High-Pressure Liquid Chromatographic Determination of Lidocaine and Its Active Deethylated Metabolites

JÉRÉMY HILL *, ANDRÉ ROUSSIN ‡, JACQUES LELORIER *‡x, and GILLES CAILLE *

Received January 22, 1980, from the **Department of Pharmacology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada,* and the *‡Department of Medicine, Hôtel-Dieu Hospital, Montreal, Quebec, Canada.* Accepted for publication May 2, 1980.

Abstract □ A simple, specific, high-pressure liquid chromatographic method for lidocaine and its active dealkylated metabolites, monoethylglycinexylidide (I) and glycinexylidide (II), is described. Recoveries were 94% for lidocaine, 98% for I, 77% for II, and 99% for the internal standard, ethylmethylglycinexylidide (III). The sensitivity of the assay is 0.04 μg/ml. Coefficients of variation for day-to-day replicate analyses of lidocaine and the metabolites were <7%.

Keyphrases □ Lidocaine—high-pressure liquid chromatographic analysis, human plasma □ High-pressure liquid chromatography—analysis, lidocaine and active dealkylated metabolites, human plasma □ Antiarrhythmics—lidocaine, high-pressure liquid chromatographic analysis, human plasma

Lidocaine, a widely used antiarrhythmic drug, undergoes extensive metabolism in the liver. Two of its principal metabolites, monoethylglycinexylidide (I) and glycinexylidide (II), often are found in significant concentrations in plasma and both possess pharmacological and toxicological properties (1–4). The pharmacokinetic parameters of lidocaine are affected by disease states (5–7) as well as by the duration of the infusion (6, 8, 9). The elimination of I was shown to be reduced in heart failure (6, 10), and that of II was reduced in both renal (11) and heart (6) failure.

The wide interpatient variability of plasma lidocaine, I, and II levels (5, 10, 12), the narrow margin between therapeutic and toxic plasma lidocaine levels, and the possible contribution of I and II to the therapeutic and toxic effects of lidocaine warrant routine monitoring of plasma lidocaine, I, and II levels in patients receiving lidocaine infusions.

A simple, rapid, accurate, and specific analytical method for quantitating lidocaine, I, and II in plasma by high-pressure liquid chromatography (HPLC) is presented.

EXPERIMENTAL

Materials—The amounts and concentrations of lidocaine[1], I[1], II[1], and ethylmethylglycinexylidide[1] (III) are expressed as the free base. Hydrochloric acid[2] was ultrex grade. Phosphoric acid[3], monobasic potassium phosphate[3], and methylene chloride[3] were reagent grade. Acetonitrile[3] was HPLC grade.

Apparatus—The analyses were performed on a liquid chromatograph equipped with a variable-wavelength UV detector[4]. Separation was carried out on an RP-8 10-μm column[5]. The instrumental conditions included a mobile phase of 18% acetonitrile in 0.1 *M* phosphate buffer adjusted to pH 3.2 at a flow rate of 2 ml/min, and the detector was set at 195 nm.

Procedure—To 2 ml of plasma contained in a culture tube were added 0.1 ml of 0.5 *N* NaOH and 50 μl of the internal standard (III, 5 μg/100 μl). The solution was vortexed for 30 sec. Following the addition of 2.5 ml of methylene chloride, the mixture was shaken for 10 min on a reciprocating shaker and then centrifuged at 3500 rpm. The aqueous phase was discarded; the organic phase was dried over anhydrous sodium sulfate and transferred to a second culture tube, to which was added 0.5 ml of 0.01 *N* HCl. The mixture was shaken for 60 min on a reciprocating shaker and centrifuged, and 150 μl of the aqueous phase was injected into the chromatograph.

Standard samples were prepared by spiking human plasma with increasing concentrations of lidocaine (0.1, 0.4, 0.8, 2, 3, 4, and 5 μg/ml) and the metabolites (0.04, 0.16, 0.32, 0.8, 1.2, 1.6, and 2 μg/ml). Calibration curves, which were identical whether prepared in plasma or in water, were constructed by plotting the ratios of the peak heights of lidocaine, I, and II to the peak height of the internal standard against the concentrations of lidocaine, I, and II.

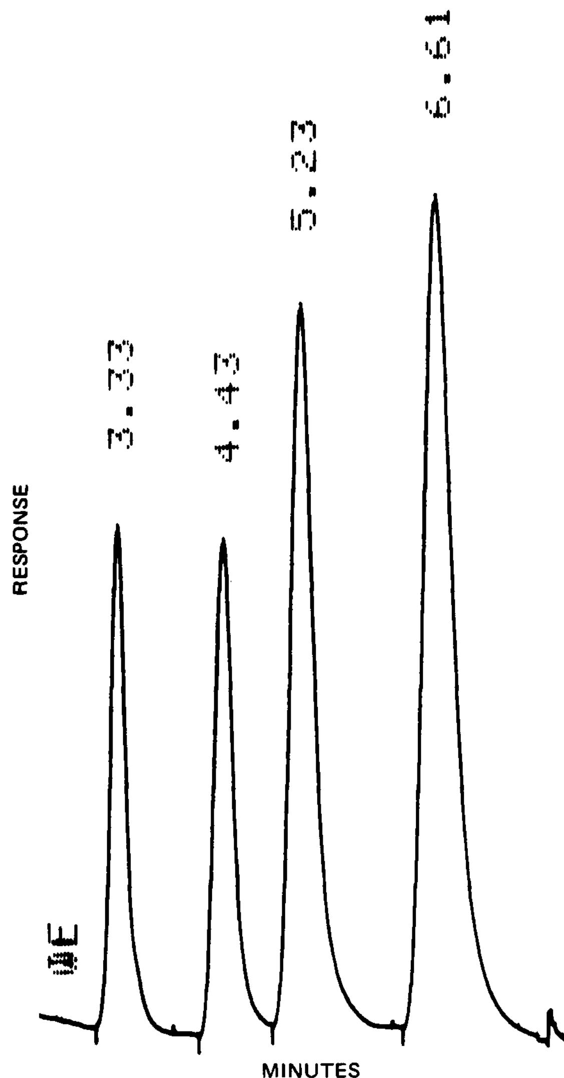

Figure 1—*Typical chromatogram. The retention times were 3.33 min for I (0.53 μg/ml), 4.43 min for II (0.60 μg/ml), 5.23 min for III, and 6.61 min for lidocaine (2.35 μg/ml).*

[1] Astra Pharmaceuticals.
[2] J. T. Baker Chemical Co.
[3] Fisher Scientific Co.
[4] Hewlett-Packard series 1084.
[5] Hewlett-Packard Co.

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Table I—Day-to-Day Reproducibility

Compound	Plasma Concentration, μg/ml	n	CV, %
Lidocaine	2.0	22	4.8
	0.8	11	3.0
I	0.8	22	2.2
	0.32	11	4.1
II	0.8	21	3.5
	0.32	10	6.8

Analytical recovery of lidocaine and I-III was determined by comparing the peak areas obtained by direct injection of standard aqueous solutions with those obtained from the injection of the same standard solutions after running them through the extraction procedure. Three samples were run at each of the seven different concentrations.

Blood samples were obtained from 75 patients receiving lidocaine for therapeutic or prophylactic indications and were analyzed by both the presently described method and by a GLC method (13).

RESULTS AND DISCUSSION

The standard curves were linear throughout the ranges studied (r^2 = 0.998, 0.999, and 0.998 for lidocaine, I, and II, respectively). The average ($\pm SD$) analytical recoveries were 94.3 ± 3.4% for lidocaine, 98.4 ± 4.1% for I, 77.2 ± 2.7% for II, and 99.7 ± 2.1% for III. The retention times were 3.33, 4.43, 5.23, and 7.01 min for II, I, III, and lidocaine, respectively. The day-to-day reproducibility of replicate analyses of standard plasma samples containing different concentrations of lidocaine, I, and II is presented in Table I. A typical chromatogram is shown in Fig. 1.

Concomitantly administered drugs included digoxin, propranolol, procainamide, nitroglycerin, carbamazepine, diazepam, flurazepam, isosorbide dinitrate, chlorpropamide, dipyridamole, allopurinol, methyldopa, furosemide, spironolactone, sulfinpyrazone, hydrochlorothiazide, hydralazine, morphine, aspirin, and oxazepam. None interfered with the assay. The sensitivity of the assay is 0.04 μg/ml, and a concentration as low as 0.01 μg/ml can be detected in the plasma.

The least-squares regression analysis of the data for the GLC method (X) *versus* the presently described method (Y) gave the following results: Y intercept, 0.08; slope, 0.99; r^2, 0.99; and S_Y, 0.57. These results suggest that the HPLC method is relatively free of significant systematic errors (14).

Plasma levels of II in one patient whose myocardial infarction was complicated by severe heart failure rose progressively, attaining 3.2 μg/ml on the 5th day of continuous lidocaine therapy. The corresponding lidocaine and I levels were 2.4 and 0.5 μg/ml, respectively. The infusion rate was 20 μg/kg/min, and there were no signs of lidocaine toxicity. The relatively low lidocaine concentration in this patient was surprising since previous work in this (9) and other (6) laboratories indicated that both heart failure and prolonged infusions should produce plasma lidocaine concentrations of 4 μg/ml at an infusion rate of 20 μg/kg/min.

The highest level of I (1.5 μg/ml) recorded was in another myocardial infarction patient with heart failure after 19 hr of continuous lidocaine infusion (35 μg/kg/min). In this patient, the plasma lidocaine concentration was 5.2 μg/ml and the concentration of II was 0.70 μg/ml. Immediately after the sample was taken, the infusion was ceased, and samples were taken hourly (Fig. 2). The lidocaine concentration fell exponentially to 0.16 μg/ml after 12 hr ($t_{1/2}$, 141 min), and the concentration of I also appeared to decline exponentially, reaching 0.23 μg/ml after 12 hr. The concentration of II was still 0.59 μg/ml 12 hr later. The half-lives cannot be determined for the metabolites from the slopes of their plasma disappearance curves since they are both synthesized from lidocaine and eliminated during the sampling period. This patient recently had suffered a stroke, rendering evaluation of possible neurological toxic effects difficult.

The elevated levels of metabolites in heart failure were reported by other investigators. Prescott *et al.* (6) reported progressively rising I and II levels in patients in whom the myocardial infarction was complicated by heart failure. Halkin *et al.* (10) found higher I levels in patients with heart failure but did not measure II levels. Narang *et al.* (15) described one patient with mild congestive heart failure whose I level was 183% of that of lidocaine (lidocaine, 1.38 μg/ml; I, 2.53 μg/ml), thus contributing more than unchanged lidocaine to the antiarrhythmic effect.

Few conclusions can be drawn concerning the relative contributions of I and II to the pharmacological and toxic effects of lidocaine therapy

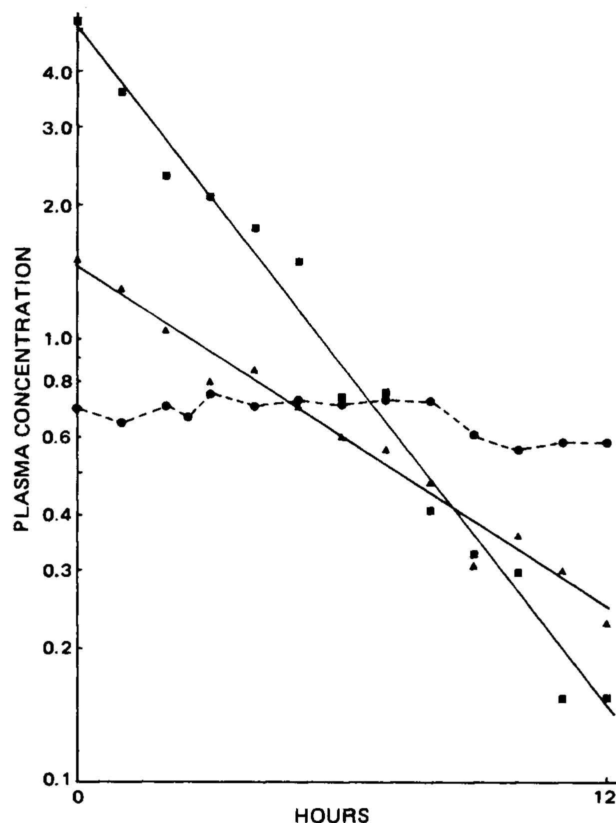

Figure 2—*Plasma concentrations of lidocaine (■), II (▲), and I (●) over the 12-hr period immediately following cessation of the lidocaine infusion.*

because their concentrations have been measured in only a limited number of patients. The availability of this method, as well as other HPLC and GLC methods (15–19) that simultaneously measure lidocaine, I, and II, should result in further studies of lidocaine and its active metabolites.

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Synthesis and Structure–Activity Relationship of a Pyrimido[4,5-*d*]pyrimidine Derivative with Antidepressant Activity

J. L. BERNIER *[x], J. P. HENICHART *, V. WARIN ‡, and F. BAERT ‡

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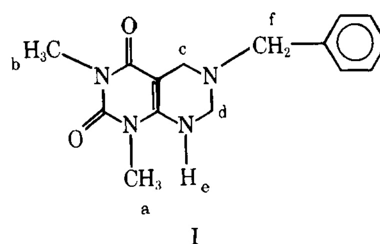
Abstract ☐ The synthesis and antidepressant properties of a new pyrimido[4,5-*d*]pyrimidine are described. Spectral data determined in solution and in the solid state allowed establishment of the relationship between the activity and the conformation of the molecule. The spatial structure seems to be in accordance with a possible binding at the presynaptic α-receptor sites.

Keyphrases ☐ Pyrimido[4,5-*d*]pyrimidines—synthesis and evaluation for antidepressant activity, binding at presynaptic α-receptor sites, structure–activity relationships ☐ Structure–activity relationships—pyrimido[4,5-*d*]pyrimidines, synthesis and evaluation for antidepressant activity, binding at presynaptic α-receptor sites ☐ Antidepressant activity—pyrimido[4,5-*d*]pyrimidines, synthesis and evaluation for antidepressant activity, binding at presynaptic α-receptor sites, structure–activity relationships

The basic assumptions in the analysis of a structure–activity relationship are that the drug and the receptor exhibit mutual complementarity and that the study of drug conformation yields information relevant to the geometry of the binding sites of the receptors. As a continuation of studies on 1,3-dimethyl-2,4-dioxo-6-substituted octahydropyrimido[4,5-*d*]pyrimidines (1), the particular behavior of the 6-benzyl derivative (I) was evaluated. This compound appears to possess significant antidepressant activity. Antidepressant drugs containing the pyrimido[4,5-*d*]pyrimidine ring system have not been described. In addition, the apparent importance of the benzyl moiety[1] in inducing such properties prompted the establishment of relationships between three-dimensional molecular structure and pharmacological response.

The mechanism of action of antidepressant drugs has been studied recently (2). It is generally accepted that the therapeutic effect of antidepressant agents may be a consequence of an increased availability of norepinephrine at postsynaptic sites. A 3-week administration of tricyclic antidepressant drugs gradually decreased the sensitivity of the presynaptic α-receptor (3), which would explain the delay in the onset of the clinical effect. On the other hand,



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the feedback inhibition of brain norepinephrine neurons by tricyclic antidepressants was due to an α-receptor mediation (4). This finding would explain the increase of the availability of norepinephrine at postsynaptic receptor sites after an antidepressant treatment. A correlation may be postulated between antidepressant activity and the action of the drug at the α-adrenergic receptors.

EXPERIMENTAL[2]

1,3-Dimethyl-2,4-dioxo-6-benzyl-1,2,3,4,5,6,7,8-octahydropyrimido[4,5-*d*]pyrimidine (I)—To an ethanolic solution of 9 g (0.06 mole) of 1,3-dimethyl-6-aminouracil were added successively 8.5 g (0.08 mole) of benzylamine and 12 ml of 35% aqueous formaldehyde (0.12 mole) solution. The mixture was stirred and heated under reflux for 2 hr and then concentrated *in vacuo* to dryness. The residual solid was washed with acetone and recrystallized from ethanol to give 14 g (81%) of white crystals. Further recrystallization from ethanol gave an analytical sample, mp 160°; IR(KBr pellets): 1630 and 1690 (C=O), 3150 (NH), and 2750 and 2810 (CH_2) cm^{-1}; PMR (dimethyl sulfoxide-d_6): δ 3.23 (s, 3H, CH_3a), 3.36 (s, 3H, CH_3b), 3.75 (s, 2H, CH_2c), 4.15 (d, 2H, CH_2d), 7.48 (t, 1H, J_{d-e} = 3 Hz, NHe), 3.56 (s, 2H, CH_2f), and 7.56 (s, 5H, phenyl g) ppm.

Anal.—Calc. for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.56. Found: C, 62.88; H, 6.33; N, 19.56.

Pharmacological Assays—Compound I was subjected to the classical tests for psychopharmacological effects. The central nervous system (CNS) activity was evaluated first as described by Irwin (5). Graded doses were given to groups of five mice, and the animals were observed con-

[1] Other pyrimido[4,5-*d*]pyrimidines with a 6-alkyl or aryl group were devoid of such activity (1).

[2] Melting points were determined with a Büchi capillary melting-point apparatus and are uncorrected. PMR spectra were obtained with a Jeol-MH 60 spectrometer using tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer 177 spectrometer. Crystallographic data were collected on a Philips PW 1100 diffractometer with graphite monochromated *M kα* radiation at room temperature. Elemental analyses were performed with a Perkin-Elmer CHN 240 instrument.

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