Simultaneous Determination of Lidocaine and Its Principal Metabolites by Liquid Chromatography on Silica Gel, with Aqueous Eluent

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We describe the simultaneous determination of lidocaine and its pharmacologically active metabolites, monoethylglycine-xylidide and glycinexylidide, in plasma by "high-performance" liquid-chromatography. By use of a bare (unbonded) silica gel with aqueous eluents, separations of organic amines such as lidocaine and its metabolites, which are very difficult and have a poor peak symmetry on bonded reversed-phase packings, were easily accomplished with a good peak symmetry. The method is sufficiently precise, sensitive, and specific. Analytical recoveries of all compounds were >95%; CVs for reproducibility were <5% for all compounds; the lower detection limits were 0.1 μg/mL or less. This method can be used to monitor the concentrations of these compounds in plasma and to prevent the concentration-related side-effect(s).

Additional Keyphrases: drug assay • monitoring therapy • active metabolites • ventricular arrhythmias

Lidocaine (2-diethylamino-2',6'-acetoxyxylidide) has been used extensively for the treatment of ventricular arrhythmias accompanying acute myocardial infarction (1, 2). The concentration of lidocaine in plasma is better related than is the dosage to the clinical response of patients requiring this type of therapy (3).

Lidocaine is metabolized predominantly by microsomal enzymes via oxidative de-ethylation in the liver, resulting in two main products, monoethylglycine-xylidide (MEGX) and glycinexylidide (GX) (4, 5). The antiarrhythmic potency of MEGX reportedly is about 80% that of lidocaine; GX appears to be only about 10% as potent as the parent drug (6). Because of their pharmacological activities (6–14), both MEGX and GX may contribute to the occurrence of the adverse reactions that sometimes arise after administration of lidocaine. Therefore, monitoring the concentrations of lidocaine, MEGX, and GX in the plasma of patients who are being treated with lidocaine may be of benefit, not only in controlling the antiarrhythmic effects but also in avoiding toxic effects (11–13).

Gas-chromatographic (15–17) and enzyme immunoassay methods (18) have been described for the quantification of lidocaine but not of its active metabolites in plasma. Several investigators (19–23) have reported methods for the simultaneous assay of lidocaine and its de-ethylated metabolites. In the field of "high-performance" liquid chromatography (HPLC), lidocaine has been assayed by a reversed-phase technique (24–27). Two groups of investigators (6, 28) have reported methods for determining lidocaine, MEGX, and GX simultaneously with a similar chromatographic technique.

In general, separations of organic amines such as lidocaine and its metabolites are considered to be very difficult, and the peaks that are produced by use of the bonded reversed-phase packings are poorly symmetrical. However, a recent paper (29) showed that many lipophilic amines that are very difficult to resolve on bonded reversed-phase packings are easily separated, with good peak symmetry, on silica.

The purpose of this report is to describe a rapid, sensitive, and specific HPLC method for the simultaneous quantification of lidocaine, MEGX, and GX in 200 μL of sample on a bare (unbonded) column of silica gel with aqueous eluents. We show that the method is applicable to pharmacokinetic studies and to clinical use or therapeutic monitoring of lidocaine and its pharmacologically active metabolites.

Materials and Methods

Reagents

Lidocaine, MEGX, GX, and ethylmethylglycine-xylidide (EMGX) all as their hydrochloride salts, were donated by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan; phenobarbital and phenytoin by Dainippon Pharmaceutical Co., Ltd., Osaka; diazepam and chloridiazepoxide by Takeda Pharmaceutical Co., Ltd., Osaka. Disopyramide, procainamide, N-acetylprocainamide, propranolol, quinidine, propranolol, salicylic acid, and theophylline were from Sigma Chemical Co., St. Louis, MO. Tetrahydrofuran (HPLC grade), methanol, dichloromethane, hydrochloric acid, and anhydrous sodium carbonate (all were of reagent grade) were from Wako Pure Chemical Industries, Ltd., Osaka.

Drug Standard Solutions

A stock solution containing lidocaine, MEGX, and GX (200 μg of each per milliliter, as the hydrochloride salt) was further diluted with methanol to produce the desired concentrations for each component. We prepared a stock 5 mg/L solution of the internal standard (EMGX hydrochloride) in distilled water. All solutions were stored at 4°C.

Apparatus

We used a high-performance liquid-chromatograph, Model LC 635 (Hitachi Co., Ltd., Tokyo), equipped with an UVILOG-5 IV variable-wavelength ultraviolet detector (Oyo Bunko Kiki Co., Ltd., Tokyo) and a Rheodyne Model 7125 syringe-loading sample injector (Rheodyne, Cotati, CA 94928). Detector output was recorded at 10 mV with a C-RIB Chromatopac recorder (Shimazu Co., Ltd., Kyoto). The 4 mm (i.d.) × 25 cm chromatographic column contained LiChrosorb Si-60 column (particle size 5 μm).

Assay Procedure

To 200 μL of plasma in a culture tube add 200 μL of internal standard solution and 10 μL of a 50 g/L sodium carbonate solution. Vortex-mix for 5 s, add 3 mL of dichloro-
methane, and shake the resulting mixture for 5 min on a reciprocating shaker. Centrifuge (1500 × g, 5 min), aspirate the aqueous (top) layer, and discard it. Transfer the organic phase to a centrifuge tube and evaporate it under a gentle stream of air at 40 °C. Reconstitute the residue in 50 µL of tetrahydrofuran containing 6 mL of 100 mmol/L HCl per deciliter. Vortex-mix for 5 s, and then inject 10 µL of the solution into the chromatograph. Use the following chromatographic conditions: flow rate, 1 mL/min; mobile phase, 6 mL of 10 mmol/L HCl per 94 mL of tetrahydrofuran; detection wavelength, 225 nm; chart speed, 2.5 mm/min.

Quantification

Prepare plasma standards by adding known amounts of the hydrochloride salts of lidocaine, MEGX, and GX to drug-free plasma to give final concentrations ranging from 0.5 to 20 mg/L. Construct a calibration curve for these compounds by using the analytic/internal standard peak-height ratios. Convert the ratios for an unknown sample to the concentrations of lidocaine, MEGX, and GX by the use of the corresponding calibration curves. Analyze solutions of these compounds in drug-free plasma in triplicate. Perform linear regression analyses within the drug concentration ranges as described above.

Results

Chromatography

With our chromatographic system, lidocaine, its de-ethylated metabolites, and the internal standard all exhibited symmetrical peaks (Figure 1) with baseline resolutions and no disturbance from endogenous component(s) of plasma from normal persons or patients. We observed no interfering peaks when the blank plasma extract was analyzed (Figure 1A).

The calibration curves for lidocaine, MEGX, and GX (Figure 2), prepared as described, were apparently linear and passed through the origin.

Figure 1B shows a chromatogram of a drug-free plasma sample to which we added lidocaine, MEGX, and GX, each in a concentration of 2 mg/L. The capacity ratios (k') for lidocaine, MEGX, and GX were 5.00, 1.61, and 1.03, respectively, and 5.45 for the internal standard. Separation factors (α) between each adjacent peak were 1.09 for the internal standard and lidocaine, 3.11 for MEGX and lidocaine, and 1.56 for GX and MEGX. Figure 1C illustrates an example of patient plasma analysis.

Analytical Variables

Recovery. Analytical-recovery data were collected by adding known amounts of lidocaine, MEGX, and GX to drug-free plasma and taking aliquots of it through the complete procedure, then comparing the peak heights of extracted compounds with those of pure standards. Recoveries at a concentration of 2 mg/L were 92.58, 95.74, and 94.12% for lidocaine, MEGX, and GX, respectively.

Reproducibility. These data were obtained by using aliquots of a pooled plasma containing 2 mg of each of the compounds per liter (n = 5 each). We evaluated the within-run precision by analyzing the plasma standard five times in one day, obtaining values for the coefficient of variation (CV) for each compound: 1.96, 4.43, and 3.56% for lidocaine, MEGX, and GX, respectively. We evaluated between-run precision by analyzing the same standard once a day for five days during a month. The respective CVs were 4.62, 2.69, and 4.95%.

Sensitivity. Although analytical recoveries of these analytes exceeded 90% and interfering peaks were absent, the sensitivity of the assay method might depend on sample size. However, with the 200-µL volume of sample used in the present study, the sensitivity is such that the compounds can be detected in plasma in concentrations as low as 0.1 mg/L.

Accuracy. To assess the accuracy of the lidocaine assay, we compared the results of our chromatographic procedure with those of a fluorescence polarization immunoassay (FPIA, TDX System; Abbott Diagnostik, South Pasadena, CA 91030) in a total of 196 plasma samples from subjects on lidocaine therapy (Figure 3). The results showed a good correlation (r = 0.986) between the two methods, indicating
that our HPLC method can be successfully used for the clinical monitoring of lidocaine. Furthermore, although we have searched for possible interferences from 12 drugs that may be occasionally coadministered with lidocaine (Table 1), we found none.

Clinical Applicability

Further to test the clinical applicability of our method, we examined plasma samples from a 37-year-old woman who was receiving lidocaine (Xylocaine®, Fujisawa Pharmaceutical Co., Ltd., Osaka) for anesthesia during the surgical treatment of endometriosis. Figure 4 illustrates the results of the analyses for these compounds in the plasma of this patient, who received a total dose of lidocaine of 800 mg, administered epidurally. Although this patient had been premedicated with diazepam, pentobarbital, and atropine, and received pentazocine and ephedrine during the surgical procedures, these drugs did not interfere with the chromatograms of lidocaine, MEGX, and GX, which were simultaneously measured. Lidocaine and its metabolites were completely resolved in the chromatograms.

![Graph](image)

**Fig. 3.** Comparison of plasma lidocaine concentrations as measured with "high-performance" liquid-chromatographic (HPLC) and fluorescence polarization immunoassay (FPIA) methods

The coefficient of correlation (r) is 0.998 and the regression equation is y = 1.02x + 0.15; n = 196

<table>
<thead>
<tr>
<th>Table 1. Capacity Ratio for Selected Drugs</th>
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<tr>
<td>Drug</td>
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<td>Phenobarbital</td>
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<td>Phenytoin</td>
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<td>Salicylic acid</td>
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<td>Propranolol</td>
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<td>Chlorzoxazone</td>
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<td>Disopyramide</td>
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<td>Lidocaine</td>
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<td>Procaine</td>
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<tr>
<td>EMGX</td>
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<td>N-Acetylprocainamide</td>
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<td>Procainamide</td>
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The elimination $t_{1/2}$ of lidocaine for this patient was calculated to be 1.8 h, which agrees with the value reported previously (1, 2).

Discussion

Several recent reports strongly recommend that the concentration of lidocaine and of its metabolites in the blood be routinely monitored during treatment of ventricular arrhythmias, both to assure that therapeutic concentrations are present and to avoid concentration-related toxicity (11–13). The therapeutic range for lidocaine is considered to be 1.5–6 mg/L (1, 2), and the risk of reversible concentration-related toxicity increases progressively as concentrations exceed 8 mg/L. Therefore, monitoring such as can be done with the present assay is important. Methods have been published for simultaneous assay by mass spectrometry (23), gas chromatography (19–21), gas chromatography–mass spectrometry (22), and, more recently, HPLC (6, 28). The methods for simultaneous HPLC previously reported (6, 28) have involved reversed-phase chromatography. However, as mentioned above, such separations of organic amines are very difficult. Our use of silica gel packing and aqueous eluents resulted in an improved peak shape for lidocaine, such that the method can be applied clinically.

Our extraction technique for the analytes in plasma is simple: dichloromethane is used after addition of a known amount of internal standard and alkalinization with sodium carbonate solution. Analytical recovery of the analytes from plasma was good, as determined by peak height analysis compared with direct injection of the authentic compounds. In the case of GX, recovery was only about 63–70% when the plasma was made basic with sodium hydroxide solution, but surprisingly, it improved to >90% when sodium carbonate was used instead.

Our chromatographic system includes nothing unusual. The sensitivity of the analysis was greatest at 225 nm, and as little as 20 ng of the authentic compounds per injection volume was required for the measurement at this wavelength with a detector range of 0.005. The extract of normal plasma showed no significant background peak in the area of lidocaine-related compounds when eluted with the eluent described, so the detection limit for plasma samples should be considered to be in this same range. However, in the usual drug monitoring one can use a more easily controllable absorbance unit full scale, because the therapeutic range of lidocaine in plasma is considered to be 1.5–6 mg/L (1, 2), which is high enough to be detected without the need for...
such a sensitive experimental condition as a detector range of 0.005.

The elution sequence of the compounds, related to their $k'$ values, was GX, MEGX, lidocaine, and EMGX—i.e., primary, secondary, and tertiary amine hydrochlorides, respectively. We can not offer any reasonable explanations for this elution sequence with silica gel packing and aqueous eluents, but retention of the basic amines on silica when reversed-phase eluents are used appears to depend on electrostatic and adsorption forces (29).

Our findings (Table 1, Figures 3, 4) indicate that our assay method is applicable for not only clinical monitoring but also for pharmacokinetic study of lidocaine and its pharmacologically active metabolites.

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References