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Note

Determination of medifoxamine in plasma and urine by high-performance liquid chromatography

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Medifoxamine is a new monoamine re-uptake-inhibiting antidepressant which is undergoing clinical investigation It is structurally unrelated to other monoamine oxidase inhibitors and tricyclic antidepressants (Fig. 1). Oral doses of 100–300 mg daily are well tolerated and appear to be of similar efficacy to clomipramine and maprotiline in controlled double-blind trials [1,2]. The relationship between the blood concentration and the antidepressant effect of various antidepressants has been extensively studied [3] Although medifoxamine has been in clinical use, its disposition has not been previously studied

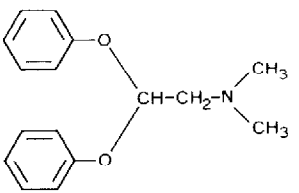

Fig 1 Structure of medifoxamine

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in man because of a lack of analytical methods to measure it in biological fluids. The pharmacokinetics of medifoxamine have been studied in animals after administration of doses of ^{14}C-labelled medifoxamine [4] A spectrofluorimetric assay has been developed for the measurement of medifoxamine, but as this method has poor sensitivity, 1 mg/l, serum drug concentrations are impossible to measure [5]

To investigate the kinetics of medifoxamine it was necessary, therefore, to develop a method to measure the drug in biological fluids at low concentrations. A method using high-performance liquid chromatography (HPLC) and solvent extraction has been developed for the measurement of medifoxamine in plasma and urine and it has been used to estimate the pharmacokinetics following an intravenous administration.

EXPERIMENTAL

Chemicals

All solvents used were of HPLC grade (Rathburn, Walkerburn, U.K.). Perchloric acid (60%) was obtained from BDH (Poole, U.K.), medifoxamine from Anphar-Rolland Labs, (Paris, France) and 5,6-benzoquinoline from Aldrich (Gillingham, U.K.).

Apparatus

The HPLC separation was carried out using a Shimadzu LC-6A liquid chromatography pump (Dyson Instruments, Tyne and Wear, U K.) with a Spectromonitor III Model 1204A UV detector (Laboratory Data Control, Milton Roy, U.K.) and a Unicam AR25 linear chart recorder (Phillips Scientific, Cambridge, U.K.) A Model 7125 injector (Rheodyne, Cotati, CA, U.S.A.) was used with an 80-μl loop Separation was achieved using a stainless-steel analytical column (150 mm$\times$5 mm I.D.) containing 5-μm Spherisorb silica (HiChrom, Reading, U K). The centrifuge used was a Centaur 2 MSE (Fisons Scientific Supplies, Loughborough, U K.).

Standard samples

Standards samples were prepared from a stock solution of 100 mg/l medifoxamine (free base) in distilled water, which was added to drug-free plasma or urine to give final concentrations of 0, 10, 25, 50, 100, 200, 500, 1000 and 2000 μg/l for plasma and 0, 50, 100, 250, 500, 1000, 2000 and 5000 μg/l for urine. Quality control samples were prepared from a 100 mg/l aqueous solution of medifoxamine which was diluted with drug-free plasma or urine to give final concentrations of 75, 250, 750 and 1000 μg/l. The internal standard solution (100 μg/l) was prepared from a 100 mg/l stock solution using distilled water

Sample extraction

To a screw-capped glass tube containing 1 ml of sample were added 50 μl of aqueous internal standard solution (5,6-benzoquinoline, 100 μg/l for plasma and 1000 μg/l for urine), 50 μl of 4 *M* sodium hydroxide and 5 ml of diethyl ether. After 10 min of gentle shaking, the tubes were centrifuged at 350 *g* for 5 min, and the ether layer was decanted into a conical glass tube containing an antibump granule. The diethyl ether was evaporated to dryness using a hot plate at 70°C. The residue was redissolved in 120 μl of the HPLC mobile phase, and 100 μl of the final extract were injected.

Chromatography

The mobile phase used was methanol modified with perchloric acid (1 l of methanol and 200 μl of 60% perchloric acid) at a flow-rate of 2 ml/min. The UV absorbance of the effluent was monitored at 266 nm and 0 002 a u.f.s Under these conditions the retention times were 3 min for medifoxamine and 5 min for the internal standard.

Data analysis

Medifoxamine concentrations in serum and urine were calculated from the peak-height ratio of medifoxamine to internal standard versus the concentration of the calibration standard using linear regression with zero intercept

RESULTS AND DISCUSSION

Using this procedure, medifoxamine and internal standard gave a chromatogram with well shaped peaks and short retention times. The retention times were very sensitive to small changes in the perchloric acid content of the mobile phase, decreasing with increasing concentrations of perchloric acid There was no interference from metabolites of the drug or other UV-absorbing endogenous substances. Chromatograms of medifoxamine and internal standard in plasma and urine are shown in Fig. 2 The amount of medifoxamine in the lipid organic phase was determined to establish the most appropriate solvent medium for extraction purposes.

Solvents studied were chloroform, diethyl ether, ethyl acetate, *n*-heptane and acetonitrile. The most suitable solvent was diethyl ether. Further studies were carried out to establish the most favourable acidic and alkaline conditions for extraction. Extraction efficiency of medifoxamine using this method was more than 80% at 100 μg/l in both plasma and urine. Quantitation of medifoxamine was performed by means of the peak-height ratio of drug to internal standard. Linear regression of peak-height ratios against medifoxamine concentrations typically gave correlation coefficients of more than 0.999 for the range tested. The coefficients of variation of the analytical method were 6.1% at 100 μg/l, 13% at 50 μg/l, 27.2% at 25 μg/l and 41% at 10 μg/l ($n=10$).

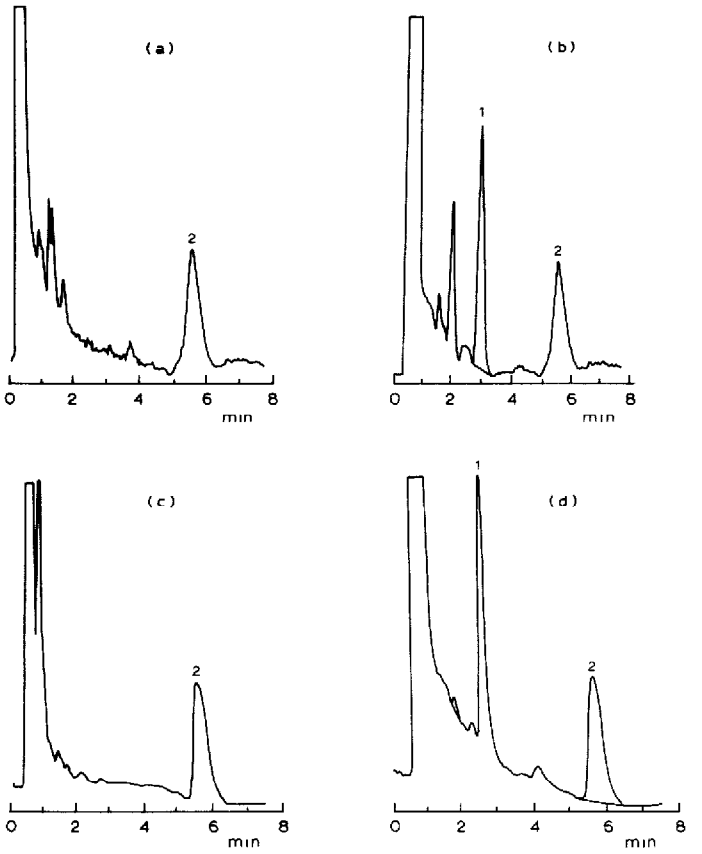

Fig 2 Chromatograms of medifoxamine (1) and internal standard (2) after extraction from (a) blank plasma, (b) 350 μg/l medifoxamine in plasma, (c) blank urine and (d) 750 μg/l medifoxamine in urine

A pharmacokinetic study was carried out to estimate the kinetic parameters of medifoxamine following intravenous administration (5–100 mg). Unchanged medifoxamine was detected in plasma and urine samples of all subjects and sufficient data for determination of kinetic parameters were obtained Fig. 3 shows the concentration–time curves of the plasma levels following a 50-mg intravenous dose of medifoxamine. The limit of detection (signal-to-noise ratio = 3) in this assay of 10 μg/l is adequate for serum sample measurement following intravenous doses, but may not be sufficiently sensitive to monitor drug levels following 50- and 100-mg single oral doses due to high first-pass extraction and metabolism The amount of drug excreted unchanged in 3 h was less than 0 5% and directly proportional with the dose administered. These kinetic data agree very well with the animal kinetics obtained by using ^{14}C-labelled medifoxamine.

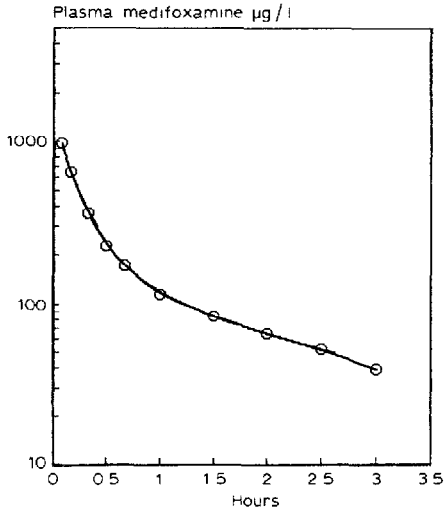

Fig 3 Mean plasma concentration–time curve following intravenous administration of 50 mg medifoxamine to four subjects each

CONCLUSION

The method described allows the measurement of medifoxamine in biological fluids with a run time of less than 7 min and limit of detection of 10 µg/l. The method is selective, rapid, utilizes widely available equipment and is simple. It has considerable advantages over the currently used radioassay for the estimation of medifoxamine in animal biological fluids and it is suitable for pharmacokinetic monitoring. Medifoxamine was measured following intravenous doses up to 100 mg, and the drug was distributed rapidly, following two-compartment open model with short half-life.

ACKNOWLEDGEMENTS

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REFERENCES

1 H Scharbach, C H Blanchard, A Grivel, Z Houri and J D Lachaud, Psychol Med , 18 (1986) 1485
2 J M Leger, D Malauzat, P Karczewski, M Pareaud, G Parmentier, M Paupry and M Soustre, Psychol Med , 18 (1986) 2295
3 L Gram, O Pedersen, C Kristensen, L Bjerrol and P Krach-Sorensan, Ther Drug Monit , 4 (1982) 17
4 J P Labaune, P Andoit and D Bolnot, in J M Aiache and J Hirtz (Editors), Proceedings of 2nd European Congress of Biopharmaceutics and Pharmacokinetics, Vol II, Salamanca, April 24–27, 1984, Lavoisier, Paris, 1984, p 626
5 A Randhawa, A Blackett and P Turner, J Pharm Pharmacol , 38 (1986) 629