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Note

Simultaneous determination of disopyramide and its mono-N-dealkylated metabolite in plasma by gas—liquid chromatography

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Disopyramide is a new anti-arrhythmic agent with a broad spectrum of efficiency. It has been comparatively well tolerated [1]. Studies correlating the plasma levels of disopyramide with clinical effects are few, and different therapeutic ranges have been reported: 1.5—6 μg/ml [2], 3—5 (6) μg/ml [1], 2—4 μg/ml [3], and even 3—8 μg/ml [4].

The major metabolic pathway of disopyramide in man is N-dealkylation; the resulting mono-N-dealkylated disopyramide (MND) represents 15—25% of the dose given [5]. At present there is no information in the literature about the plasma levels of the metabolite during long-term therapy with disopyramide. In animal studies the metabolite has been shown to be active and possibly to have a positive inotropic effect [6]. The early fluorimetric assay [7] did not differentiate between the parent drug and the metabolite. Thereafter, several gas—liquid chromatographic methods have been described [2, 8—11]. Only one of these, the method of Hutsell and Stachelski [8], includes an assay for N-dealkylated disopyramide. The latter usually breaks down and is eluted as three poorly separated peaks under gas chromatographic conditions suitable for disopyramide. This can be avoided by acetylation [8]. A high-performance liquid chromatographic assay of disopyramide [12, 13] measures MND without derivatization, but the equipment is not yet available everywhere.

The method described in this paper was developed for the routine laboratory use for the simultaneous analysis of disopyramide and MND from the same sample with a single injection.

EXPERIMENTAL

Materials

Disopyramide [4-diisopropylamine-2-phenyl-2-(2-pyridyl)butyramide] phosphate was supplied by Leiras, Turku, Finland, and was used as an aqueous solution of 0.1 mg/ml in 0.1 *M* hydrochloric acid. Mono-N-dealkyldisopyramide [MND, 4-isopropylamino-2-phenyl-2-(2-pyridyl)butyramide] and the internal standard, *p*-chlorodisopyramide [4-diisopropylamino-2-*p*-chlorophenyl-2-(2-pyridyl)butyramide] were gifts from Roussel Laboratories, Wembley, Great Britain. Both of these were used as 0.05 mg/ml solutions in 0.1 *M* hydrochloric acid. Diethyl ether, chloroform, sodium hydroxide and sulphuric acid were of analytical reagent grade. Glass microfibre paper GF/A was purchased from Whatman, Maidstone, Great Britain, cut to 7 × 7 cm pieces, turned into rolls and heated at 570° for 20 min, to remove impurities, before use.

Gas—liquid chromatography

A Varian Model 2100 gas chromatograph equipped with an alkaline flame ionization detector and linked to W+W 1200 recorder was used. Integration of peak areas was performed using a Spectra-Physics integrator. The column, detector and injector temperatures were 250, 285 and 275°, respectively. The nitrogen (carrier gas) flow-rate was 28 ml/min and those of air and hydrogen approximately 300 and 50 ml/min, respectively. A glass column 3 ft. long was silanized by dichloromethylsilane overnight, rinsed in toluene and ethanol and dried. The column was packed with 3% OV-17, 100—120 mesh, on Chromosorb W.

Extraction procedures

Sample, or standard (1 ml of EDTA plasma), with 100 μl (5 μg) of the internal standard solution, was pipetted into 20-ml glass tubes. The mixture was made alkaline by the addition of 100 μl of 2 *M* sodium hydroxide. A glass microfibre paper roll, cut in two halves, was dropped into the tube containing the sample. Within 1 min the plasma was totally absorbed into the paper; thereby, clotting of the plasma was completely avoided*. Diethyl ether (3 ml) was added and the tube contents were mixed. After pouring the ether into another tube, the extraction was repeated. The combined ether phases were acidified (500 μl of 0.05 *M* sulphuric acid) in tapered, stoppered tubes. After mixing, the phases were separated by centrifugation. The organic layer was removed very carefully. The remaining aqueous phase was again made alkaline (300 μl of 2 *M* NaOH), and 3 ml of chloroform were added. The tube contents were mixed, and the layers separated. The aqueous layer was discarded, and the chloroform layer was poured into a stoppered tube containing 25 μl of acetic anhydride, in order to make an acetate derivative of MND. After evaporation to dryness under a stream of nitrogen, the residue was redissolved in 25 μl of ethanol, and 2-μl aliquots were injected onto the column.

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*Use of glass microfibre paper in the extraction was a suggestion by Torsti Yrjänä, Ph.D., from the Central Laboratory of Turku University Hospital, which is gratefully acknowledged.

All pipetting was performed manually, and all mixing was done in a vortex-type mixer (30 sec.).

Calibration

Calibration graphs were obtained by adding known amounts of disopyramide and MND to human plasma samples. They were straight lines in the range of 0.5—12.5 μg/ml for disopyramide and 0.5—7.5 μg/ml for MND. The regression lines of the calibration graphs were $y = 0.257x - 0.005$ and $y = 0.179x + 0.023$, for disopyramide and MND, respectively. The correlation coefficient was 0.999 in both cases.

RESULTS AND DISCUSSION

Disopyramide, *p*-chlorodisopyramide, and the acetyl derivative of MND gave sharp well-separated peaks with retention times of 1.5, 2.4, and 4 min, respectively (Fig. 1). Both disopyramide and acetyl-MND could be determined from a single chromatographic injection. The short retention times allow analysis of several samples in a short period of time, making this method suitable for routine laboratory analysis. The back-extraction of Hayler and Flanagan [11] removed an endogenous interfering peak which is probably cholesterol — authentic cholesterol appears as a coincident peak that has a retention time very close or similar to that of acetyl-MND (Fig. 1). Although a nitrogen-sensitive detector was used, this peak might constitute a problem. Therefore, the ether layer must be aspirated very carefully to remove any residual ether which contains the interference peak.

The extraction efficiency of the method for disopyramide was 71.6 ± 7.8% (S.D.) (*n* 22), at disopyramide levels of 1.5, 5.0 and 10.0 μg/ml. The efficiency is somewhat low compared to other methods [8, 9]. This is obviously due to the use of the glass-fibre paper: about 1 ml of ether remains in the paper after the extraction. The glass-fibre paper, however, has many advantages: all emulsification of the plasma is avoided; clotting of plasma that is occasionally seen upon addition of concentrated sodium hydroxide does not occur; no centrifugation after extraction is needed, and after extraction, the organic phase can simply be poured into another tube.

Both within-batch and between-batch precisions of the assay procedure were determined (Tables I and II); both were clearly better for disopyramide than MND. This difference most probably is not due to variability in acetylation, because in the very recent high-performance liquid chromatographic determination of disopyramide and MND, not using acetylation, the same difference was seen [13]. With disopyramide the precision was reasonable at drug plasma levels with clinical significance, whereas at low plasma levels a larger coefficient of variation was seen. When duplicate samples were assayed, at therapeutic plasma levels the coefficient of variation for disopyramide was 4—6%, reasonably good for a gas—liquid chromatographic assay procedure. On the other hand, the precision of the assay for MND was rather poor at the levels ordinarily encountered, that is at levels below 1 μg/ml. At these plasma levels, however, MND most probably has no clinical significance: in animal studies [6] MND was about four times less active than disopyramide. On the

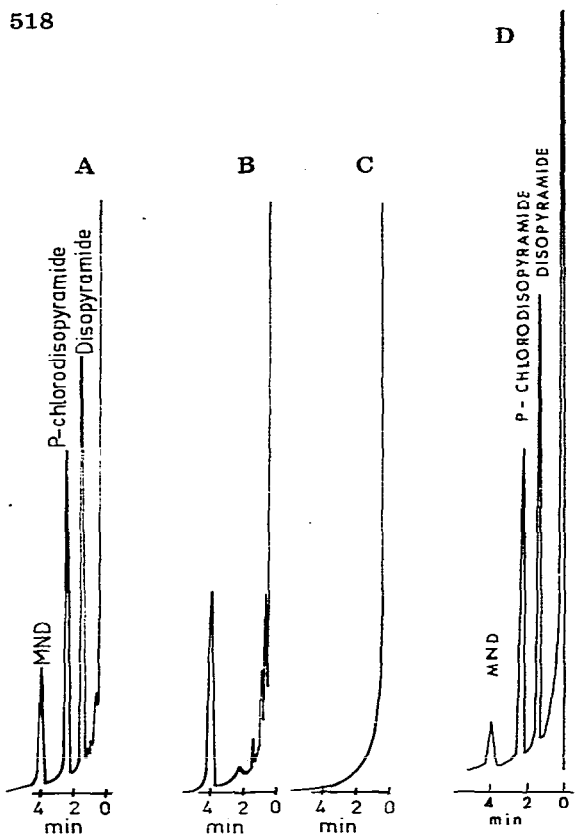

Fig. 1. Gas chromatographic tracings of plasma extracted as described in the methods. (A) Plasma spiked before the extraction process with disopyramide (3 μg/ml), mono-N-dealkyldisopyramide (3 μg/ml), and the internal standard (*p*-chlorodisopyramide). (B) Blank plasma extracted and analyzed without the back-extraction to sulphuric acid. (C) The same blank plasma extracted and analyzed as described in the methods. (D) Plasma sample of a patient undergoing disopyramide therapy, 600 mg daily (disopyramide 3.6 μg/ml, MND 1.4 μg/ml).

TABLE I

WITHIN-BATCH PRECISION OF THE ASSAY OF DISOPYRAMIDE AND MONO-N-DEALKYLDISOPYRAMIDE (MND)

Values were calculated from duplicates of all patient samples analyzed during three months. The analyses were performed by two persons.

Drug concentration (μg/ml)	Coefficient of variation (%)	No. of assays
Disopyramide		
<2.5	5.0	16
2.5—4.0	5.7	47
>4.0	4.1	27
MND		
All determinations	13.2	80
>1.5	10.9	17

TABLE II

BETWEEN-BATCH PRECISION OF THE ASSAY OF DISOPYRAMIDE AND MONO-N-DEALKYLDISOPYRAMIDE (MND)

The figures represent mean ± S.D. of ten successive analyses, during three months, performed by two persons, of plasma samples spiked with the indicated amounts of disopyramide and MND, and of a sample from a patient given disopyramide. The analyses were carried out in duplicate.

Expected value (μg/ml)	Analytical result (μg/ml ± S.D.)	Coefficient of variation (%)
Disopyramide		
1.0	1.10 ± 0.097	8.83
4.0	4.01 ± 0.220	5.53
8.0	8.60 ± 0.319	3.71
Unknown	3.38 ± 0.128	3.78
MND		
1.0	1.02 ± 0.177	17.3
4.0	3.89 ± 0.488	12.8
8.0	8.09 ± 0.711	8.8
Unknown	0.52 ± 0.149	28.7

other hand, I have seen patients with exceptionally high MND levels, with simultaneous, relatively low levels of the parent drug [14]. It is in these cases, when it becomes important to know the plasma level of MND, and the present assay, although admittedly not as good for MND as for disopyramide, is reasonably good at MND levels exceeding 2 μg/ml (Tables I and II). The precision of a method using high-performance liquid chromatography was similar to that of the present study [13].

Hutsell and Stachelski [8] were the first to describe a gas chromatographic assay for both disopyramide and MND. Their extraction procedure was more tedious, and included a florisil separation. They also needed larger sample sizes and big volumes of organic solvents.

N-Acetylprocainamide and diazepam have been reported to interfere in this type of assay [15]. Procainamide and disopyramide are very seldom used in combination. N-Acetylprocainamide has, in this assay, a retention time similar to the internal standard, *p*-chlorodisopyramide. Approximate levels of disopyramide can be determined, however, by assaying samples with and without the internal standard. Diazepam has the same retention time as disopyramide, but even an unusually high plasma level of 500 ng/ml of diazepam appeared as a peak corresponding to an apparent level of disopyramide below 0.5 μg/ml; therefore, possible error due to diazepam levels usually encountered is no serious handicap in the present assay procedure.

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