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Note

Determination of lidocaine in postmortem fluids and tissues

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Lidocaine has long been used as a local anesthetic[1], and in recent years it has been widely employed intravenously in the treatment of ventricular arrhythmias, especially in heart attack victims[1] in both coronary care units and in emergency room situations[2]. In 1980, in Los Angeles County Coroner's cases, lidocaine was found in the blood of 84 victims, including 17 drug overdose victims and 16 drug-related death victims. In most cases, the lidocaine was probably administered in an attempt to revive the persons and was not considered to be the cause of death or even a contributing factor in any case. Thus, lidocaine does not appear to be a drug of self-abuse.

However, in 1981 there was extensive controversy in Southern California concerning the suspected administration of lidocaine by hospital personnel in other counties to end the lives of very ill patients. It has, therefore, become extremely important in this region to determine accurately the postmortem levels of lidocaine to determine whether a therapeutic or overdose amount was given.

Hence there is great interest in the determination of lidocaine in biological material for toxicological and clinical laboratories. Several gas–liquid chromatographic (GC) methods using flame-ionization and nitrogen-sensitive detectors for the quantitative determination of lidocaine have been described[3–7]. Quantification of lidocaine and several metabolites utilizing chemical ionization mass spectrometry (MS) and high-performance liquid chromatography have also been described[8,9].

In this work we used computerized GC–MS to analyze for lidocaine. This is the most powerful tool for the detection and identification of drugs in biological systems at present and allows the greatest sensitivity, accuracy and specificity.

This method was used to determine the lidocaine levels in the tissue of six exhumed postmortem cases so that comparisons and correlations between these levels could be made.

Additionally, the method can be adapted for use with a gas chromatograph with nitrogen–phosphorus detection (NPD), which responds selectively to nitrogen and phosphorus in the molecules of a substance. The method is accurate and sensitive, as lidocaine contains two nitrogen atoms in its molecular structure.

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EXPERIMENTAL

Chemicals

Lidocaine hydrochloride was obtained from Astra Pharmaceutical Products and SKF-525A from Smith Kline and French Labs.

Sample preparation

Add 15 ml of biological fluid (blood, urine, etc.) or 15 ml of tissue homogenate [tissue–distilled water (1:1)] to a 250-ml bottle containing about 6 g of sodium hydrogen carbonate. Check the pH to make sure it is above 10. Add 200 ml of diethyl ether containing 0.375 μg/ml of internal standard (SKF-525A). Shake the bottle for 15 min on a shaker at a slow speed. Transfer the ether layer into a 250-ml separating funnel and discard the tissue or blood. Add 15 ml of 0.5 *N* sodium hydroxide solution and manually shake 100 times. Discard the bottom layer, add 25 ml of distilled water and shake 100 times. Filter the ether through No. 2 filter-paper into a clean separating funnel containing 3 ml of 2 *N* sulfuric acid and shake 200 times. Transfer the sulfuric acid extract into a 12-ml centrifuge tube, then place the tube in an oven at 100°C for 10 min. After the tube has cooled, add 0.7 ml of 40% sodium hydroxide solution for neutralization, and check the pH to make sure it is above 10. Then, add 0.2 ml of chloroform to the tube and vortex vigorously for 1 min. Centrifuge the tube, then inject 2 μl of the chloroform extract into the GC–MS system. Under the conditions used, lidocaine has a retention time of 1.12 min and SKF-525A 4.12 min. Alternatively, an aliquot of the extraction chloroform can be injected into the gas chromatograph–nitrogen–phosphorus detector.

Computerized GC–MS

A Finnigan 1020 automated computerized gas chromatograph–mass spectrometer equipped with a Nova 4-based Finnigan data system was used for the sample analysis. The experimental conditions were as follows: GC column, 1.8 m × 2 mm I.D. silanized glass column packed with 3% OV-1 on Gas-Chrom Q (100–200 mesh); carrier gas, helium at 18–20 ml/min; injector temperature, 250°C; column tempera ture, 230°C; and GC–MS separator temperature, 250°C.

The complete spectra were compared with stored spectra of drugs for identification and quantitation. The major ions are as follows for lidocaine, *m/e* 86; for norlidocaine, *m/e* 58; and for SKF-525A, *m/e* 86.

For sample analysis, inject 2 μl of chloroform containing the extracted lidocaine and SKF-525A. The peak areas are used for quantitation of positive results.

GC–NPD analysis

A Hewlett-Packard Model 5840A gas chromatograph equipped with a nitrogen–phosphorus detector was used for sample analysis. The experimental conditions were as follows: GC column, 1.8 m × 2 mm I.D. silanized glass column packed with 3% OV-1 on Gas-Chrom Q (100–120 mesh); carrier gas, helium at 30 ml/min; injector temperature, 250°C; column temperature, 223°C; and detector temperature, 300°C.

For sample analysis, inject 2 μl of chloroform containing lidocaine and SKF-525A. Peak heights are used for quantitation.

TABLE I

LIDOCAINE BLOOD LEVELS IN 74 CORONER'S CASES FOR 1979–81

Therapeutic level, 1.5–2.5 μg/ml; toxic level, 8–10 μg/ml; fatal level, 12 μg/ml.

Level (μg/ml)	*No. of cases*	*%*
0.01– 2.0	45	60.8
2.1 – 5.9	21	28.4
6.0 –10.0	5	6.8
⩾11.0	3	4.1

RESULTS AND DISCUSSION

Lidocaine can be readily determined in any type of biological sample at concentrations down to 0.1 μg/ml, and the method can readily detect lidocaine at fatal levels (12 μg/ml)[10], toxic levels (8–10 μg/ml)[11], therapeutic levels (1.5–2.5 μg/ml)[12] and even sub-therapeutic levels (Table I). SKF-525A is used as an internal standard both to monitor the extraction procedure and to provide a means of quantitation. The GC–NPD data (Table II) show that an excellent recovery is obtained (average 82.8%) and GC–MS data shows that the quantitation is linear up to at least 100 μg/ml (Fig. 1, Table III) and that excellent accuracy and precision of results are obtained (Table IV).

The GC–MS and GC–NPD methods thus yield excellent results for lidocaine determinations. Lidocaine is separated from other drugs by the extraction procedure, which removes opiates and acidic and neutral drugs, and the GC columns, which separate most other basic drugs, including SKF-525A internal standard (Fig. 2).

In the GC–MS procedure the distinctive lidocaine mass spectrum (Fig. 3) in the computer is compared with that produced by any peak near the lidocaine retention time. In the GC–NPD procedure only substances with nitrogen or phosphorus in their molecular structures are detected. Thus both methods yield almost no false positive results.

Table V illustrates the lidocaine levels in various tissues in six exhumed postmortem cases where the victims had received lidocaine prior to death. Low levels were found in the heart blood, brain, spleen, adipose tissue and kidney. The levels

TABLE II

LIDOCAINE RECOVERY STUDY

Extracted lidocaine standards compared with a non-extracted standard by GC–NPD.

Added lidocaine concentration (μg/ml)	*Number of samples*	*Mean measured lidocaine recovery (μg/ml)*	*Standard deviation (μg/ml)*	*Coefficient of variation (%)**	*Lidocaine recovery (%)*
10.0	7	8.229	0.174	2.11	82.29
20.0	7	16.679	0.577	3.46	83.39

* Coefficient of variation (C.V.) $= \frac{\text{standard deviation}}{\text{mean}} \times 100\%$.

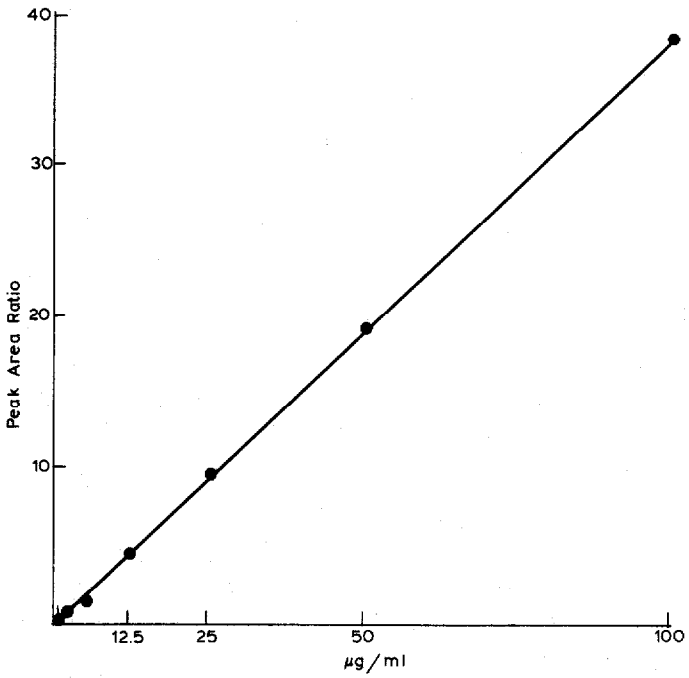

Fig. 1. Calibration graph for determination of lidocaine in blood by GC–MS.

in the spleen and heart blood were very close, as might be expected. Higher levels were found in the liver, lung and heart muscle. The relatively high concentration of the drug in the heart muscle, of course, may be partly responsible for the useful effects of the drugs in treating heart arrhythmias. In all cases, though, the bodies were embalmed and it is not clear how this affected the lidocaine contrations, but the effects would have been consistent from case to case (note that in a seventh

TABLE III

LIDOCAINE LINEARITY STUDY

Concentration of lidocaine in blood (μg/ml)	*Peak area of lidocaine/ peak area of SKF 525A*	*Standard deviation*	*C.V. (%)*
100 (7)*	38.04	2.34	6.17
50 (5)	18.46	0.78	4.25
25 (7)	9.60	0.33	3.46
12.5 (7)	4.10	0.11	2.82
6.25 (7)	1.51	0.06	4.16
3.12 (7)	0.85	0.05	5.98
1.56 (7)	0.40	0.014	3.56

* Number of times analyzed given in parentheses.

TABLE IV

ACCURACY AND PRECISION DATA FOR DETERMINATION OF LIDOCAINE IN BLOOD BY GC–MS (n = 7)

No.	*Concentration of lidocaine in blood (μg/ml)*	*Lidocaine concentration detected (μg/ml)*	*Detection (%)*	*Standard deviation (%)*	*C.V. (%)*
1	100.00	94.40	94.40	6.295	6.07
2	50.00	44.30	88.62	4.220	4.76
3	25.00	21.20	84.60	3.100	3.70
4	12.50	9.40	74.80	2.320	3.12
5	6.25	4.70	75.71	4.020	5.31
6	3.12	2.40	77.50	5.030	6.47
7	1.56	1.10	72.01	3.310	4.60

exhumed case, where no lidocaine had been administered, the fluid and tissues were analyzed and found to contain no lidocaine).

Norlidocaine (monoethyglycinexylidide), which is the desethylated, major metabolite of lidocaine[8], was searched for in the tissue analyses of each of the exhumed cases, but was not found. It has been found in some postmortem blood specimens, in 5 out of 100 cases found positive for lidocaine. It was not detected unless

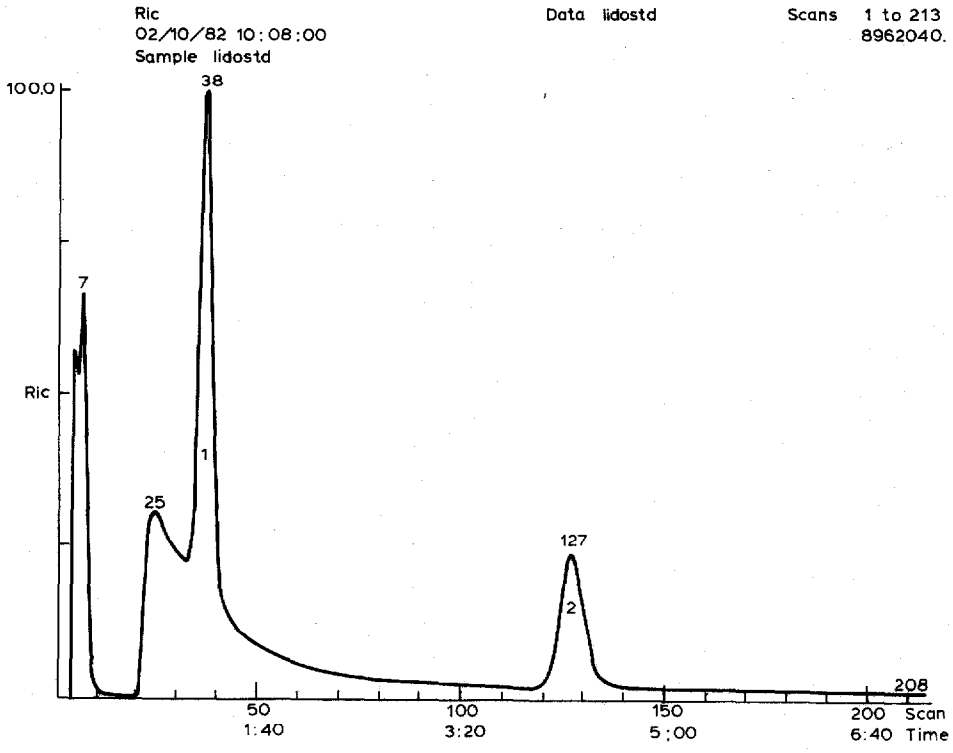

Fig. 2. Reconstructed ion chromatogram (RIC) of (1) lidocaine and (2) SKF-525A.

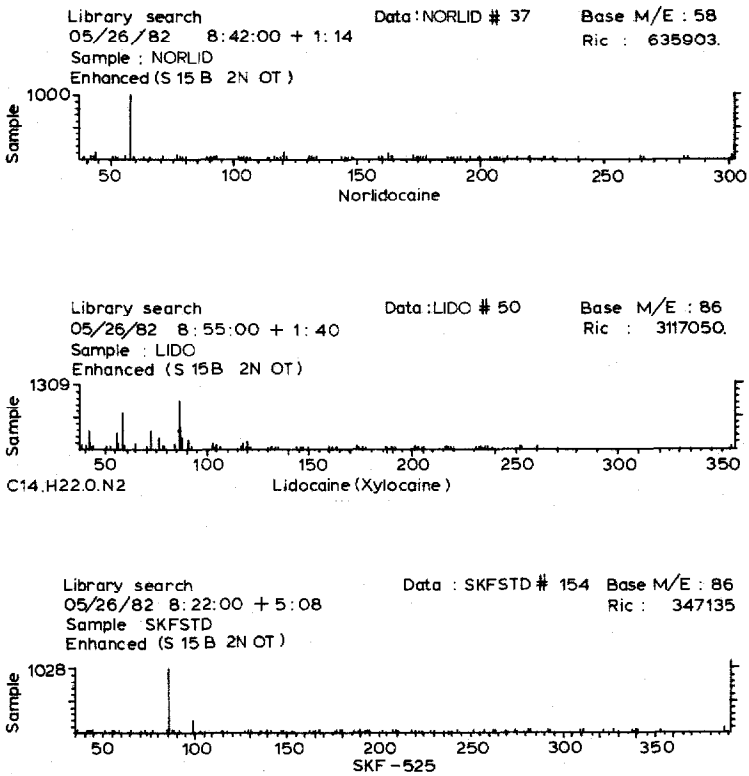

Fig. 3. Mass spectra of lidocaine (LIDO), norlidocaine (NORLID) and SKF-525A (SKFSTD).

TABLE V

COMPARISON OF THE CONCENTRATIONS OF LIDOCAINE AMONG POSTMORTEM TISSUES AND BODY FLUIDS FOR SIX EXHUMED CASES

Sample	*Case No.*					
	1	*2*	*3*	*4*	*5*	*6*
Blood*	–**	7.40	1.40	3.30	–	–
Brain	2.80	9.00	2.90	1.90	0.90	–
Liver	4.80	6.08	5.60	10.30	0.70	1.40
Heart muscle	5.80	10.44	0.88	4.00	–	–
Kidney	3.84	4.88	ND***	3.30	–	–
Lung	9.80	–	2.86	–	0.70	17.70
Spleen	4.25	6.02	0.94	3.10	–	0.30
Urine	–	–	0.30	–	–	–
Adipose tissue	2.30	2.80	ND	–	–	–
Stomach contents	0.204	–	Trace	–	–	–
Bile	ND	43.10	ND	–	–	–

* Clotted heart blood.
** – = Not available.
*** ND = Not detected.

TABLE VI

BLOOD LIDOCAINE AND NORLIDOCAINE LEVELS DETERMINED USING GC–NPD

Case No.	*Lidocaine level (μg/ml)*	*Norlidocaine level (μg/ml)*	*Lidocaine:norlidocaine ratio*
1	0.06	0.07	0.86
2	0.027	0.004	6.75
3	0.22	0.22	1.00
4	4.20	0.40	10.50
5	3.40	0.80	4.25

lidocaine was also present. Its concentration ranged from 0.004 to 0.8 μg/ml in blood and was generally less than that of the lidocaine (Table VI). It is not clear why it is present in such a low percentage of the lidocaine-positive cases.

Norlidocaine is readily detected by both the GC–MS and GC–NPD analysis procedures. It has a retention time slightly less than that of lidocaine (1.03 min compared with 1.12 min for lidocaine) on the 3% OV-1 GC–MS column.

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