Rapid Gas Chromatographic Measurement of Plasma Procainamide Concentration

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A gas-chromatographic method has been developed for measuring procainamide in plasma. This simple analysis can be performed in less than an hour with a standard deviation of less than 8% for concentrations of procainamide in the range that is commonly encountered in clinical practice (1–20 mg/liter).

Additional Keyphrases Pronestyl • ventricular arrhythmias

Over the past two decades, familiarity with the clinical use of procainamide ("Pronestyl," Squibb) has increased. The dose of this drug that is effective in suppressing ventricular arrhythmias varies widely in different patients. The extent of this variability is most notable in reports of patients who required exceptionally large doses of procainamide to control recurrent ventricular tachycardia (1, 2). Plasma concentrations of procainamide were not determined in these patients and the causes for this increased dosage requirement were unclear. Individual differences in drug absorption and metabolism are plausible explanations for this variation, because recent pharmacokinetic investigations have established that steady-state plasma concentrations of procainamide differ by as much as sevenfold for a given daily dose (3, 4).

As with many therapeutic agents (5), plasma concentrations of procainamide appear to relate more predictably than dose to the therapeutic and toxic responses of patients treated with this drug (3, 4, 6): concentrations between 4 and 8 mg/liter are therapeutic, those between 8 and 16 mg/liter potentially toxic, and those greater than 16 mg/liter generally associated with hypotension or major disturbances in cardiac rhythm. Therefore, measurement of plasma procainamide concentration should help control patients who require this drug. However, for these determinations to be used widely and to be of more than retrospective value, rapid and simple analytical procedures are necessary. A gas-chromatographic analysis has been developed for this purpose and is described below.

Materials and Methods

Reagents

Procainamide hydrochloride (mp 163°–164°C), N-acetylprocainamide (mp 188°–191°C), and the dipropyl analog of procainamide, p-amino-N-(2-dipropylaminoethyl)benzamidine hydrochloride (mp 188°–190°C), were generously supplied by Dr. Thomas Q. Spitzer of E.R. Squibb & Sons, Inc., New Brunswick, N.J. A solution of this last compound, used for an internal standard in the procainamide assay, was prepared in a concentration of 5 mg/liter of water.

Procedure

Blood samples were collected in heparinized polyethylene syringes ("Peel-A-Way Blood Sampler"; Scientific Products, Evanston, Ill.) and the plasma was separated by centrifugation for 10 min at 1600 × g. Plasma should be stored in glass tubes at −4°C until analyzed or directly assayed in duplicate as follows.

One milliliter of plasma, 1 ml of internal stan-
standard solution, 0.2 ml of 5.0 molar NaOH, and 3 ml of methylene chloride were mixed by shaking in a 15-ml glass-stoppered conical centrifuge tube. The tube was centrifuged at 1600 × g for 3 min and the methylene chloride phase then was transferred to a 5-ml pear-shaped flask. Occasionally a gel formed in this phase, but was not transferred to the flask and did not interfere with the analysis. The methylene chloride phase was then evaporated at 47 × 10³ Pa (350 mm Hg) in a water bath set at 25°C. The residue was dissolved in 50 µl of ethyl acetate and a 5-µl aliquot injected into the gas chromatograph (Figure 1). The ratio of the heights of the peaks corresponding to procainamide and the internal standard was calculated.

Blood bank plasma was used to prepare a series of standard samples containing from 0.4 to 20 µg of procainamide base per milliliter of solution. These samples were analyzed by the above procedure. A standard curve was drawn by plotting the peak-height ratio on the ordinate and the known procainamide concentration of each standard sample on the abscissa (Figure 2). This standard curve was used to calculate the concentration of procainamide in each sample after the peak-height ratio for the sample was determined.

Instrumental Details

Gas chromatography. A Varian Series 1440 gas chromatograph equipped with a flame-ionization detector was used with an Hitachi–Perkin-Elmer Model 165 strip-chart recorder. The column was a 2 m × 2 mm (i.d.) glass coil packed with 0.2% OV-17 liquid phase on a solid support of Corning GLC-110 textured glass beads, 100/120 mesh (Chemical Research Services, Addison, Ill. 60101). The temperature of the injection port was 230°C, of the column 225°C, and of the detector 240°C. A flow rate of 30 ml/min was used for the nitrogen carrier gas and hydrogen. The air flow for the detector was maintained at 300 ml/min.

Gas chromatography–mass spectrometry. A Finnigan Model 3000 gas chromatograph–mass spectrometer, fitted with a Gohlke Separator, was used to check the identity of the procainamide and internal standard peaks. For chromatography, a 2 m × 2 mm (i.d.) stainless-steel coil was packed with 3% OV-17 coated on a solid support of Chromosorb W-HP, 80/100 mesh (Chemical Research Services). The flow of helium carrier gas was 20 ml/min. The temperature of the injection port was 250°C, of the column 230°C, and of the transfer line 225°C. The ionizing energy was 70 eV.

Results and Discussion

A spectrophotometric assay for procainamide was introduced in 1951 (7). More recently, extracted procainamide has been assayed directly by spectrophotofluorometry (3, 6). Both these methods are adequately sensitive and precise for clinical work, but are not yet widely used (3). However, unless complex derivatization procedures are required, gas chromatography could be expected to offer major advantages in simplicity and speed for analysis of single samples.

The first step in developing the gas-chromatographic method was to show that both procainamide and the internal standard were detectable when injected into the chromatographic unit and were stable under the chromatographic conditions. A
single, well-defined, symmetrical peak was observed when the free base of either compound was injected. The identity of the material comprising the peaks was confirmed on the basis of molecular weight by gas chromatography–mass spectrometry (Figure 3). Mass spectral fragmentation patterns for these compounds have not been proposed previously and are shown in Figure 3. The most prominent ion, or base peak of each compound, m/e 86 for procainamide and m/e 114 for the internal standard, appears to result from cleavage of a carbon–carbon bond that is in the beta position with respect to the tertiary amine nitrogen atom (8). The high relative intensity of this fragment ion is expected, because the free electron pair of this nitrogen atom is able to stabilize a positive charge on the adjacent carbon atom (9).

A number of other major ions of the spectra also appear to be formed by simple cleavage of the parent molecules. In addition, the m/e 99 fragment of procainamide and the m/e 127 fragment of the internal standard are apparently the result of a McLafferty rearrangement (9).

Quantitation of procainamide was obtained by the internal standard method, which also was used to correct for variations in the extraction and chromatography of this drug. Plasma standards containing known amounts of added procainamide were analyzed and a standard curve was drawn relating the ratio of the heights of the procainamide and internal standard peaks to procainamide concentration. Initially, the standard curve was nonlinear at procainamide concentrations of less than 4 mg/liter. The extent of this curvature was successively decreased, but not entirely eliminated, by changing from metal to glass columns and then from a diatomaceous earth solid support to textured glass beads (Figure 2). These results suggested that procainamide was partially adsorbed to the chromatographic column. A similar problem has been described in a method developed recently for the gas-chromatographic analysis of barbiturates and glutethimide (10).

N-Acetylprocainamide has been found in the urine of human subjects treated with procainamide (11). This metabolite was added to plasma and could be carried through the analytical procedure. However, it did not interfere with the determination of procainamide because it had a retention time 3.9 times longer than that of this drug. In addition, plasma from 20 patients receiving various sedative, analgesic, antibiotic, and antiarrhythmic drugs other than procainamide was analyzed without internal standard. No gas-chromatographic peaks from these drugs or from endogenous or drug metabolites were found that would interfere with the assay of procainamide. At the same time it was found that rubber-stoppered blood-collecting tubes ("Vacutainer"; Becton-Dickinson) contained materials that did interfere with this method. A somewhat similar interference, owing to silicone adsorbed by blood from the rubber stoppers of vacuum tubes, has been encountered in the development of a method for the gas-chromatographic measurement of lidocaine (12). However, the material interfering with the determination of procainamide behaved differently in that it could not be removed by extracting acidified plasma with organic solvents. For this reason, heparinized plastic syringes were chosen for blood collection.

The efficiency of the extraction of procainamide from plasma was 80%. The precision of the method, determined by analyzing standard plasma solutions of procainamide at least five times in duplicate, averaged less than 8% for procainamide concentrations ranging from 1 to 20 mg/liter. This standard deviation is comparable to the precision for the most widely used gas-chromatographic method for measuring lidocaine (12), but is greater than the standard deviation (1.8% to 3.2%) reported for the spectrophotometric and spectrofluorometric methods for procainamide (3).

In routine operation, plasma samples from patients can be analyzed in duplicate by gas chromatography in less than 1 h. The performance of the method is checked weekly by analyzing standard samples. Although procainamide will deteriorate when kept in plasma at room temperature, these standards are stable for at least three months when stored frozen. The use of textured glass beads and a 0.2% loading of liquid phase results in a column that tolerates prolonged bake-out at temperatures of 250°–275°C less well than columns packed with diatomaceous earth solid supports and higher loadings of liquid phase. However, lower column adsorption compensates
for this, and with proper care these columns maintain good performance with daily use for several months.

Initial experience has shown that this method of determining plasma procainamide concentration has the characteristics of speed and reliability that are essential if an analytical method is to be of value in the care of patients, and the correlation between plasma concentration and patient response seems to be the same as has been reported (3). Specific data that would permit a rigorous comparison between this gas-chromatographic procedure and spectrophotometric and fluorometric methods for procainamide analysis are not available. An estimated 1.5 to 2 h are required for the spectrophotometric assay (7), and that method is technically more difficult because procainamide must be extracted, diazotized, and then coupled with N-(1-naphthyl)ethylenediamine to form a dye that can be measured spectrophotometrically. The fluorometric method may have a higher daily capacity for sample analysis, but would not seem to have the specificity, simplicity, or dependability of gas chromatography for rapid analysis of single samples that are obtained at unpredictable times for on-line monitoring of patient therapy.

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