Serum Quinidine Concentrations: Comparison of Fluorescence, Gas-Chromatographic, and Gas-Chromatographic/Mass-Spectrometric Methods

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Serum quinidine concentrations were determined in patients on chronic therapeutic doses. Although results were higher by a protein precipitate–fluorescence method as compared to a specific extraction fluorescence method, there was substantial correlation between results by the two methods (r = 0.945, P < 0.001). We established the specificity of the extraction method by a methylation gas-chromatographic method and by a gas-chromatographic/mass spectrometric method in which the base peak in the mass spectra of the methylated products of both quinidine and cinchonidine, the internal standard, was monitored. We conclude that the protein precipitate method should be discarded.

Additional Keyphrase: cardiac arrhythmias

Knowledge of serum quinidine concentrations assists the physician in the management of patients who are receiving quinidine (1–4). Until recently, quinidine concentrations have been determined by a protein precipitate–fluorescence method (5). For this method, a therapeutic range of 3–6 mg/liter has been suggested (6). Hartel and Korhonen (7) identified several polar metabolites of quinidine and dihydroquinidine by thin-layer chromatography, and modified an extraction method (8) to measure quinidine concentrations, in which benzene is used as the organic solvent. In a further modification of this method, Kessler et al. (9) studied the pharmacokinetics of quinidine in patients with renal failure and congestive heart failure, and recommended that the protein-precipitate method be abandoned in favor of the more specific extraction method.

We have determined quinidine concentrations in the sera of 27 patients who were receiving quinidine sulfate as therapy for cardiac arrhythmias. This report compares the quinidine concentrations determined by the two fluorescence methods with those by a specific gas-chromatographic method (10), the validity of which we have confirmed by gas chromatography/mass spectrometry (GC/MS).

Materials and Methods

Patients

All patients were hospitalized on a general medical service and had been receiving quinidine sulfate for at least three days. Blood for quinidine concentration was obtained by venipuncture 4–6 h after the last oral dose of quinidine sulfate. The blood was centrifuged, and the serum was either promptly assayed or frozen at −20 °C until assay. Creatinine clearances for the patients were either measured or were estimated with use of a nomogram involving the patient’s serum creatinine value, age, sex, and weight (11).

Fluorescence Assays

Fluorescence method I was the protein precipitate method described by Brodie and Udenfriend (5). Fluorescence method II was the extraction method of Hartel and Kohrnonen (7) as modified by Kessler et al. (9).

For both methods fluorescence was measured in a Farrand spectrophotofluorometer (activation wavelength, 350 nm; fluorescence wavelength, 445 nm) and compared with quinidine standards that had been carried through the entire procedure. All quinidine concentrations are given in micrograms of quinidine base per milliliter of serum.

Gas Chromatography

We used a modification of the flash methylation procedure of Midha and Charette (10). To duplicate 1-ml serum samples, 1 ml of water containing the internal standard, cinchonidine (2 µg/ml), 0.5 ml of NaOH (1 mol/liter), and 5 ml of benzene were added. The mixture was shaken for 10 min, centrifuged, and the benzene layer transferred to a conical tube and evaporated at 70 °C under air. The residue was reconstituted
in 25 μl of trimethylanilinium hydroxide (0.2 mol/liter in methanol; "Methelute"; Pierce Chemical Co., Rockford, Ill. 61105). Two microliters of the Methelute solution were injected into a Varian Aerograph, Model 2400, equipped with a hydrogen flame detector. Nitrogen was the carrier gas, with a flow rate of 30 ml/min. The column was a 180-cm glass column packed with 3% OV-17 on Gas Chrom Q, 80–100 mesh. The temperatures of the injection port, column, and detector were 320, 245, and 285 °C, respectively. Under these conditions the retention times for methylated cinchonidine and quinidine were 4.25 and 7.2 min, respectively. There were no interfering peaks in blank plasma carried through the entire procedure, but two later-eluting peaks were present in the blank plasma. The sensitivity limit for serum quinidine concentration was 50 ng/ml. Quinidine concentrations were determined by the peak-height ratio for quinidine and the internal standard. Four quinidine standards (1, 3, 5, and 7 μg/ml) were prepared in blank plasma and assayed together with the patient’s sera. Because quinidine sulfate was used to prepare the standards, it was necessary to multiply the determined quinidine concentrations by 0.82, to obtain the quinidine concentrations as micrograms of quinidine base per milliliter of serum. The coefficient of variation for 10 determinations on the same day was 4.7%.

Gas Chromatography/Mass Spectrometry

To substantiate the results determined by GC analysis, portions of selected samples were also analyzed by GC/MS. The GC/MS computer system was the Finnigan 3300, operated on line with a Finnigan 6000 data system. The electron impact mass spectra were continuously monitored throughout the complete temperature-programmed gas chromatographic analysis, 5–10 mass spectra being recorded for each effluent component. The mass chromatogram for m/e 136, the base peak in the mass spectra of the methylated products of both quinidine (Q) and cinchonidine (IS) for a 5 μg/ml quinidine standard (panel A), and two patients (panels B and C).

Column conditions identical to the GC analyses except that helium was the carrier gas (30 ml/min) and the column temperature was programmed from 220 to 320 °C at 10 °C/min.

**Results and Discussion**

In agreement with earlier reports (1, 7–9), quinidine concentrations as determined by the protein precipitate method (fluorescence method I) are higher than those determined by the extraction method. Despite these differences there was a substantial correlation (Figure 1) between results by the two methods \( r = 0.945, P < 0.001 \). To determine which method was the more nearly accurate, the same serum samples were assayed by the GC method. As demonstrated in Figure 2, there was an excellent correlation between results by gas chromatography and by fluorescence method II \( r = 0.959, P < 0.001 \), which indicated that the extraction method was more specific than the protein precipitate method.
To further substantiate the accuracy and specificity of the second fluorescence method, we used the GC/MS system. Methylated quinidine and cinchonidine both have base peaks at m/e 136, and so we could use a single mass chromatogram for quantitation. The correct peaks for each compound in the mass chromatogram were chosen on the basis of both retention times and examination of the complete mass spectra. Figure 3 shows three such mass chromatograms. The areas of the peaks corresponding to quinidine and the internal standard were determined with the aid of the computer. Panel A was the mass chromatogram of m/e 136 for a 5 μg/ml quinidine standard. Panels B and C were mass chromatograms of m/e 136 for sera from two of the patients. Quinidine concentrations for these patients were 2.57 and 0.63 mg/liter. This related well to concentrations of 2.75 and 0.60 mg/liter, as determined by the extraction fluorescence method. A similar close comparison was obtained for a third patient (2.63 vs. 2.75 for the GC/MS and fluorescent method II, respectively).

From these data, we conclude that the extraction fluorescence method (II) is both accurate and specific and should therefore be used when the pharmacokinetics of quinidine are to be studied. However, under certain circumstances, the protein precipitate method may be preferred for monitoring clinical response to quinidine in patients: it is easier than the extraction method and, after the initial protein precipitation step, could be done by an automated procedure. However, if the protein precipitate method is used, the resulting quinidine concentrations should be divided by two to obtain an estimate of the actual quinidine concentration.

An objection to using the protein precipitation method was raised by Kessler et al. (9). They determined the ratio of quinidine concentrations determined by the two methods (I/II), related this ratio to the renal function of that patient, and found a significant inverse correlation between these variables. That is, as creatinine clearance decreased, the ratio (I/II) increased. They interpreted this to indicate a greater retention of the water-soluble metabolites of quinidine and dihydroquinidine relative to quinidine in renal failure. The varying amounts of these metabolites changed the ratio of the quinidine concentrations determined by the two methods, and this was the basis of their recommendation that the protein precipitate method be discarded.

In our experience, however, this relationship was not as evident. The mean (±SD) ratio for quinidine concentration determined by the two methods (I/II) was 2.0 ± 0.29 (range, 1.55 to 2.54). The endogenous creatinine clearances of these patients varied from 15 to 85 ml/min, and there was no correlation between the ratio and creatinine clearance. It is possible, however, that an increased ratio (I/II) might occur in patients with more severe renal-function impairment. Because results of the fluorescence method II agree well with those of GC and GC/MS methods, and because fluorescence method I gives quinidine concentrations that average twice those obtained by fluorescence method II, then fluorescence method I, the protein precipitate method, gives erroneous results and should be discarded.

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References