GLC Analysis of Lidocaine in Plasma Using a Novel Nitrogen-Sensitive Detector

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Abstract □ A simple, rapid GLC method for the determination of therapeutic levels of lidocaine in plasma or blood is described. After extraction with benzene from alkalinized plasma, the compound is analyzed by GLC using a nitrogen-sensitive detector. Quantitation is accomplished using an internal standard and a standard curve, which was linear over the 0.1–10-μg range. The limit of detection was approximately 10 ng/ml. Recovery of the parent compound was essentially quantitative. Plasma data are presented to demonstrate the utility of the method.

Keyphrases □ Lidocaine—GLC analysis, plasma or blood □ GLC—analysis, lidocaine, plasma or blood □ Anesthetics, local—lidocaine, GLC analysis, plasma or blood

Lidocaine has long been used as a local anesthetic agent and, in recent years, has been widely employed intravenously in the treatment of ventricular arrhythmias (1). Since desirable pharmacological effects require blood levels of approximately 1.5 μg/ml, whereas toxic side effects are observed at concentrations of 3 μg/ml (1), it is important to monitor lidocaine concentrations in patients undergoing therapy.

Several GLC methods using flame-ionization detection for the quantitative determination of lidocaine have been described (2–5). Assay of the drug by mass fragmentography also has been demonstrated (6). However, the use of a nitrogen detector to measure lidocaine in the GLC effluent appeared to offer advantages of sensitivity, rapidity, and specificity; a procedure for lidocaine using this technology is presented here.

EXPERIMENTAL

Reagents—Benzene[1], lidocaine hydrochloride, and ethylmethylglycinexylidide hydrochloride[2] were used. Drug concentrations were calculated as the free base.

Apparatus—A gas chromatograph fitted with a 1.8-m (6-ft) × 2-mm (i.d.) glass column, packed with 3% OV-17 on Gas Chrom Q (100–120 mesh), and a nitrogen–phosphorus detector were used[3]. Operating temperatures were 200, 260, and 265° for the column oven, injector, and interface (detector), respectively. Flow rates were 50, 4, and 100 ml/min for the carrier gas (helium), hydrogen, and air, respectively. A 1-mv recorder was used[4].

Procedure—Dog or human plasma (1 ml), the internal standard (ethylmethylglycinexylidide, 1 or 5 μg in 0.1 ml of water), 0.5 *N* NaOH (1 ml), and benzene (1 ml) were shaken in a 13-ml glass-stoppered centrifuge tube for 5 min. After centrifugation, 5 μl of the organic phase was injected directly into the chromatographic column[5].

Six male dogs (7.6–10 kg) were anesthetized with pentobarbital and given a continuous intravenous infusion of either 0.06 or 0.25 mg/kg/min of lidocaine in 5% dextrose in water. Blood samples were drawn at specified times into heparinized syringes and immediately centrifuged, and the plasma was separated. The concentration of lidocaine in the plasma was measured by using a calibration curve constructed by analyses of normal plasma spiked with known amounts of lidocaine and plotting the peak height ratio (lidocaine–internal standard) *versus* the lidocaine concentration. The peak height ratio was linearly related to the concentration of lidocaine over a range of 0.1–10 μg of lidocaine/ml of plasma.

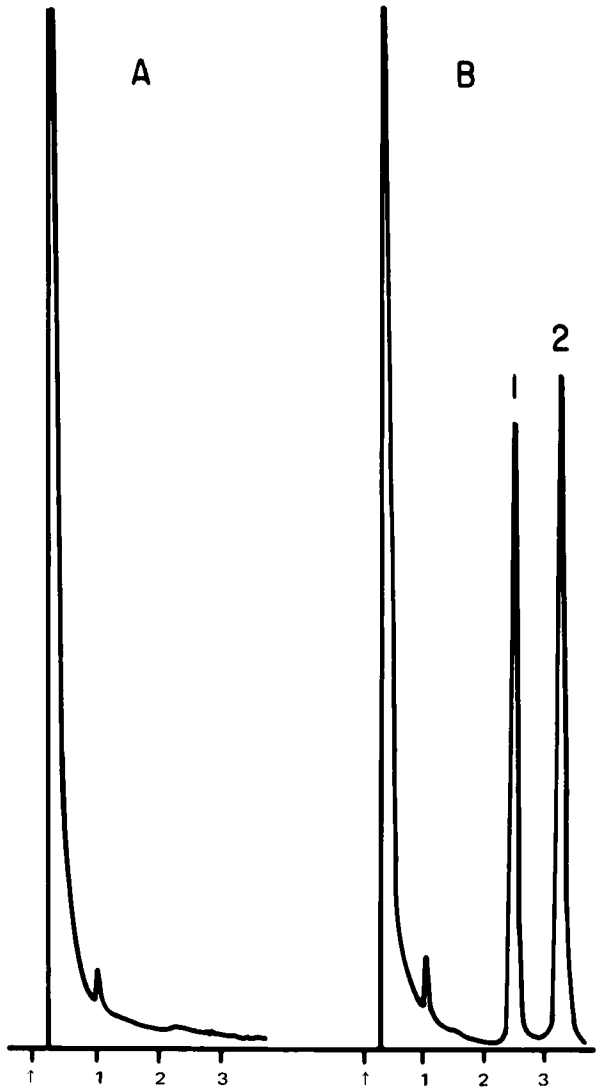

Figure 1—*Chromatograms of: A, a blank plasma extract; and B, an extract of plasma from a dog infused with lidocaine (0.06 mg/kg/min). Sample represents a concentration of 1.20 μg/ml with the equivalent of 6 ng injected. Key: 1, methylethylglycinexylidide (internal standard); and 2, lidocaine. Recorder attenuation was 10 × 5.*

RESULTS AND DISCUSSION

The reproducibility of the method was examined by analysis of plasma samples (1 ml) containing known concentrations of lidocaine (0.1–10 μg/ml) (Table I). The recovery of lidocaine was estimated by

[1] Burdick and Jackson Laboratories, Muskegon, Mich.
[2] Astra Pharmaceutical Products, Worcester, Mass.
[3] Model 3920, Perkin-Elmer Corp., Norwalk, Conn.
[4] Model 56, Leeds & Northrup Co., North Wales, Pa.
[5] Mass spectra were obtained on an LKB-9000S combined gas chromatograph–mass spectrometer. The spectra of the 3.3-min peak attributed to lidocaine in dosed dog plasma were essentially identical to those of authentic drug. A molecular ion was observed at *m/e* 234, and characteristic fragment ions were at *m/e* 219, 163, 148, 120, and 86 (base peak), in accordance with previously reported results (6).

Table I—Precision of Lidocaine Assay by GLC with a Nitrogen Detector

Lidocaine, μg/ml	Peak Height Ratio ± *SD*
0.1	0.08 ± 0.00 (n = 4)
0.5	0.44 ± 0.01 (n = 4)
1.0	0.85 ± 0.04 (n = 6)
2.5	2.10 ± 0.21 (n = 4)
5.0	4.00 ± 0.21 (n = 2)
10.0	8.28 ± 0.10 (n = 2)

comparison of peak heights of lidocaine standards with those obtained after extraction of the drug added to plasma over a concentration range of 0.1–5 μg/ml and was found to be quantitative (100.7 ± 6.6% *SD* for dog plasma and 103.2 ± 6.4% for human plasma). No interfering peaks were present in blank plasma extracts (Fig. 1); the retention times of lidocaine and internal standard were 3.3 and 2.5 min, respectively. The limit of lidocaine detection was approximately 10 ng/ml. Whole blood was as satisfactory for analysis as plasma.

Plasma levels of lidocaine in dogs given the drug by continuous intravenous infusion of different doses are shown in Table II. The levels in each group approached a constant value at 70 min, indicating that steady-state levels had nearly been achieved. Dose and plasma level appeared to be correlated, since increasing the dose by a factor of four caused a nearly proportional rise in plasma concentration.

The results indicate that the present method is satisfactory for routine clinical use, since the blood levels required in humans to achieve antiarrhythmic activity, 1.5 μg/ml (1), are the same as those measured in dogs by the new procedure. The present method would permit rapid monitoring of clinical samples; by avoiding an evaporation step, the entire analysis (using whole blood) may be completed in 10 min or less.

Although the use of a thermionic detector for analysis of other nitrogen-containing drugs was reported previously (7, 8), the nitrogen detector used in the present study is of a substantially different design than previous apparatus; it was described in detail elsewhere (9, 10). The thermionic source, a rubidium silicate glass bead in the new detector, is electrically heated and thus freed from dependence upon the flame as a source of thermal energy. Consequently, detector performance is independent of the heretofore critical factors of shape and temperature of the flame and the area of its contact with the alkali surface (11, 12), with an attendant increase in stability (10). Additional sensitivity is also gained since the hydrogen flow requirement is greatly reduced.

These advantages indicate that GLC analysis of many drugs using the new nitrogen detector is worth investigation. A preliminary report on the analysis of stimulants in this manner appeared previously (13).

During the preparation of this manuscript, two procedures for the determination of lidocaine using a nitrogen detector of conventional design appeared (14, 15). Both procedures entailed more steps than are required using the method described in the present paper.

Table II—Plasma Levels of Lidocaine in Dogs after Continuous Intravenous Infusion of Lidocaine

Minutes	Lidocaine Plasma Concentration[a]	
	Group 1	Group 2
10	3.22 ± 0.14	0.95 ± 0.11
20	3.86 ± 0.11	1.24 ± 0.14
30	4.48 ± 0.25	1.35 ± 0.19
40	4.74 ± 0.29	1.41 ± 0.23
50	4.86 ± 0.11	1.53 ± 0.25
70	5.56 ± 0.84	1.61 ± 0.29

[a] Expressed as micrograms of lidocaine per milliliter of plasma ± *SD*. Group 1 (two dogs) received 0.25 mg of lidocaine/kg/min; Group 2 (three dogs) received 0.06 mg/kg/min.

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