) and heptane containing 1.5% (v/v) amyl alcohol (5 ml) were added and the capped tubes shaken mechanically for 30 min. After centrifugation the organic phase (4.5 ml) was transferred to tubes containing hydrochloric acid (0.1 *N*, 2 ml), shaken for 15 min and centrifuged. The acid layer (1.8 ml) was transferred to pointed tubes, made alkaline with sodium hydroxide (2 *N*, 0.2 ml) and extracted with 15% (v/v) amyl alcohol in toluene (0.05 or 0.10 ml).

The aqueous phase was removed in a series of centrifugation and aspiration steps. Aliquots (5—20 μl) of the organic phase were injected into the chromatograph.

Standard solutions were prepared containing 20, 10, 5, 2, 1, 0.5, 0.2 and 0 ng ml^{-1} trifluoperazine dihydrochloride in drug-free plasma. Samples (4 ml) were extracted as above.

Recovery experiment

The purity of [^{3}H]trifluoperazine was checked by thin-layer chromatography [chloroform—ethanol—0.88 S.G. ammonia (80:10:1); silica gel] and found to be > 97%. The material was used without further purification. Radioactive trifluoperazine was diluted to 1 μg ml^{-1} in ethanol and 0.1 ml added to 4-ml plasma samples. The plasma was extracted as described above and 0.05 ml toluene—amyl alcohol sampled for liquid scintillation counting. Two batches of blood bank plasma gave recoveries (after correction for aliquot losses) of 78.1 and 69.3%, n = 5, with coefficients of variation of 8.5 and 6.5%, respectively.

Gas chromatography—mass spectrometry

A Pye 104 gas chromatograph coupled to an LKB 9021 mass spectrometer was used. The silanized glass column, 1 m × 4 mm I.D., packed with 3% OV-225 on 80—100 mesh Chromosorb W HP, was maintained at 245°C. Helium carrier gas flow-rate was 20 ml min^{-1}.

The separator and ion source temperatures were 290°C and 190°C, respectively. The ionisation potential was 20 eV and the trap current 50 μA. The magnetic field was adjusted to *m/e* 407 (the molecular ion of trifluoperazine) and the accelerating voltage varied to allow measurements at *m/e* 407 and *m/e* 410.

RESULTS AND DISCUSSIONS

Precision and sensitivity

An isotope dilution assay of the type described should have reasonable precision as the problems of adsorption, oxidation, etc., mentioned in the introduction are compensated for by the isotopically labelled internal standard. The addition of 100 ng trifluoperazine-d_3 dimaleate (equivalent to 63 ng trifluoperazine base) maintains a high and almost constant concentration. An indication of the precision of the method can be obtained from the coefficients of variation obtained on repeated assay. Coefficient of variation (C.V.) or relative standard deviation is the mean value divided by the standard deviation. The intra-assay coefficients of variation at 20, 2 and 0.2 ng ml^{-1} trifluoperazine dihydrochloride (TFP·2HCl) were 1.6, 3.3 and 13%, respectively, when five plasma samples of each concentration were assayed. Comparison with C.V. values for the recovery experiment demonstrates how the inclusion of the internal standard reduces the variance between extractions. Omission of internal standard solution from plasma samples spiked with 1 ng ml^{-1} TFP·2HCl resulted in a GC—mass spectrometric (GC—MS) chromatogram devoid of a peak corresponding to trifluoperazine. Presumably, adsorptive losses etc. reduced the amount of drug recovered to below the sensitivity of the method.

The sensitivity of a drug assay is not a fixed value but varies with day-to-day changes in the instrument conditions and from sample to sample, depending on the amount of interfering substances and the recovery of the assay material.

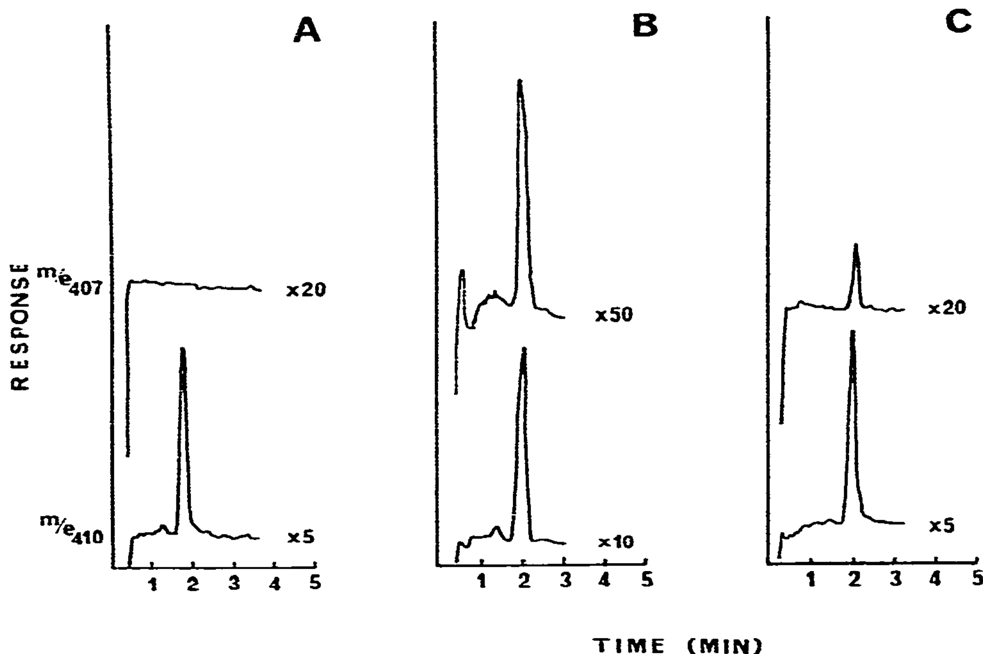

Fig. 1. Chromatogram of trifluoperazine. (A) Blank plasma (B) plasma spiked with trifluoperazine at 2 ng ml^{-1}, and (C) plasma from treated patient. Trifluoperazine, *m/e* 407 and trifluoperazine-d_3 dimaleate, internal standard, *m/e* = 410.

Electronic noise was a major factor influencing the sensitivity. When 10-μl aliquots from a final extraction volume of 100 μl were injected the limit of detection was about 0.5 ng ml^{-1} TFP·2HCl (0.4 ng base). Injection of 20 μl from a final volume of 50 μl increased the sensitivity to about 0.2 ng ml^{-1} (i.e. 160 pg ml^{-1} of trifluoperazine base). Extraction of TFP·2HCl free plasma resulted in a chromatogram free from interfering peaks at the retention time of the compound of interest (Fig. 1A).

The calibration between 0.5 and 200 ng ml^{-1} was linear; for example, least square linear regression of two calibration curves gave $y = -0.0118 + 0.0634x$ ($n = 6$, $r = 0.9996$) and $y = -0.0084 + 0.0567x$, $n = 6$, $r = 0.9999$, where y is the peak height ratio 407 response/410 response and x the (TFP·2HCl) concentration (ng ml^{-1}).

Plasma concentrations

The concentrations of TFP determined in rat and human plasma following oral doses are shown in Tables I and II. These data are presented to demonstrate the applicability of the GC—MS method to plasma from treated animals or subjects as well as to blank plasma to which drug has been added. The rather slow rise in plasma concentrations in the human subject is similar to that reported by Curry et al. [6] using LC to monitor trifluoperazine concentrations after multiple dosing with 80 mg. In the LC study, the pre-dose concentration was 2.1 ng ml^{-1} rising to 27.5 ng ml^{-1} at 8 h. Using radioimmunoassay, Midha et

TABLE I

PLASMA CONCENTRATIONS (ng ml^{-1}) IN RATS CHRONICALLY DOSED WITH TRIFLUOPERAZINE

Each value from one rat.

Duration (months)	Dose (mg kg^{-1} day^{-1})	
	0.7—0.9	2.5—3.5
3	< 1	4
	< 1	11
6	3	4
	1	6
12	< 1	23
	2	90

TABLE II

CONCENTRATIONS OF TRIFLUOPERAZINE IN HUMAN PLASMA IN A SUBJECT RECEIVING 15 mg (THREE 5-mg TABLETS) TRIFLUOPERAZINE EVERY 12 h

Time (h)	Concentration (ng ml^{-1})
Before treatment	4.2
0.5	3.6
1.0	4.0
1.5	4.0
2.0	4.2
4.0	5.1
6.0	5.3

al. [11] observed a peak concentration of 2.7 ng ml^{-1} at 3 h following a single oral dose of 5 mg. These studies suggest that peak plasma concentrations following single oral doses of 5 or 10 mg TFP·2HCl will be in the order of a few nanograms per millilitre in plasma. The GC—MS method appears capable of assaying plasma samples following single oral doses of TFP·2HCl for sufficient time to derive kinetic data. A study along these lines is currently being undertaken.

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