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Note

Assay of disopyramide in plasma by high-pressure liquid chromatography

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Disopyramide [4-diisopropyl-amino-2-phenyl-2-(2-pyridyl)-butyramide] is an antiarrythmic drug which is used in the treatment of both atrial and ventricular arrhythmias in man and has shown particular promise in suppression of ventricular arrhythmias following myocardial infarction[1]. Its half-life in normal volunteers ranges between 3.4 and 10.8 h[2] and prolongation of its half-life has been reported in patients with severe renal failure[3]. In addition, studies by Ward and Kinghorn[4] indicate that the volume of distribution of disopyramide may be decreased following myocardial infarction. Such variation in half-life and volume of distribution makes measurement of plasma levels of disopyramide essential to ensure that the dosage regimen is appropriate to maintain plasma levels within the therapeutic range (2–4 μg/ml)[5]. Monitoring plasma levels may also be helpful in decreasing the urinary retention and other anticholinergic side effects which commonly occur at higher dose levels of the drug.

Specific measurements of disopyramide in plasma have usually been made by gas chromatography (GC)[6–9]. These GC methods have mostly employed complex and time consuming sample preparation procedures followed by chromatography on SE-30 or OV-17. In this laboratory, a single extraction/evaporation sample preparation step, followed by GC on OV-17 has been used, but some limitations have been encountered in this assay. Firstly, the slope and intercept of the plot of peak area disopyramide/peak area internal standard *versus* plasma level of disopyramide has been found to vary from day to day, thus making it necessary to have a complete standard curve for each batch of samples. Secondly, the N-acetyl metabolite of procainamide, another antiarrythmic drug, sometimes used in combination with disopyramide, interferes in the assay for disopyramide since it has a retention time similar

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to that of *p*-chlorodisopyramide which is used as an internal standard. In addition, the commonly used minor tranquillizer diazepam also interferes in the assay as it has an almost identical retention time to that of disopyramide. Thirdly, cholesterol is also extracted by the assay procedure and chromatographs with a retention time of around 10 min thus making an effective total analysis time of some 12 min for each disopyramide sample.

More recently[10], a high-pressure liquid chromatographic (HPLC) method for analysis of both disopyramide and its major (mono-dealkylated) metabolite in plasma has been described. The method used ion-pair chromatography on an octadecylsilane reversed-phase column and although it has good sensitivity it still requires a double extraction step in sample preparation and has a minimum analysis time of around 8 min per sample.

The present paper describes an assay for disopyramide in plasma using a single extraction/evaporation sample preparation step followed by quantification of the drug by HPLC on a reversed-phase octadecyltrichlorosilane column and compares it with a commonly used GC procedure. The new method overcomes problems encountered with the GC procedure and is simpler and quicker than the only other published HPLC procedure.

METHODS

Extraction of disopyramide from plasma

Plasma (1 ml) to which 50 μl of a 0.2-μg solution of *p*-chlorodisopyramide in ethanol had been added (internal standard) was diluted with 1 ml of water, was made alkaline by the addition of 0.2 ml 5*M* NaOH and extracted with 5 ml dichloromethane by gentle shaking for 10 min. After centrifugation at 1000 *g* for 5 min, 3 ml of the organic phase was removed and evaporated to dryness under a stream of dry nitrogen. For GC analysis the residue was redissolved in 50 μl $CHCl_3$ and 2-μl aliquots were injected directly onto the column. For HPLC analysis the residue was redissolved in 200 μl of 35% acetonitrile in 0.05 *M* potassium phosphate buffer (pH 3) and 10-μl aliquots were injected.

Gas chromatography

All analyses were performed in duplicate using a gas chromatograph with flame ionization detector (Hewlett-Packard Model 5710A) coupled to a digital integrator (Hewlett-Packard Model 3380A). A 1 m × 4 mm I.D. glass column was uniformly packed with 3% OV-17 on Chromosorb 750 (80–100 mesh). The operating conditions were: oven, 265°; injector 250°; detector, 300° and a nitrogen flow-rate of 60 ml/min. Under these conditions disopyramide and *p*-chlorodisopyramide have retention times of around 3.7 min and 5.9 min respectively. Peak area ratios (disopyramide/*p*-chlorodisopyramide) were used to quantify disopyramide concentration in unknown plasma samples.

High-pressure liquid chromatography

All analyses were performed in duplicate using a Waters Assoc. high-pressure liquid chromatograph (Model M6000A) equipped with a variable wavelength UV detector set at 258 nm. A 30 cm × 4 mm I.D. stainless-steel column packed with 10-

μm diameter silica beads coated with octadecyltrichlorosilane was used (μBondapak C_{18}, Waters Assoc.). The operating conditions were: solvent 35% acetonitrile in 0.05 *M* potassium phosphate buffer (pH 3), flow-rate 2 ml/min, pressure 2000 p.s.i., detector sensitivity 0.02 a.u.f.s. and chart speed 0.5 cm/min. Peak height ratios (disopyramide/*p*-chlorodisopyramide) were used to quantify disopyramide concentration in unknown plasma samples.

Materials

Disopyramide [4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butyramide] and the internal standard [4-diisopropylamino-2-*p*-chlorophenyl-2-(2-pyridyl) butyramide] were kindly supplied by Roussel Lab. (Sydney, Australia). Dichloromethane was purified by shaking with a small volume (10%) of conc. H_2SO_4 for 2 h, washing several times with water, drying with anhydrous Na_2SO_4 followed by redistillation. All other reagents were of analytical grade. Glassware was soaked in chromic acid solution for 12 h prior to the routine laboratory washing procedure.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of the analysis of disopyramide by HPLC. Under the conditions used, disopyramide and *p*-chlorodisopyramide gave symmetrical peaks with retention times of 2.8 and 4.2 min respectively. There was

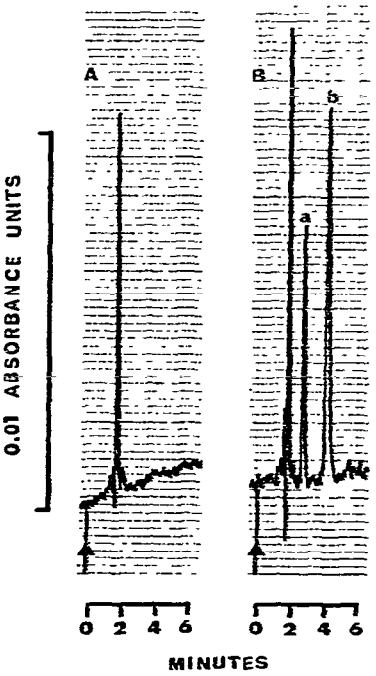

Fig. 1. HPLC analysis of disopyramide. (A) Chromatogram of an extract of a blank plasma sample. (B) Chromatogram of an extract of plasma from a patient; peak a is disopyramide (4.1 μg/ml plasma) and peak b is *p*-chlorodisopyramide (internal standard). The arrows indicate the time of sample injection and the vertical bar indicates 0.01 absorbance units.

no interference from endogenous cholesterol or from the likely interfering drugs, lignocaine, propranolol, procainamide, N-acetylprocainamide, quinidine and diazepam. Moreover, the HPLC assay can be performed in approximately one half the time required for assay by GC or by the only other published HPLC method[10]. Thus, the new method enables analysis of twice as many samples in a given time.

Both the GC and HPLC assays were found to give a linear response over the concentration range 1–10 μg/ml plasma, and a good correlation ($r = 0.98$) between plasma concentrations measured by the two methods was observed (Fig. 2).

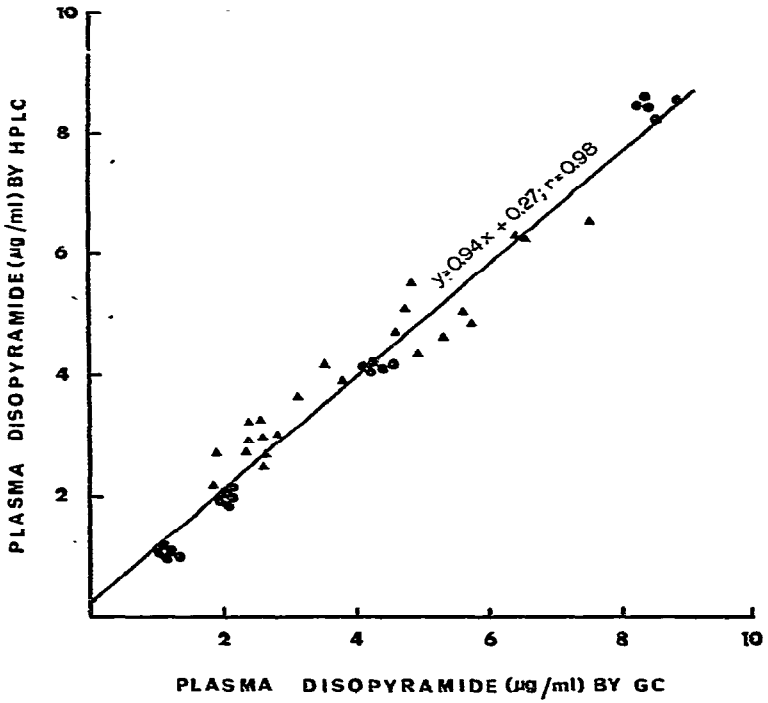

Fig. 2. Correlation between plasma disopyramide concentrations determined by HPLC (ordinate: μg/ml) and GC (abscissa: μg/ml). Key: ●, blank plasma spiked with disopyramide; ▲, plasma samples from patients taking disopyramide.

Variation between duplicate determinations for six samples selected at random was 8.2% for GC compared to 3% for the same samples by HPLC. At a signal-to-noise ratio of 5:1 the minimum detectable amount of disopyramide was 0.01 μg by GC and 0.02 μg by HPLC. In this respect, the new HPLC method is similar to that reported by Meffin *et al.*[10], which has a detection limit of 0.025 μg. The coefficients of variation (standard deviation as percent of mean) for the HPLC and GC assays are shown in Table I. At all concentrations tested, the coefficient of variation was smaller for the HPLC procedure. This reflects the greater reproduceability of the HPLC assay and indicates that HPLC will be the method of choice for the measurement of plasma disopyramide in pharmacokinetic studies.

The most serious limitation of the GC assay for disopyramide is the day-to-day variability encountered in the slope and intercept (x-axis) of the plot of peak area or height disopyramide/peak area or height internal standard (y-axis) *versus* plasma disopyramide concentration (x-axis). The coefficient of variation for the slop

TABLE I

COEFFICIENT OF VARIATION* FOR DISOPYRAMIDE ASSAY BY GC AND HPLC

Assay procedure	*Coefficient of variation (%) at*			
	1.05 μg/ml	*2.1 μg/ml*	*4.2 μg/ml*	*8.4 μg/ml*
GC	9.4	5.0	4.1	2.8
HPLC	5.3	4.3	1.4	2.0

* Standard deviation expressed as percent of the mean; four separate spiked plasma samples were assayed at each level.

of this plot for the HPLC assay was only 3% compared to 6% for the GC procedure ($n = 4$). The HPLC assay has the additional advantage that the plot of peak height ratio *versus* plasma disopyramide concentration passes through the origin. Thus, with the HPLC procedure it should not be necessary to have a complete standard curve for each batch of samples.

The HPLC method described in this paper does not enable analysis of the monodealkylated metabolite of disopyramide. However, this does not appear to be a significant limitation since in animal experiments the metabolite has only a quarter to a fifth of the antiarrhythmic potency of the parent drug[11] and has been detected (plasma concentration <0.4 μg/ml) in only 15% of patients given a single therapeutic dose (3 mg/kg) of disopyramide[6].

In summary, the HPLC method for estimation of plasma disopyramide described in this paper offers significant advantages in terms of rapidity, simplicity, reproducibility and non-interference from other drugs, over the most widely used GC method. It has a similar sensitivity to the recently published ion-pair HPLC method of Meffin *et al.*[10], but employs a simpler sample preparation procedure and is more rapidly performed.

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REFERENCES

1 G. Jennings, M. B. S. Jones, E. M. M. Besterman, D. G. Model, P. P. Turner and P. H. Kidner, *Lancet*, i (1976) 51.
2 R. E. Ranney, R. R. Dean, A. Karim and F. M. Radzialowski, *Arch. Int. Pharmacodyn. Ther.*, 191 (1971) 162.
3 B. Whiting, in *Proceedings Disopyramide Seminar, St. John's College, Cambridge, March 24–25, 1977*, Roussel, Wembley Park, 1977, p. 11.
4 J. W. Ward and G. R. Kinghorn, *J. Int. Med. Res.*, 4 (1976) Suppl (1) 49.
5 P. Danilo and M. R. Rosen, *Amer. Heart. J.*, 92 (1976) 532.
6 T. C. Hutsell and S. J. Stachelski, *J. Chromatogr.*, 106 (1975) 151.
7 A. M. J. A. Duchateau, F. W. M. H. Merkus and F. Schobben, *J. Chromatogr.*, 109 (1975) 432.
8 J. W. Daniel and G. Subramanian, *J. Int. Med. Res.*, 4 (1976) Suppl (1) 2.
9 A. M. Hayler, in *Proceedings Disopyramide Seminar, St. John's College, Cambridge, March 24–25, 1977*, Roussel, Wembley Park, 1977, p. 7.
10 P. J. Meffin, S. R. Harapat and D. C. Harrison, *J. Chromatogr.*, 132 (1977) 503.
11 R. Marshall, *J. Int. Med. Res.*, 4, *Suppl.* 1 (1976) 13.