Gas-Chromatographic Determination of Disopyramide in Serum, with Use of a Nitrogen-Selective Detector

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In this procedure for disopyramide in serum, the drug is extracted into n-heptane/isobutanol (96/4 by vol), then back-extracted into 1 mol/L H2SO4. The acidic solution is made basic with sodium hydroxide, extracted with diethyl ether, and the extract evaporated. The residue is redissolved in ethanol and analyzed by gas-chromatography, with use of a nitrogen-selective detector. p-Chlorodisopyramide is used as internal standard. Concentration and instrument response for serum extracts are linearly related from 1 to 5 mg/L, the slope being 0.61, the y-intercept -0.10, the standard error of estimate 0.01, and the correlation coefficient 0.99. Within-run precision was 6 and 4% for 3 and 5 mg/L concentrations, respectively, with a between-run precision of 7% at the 3 mg/L concentration. Diazepam interferes, but procainamide, chlordiazepoxide, quinidine, lidocaine, propranolol, sulfanilamide, and many other basic drugs do not.

Disopyramide phosphate ("Norpace," Searle), a newly marketed anti-arrhythmic drug, is currently available for oral administration. Although the structure of disopyramide is not chemically related to that of procainamide or quinidine, it similarly affects cardiac action potential.

The drug has a biological half-life of 5 to 7 h, most (about 80%) being excreted in the urine unchanged (1, 2). The predominant metabolite of disopyramide, the mono-n-dealkylated metabolite, possesses about half the anti-arrhythmic properties of the parent compound and is present at <0.1 its concentration in serum (1, 3).

For maximum therapeutic efficacy, it is thought that plasma concentrations should be 2 to 4 mg of disopyramide per liter; these are achieved with an oral dose of 100 to 200 mg four times a day (1, 4, 5). Toxic symptoms and adverse reactions owing to the drug's anticholinergic properties are thought to occur when concentrations exceed 5.5 mg/L (1, 4).

Several chromatographic procedures for determining disopyramide and its monoalkyl metabolite have been reported (3, 6-8).

This report describes a sensitive, rapid, and precise procedure for the routine clinical laboratory determination of disopyramide in serum. Gas-chromatographic conditions, extracting solvents, and drug interferences were optimized and a nitrogen-selective detector was used.

Materials and Methods

We used a Model 3920 gas chromatograph equipped with a flame ionization and a nitrogen-phosphorus-selective detector (all from Perkin-Elmer Corp., Norwalk, CT 06856). The coiled glass column was 1.8 m by 2 mm (i.d.), packed with 3% OV-17 on 100/120 mesh Gas Chrom Q (Applied Science Labs., State College, PA 16801).

We also used a Model 5840A gas chromatograph with dual nitrogen-phosphorus detectors and a flame ionization detector for some of the analyses. Coiled glass columns, 1.2 m by 2 mm (i.d.), packed with 2% SP-2250 and 2% OV-101 on Chromosorb W-HP 100/120 mesh, were used (Hewlett Packard, Avondale, PA 19311).

Gas chromatographic–mass spectrometric analyses were performed on a Model 5985A quadrupole system (Hewlett Packard, Palo Alto, CA 94304), used in the electron impact mode. The system consisted of a Hewlett Packard 5840A gas chromatograph interfaced to the mass spectrometer. The glass column was 1.2 m by 2 mm (i.d.), packed with 2% SP-2250 Chromosorb W-HP, 100/120 mesh. The ionization energy was 112.14 × 10^-19 J (70 eV).

Reagents

All reagents used were analytical reagent (AR) grade or "spectral" grade: heptane, chloroform, isopropanol, isobutanol. Absolute ethanol was used. Anesthetic-grade ether (J. T. Baker Chemical Co., Phillipsburg, NJ 08805) was used. Sodium hydroxide, 0.5 mol/L.

Sulfuric acid, 1 mol/L.

n-Heptane/isobutanol, 96/4 by volume.

Hydrochloric acid, 0.1 mol/L.

Sodium sulfate, anhydrous.

Standards

Disopyramide phosphate, its mono-n-dealkylated metabolite, and parachlorodisopyramide were obtained from G. D. Searle and Co., Chicago, IL 60680.

p-Chlorodisopyramide (internal standard). Dissolve 10 mg of p-chlorodisopyramide in 0.1 mol/L HCl. Dilute to 100 mL with 0.1 mol/L HCl. Prepare a 10 mg/L solution from the above.

Disopyramide. Dissolve 32.2 mg of disopyramide phosphate to 25 mL with de-ionized distilled water. Prepare an intermediate solution of 0.1 g/L and then aqueous standards of 1, 3, and 5 mg/L. Reconstitute lyophilized normal human serum to volume with the aqueous 1, 3, and 5 mg/L disopyramide standards.

Disopyramide injection standard. Take 5 mL of the 1 g/L
disopyramide solution, make it basic and extract it into ether. Evaporate the ether to dryness. Dissolve the residue in 5 mL of ethanol.

p-Chlorodisopyramide injection standard. Dissolve 10 mg of p-chlorodisopyramide in ethanol and dilute to 10 mL.

Operating Conditions

We used the following chromatographic conditions. For the 3% OV-17 column: helium carrier gas with a flow rate of 40 mL/min, injector temperature 275 °C, interface temperature of 275 °C, and column temperature isothermal at 250 °C. For the 2% OV-101 column: nitrogen carrier gas at 30 mL/min, injector temperature 275 °C, detector temperature 275 °C, and column temperature isothermal at 230 °C. For the 2% SP-2250 column the conditions were identical to the OV-101 column except that the column temperature was 250 °C. The nitrogen-selective detector settings were 5.5 to 6.5 on the Perkin-Elmer 3920 gas chromatograph with air flow at 40 psi and hydrogen flow at 8 psi. On the Hewlett-Packard 5840A gas chromatograph, the nitrogen detector was set at 14 or 15 V (equivalent to a recorder offset of 80 to 90 mm) with a hydrogen flow of 3 mL/min and an air flow of 50 mL/min.

Procedure

Acid wash and rinse the glass ware with de-ionized distilled water before use. Place 2–3 mL of serum (blank, standard, control, and patient's sample) into a 50-mL centrifuge tube. Add 0.8 mL of the 100 mg/L internal standard solution, 4 mL of 0.5 mol/L NaOH, and vortex-mix. Add 25 mL of n-heptane/isobutanol. Shake with a mechanical shaker for 5 min. Centrifuge for 5 min and transfer 20 mL of the top layer, filtering it through phase separation paper or Whatman No. 1 filter paper. Add 4 mL of 1 mol/L H₂SO₄ shake for 2 min, and then centrifuge for 2 min. To 3 mL of H₂SO₄, add 2.5 mL of 4.4 mol/L NaOH, vortex-mix, and then add 10 mL of diethyl ether. Shake for 2 min, centrifuge, and transfer the ether layer. Add 1 g of anhydrous sodium sulfate, mix, and then filter the ether layer through phase separation paper. Evaporate the ether and dissolve the residue in 25 μL of absolute ethanol. Inject 1 μL for analysis. Inject pure standards of disopyramide and p-chlorodisopyramide, to confirm the retention time of the drugs before gas-chromatographing the serum extracts.

Calculate the peak-height ratio of disopyramide to the internal standard for each sample, then calculate the concentration of the unknowns by using the closest standard.

Results

Chromatograms for a serum blank, standard, and a patient's sample are given in Figure 1. Blank sera assayed by this procedure gave no significant peaks that might interfere with the analysis. Gas chromatography–mass spectrometry of serum extracts confirmed the presence of disopyramide (m/e, 195,212,194) and the internal standard p-chlorodisopyramide (m/e, 229,114,246) at their respective retention times.

Sera containing 1, 3, and 5 mg of disopyramide per liter, determined in duplicate on three separate days, gave a slope of 0.61, a y-intercept of −0.10, a standard error of estimate Sᵧₓ = 0.01, and a correlation coefficient of 0.99.

The relative percent recovery of sera extracts of 2, 4, 6, and 8 mg of disopyramide per liter was 97 ± 3 (n = 4), 105 ± 7 (n = 3), 100 ± 6 (n = 3), and 95 ± 6% (n = 3).

Within-run precision of the assay (CV) for the 3 and 5 mg/L concentrations was 6% (n = 4) and 4% (n = 3), respectively, with a 108 ± 6 and 92 ± 4% recovery. The between-run precision (CV) for a 3 mg/L control was 7% (n = 9) with a 105 ± 7% recovery. The between-run recovery at the 1, 3, and 5 mg/L concentration was 95 ± 13 (n = 6), 101 ± 9 and 103 ± 9% (n = 3).

Interference studies proved that diazepam has a retention time similar to that for disopyramide, and that it will interfere with disopyramide in this procedure. The following drugs, which all can be extracted from basic solution, did not interfere: procaïnamide, quinidine, lidocaïne, propranolol, propyoxphene, sulfanilamide,loxapine, methaqualone, amitryptiline, nortriptyline, doxepin, imipramine, desipramine, oxazepam, and flurazepam. Interference was also not observed from more acidic drugs such as glutethimide, barbiturates, mepramamate, etchloxyvynol, phenytoïn, and salicylate.

Discussion

The observed interference by diazepam in this procedure can be eliminated by extracting at pH 7.4 instead of a more basic pH and then following the procedure we described above. An ultraviolet spectrophotometric procedure, with extraction at pH 7, has been reported for disopyramide (9). The problem in using ultraviolet spectrophotometry for disopyramide is that acetaminophen and chlor Diazepoxide are co-extracted under the same conditions and would interfere. However, if this extraction procedure (9) is followed by gas-chromatographic analysis, interference from acetaminophen and chlor Diazepoxide is eliminated.

A pH >10 is preferred for the optimal extraction of disopyramide (3). Chloroform extraction at pH 14 gives a 20% increase in recovery of disopyramide as compared to extraction at pH 7 (3). Specificity as well as sensitivity was improved by extraction at a basic pH, since gas-chromatography of the pH 7 extract gave an interference peak close to that of disopyramide.

We compared the effect of using chloroform/isopropanol (20/1 by vol) and heptane/isobutanol (96/4 by vol) as extracting solvents for disopyramide. Sera blanks and standards of 1, 3, and 5 mg/L, run in duplicate, gave almost identical results with the two solvent systems. Thus there is no advantage in using chloroform instead of n-heptane as the extracting solvent. We prefer to use heptane because during extraction it forms the top layer and permits more convenient transfer of material.

Extracting disopyramide in a basic pH and then back extracting it in an acidic medium to eliminate interference is an important step in this determination. Although nitrogen-selective detectors are known to have a greater discriminating power in detecting nitrogen-containing compounds over non-nitrogen-containing compounds, a better chromatogram is obtained from a cleaner sample (7). In addition, back ex-
Table 1. Concentrations of Disopyramide Phosphate (Norpace) in Sera of Three Patients

<table>
<thead>
<tr>
<th>Dose</th>
<th>Date</th>
<th>Time, h</th>
<th>Conc., mg/L</th>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>200 mg</td>
<td>7/26</td>
<td>0800</td>
<td>5.8</td>
</tr>
<tr>
<td>every 6 h</td>
<td>7/26</td>
<td>0849</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>7/27</td>
<td>0554</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>7/27</td>
<td>0800</td>
<td>5.6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>7/19</td>
<td>1701</td>
<td>5.4</td>
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<tr>
<td>every 6 h</td>
<td>7/28</td>
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</tr>
<tr>
<td></td>
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<tr>
<td>every 6 h</td>
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<td>107</td>
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<td></td>
<td>8/18</td>
<td>1107</td>
<td>3.8</td>
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<td></td>
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<td>3.9</td>
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<tr>
<td></td>
<td>10/23</td>
<td>1330</td>
<td>5.0</td>
</tr>
<tr>
<td>Patient A.M.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg</td>
<td>10/16</td>
<td></td>
<td>3.1</td>
</tr>
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<td>every 6 h</td>
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<td>0900</td>
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*Blood sampled just before next dose or at time indicated; single analysis.

tracting in H₂SO₄ and then ether eliminates possible interferences from certain drugs such as procarbazine (8).

Gas-chromatography of the pure mono-n-dealkylated metabolite showed that the compound decomposes under the gas-chromatographic conditions of study, yielding two peaks. "High pressure" liquid chromatography of the pure metabolite showed only one peak, indicating that a direct gas-chromatographic method could not be used to determine the metabolite.

The mono-n-dealkylated metabolite is only half as active as disopyramide and it is present in serum in <0.1 the concentration of the parent drug, so knowledge of the metabolite concentration in patients with normal renal function is not critical. However, should such a determination be needed the procedure could be modified to include the metabolite by forming an acetate derivative (3). A more desirable method of analysis, however, would be reversed-phase liquid chromatography. Such a procedure has been reported for disopyramide and its metabolite (6). By dissolving the residue of the extraction procedure as described above in 0.05 mol/L H₂SO₄ and submitting it to such chromatography, an excellent separation was obtained for mono-n-alkylated disopyramide, disopyramide, and p-chlorodisopyramide. In this laboratory, for reversed-phase chromatography, we used 5 mmol of sodium octane sulfonate per liter for ion pairing and a 60/40 mixture of methanol/water as the eluting solvent at 1.5 mL/min. Retention times relative to that for p-chlorodisopyramide are 0.56 and 0.70 for mono-n-alkyl-disopyramide and disopyramide; retention time of p-chlorodisopyramide is 8.5 min.

Table 1 illustrates the usefulness of disopyramide monitoring. Patient B.B., who was started on 100 mg of Norpace, was well under control for some time; then there was a sudden decline in the serum drug concentration, even though the patient was still on the same dosage of drug. Increasing the dose to 200 mg again brought the patient under therapeutic control—an example of the unpredictability of serum concentrations of disopyramide. The reason for this change in this case is not known. However, the patient was under adequate anti-arrhythmic control after the dosage of Norpace was increased.

In therapeutic monitoring, at steady state there should be a linear relationship between the total drug present in the body and the concentration of the parent drug and all of its possible metabolites in the blood. The determination of the concentrations of parent drug is thus quite appropriate under these conditions. This relationship may not hold true in cases where steady-state cannot be assumed, as in a patient with changing drug distribution or rate of elimination or metabolism.

Several different gas-chromatographic packings can be used in determining disopyramide by our method. Columns of 2% OV-101 at 230 °C and of 2% SP-2250 at 250 °C gave good analytical results. If one does not have a nitrogen-selective detector, the analysis can be performed with use of a flame-ionization detector (8). However, the internal standard will have to be increased to about threefold its suggested concentration, and the chromatographs will not be as "clean" as those obtained with a nitrogen-selective detector.

With the present procedure, good between-day linearity was observed, with close to 100% recovery of the parent drug from a serum matrix.

References