Journal of Chromatography, 232 (1982) 180—185
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1342

Note

Lidocaine determination in human plasma with application to single low-dose pharmacokinetic studies

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(First received February 9th, 1982; revised manuscript received March 26th, 1982)

Lidocaine, a widely used local anesthetic [1] and antiarrhythmic [2], has been measured by specific gas—liquid chromatography (GLC) using flame ionization detection [3—5], GLC using mass spectroscopy (MS) [6, 7], liquid chromatography [8—10], and by enzyme immunoassay (EMIT) [5, 11]. Though specific, with the exception of GLC—MS determinations which are limited by the number of analyses that can be performed, these methods lack adequate sensitivity to detect very low lidocaine plasma levels. This is of importance since lidocaine kinetics are dose-dependent, with only very small doses exhibiting first-order processes, and higher doses becoming zero-order as metabolizing enzyme saturation occurs, thus being described by Michaelis—Menten kinetics [12]. To evaluate lidocaine pharmacokinetics between populations and identify potential differences in the first-order processes, we describe here a method that is specific, separating lidocaine from metabolites and plasma contaminants, and sensitive to 2.5 ng/ml using GLC with a nitrogen—phosphorus detector (NPD). In addition, when coupled with an automated injection system, up to 125 samples per 24 h may be analyzed after a straightforward plasma extraction which requires only 3—4 h technical time.

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0378-4347/82/0000—0000/$02.75

EXPERIMENTAL

Apparatus and chromatographic conditions

The analytical instrument is a Hewlett-Packard Model 5840A gas chromatograph equipped with an NPD and an electronic integrator. The column is coiled glass, 1.83 m × 2 mm I.D., packed with 5% OV-101 on 80—100 mesh Chromosorb W HP (Lot No. 17186; Supelco, Bellefonte, PA, U.S.A.). The carrier gas is ultra-high-purity helium (Matheson Gas Products, Gloucester, MA, U.S.A.) at a flow-rate of 30 ml/min. The detector purge is ultra-high-purity hydrogen (Matheson) at 3 ml/min mixed with dry air (Matheson) at 50 ml/min. Operating temperatures are: injection port, 310°C, column, 190°C, detector, 275 °C. Before being connected to the detector, a new column is conditioned at 270°C for 48 h with a carrier flow-rate of 30 ml/min.

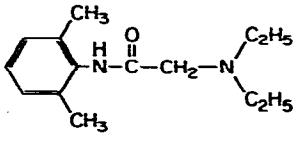
At the beginning of each working day, the column is primed with 2 μg phospholipid (asolectin) in benzene.

Reagents

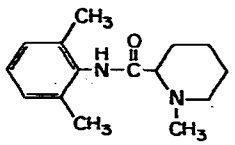
The following reagents are used: certified 99% pure *n*-hexane (Fisher Scientific, Fair Lawn, NJ, U.S.A.), analytical-reagent grade toluene (Mallinckrodt, St. Louis, MO, U.S.A.), certified isoamyl alcohol and analytical-reagent grade methanol (Fisher); analytical-reagent grade concentrated hydrochloric acid, analytical-reagent grade sodium hydroxide and analytical-reagent grade sodium carbonate and sodium bicarbonate all from Mallinckrodt. Isoamyl alcohol is glass distilled prior to use. Other organic solvents are used without further distillation. All aqueous solvents (0.25 *M* sodium hydroxide, 0.1 *M* hydrochloric acid, 1 *M* carbonate—bicarbonate buffer) are washed five times with hexane—isoamyl alcohol (98:2) prior to use.

Reference standards

Pure standards of lidocaine hydrochloride (Fig. 1) and metabolites monoethylglycinexylidide (MEGX) and glycinexylidide (GX) were kindly provided by Astra Pharmaceutical, Worcester, MA, U.S.A.). Mepivacaine hydrochloride (Fig. 1) was supplied by Sterling-Winthrop Research Institute (Rensselaer, NY, U.S.A.). Standards of each are prepared by dissolving the appropriate quantity of the hydrochloride salt to yield 100 mg base in 100 ml methanol. Sequential dilutions to 1 μg/ml are made. The solutions are stored in the dark in glass-stoppered bottles at 4°C and are stable for at least four months.



LIDOCAINE



MEPIVACAINE

Fig. 1. Structural formulae of lidocaine and the internal standard, mepivacaine.

Preparation of samples

Mepivacaine is used as the internal standard for all analyses. A 50-μl volume of stock solution (10 μg/ml), containing 500 ng mepivacaine, is added to a series of 15-ml round-bottomed glass culture tubes, with PTFE-lined screw-top caps. A 0.25—2.0 ml sample of unknown plasma is added to each tube. Calibration standards for lidocaine are prepared by adding 5, 10, 25, 50, 100, 200, 300, 400 and 500 ng of drug to consecutive tubes. Drug-free control plasma is added to each of the calibration tubes. One blank sample, taken from the subject prior to drug administration, is analyzed with calibration standards and each set of unknown samples.

Extraction procedure

A 1-ml volume of 0.25 *M* sodium hydroxide solution is added to each tube. To this is added 5 ml hexane—isoamyl alcohol (98:2) and the tubes are agitated gently in the upright position on a vortex mixer for 15 min. The samples are centrifuged at room temperature for 5 min at 400 *g* (Portable Refrigerated Centrifuge Model PR-2; Head No. 269, International Equipment, Boston, MA, U.S.A.). The organic layer is transferred to another 15-ml glass culture tube which contains 1.2 ml 0.1 *M* hydrochloric acid. This mixture is agitated gently in the upright position on a vortex mixer for 10 min. The samples are again centrifuged at room temperature for 5 min at 400 *g*. The upper, organic layer is discarded. The aqueous layer is transferred by a 9-in. pipet to a conical 13-ml screw-top centrifuge tube. Great care is taken to transfer only the aqueous layer uncontaminated with organic residue. To this 0.5 ml of 1 *M* carbonate—bicarbonate (pH 9.8) buffer is added. The final organic extraction is done by adding 300 μl toluene—isoamyl alcohol (85:15) to the conical centrifuge tube. This mixture is agitated gently in the upright position on a vortex mixer for 15 min. The samples are again centrifuged at room temperature for 5 min at 400 *g*. Using a 9-in. disposable pipet passed through the organic layer, the entire aqueous layer is removed leaving the small volume (300 μl) of organic phase containing lidocaine, metabolites, and internal standard. This is transferred to a 2-ml Wheaton automatic sampling vial (Wheaton Scientific, Millville, NJ, U.S.A.). A 6-μl aliquot of this is injected into the gas chromatograph using the automatic injection sampling system.

Single-dose pharmacokinetic study

Four healthy, young volunteers participated after giving written informed consent. While being electrocardiographically monitored, 25 mg lidocaine hydrochloride (Xylocaine) was administered by intravenous bolus infusion. The drug solution (10 mg/ml) was administered through a glass syringe. Multiple venous blood samples were drawn into Venoject heparin-containing tubes over the following 8 h. Concentrations of lidocaine were determined by the method described above.

Plasma lidocaine concentrations were analyzed by iterative weighted nonlinear least-squares regression analysis [13, 14]. After correction of the dose for the quantity of free base given, the following pharmacokinetic variables were determined: distribution half-life, elimination half-life, total volume of distribution, and total clearance.

RESULTS

Evaluation of the method

Under the described conditions, retention times for lidocaine and metabolites MEGX and GX are shown in Table I. The chromatogram of a plasma sample obtained 2 h after a subject received 25 mg lidocaine hydrochloride intravenously is shown in Fig. 2.

The relation between lidocaine concentrations and the area ratio (versus internal standard) is linear at least to 500 ng/ml. Analysis of more than 50 standard curves over a 6-month period indicates that the correlation coefficient is always 0.99 or greater. Day-to-day coefficient of variation in the slopes of the calibration curves was 8.1%.

The sensitivity limit of the method is 2.5 ng/ml of a 2-ml extracted plasma sample. Within-day coefficients of variation for identical samples were: at 500 ng/ml, 4.0% (n = 6); 200 ng/ml, 6.5% (n = 6); 100 ng/ml, 4.1% (n = 6); 50 ng/ml, 3.9% (n = 6); 25 ng/ml, 5.7% (n = 6); and 5 ng/ml, 1.0% (n = 4).

TABLE I

RETENTION TIMES OF LIDOCAINE, METABOLITES MONOETHYLGLYCINEXYLIDIDE (MEGX), GLYCINEXYLIDIDE (GX), AND MEPIVACAINE, USED AS THE INTERNAL STANDARD

Drug	Retention time (min)
Lidocaine	4.73
MEGX	3.98
GX	3.15
Mepivacaine	8.39

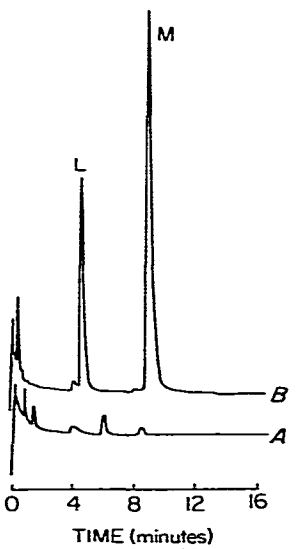


Fig. 2. Gas—liquid chromatograms of (A) extract of 1 ml plasma obtained from subject prior to receiving lidocaine and (B) 2 h after receiving 25 mg lidocaine hydrochloride intravenously. Peaks: L = lidocaine, M = mepivacaine (internal standard).

Residue analysis indicated the extraction of lidocaine is greater than 95% at low (25 ng/ml) and relatively high (200 ng/ml) lidocaine plasma concentrations.

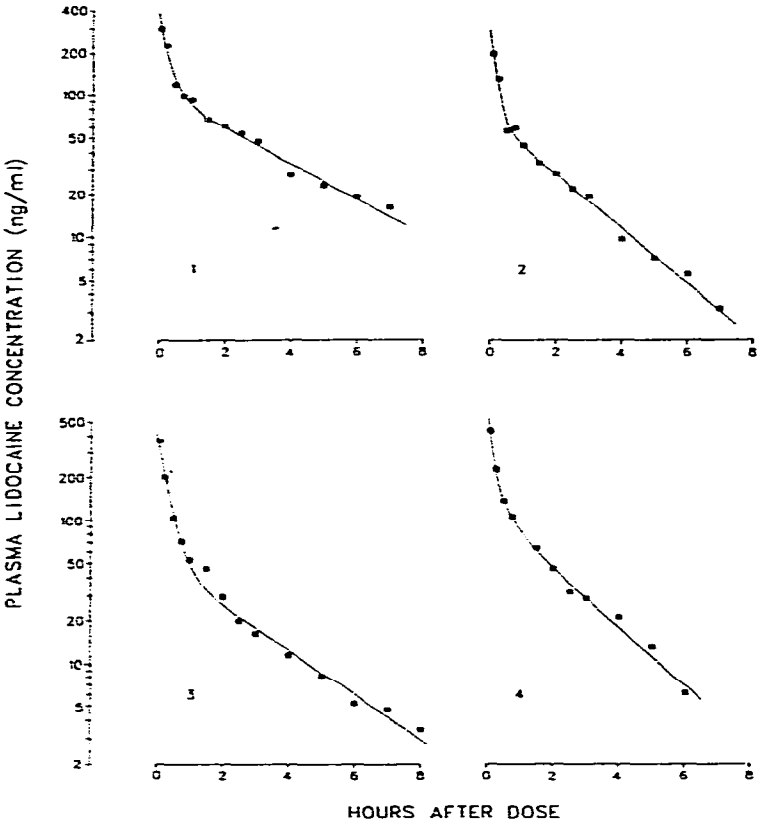


Fig. 3. Plasma lidocaine concentrations and pharmacokinetic functions following intravenous lidocaine administration to four healthy, young subjects. See Table II for derived kinetic variables.

TABLE II

DERIVED LIDOCAINE PHARMACOKINETIC PARAMETERS AFTER A SINGLE 25-mg INTRAVENOUS DOSE ADMINISTERED TO FOUR HEALTHY, YOUNG SUBJECTS

	Subject No.			
	1	2	3	4
Subject characteristics				
Age/sex	31/M	25/F	42/F	24/M
Weight (kg)	70.5	54.5	71.4	70.5
Lidocaine kinetic variables				
Distribution half-life (min)	4.4	12.6	10.6	7.4
Elimination half-life (h)	1.47	1.91	2.37	1.58
Central compartment volume (l/kg)	0.442	0.948	0.785	1.05
Total volume of distribution (l/kg)	1.86	4.29	2.38	3.64
Total metabolic clearance (ml/min/kg)	14.60	25.91	11.58	26.69

Pharmacokinetic study

Fig. 3 shows plasma lidocaine concentrations and pharmacokinetic functions for the subjects. Derived pharmacokinetic parameters are listed in Table II.

DISCUSSION

This report describes a reliable, specific method for the quantitation of lidocaine in plasma using GLC—NPD. Sensitivity is adequate to carry out single-dose pharmacokinetic studies with very low doses to permit study of factors which may influence first-order elimination processes of lidocaine in humans. A basic extraction from plasma, acidic back-extraction, subsequent adjustment of the aqueous phase to a basic pH, and final organic extraction into a small volume for direct injection into the gas—liquid chromatograph is the method employed. This method produces blank plasma samples that are consistently free of contaminants in the areas corresponding to the retention time for lidocaine and efficiently separates the known metabolites, MEGX and GX, from the parent drug.

The value of this GLC—NPD method includes the reasonable time required for sample preparation, as well as its sensitivity which is adequate for single low-dose pharmacokinetic studies.

ACKNOWLEDGEMENTS

We are grateful for the assistance of Christopher Willis, Ann Locniskar, Jerold S. Harmatz, Dr. Richard I. Shader, and the Staff of the Clinical Study Unit, New England Medical Center Hospital (Supported by USPHS Grant RR-24040).

This work was supported in part by Grant MH-34223 from the United States Public Health Service and by Grant Oc 10/6-3 from the Deutsche Forschungsgemeinschaft.

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