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Note

Quantification of imipramine and desipramine in plasma by high-performance liquid chromatography and fluorescence detection

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Although imipramine is a widely prescribed tricyclic antidepressant, little is known of the drug's pharmacokinetics in man. The lack of suitable methods for precisely quantitating plasma levels of imipramine and desipramine following administration of single doses is the probable explanation for this deficiency. Although many of the available assay methods [1—10] are adequate for the monitoring of plasma levels during chronic therapy they are either time consuming or lack the sensitivity required to measure the low plasma concentrations following single oral doses.

Gas chromatography—mass spectrometry [1—3] and gas chromatography with alkali-flame detection [4—7] have provided the most sensitive means of measuring plasma levels of imipramine and its major metabolite, desipramine although both procedures involve a time consuming preparation of samples before injection. Adsorption [9] and reversed-phase [10] high-performance liquid chromatographic methods employing ultra-violet detection have also been reported although this method of detection has limited the assay sensitivity to 5—10 ng/ml (15—30 nmole/l). This may be insufficient to measure plasma levels at times greater than 12 h after a single 50-mg oral dose of imipramine hydrochloride. In the present method, the inherent fluorescence of imipramine and desipramine allowed the use of the sensitive fluorescence detector with the high-performance liquid chromatograph to conveniently

measure plasma levels of these compounds and extend the range of their detection in plasma to 2—3 nmole/l.

MATERIALS AND METHODS

Reagents

All reagents were analytical-reagent grade and aqueous solutions were prepared using glass-distilled water. The extracting solvent was a hexane mixture (Nanograde Hexanes, Mallinckrodt, St. Louis, Mo., U.S.A.). Specially purified acetonitrile (Unichrom, Ajax Chemicals, Melbourne, Australia) was used for the high-performance liquid chromatography. Imipramine hydrochloride was obtained from Protea (Sydney, Australia), desipramine hydrochloride from Ciba-Geigy (Sydney, Australia) and trimipramine maleate from May and Baker (Melbourne, Australia).

Standards

A standard solution of imipramine and desipramine hydrochloride (10 μmole/l of each) was prepared in water and stored at 4° (stable for at least 2 months). This solution was then diluted as necessary and used to prepare the appropriate plasma standards for each assay run. The internal standard solution of trimipramine was also prepared in water (10 μmole/l) and stored at 4°. Peak height ratios of imipramine and desipramine to trimipramine were determined for plasma standards and unknowns and quantitation performed by reading unknown values from a plotted standard curve.

Extraction procedure

To 2 ml of plasma (either patient sample or standard) were added 200 μl of the internal standard solution followed by 2 ml of 1 *N* sodium hydroxide solution. The basified plasma was then extracted with 5 ml of hexane—isoamyl alcohol (99:1) by shaking at 200 rpm for 10 min. After separation of the phases by centrifugation at 20°, the organic layer was transferred to a 15-ml tapered centrifuge tube containing 0.2 ml of 0.05% orthophosphoric acid. The mixture was vortexed for 2 min (or shaken for 10 min at 200 rpm) and the phases again separated by centrifugation. A 100-μl aliquot of the aqueous layer was injected into the high-performance liquid chromatograph.

High-performance liquid chromatography

The chromatograph was a Spectra-Physics (Model SP 8000) instrument equipped with a 10-μm alkyl phenyl reversed-phase column (μBondapak/Phenyl from Waters Assoc., Milford, Mass., U.S.A.) and a 100-μl injector loop. The column oven temperature was 50° and the eluting solvent a helium-degassed mixture of acetonitrile and 0.015% aqueous phosphoric acid (71:29) at a flow-rate of 2 ml/min. The instrument was operated in the constant flow mode and all solvent lines from the column to the detector were carefully thermally insulated. The fluorescence of the eluent was monitored using a Schoeffel Model 970 fluorometer at an excitation wavelength of 252 nm with an emission cut-off filter allowing 90% transmission at 360 nm. The fluorometer sensitivity setting was 3.5, range 0.04 μA full scale and time constant 9.0 sec.

Recovery and reproducibility

Recovery of the extraction procedure was determined at concentrations of 10, 25, 100, 500 and 1000 nmole/l in plasma by comparison of the peak heights with those obtained for an aqueous solution containing known concentrations of imipramine and desipramine. Reproducibility was determined at concentrations of 10, 25, 100, 500 and 1000 nmole/l by assaying five plasma samples at each concentration.

RESULTS

High-performance liquid chromatography with fluorescence detection was found to be a convenient and sensitive means of quantitating plasma levels of imipramine and desipramine. The extraction time for five standards and twenty samples was approximately 1 h and the run time for each chromatogram was approximately 15 min. The minimum detectable level (determined at peak height twice noise) was 2 and 3 nmole/l for imipramine and desipramine, respectively. The reproducibility of the assay determined by replicate analyses of known concentrations of imipramine and desipramine over the range 10—1000 nmole/l is shown in Table I. Recovery of imipramine and desipramine was 90% for both and independent of concentration over the range 10—1000 nmole/l.

TABLE I

COEFFICIENTS OF VARIATION (C.V., %) FOR IMIPRAMINE AND DESIPRAMINE DETERMINATIONS

Five determinations at each concentration.

Concentration (nmole/l)	C.V. (%)	
	Imipramine	Desipramine
1000	2	3
500	2	3
100	2	3
25	5	5
10	10	15

No interference was observed from the tricyclic antidepressants amitriptyline, nortriptyline and protriptyline and the following fluorescent drugs and metabolites: propranolol, 4-hydroxypropranolol, propranolol glycol, N-desisopropylpropranolol, quinidine, dihydroquinidine and 3-hydroxyquinidine all of which eluted before imipramine and desipramine. The assay of plasma samples from a number of patients and volunteers not taking imipramine showed a plasma peak eluting at approximately 3 min with no potentially interfering peaks eluting after that time.

Chromatograms obtained by analysis of samples from a volunteer prior to taking an imipramine hydrochloride dose (50 mg), 4 h after the dose and from a patient on chronic oral medication are shown in Fig. 1a, b and c, respectively. The retention times for the eluted components were 3.0 min (plasma peak), 9.8 min (desipramine), 11.3 min (imipramine) and 14.1 min (trimipramine).

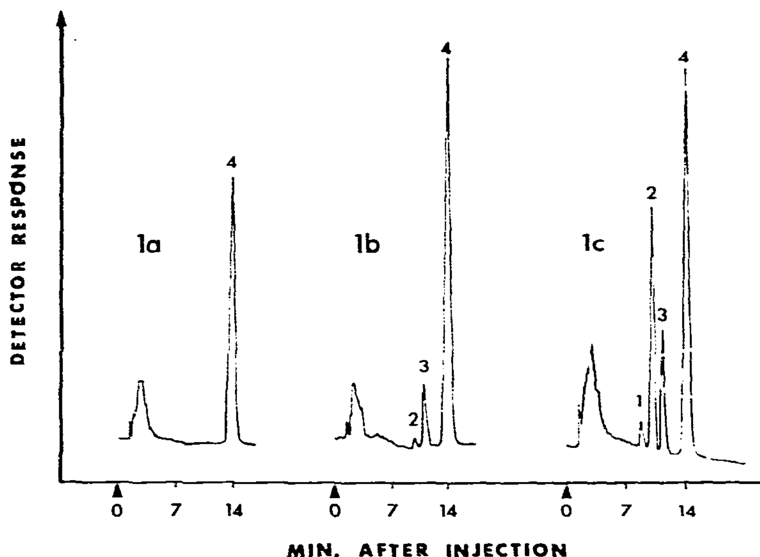

Fig. 1. Chromatograms obtained for the assay of plasma samples from a volunteer prior to taking an imipramine hydrochloride dose (50 mg) (1a), 4 h after the dose (1b) and from a patient on chronic oral therapy (1c). Peaks: 1 = unknown metabolite, 2 = desipramine, 3 = imipramine and 4 = trimipramine. In Fig. 1b, peak 2 = 20 nmole/l and 3 = 90 nmole/l; in Fig. 1c, peak 2 = 590 nmole/l and 3 = 190 nmole/l.

The small additional peak (peak 1) in Fig. 1c was only observed for patients taking imipramine and is probably attributable to an additional imipramine metabolite.

The plasma level time course following administration of a single 50-mg oral dose of imipramine hydrochloride to a healthy volunteer is shown in Fig. 2. The imipramine concentration peaked at 3.5 h (90 nmole/l) and then declined to a level of 8.0 nmole/l 28 h after the dose. The desipramine concentration also peaked at 3.5 h (30 nmole/l) but the levels at times 1—3 h and 12—28 h after the dose were too small to allow precise quantification (C.V.$>$15%).

DISCUSSION

The presently described method is applicable to the assay of plasma levels of at least two tricyclic antidepressants, imipramine and desipramine and possibly trimipramine, clomipramine and demethylclomipramine. Since trimipramine was used as the internal standard it is apparent that plasma levels of this drug could also be measured if another internal standard such as imipramine or desipramine was used.

The procedure involves a single hexane—isoamyl alcohol extraction of the drug and metabolite from basified plasma, back extraction into phosphoric acid and injection into the chromatograph. This allows approximately 20—30 plasma samples to be assayed per day. A combination of the separative power of reversed-phase high-performance liquid chromatography and the sensitivity of fluorescence detection has extended the separate detection of these compounds in plasma down to 2—3 nmole/l with a high degree of reproducibili-

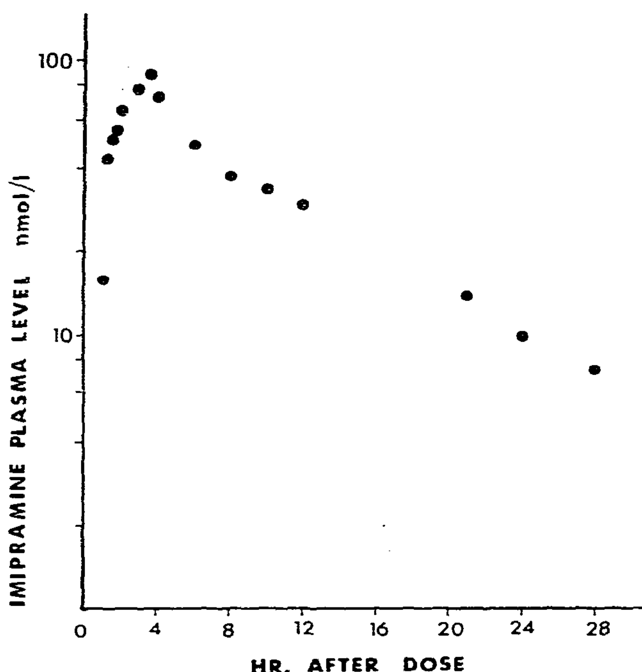

Fig. 2. Imipramine plasma level time course following administration of a single 50-mg oral dose of imipramine hydrochloride to a healthy volunteer.

ty. The sensitivity is sufficient to measure the very small levels of drug present in plasma more than 24 h after a single oral dose of imipramine. The convenience and speed of the procedure makes it suitable for the routine monitoring of steady-state plasma levels during chronic therapy.

REFERENCES

1 J. Dubois, W. Kung, W. Theobald and B. Wirz, Clin. Chem., 22 (1976) 892.
2 G. Belvedere, L. Burti, A. Frigerio and C. Pantarotto, J. Chromatogr., 111 (1975) 313.
3 A. Frigerio, G. Belvedere, F. De Nadai, R. Fanelli, C. Pantarotto, E. Riva and P.L. Morselli, J. Chromatogr., 74 (1972) 201.
4 M. Bertrand, C. Dupuis, M. Gagnon and R. Dugal, Clin. Biochem., 11 (1978) 117.
5 T.B. Cooper, D. Allen and G.M. Simpson, Psychopharmacol. Comm., 1 (1975) 445.
6 S.F. Reite, Medd. Nor. Farm. Selsk., 37 (1975) 76.
7 L.A. Gifford, P. Turner and C.M.B. Pare, J. Chromatogr., 105 (1975) 107.
8 G. Nyberg and E. Mårtensson, J. Chromatogr., 143 (1977) 491.
9 F.L. Vandemark, R.F. Adams and G.J. Schmidt, Clin. Chem., 24 (1978) 87.
10 H.J. Lohmann, H.F. Proelss and D.G. Miles, Clin. Chem., 24 (1978) 1006.