Enzyme Immunoassay for Serum Lidocaine in Antiarrhythmic Therapy

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We have adapted the commercially available EMIT® kit [Clin. Chem. 23, 1161 (1977)] to the Gilford System 3500 Analyzer. Sample volume is 10 µL. We compare reagent blank-corrected absorbance changes at 340 nm between 15 and 55 s for samples and a series of calibrators, and calculate results with use of a logarithmic transformation. Within-run precision (CV) for a serum pool with 4.0 mg of added lidocaine per liter was 2.7% (n = 45); day-to-day precision was 3.3% (n = 15). Analytical recoveries of 2 to 6 mg of lidocaine per liter were 90–102% (average, 97.3%). Results correlated significantly with those by a gas-chromatographic technique. No clinically significant interferences by concurrently administered medications were observed. The procedure is rapid (42 samples per hour) and is well suited to the fast response required in monitoring lidocaine therapy. Usefulness of the assay data in the management of arrhythmias in the coronary care unit is discussed.

Lidocaine (diethylaminoacet-2,6-xylylamine; lidocaine) has long been used for the production of local or regional anesthesia, and in recent years has been widely used in the management of cardiac arrhythmias, particularly those of ventricular origin such as occur with acute myocardial infarction. Use of lidocaine is usually indicated when the infarction is complicated by ventricular premature beats (1). Recently, routine prophylactic lidocaine administration to all patients during the first 24 h of acute infarction has been advocated (2).

In the therapeutic use of lidocaine, many clinical situations arise in which laboratory monitoring of lidocaine concentrations in blood is desirable. Lidocaine kinetics usually are in accord with a linear two-compartment model (3): a rapid vascular distribution phase (half-life, 9 min) and a slower elimination from the “central” compartment (half-life, 100 min). This elimination half-life is the shortest of any of the commonly used antiarrhythmic agents; moreover, the therapeutic range for lidocaine concentrations in serum is rather narrow, 2 to 6 mg/L in adult subjects (4–6). Because lidocaine is extensively metabolized by the liver, hepatic blood flow and function are crucial variables in controlling concentrations of lidocaine in blood. In severe liver disease, lidocaine half-life may be increased fivefold, because of impaired drug elimination (7, 7). An increased elimination half-life of lidocaine is also observed in patients with diminished cardiac output, in middle-aged and elderly patients, and in cases where the drug has been infused for longer than 24 h (5, 6).

Lidocaine in serum can be assayed by gas- (5, 9, 10) or liquid-chromatography (11, 12), but neither approach is as simple and rapid or as specific and sensitive as the recently available EMIT lidocaine assay (Syva Corp., Palo Alto, CA 94304). This nonisotopic immunoassay is based on competitive protein binding with glucose-6-phosphate dehydrogenase (EC 1.1.1.49) to label the drug and antibody to lidocaine as the specific binding protein. The enzyme activity, monitored as a change in absorbance at 340 nm due to conversion of NAD+ to NADH, correlates directly with the concentration of lidocaine in the serum sample (13–15).

This report describes a totally mechanized adaptation of the lidocaine immunoassay to the Gilford System 3500 Analyzer. In addition, a clinical study by the Department of Medicine on patients with arrhythmias in the coronary care unit determined optimum lidocaine dosage by correlating therapeutic effectiveness with concentrations of lidocaine in serum.

Materials and Methods

Enzyme Immunoassay

Instrumentation: A System 3500 Computer-Directed Analyzer (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074) was used throughout the study. The analyzer was programmed with the EMIT Antiepileptic Drugs magnetic card, and instrument settings were as follows:

- Wavelength: 340 nm
- Temperature: 30 °C
- Dispenser A volume: 0.25 mL (EMIT buffer)
- Dispenser B volume: 0.30 mL (EMIT Reagent A)
- Dispenser C volume: 0.30 mL (EMIT Reagent B)
- Sample volume: 10 µL

Reagents: EMIT reagent A, containing the lidocaine antibodies, glucose 6-phosphate, NAD+, monoethylglycinexylidide, and 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride (Tris-HCl) buffer, pH 5.0, was reconstituted to 3.0 mL with de-ionized water and allowed to stabilize for 8 h at room temperature before use. This stock reagent, when stored at 4 °C, is stable for as long as 12 weeks. EMIT reagent B, containing lidocaine coupled to glucose-6-phosphate dehydrogenase and Tris-HCl, pH 7.8, was reconstituted to 3.0 mL with de-ionized water. This stock reagent was also allowed to stabilize for 8 h at room temperature before use and, when refrigerated, is stable for as long as 12 weeks.
Upon reconstitution, the Tri-HCl buffer concentration in both stock reagents A and B was 55 mmol/L.

EMIT buffer solution (55 mmol of Tri-HCl per liter, pH 7.9) was prepared by diluting 10 mL of buffer concentrate to 150 mL with de-ionized water, and stored at room temperature.

To make working solutions of EMIT reagents A and B, we diluted each fivefold in Tri-HCl buffer, 55 mmol/L. These working solutions were allowed to stabilize for 4 h at room temperature before use; they then were stable for 36 h when refrigerated.

Procedure: Calibrators and undiluted patients' sera were sampled automatically by the analyzer, with an effective rate of 42 samples per hour. Zero calibrator values were subtracted from values obtained from calibrator standards (1.0 to 12.0 mg/L) and sera ($\Delta A-\Delta A_0$). Unknown lidocaine concentrations were determined from a logarithmically derived calibration curve.

Gas Chromatography

The gas-chromatograph procedure was a modification of that of Hucker and Stauffer (9). Serum was deproteinized with trichloroacetic acid, 300 g/L, and the supernate was made alkaline with sodium hydroxide, 10 mol/L. Lidocaine was extracted by agitation with dichloromethane, and the organic phase was dried by passage over anhydrous sodium sulfate. After evaporation of the dichloromethane under nitrogen at 40 °C, the residue was reconstituted with an equivalent mixture of methanol/toluene/hexane, and a suitable aliquot was injected into the gas chromatograph. Methylethylglycinexylidide (Astra Pharmaceutical Products, Inc., Worcester, MA 01606) was used as internal standard.

A Model 900 gas chromatograph (Perkin-Elmer Corporation, Norwalk, CT 06856) fitted with a nitrogen-sensitive detector was used, with a 3.0 m x 2 mm (i.d.) glass column packed with 3% OV-101 on 100/120 Gas-Chrom Q (Applied Science Laboratories, State College, PA 16801). The injection port heater and detector oven temperatures were 260 and 280 °C, respectively; the oven temperature was programmed from 215 to 250 °C, at 16 °C/min. After elution of internal standard and lidocaine, the column was purged by heating at 280 °C for 4 min. Quantitation was by peak height measurement.

Results

Reproducibility

Precision of the immunoassay was determined by use of a lidocaine-supplemented pool of human serum, with a concentration of 4.0 mg of lidocaine per liter. The within-run reproducibility study gave a mean concentration of 3.95 mg/L, a standard deviation (SD) of 0.107 mg/L, and a coefficient of variation (CV) of 2.72% (n = 45). Day-to-day precision studied for three months gave a mean concentration of 4.13 mg/L, an SD of 0.142 mg/L, and a CV of 3.43% (n = 15).

Accuracy

Lidocaine concentrations as measured by immunoassay and by gas chromatography were compared. Regression analysis of 25 pairs in the range of 0.0 to 6.5 mg/L gave: slope, 0.980; y-intercept, 0.012; and correlation coefficient, 0.995. Analytical recoveries by immunoassay of lidocaine added to pooled drug-free serum to give concentrations of 2.0, 3.0, 4.0, and 6.0 mg/L were 90.0, 101.7, 97.5, and 100.0%, respectively (mean, 97.3%).

Specificity

The first column of Table 1 lists 12 commonly used drugs that were added to drug-free serum. Analysis of the supplemented samples showed that these compounds did not interfere with the lidocaine immunoassay. Sera selected without conscious bias from patients not being administered lidocaine were analyzed for the drug. These patients were in the coronary care unit and received many other medications, listed in the second column of Table 1, none of which showed any response in the immunoassay.

Certain other medications and active metabolites of antiarrhythmic agents have been tested for possible cross-reactivity in the lidocaine immunoassay (14); no clinically significant interferences were found. Monoethylglycinexylidide, the major metabolite of lidocaine, is added to the lidocaine–antibody preparation to desensitize the assay to this pharmacologically active compound.

Clinical Observations

A total of 65 sera from 35 patients receiving lidocaine in the coronary care unit was assayed for the drug by the nonisotopic immunoassay. These patients generally received a loading dose of 50 to 100 mg of lidocaine followed by a continuous intravenous infusion of 2 to 4 mg/min. Blood sampling 30 min after the bolus demonstrated a high incidence of subtherapeutic lidocaine levels; however, sampling after 4.5 h of continuous lidocaine infusion (half-life X 3) reflected reliable steady-state concentrations. Later in the series, lidocaine dosage was altered to adhere to the pharmacokinetic guidelines of Greenblatt et al. (16), in which the initial bolus of lidocaine is followed by a second bolus after a 30-min interval. The incidence of subtherapeutic lidocaine levels was significantly lessened in the blood specimens drawn after the loading doses. In the entire series, only one frankly toxic lidocaine concentration (10.0 mg/L) was recorded. Although clinical symptoms of toxicity were not observed, the patient had received 3435 mg of lidocaine over a 24-h period.

Discussion

The clinical study showed that laboratory monitoring of lidocaine concentrations in serum was useful in the following cases: (a) when arrhythmia control was unsatisfactory and drug ineffectiveness needed to be distinguished from inadequate dosage; (b) when a diagnosis of possible lidocaine toxicity needed to be confirmed, or potential toxicity guarded against where continuous lidocaine infusion approached a 24-h span; (c) when intercurrent illness would markedly alter the biotransformation and elimination of lidocaine.

In monitoring lidocaine therapy, analytical methods must

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<th>Drugs tested for interference in nonisotopic immunoassay</th>
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<tr>
<td><strong>Drugs added to drug-free serum</strong></td>
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<tr>
<td>Amitriptyline, 1 mg/L</td>
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<td>Amoxapine, 4 mg/L</td>
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<td>Carbamazepine, 20 mg/L</td>
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<td>Ethosuximide, 150 mg/L</td>
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<td>Imipramine, 1.5 mg/L</td>
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<td>Nortriptyline, 1 mg/L</td>
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<td>Phenobarbital, 80 mg/L</td>
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<td>Phenytoin, 30 mg/L</td>
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<td>Primidone, 20 mg/L</td>
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<td>Procaainamide, 10 mg/L</td>
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<td>Quindine sulfate, 5 mg/L</td>
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<td>Theophylline, 20 mg/L</td>
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Table 1. Drugs Tested for Interference in Nonisotopic Immunoassay
be capable of rapid response. This mechanized enzyme immunoassay is adaptable to emergency situations as well as to heavy workloads. The method displays excellent reproducibility and accuracy, and requires only 10 μL of serum. This reliable assay should be invaluable in guiding a physician’s approach to arrhythmia control, and obviates indirect guides to lidocaine infusion doses, such as measurement of indocyanine green (17).

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References