Liquid Chromatographic Analysis of Disopyramide and Its Mono-N-dealkylated Metabolite

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We describe a rapid, sensitive, and specific "high performance" liquid chromatographic analysis for disopyramide and its mono-N-dealkylated metabolite in serum, urine, and saliva. We used a μ-Bondapak CN column and an acetate buffer mobile phase containing methanol. Retention times for the two compounds and the internal standard, p-chlorodisopyramide, were 3.4, 4.1, and 6.3 min, respectively. The lower limits of sensitivity for drug and metabolite were 50 and 80 μg/L, respectively, with maximum coefficients of variation of 4.8 and 12%, respectively. Currently used antiarrhythmic drugs did not interfere with the analysis of disopyramide, and the pharmacokinetics of the drug, obtained from studies of one subject, agree well with reported values.

Disopyramide (DP),1 α-[2-(diisopropylamino)ethyl]-α-phenyl-2-pyridineacetamide, is a new drug currently being marketed for long-term suppression of ventricular arrhythmias.2 It reportedly is less toxic than quinidine.2 With a longer biological half-life than procainamide (1), DP need not be given as often as procainamide.

DP is eliminated from the body by both renal and nonrenal pathways (2). N-Dealkylation of DP to its mono-N-dealkylated metabolite (MND) is a major metabolic pathway; 50–70% of a dose is cleared renally (3). Because these clearance pathways may be reduced in cardiac patients, and because the drug has a low therapeutic index, clinical monitoring of DP is necessary to maintain serum concentrations in the therapeutic range of 2–4 mg/L (3).

Previously reported techniques for measuring DP concentrations in biological fluids include fluorescence and gas chromatography. Because of its high specificity, sensitivity, and speed of assay, liquid chromatography is often the assay method of choice in a clinical laboratory. This report describes a "high performance" liquid chromatographic method (HPLC) for analyzing concentrations of DP and MND in biological fluids, and its application in a clinical pharmacokinetics laboratory.

Materials and Methods

Reagents and Apparatus

The phosphate salt of DP, MND, and the p-chlorodisopyramide used as an internal standard were generously supplied by Dr. D. J. McDermott, G. D. Searle & Co. (Chicago, IL 60680). All reagents used in the analysis of DP and MND were of analytical grade.

We used a DuPont (Wilmington, DE 19898) HPLC Model 841 with a DuPont Model 842 UV-detector–photometer for measurement at 254 nm. The column used was the Waters Associates (Milford, MA 01757) μ-Bondapak CN (0.5 cm × 0.25 m). The mobile phase consisted of an aqueous solution (pH 3.5) containing 4 g of sodium acetate, 40 g of acetic acid, and 150 mL of methanol per liter. Column operating conditions were an eluent flow rate of 1.9 mL/min at 1500 psi.

Extraction

To 1.0 mL of serum, plasma, urine (diluted 25-fold with water), or saline in 12 × 75 mm polypropylene tubes, add 50 μL of sodium hydroxide (1 mol/L), 50 μL of internal standard (40 mg/mL), and 1.2 mL of chloroform. Vigorously shake the mixture on an Eberbach metabolic shaker for 10 min and centrifuge for 2 min at 7500 rpm. Aspirate and discard the aqueous phase and evaporate the chloroform phase under a mild stream of nitrogen. Dissolve the residue in 100 μL of mobile phase and inject 20 μL onto the column.

Prepare standard solutions of DP and MND in serum at concentrations ranging from 0.25 to 20 mg/L. To determine the efficiency of extraction of DP and MND, compare the detector response for known concentrations of both compounds extracted from serum to that obtained from similar concentrations dissolved in mobile phase.

Reproducibility. We assessed within-day and day-to-day variation of our method by adding approximately 0.15 and 0.05 mg of DP and MND to 50 mL of serum and analyzing 10 times on one day and then once daily for 10 consecutive days. For each daily analysis we constructed standard curves of DP and MND from concentrations of each compound of 0.5, 1, 2, and 4 μg/mL of serum and sodium phosphate buffer (pH 7.4).

Interferences. We analyzed one sample of serum containing procainamide, its N-acetylated metabolite (NAPA), quini-
dine, and lidocaine at a concentration of 5 mg/L each, and DP and MND at concentrations of 2 and 1 mg/L, respectively, to assess whether these antiarrhythmic drugs interfered with the assay of DP and MND. We also analyzed sera from cardiac patients who were taking digoxin, furosemide, diazepam, and other drugs commonly used in the treatment of cardiac disease.

Applicability. One volunteer subject took 150 mg of DP phosphate (Norpace, G. D. Searle & Co.). Samples of serum and saliva were obtained at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after ingestion. Urine was collected before and 60 h after ingestion.

Results

Figure 1 shows two typical chromatograms for serum. The left chromatogram was obtained from the analysis of a pooled serum sample from patients not receiving DP; no interfering peaks were detected. The right chromatogram is for serum from a patient who was taking 150 mg of DP phosphate every 6 h. The detector response was to 1.24 and 3.39 μg/mL of MND and DP, respectively. The retention times for MND, DP, and the internal standard were 3.4, 4.1, and 6.3 min, respectively.

Extraction Efficiency. Analytical recoveries (and SD) for MND and DP extracted from serum were 108 (0.01) and 105 (2.6)%, respectively. The detector response was linearly related to concentrations of MND and DP over a range of 0.25–20 μg/mL.

Reproducibility. Table 1 summarizes the results of our assessment of the reproducibility of the method. The mean concentration of DP analyzed on any given day was within 5% of the mean concentration for 10 consecutive days. Similar analysis of MND concentrations indicated that they did not differ by more than 15%.

The mean (and SD) of the ratio of peak heights of MND and DP to internal standard, as obtained from standards in plasma analyzed on 11 days, is depicted in Figure 2. The slopes of the standard curves for MND and DP were 0.515 and 0.443, respectively; their respective intercepts were 0.002 and 0.018, not significantly different from zero. The slope and intercept for DP concentrations in phosphate buffer were identical to those for plasma, but were about 20% higher for MND. Using the criterion of the ratio of peak-height response to a baseline of 5, we determined the lower limits of sensitivity for MND and DP to be 80 and 50 μg/L, respectively.

Interferences. Retention times for procarainamide, NAPA, and quinidine were 2.2, 2.8, and 4.8 min, respectively, and did not interfere with the analysis of DP, MND, or the internal standard. In contrast, the retention time for lidocaine was identical to that for MND. Extracts of sera of cardiac patients receiving digoxin, furosemide, diazepam, and other commonly used drugs were found not to interfere with the analysis of DP and MND.

Applicability. Figure 3 shows results of the analyses of serum, saliva, and urine from a subject given a single 150-mg dose of DP phosphate. The decline of serum, salivary, and urinary drug concentrations were log linear, and their respective half-lives were 6.2, 3.8, and 6.9 h. During a 60-h collection period, 43% of the dose was recovered in the urine as the parent compound, and 13% was recovered in the urine as MND.

Discussion

Measurement of drug concentrations in biological fluids of patients is often necessary for drugs that have a low therapeutic index. DP has been reported2 to be effective in suppressing ventricular dysrhythmic activity at serum concentrations of 2–4 mg/L (3). The excellent reproducibility, the absence of interferences from commonly used cardiac drugs, and the rapidity of chromatography make our system suitable for monitoring serum concentrations of DP in patients. Standards of DP in buffer or serum may be constructed daily, and the results from patients' samples are available in less than 30 min.

Other methods reported for determining DP concentrations include fluorescence (4), which also measures MND; gas chromatography (5, 6); liquid chromatography (7); and HPLC (8). The gas-chromatography methods have non-zero intercepts, one includes multiple extractions and separate chromatography for the analysis of DP and MND (5), and one does not measure MND (6). The liquid chromatographic method requires special treatment to avoid nonlinearity at low con-

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**Table 1. Reproducibility of the Analysis of Disopyramide and its Mono-N-Dealkylated Metabolite (MND)**

<table>
<thead>
<tr>
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<th>Disopyramide</th>
<th>MND</th>
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<tbody>
<tr>
<td>Within-day</td>
<td>2.85</td>
<td>0.956</td>
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<tr>
<td>Between days</td>
<td>2.95</td>
<td>1.12</td>
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<tr>
<td>SD, mg/L</td>
<td>0.034</td>
<td>0.056</td>
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<tr>
<td>CV, %</td>
<td>2.60</td>
<td>0.138</td>
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centrations of DP, and no quantitative data on MND concentrations were given (7). The HPLC method of Meffin et al. (8) shares with ours the advantages of greater sensitivity, specificity, and rapidity over most of the other methods. Our method, however, is capable of analyzing procainamide, NAPA (9), and most likely quinidine as well, with only minor modifications in the mobile phase. This is particularly advantageous in the setting of a clinical pharmacokinetics laboratory, which commits a large part of its efforts to monitoring serum concentrations of antiarrhythmic drugs in cardiac patients.

Our HPLC method of analyzing DP and MND in biological fluids is sufficiently sensitive and specific that pharmacokinetic studies of single or multiple doses of DP may be easily performed. Utilizing gas-chromatography, Hinderling and Garrett (2) and Karim (1) reported that the biological half-life of DP was between 4.5 and 6 h. Cunningham et al. (3) collected 47–67% of a dose of DP in urine during a 72-h collection period. These data are in reasonable agreement with the pharmacokinetics of DP obtained in our one subject, and suggest that our method is accurate for pharmacokinetic studies as well as clinical monitoring.

To our knowledge there are no reports of investigation of salivary concentrations of DP in the literature. Analysis of salivary concentrations of drugs has received more interest recently partly because of its less invasive nature. In at least one situation, the salivary concentration of a drug was better correlated with its pharmacodynamic activity than was plasma concentration (10). Furthermore, drug concentration in saliva reportedly reflects its unbound, pharmacodynamically active fraction in serum, which is important for drugs that are highly or variably protein bound. Hinderling and Garrett (2) and Cunningham et al. (3) have reported that DP is variably bound between 5 and 70% at serum concentrations encountered clinically. Cunningham et al. (3) further reported that the biological half-life of the free concentration of DP in serum was shorter than the half-life of total (free and bound) concentration of DP. The half-life of DP in saliva of our subject was shorter than its serum half-life and is consistent with concentration-dependent changes in protein binding. This may be of clinical importance because increasing the dose of DP in a patient with subtherapeutic serum concentrations may result in a disproportionate increase in concentrations of unbound, active DP, which may result in toxicity. Further studies of the protein binding of DP, relating its free fraction in serum to saliva, and of its pharmacodynamic action are clearly indicated.

Supported in part by Grant No. GM-20852 from the National Institute of General Medical Sciences, NIH.

References


