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Note

Simultaneous determination of lidocaine and its deethylated metabolites using gas—liquid chromatography with nitrogen—phosphorus detection

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Lidocaine is extensively used in the treatment of cardiac arrhythmias and as a local and regional anesthetic agent [1]. Biotransformation of lidocaine in vivo leads to two major metabolic products, monoethylglycinexylidide (MEGX) and glycinexylidide (GX), both of which have pharmacologic acitivity [2, 3].

Gas chromatography (GC) [4—11], GC—mass spectroscopy [3, 11] and liquid chromatography [12—15] have been used to quantitate lidocaine in plasma, and some of these methods simultaneously measure one or both metabolites. The present report describes an improved GC methodology using nitrogen—phosphorus detection (NPD) which can simultaneously quantitate lidocaine, MEGX, and GX in plasma without derivitazion or sample clean-up. An automated injection system allows analysis of up to 100 samples per 24 h.

EXPERIMENTAL

Apparatus and chromatographic conditions

The analytic instrument was a Hewlett-Packard Model 5840A gas chromatograph equipped with a nitrogen—phosphorus detector, electronic data processor integrator and automatic sampler (Model 7672A). The column was coiled glass, 3.05 m × 2 mm I.D., packed with 3% SP-2250 on 80—100 mesh Supelcoport (Packing 1-1767, Supelco, Bellefonte, PA, U.S.A.). The carrier gas was ultra high purity helium (Matheson Gas Products, Gloucester, MA, U.S.A.) at a flow-rate of 30 ml/min. The detector purge was ultra high purity hydrogen

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(Matheson) at a flow-rate of 3 ml/min mixed with dry air (Matheson) at a flow-rate of 50 ml/min. Operating temperatures were: injection port, 310°C; column, 200°C; detector, 275°C. Before being connected to the detector, a new column was conditioned at 220°C for 48 h with a carrier flow-rate of 30 ml/min. At the beginning of each work day the column was primed with 2—4 μg of purified soy phosphatides (asolectin) in benzene [16].

Reagents

The following reagents were used: analytical reagent-grade ethyl acetate; analytical reagent-grade toluene; certified isoamyl alcohol; HPLC-grade methanol, all obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.); analytical-reagent grade sodium hydroxide (Mallinckrodt, St. Louis, MO, U.S.A.); and Baker-Analyzed acetone (J.T. Baker, Phillipsburg, NJ, U.S.A.).

Reference standards

Pure standards of lidocaine, MEGX and GX, as well as the internal standard ethylmethylglycinexylidide (EMGX), were kindly provided by Astra Pharmaceutical Products (Worcester, MA, U.S.A.). Standards of each were prepared by dissolving 100 mg of free base in 100 ml methanol. Sequential dilutions were made to 10 μg/ml. Solutions were stored in the dark in glass-stoppered bottles at 4°C and were stable for at least two months.

Preparation of samples

Extraction tubes were 15-ml round-bottomed glass culture tubes with PTFE-lined screw-top caps. Tubes were rinsed with acetone and air-dried prior to use. To each tube, 0.5 μg (50 μl of the 10 μg/ml solution) of EMGX was added as the internal standard. Calibration standards for lidocaine, MEGX and GX were prepared by adding varying amounts (0.1 to 3 μg) of all three compounds to consecutive tubes. The tubes were placed in a vacuum oven and the solvent evaporated to dryness at 40—45°C under reduced pressure. Drug-free control plasma (1 ml) was added to each calibration tube and 1 ml of unknown plasma added to all other tubes containing only internal standard.

Extraction procedure

To each tube 200 μl of 5 *M* sodium hydroxide and 2 ml of ethyl acetate were added. The tubes were capped and agitated gently in the upright position on a vortex mixer for 30 sec. The samples were centrifuged at room temperature for 5 min at 400 *g*. The organic layer was transferred to standard 2-ml Wheaton automatic sampling vials (Wheaton Scientific, Millville, NJ, U.S.A.), which were then placed in a vacuum oven and evaporated to dryness at room temperature. The residue was reconstituted with 250 μl of a toluene—isoamyl alcohol mixture (85:15), capped with aluminum foil and gently vortexed. The automatic sampler was programmed to inject 2 μl of each sample.

Pharmacokinetic studies

A healthy male volunteer participated after giving written informed consent. A 300-mg oral dose of lidocaine hydrochloride, as 15 ml of a 2 g per 100 ml solution (Xylocaine Viscous, Astra) was administered every 3 h for eight

consecutive doses. Venous blood samples were drawn into heparinized Venoject tubes prior to each dose, and at multiple points after the first and last doses. Plasma samples were separated and frozen until the time of assay as described above.

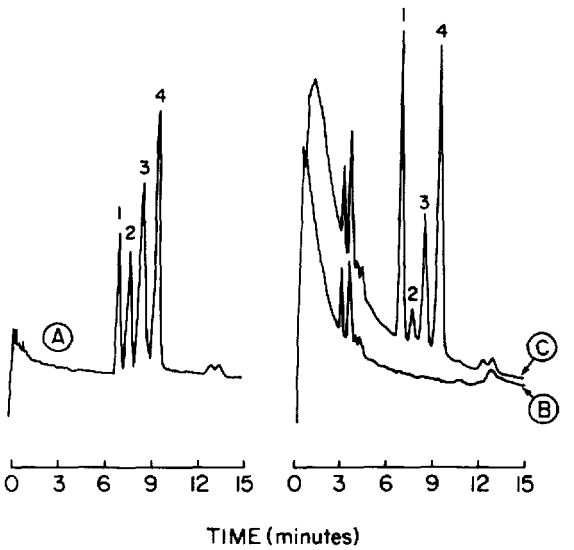

Fig. 1. (A) Chromatogram of an extract of a calibration standard containing 0.5 μg/ml of EMGX (the internal standard) and 1.0 μg/ml of GX, MEGX, and lidocaine; (B) chromatogram of a drug-free control plasma extract; (C) chromatogram of a sample from a subject after oral administration of lidocaine. Peaks and concentrations determined were: 4 = lidocaine, 0.56 μg/ml; 3 = MEGX, 0.32 μg/ml; 2 = GX, 0.09 μg/ml; 1 = EMGX, internal standard.

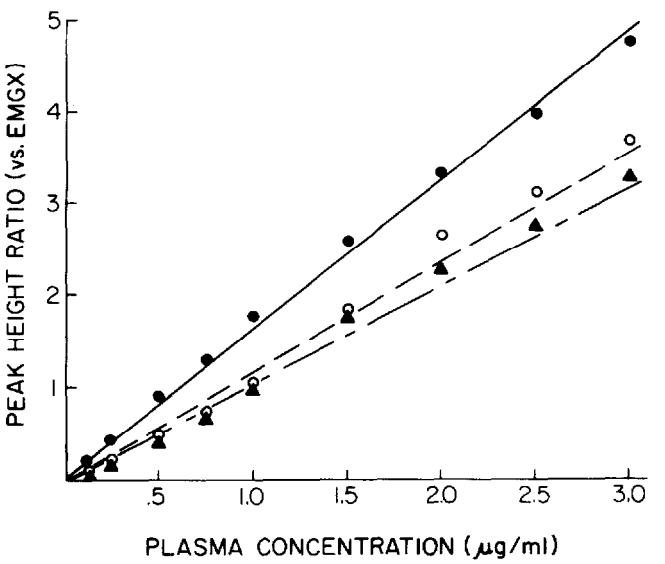

Fig. 2. Calibration curve showing relation of plasma concentration of lidocaine and its two metabolites versus the peak height ratio of drug to internal standard. (•——•), Lidocaine; (○ - - ○), MEGX; (▲— — ▲), GX.

RESULTS

Evaluation of the method

Under the described chromatographic conditions, lidocaine, its two metabolites, and the internal standard gave well resolved chromatographic peaks (Fig. 1). Drug-free blank plasma samples were free of contaminating peaks (Fig. 1). Plasma concentrations of lidocaine, MEGX, and GX were linearly related to the peak height ratio of each compound versus the internal standard (Fig. 2). The sensitivity limits are approximately 0.05 μg of each compound per ml of plasma. Table I shows replicability of identical samples at various concentrations. Residue analysis indicated greater than 90% recovery of all three compounds.

After extraction and reconstitution, lidocaine and GX are stable at room temperature for at least 48 h. However, degradation of MEGX is noted after 24 h. Therefore samples should be chromatographed within 24 h of extraction.

TABLE I

REPLICABILITY OF IDENTICAL SAMPLES AT VARIOUS CONCENTRATIONS

At each concentration $n = 4$—6.

Plasma concentration (μg/ml)	Coefficient of variation* (%)		
	Lidocaine	MEGX	GX
0.1	7.1	16.5	13.6
0.25	2.6	5.9	8.3
0.5	4.8	8.0	3.9
0.75	2.9	4.0	7.4
1.0	2.8	6.7	11.3
1.5	3.9	7.4	3.6
2.0	1.0	3.8	3.8
2.5	4.0	7.8	3.0
3.0	1.0	5.2	4.3

*Standard deviation divided by mean, in percent.

Pharmacokinetic results

Fig. 3 shows plasma concentrations of lidocaine, MEGX, and GX in the volunteer subject. Concentrations of lidocaine and MEGX reached similar levels during multiple-dose therapy. After termination of treatment, washout of MEGX was slower than that of lidocaine. GX concentrations were considerably lower than those of other two compounds.

DISCUSSION

The present report describes a rapid, sensitive, automated method for simultaneous quantitation of lidocaine and its two metabolites in plasma. The straightforward extraction procedure allows one person to prepare a large number of samples in a standard working day. With the automated sampler, 100 or more samples can be chromatographed in a 24-h period with no

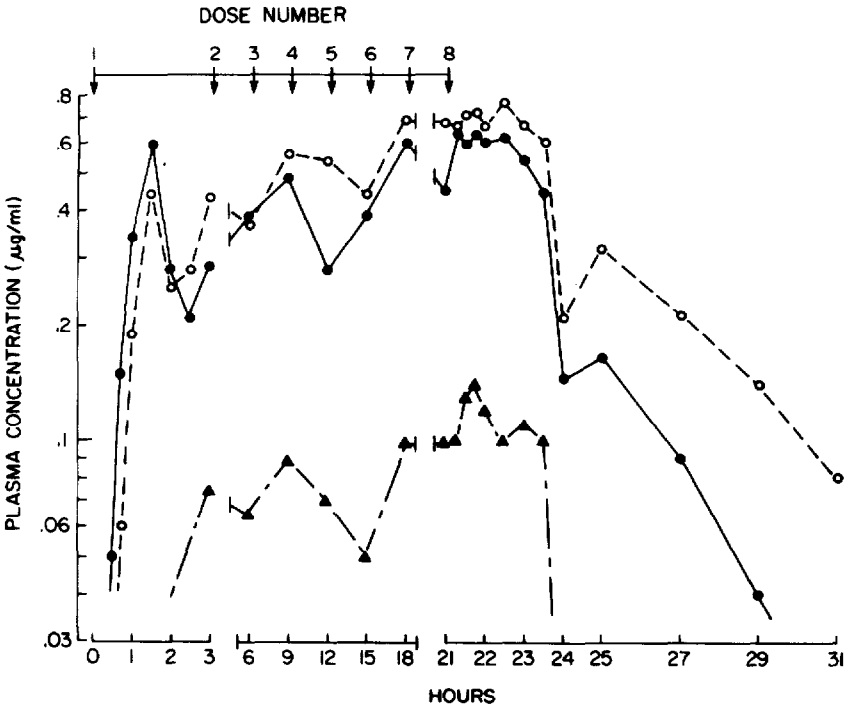

Fig. 3. Plasma concentrations of lidocaine and its two metabolites in the volunteer subject. (•——•), Lidocaine; (○ - - ○), MEGX; (▲— — ▲), GX.

technical personnel in attendance. The method is sensitive enough for therapeutic monitoring and for most pharmacokinetic studies. The two metabolites of lidocaine, MEGX and GX, both have pharmacologic activity [3, 4]. MEGX, in particular, has activity similar to that of the parent compound, and may contribute to antiarrhythmic activity and/or toxicity. Thus the capacity for quantitation of lidocaine metabolites as well as the parent compound is of considerable importance.

A parallel clinical pharmacokinetic study in eighteen volunteers confirmed previous reports that concentrations of lidocaine in the systemic circulation after oral administration are relatively low [17—19]. This is probably explained by extensive first-pass hepatic extraction. During repeated oral dosage with lidocaine, levels of the principal metabolite, MEGX, were similar to those of the parent compound. Levels of GX were considerably lower than those of either lidocaine or MEGX, confirming that GX is a quantitatively less important metabolic product [15].

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