GLC Determination of Lidocaine in Human Plasma

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Abstract □ A specific, sensitive, rapid, and reproducible analytical GLC method for lidocaine in human plasma, including pharmacokinetic parameters, is described. Aminopyrine is the internal standard. The method was used to study pharmacokinetics in four healthy volunteers following the administration of a lidocaine bolus at a dose of 1 mg/kg iv and in one patient with cardiac arrhythmias who had been given a 50-mg bolus followed by a prolonged intravenous infusion for 30 hr.

Keyphrases □ Lidocaine—GLC analysis in human plasma, pharmacokinetics ■ GLC—analysis, lidocaine in human plasma ■ Pharmacokinetics—lidocaine in humans □ Anesthetics, local—lidocaine, GLC analysis in human plasma, pharmacokinetics

The administration of a lidocaine bolus (defined as a rapid intravenous injection) followed by a fairly long intravenous infusion has become a routine practice in the treatment of patients with myocardial infarcts. Yet, there is very little information concerning the pharmacokinetic behavior of lidocaine following a prolonged infusion, and this information is controversial. For example, some investigators (1, 2) observed a mean half-life of 90 min following an infusion of approximately 12 hr. However, after a prolonged infusion of more than 24 hr, half-lives of approximately 3.3 (3) and 10 (4) hr were found. The combined factors of this slower rate of elimination followed by hemodynamic changes in the patients and higher blood concentrations can increase the drug's toxicity.

To determine the pharmacokinetics of lidocaine following a prolonged infusion of more than 24 hr, a specific and reproducible analytical technique that provides an accurate picture of what occurs in the body is necessary. For this reason, the present paper emphasizes the analytical data, whereas subsequent studies will concentrate on the metabolism, pharmacokinetics, and distribution of lidocaine in humans and animals.

Several equally valid analytical methods (6–9) previously used GLC and an internal standard for quantitative determinations in biological media. However, none of these reports discussed possible interference from pharmacological agents given concomitantly with lidocaine, such as sedatives, opiates, diuretics, bronchodilators, and antispasmodics. Therefore, the aim of this study was to develop a simple and rapid analytical method for lidocaine that would remain specific in the presence of drugs routinely administered to patients with ventricular arrhythmias.

EXPERIMENTAL

Materials—Lidocaine[1] and aminopyrine[2] were not less than 98% pure. All reagents used for extraction were analytical grade[3]. Freshly drawn human blood[4], 300 ml, was centrifuged, and the plasma was analyzed initially to make certain that it contained no interfering substances.

Subjects—Four healthy volunteers whose body weight did not vary more than ±10 kg from the mean received a 1-mg/kg bolus of lidocaine. Heparinized blood samples were collected at 0.25, 0.50, 1, 2, 3, 4, and 6 hr following drug administration.

Apparatus—The determinations were performed by GLC using a flame-ionization detector[5]. A 1.8-m, 80–100-mesh, 3% OV-17 Gas Chrom Q column was used. The column, detector, and injection port temperatures were 210, 275, and 250°, respectively. The nitrogen gas flow rate was 56 ml/min.

Extraction Procedures—Distilled water, 2 ml, and 0.5 ml of 5 *N* NaOH were added to 2 ml of plasma containing 25 μg of aminopyrine internal standard in a 50-ml test tube. The mixture was shaken for 30 sec using a test tube mixer. Following the addition of 10 ml of methylene chloride, the sample was shaken for 5 min mechanically and centrifuged at 2000 rpm. After being extracted a second time, the organic phases were combined and dried over anhydrous sodium sulfate. The organic phase was then evaporated to dryness under nitrogen, and the residue was redissolved in 0.5 ml of chloroform and transferred into a 1-ml conical test tube. The solution was again evaporated to dryness under nitrogen, and the residue was dissolved in 50 μl of chloroform. Aliquots of 1.5 ± 0.2 μl were then analyzed.

The standard plasma samples were prepared by adding quantities equivalent to 0.5, 1.25, 2.5, 5, 12.5, and 25 μg of lidocaine to the internal standard of each tube. The retention times for lidocaine and the internal standard were 4.45 and 6.70 min, respectively.

The calibration curves were constructed using the ratio of the areas under the lidocaine and aminopyrine curves along the ordinate and the concentrations of lidocaine along the abscissa.

RESULTS AND DISCUSSION

The ratio of the areas under the lidocaine and aminopyrine curves was linear for concentrations from 0.10 to 12.5 μg/ml of plasma, concentrations normally found during treatment. The standard curves *in vitro* and following the lidocaine extraction from the plasma could be superimposed on each other; the slopes corresponded to an r^2 of 0.999, indicating that the extraction of lidocaine and aminopyrine was reproducible for the different concentrations studied.

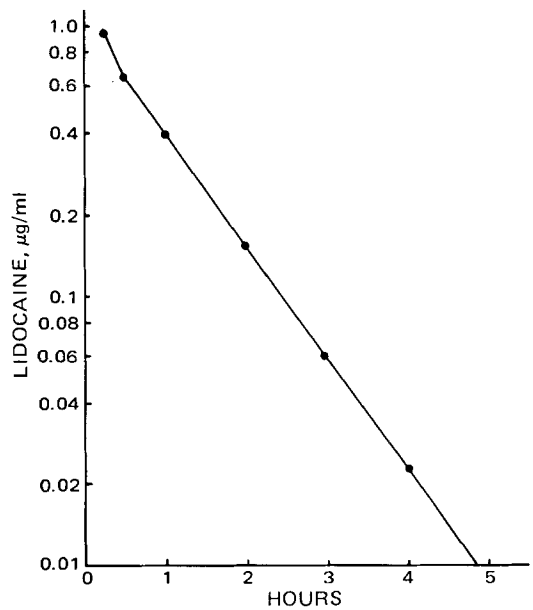

Figure 1—*Mean pharmacokinetic profile of lidocaine in a healthy volunteer after a rapid intravenous administration of a 1-mg/kg bolus.*

[1] Astra Pharmaceutical Products.
[2] British Drug House Chemicals.
[3] Fisher Scientific Co.
[4] American Red Cross.
[5] Model 5830-A, Hewlett-Packard, Montreal, Canada.

Table I—Plasma Lidocaine Concentrations (μg/ml) in Healthy Volunteers following Administration of a 1-mg/kg iv Bolus

Subject	Weight, kg	Hours[a] 0.25	0.50	1	2	3[b]	4[b]
A	52.16	1.15	0.60	0.40	0.15	0.06	0.02
B	68.04	0.85	0.69	0.50	0.22	0.10	0.04
C	62.00	0.82	0.59	0.28	0.11	0.04	0.01
D	54.34	1.01	0.71	0.41	0.14	0.05	0.01
Mean ± *SE*	59.14 ± 3.65	0.96 ± 0.08	0.65 ± 0.03	0.40 ± 0.03	0.16 ± 0.02	0.06 ± 0.01	0.02 ± 0.01

[a] At 6 hr postadministration, a 5-ml plasma sample was used and no lidocaine was detected. [b] A 5-ml plasma sample was used at these intervals.

Each point on the curves represents the mean of five different samples. Total recoveries of lidocaine and aminopyrine from the plasma were 99.04 ± 6.5 and 98.60 ± 4.2%, respectively.

This analytical method was specific, since none of the drugs administered concomitantly with lidocaine interfered. These drugs were propranolol, procainamide, furosemide, insulin, aspirin, methyprylon, cephalexin, digoxin, phenobarbital, quinidine, diazepam, meperidine, phenytoin, diphenhydramine, acepifylline, isosorbide, morphine, spironolactone, nitroglycerin, atropine, flurazepam, allopurinol, and hydrochlorothiazide. Several were eliminated during the extraction procedure in an alkaline medium; others, such as the benzodiazepines and narcotics, appeared on the chromatogram only with much higher column temperatures. The temperature of the column must be raised to 250° for 10 min after each determination to eliminate any interfering substances from the column.

The pharmacokinetic differences in the plasma clearance of lidocaine given as a rapid intravenous injection to healthy volunteers or as a rapid intravenous administration followed by a prolonged infusion of 24 hr or more to a patient suffering from ventricular arrhythmia were studied using the developed method. The pharmacokinetic behavior of lidocaine in both cases conformed to the two-compartment open model represented by the following equation (10):

$$C_{p(t)} = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

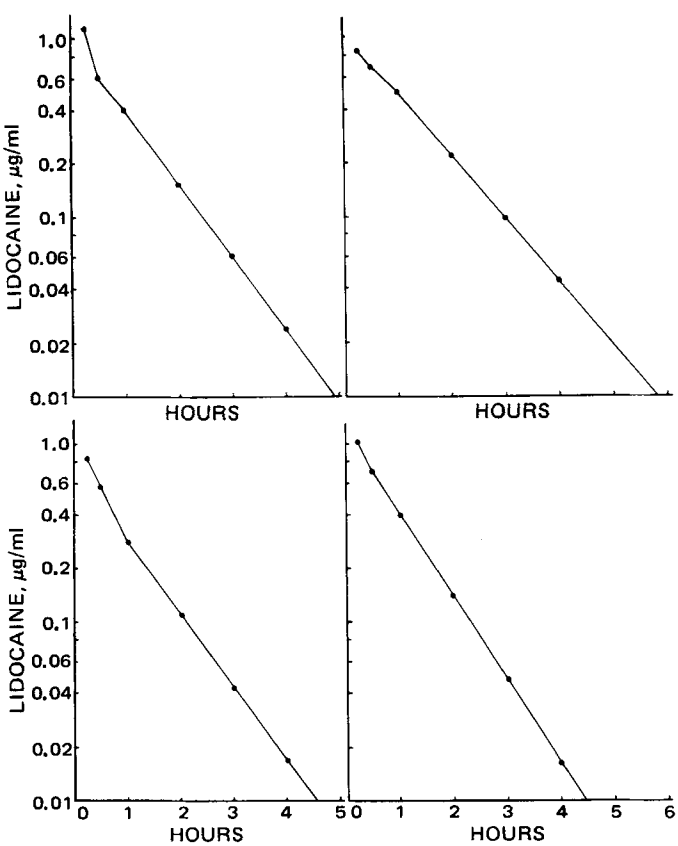

Figure 2—*Pharmacokinetic profile of lidocaine in four healthy volunteers after a rapid intravenous administration of a 1-mg/kg bolus. Key: top left, Subject A; top right, Subject B; bottom left, Subject C; and bottom right, Subject D.*

One can assume that all of the drug is in the central compartment, V_1, at the time of injection, so that:

$$V_1 = \frac{\text{dose}}{C_p{}^0} \qquad \text{(Eq. 2)}$$

Also it is assumed that its elimination is quantitative beginning with the central compartment and that an equilibrium is established between the central compartment and the peripheral tissue compartment, V_2:

$$V_1 + V_2 = V_{d_{ss}} = \frac{K_{12} + K_{21}}{K_{21}} V_1 \qquad \text{(Eq. 3)}$$

In Eq. 1, α and β are hybrid rate constants permitting the calculation of the plasma half-lives of phases α and β by the following equations:

$$T_{p1/2(\alpha)} = 0.693/\alpha \qquad \text{(Eq. 4)}$$

$$T_{p1/2(\beta)} = 0.693/\beta \qquad \text{(Eq. 5)}$$

where α is determined by the method of residuals, and β is determined by linear regression of a monoexponential equation.

The pharmacokinetics for the healthy volunteers were calculated using the mean results shown in Table I. The pharmacokinetic parameters in healthy volunteers following the administration of a bolus are fairly well known, and the results confirm the published data (11). However, since the α-phase is very short (less than 10 min) and the first sampling time was at the 15-min mark, a rather large error was introduced in its estimation. Experimentally, an α of $5.99^{h^{-1}}$, which gives a half-life of 7 min for this period, was obtained.

The β-phase was calculated to be $0.95^{h^{-1}}$, and the half-life for this phase of lidocaine clearance was 44 min. Figures 1 and 2 illustrate the individual and mean lidocaine elimination curves for the four healthy volunteers.

Figure 3 and Table II illustrate the response of a patient who received a 50-mg bolus of lidocaine by the intravenous route, followed by a prolonged infusion over 30 hr at 120 mg/hr. Two weeks after his first attack, the patient was readmitted to the hospital and given the same treatment but only for 24 hr.

The results presented in Fig. 3 demonstrate both the excellent repro-

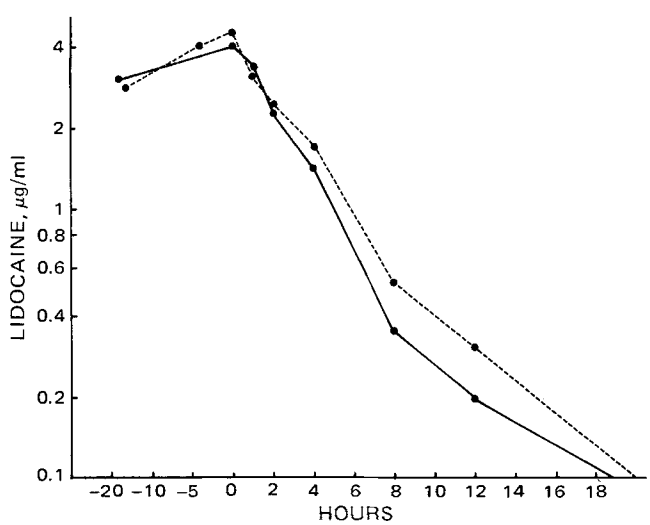

Figure 3—*Pharmacokinetic profile of a slow lidocaine infusion in a hospitalized patient. The two infusions, 120 mg/hr for 30 (—) and 24 (- - -) hr, respectively, were 2 weeks apart.*

Table II—Pharmacokinetics of Lidocaine in a Patient following a Prolonged Infusion[a]

Hours	First Infusion, μg/ml	Second Infusion, μg/ml
−29.45	0	0
−17.45	2.98	2.85
0	4.04	4.55
1	3.39	3.15
2	2.28	2.39
4	1.43	1.72
8	0.35	0.54
12	0.20	0.31
19	0.10	—
20	—	0.10
23	N.D.[b]	N.D.

[a] The first and second infusions were separated by 2 weeks. [b] N.D. = not detectable.

ducibility of this analytical method and the difference in the pharmacokinetic parameters of lidocaine following a prolonged infusion. The two phases, α and β, and their values were virtually identical for the two slow infusions. The first infusion resulted in a $T_{p1/2}(\alpha)$ of 2.08 hr and a $T_{p1/2}(\beta)$ of 6.18 hr; the second infusion resulted in a $T_{p1/2}(\alpha)$ of 2.14 hr and a $T_{p1/2}(\beta)$ of 4.93 hr.

Therefore, it can be concluded that the analytical method described confirms that the pharmacokinetic parameters of lidocaine are identical for any given subject on the same treatment. However, compared to the healthy volunteers, the pharmacokinetic parameters were quite different. A true equilibrium was not reached, since the drug concentrations increased continuously during the infusion. This phenomenon can be explained by the fact that lidocaine inhibits its own metabolism *via* its acetylated metabolite (5); moreover, its renal excretion is slower, due to a reduction in the cardiac inotropic form and rhythm which significantly reduce blood flow. The marked lengthening of the α- and β-phases indicates that considerable caution should be exercised when lidocaine is administered by slow infusion, because the toxic effects with lidocaine are greater when the blood levels are too high.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 17, 1976, from the *Department of Pharmacology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada, and the ‡Department of Medicine, Hotel-Dieu Hospital, Montreal, Quebec, Canada.

Accepted for publication September 16, 1976.

Supported by a grant from the MacDonald Stewart Foundation.

The authors thank Mrs. M. Pharand for technical assistance and the nurses and residents of the Coronary Care Unit of the Hotel-Dieu Hospital, Montreal, for cooperation.

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Kinetics and Mechanism of Blue Tetrazolium Reaction with Corticosteroids

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Abstract □ The kinetics of the reaction of blue tetrazolium with corticosteroids were investigated under pseudo-first-order conditions. The reaction rates for various corticosteroids were determined at various temperatures, and the enthalpy and entropy of activation for these compounds were determined. A mechanism is proposed in which an electron pair and a proton are transferred to blue tetrazolium from the anion formed by the action of tetramethylammonium hydroxide on the α-carbonyl moiety of the corticosteroid. The proposed mechanism is consistent with previous experimental results.

Keyphrases □ Blue tetrazolium—kinetics of reaction with various corticosteroids, effect of temperature, mechanism proposed □ Corticosteroids, various—kinetics of reaction with blue tetrazolium, effect of temperature, mechanism proposed □ Glucocorticoids, various—kinetics of reaction with blue tetrazolium, effect of temperature, mechanism proposed

Various forms of the blue tetrazolium reaction have been used for the quantitative determination of corticosteroids (1–4). The most widely used method is a slightly modified procedure of Mader and Buck (2), which is the official USP (5) and NF (6) method.

In strongly alkaline solution, blue tetrazolium [3,3′-[3,3′ - dimethoxy(1,1′-biphenyl)-4,4′-diyl]bis(2,5-diphenyl-2*H*-tetrazolium) dichloride] (I) oxidizes the α-carbonyl moiety of the C-17 side chain of the corticosteroid and is reduced quantitatively to a highly colored formazan whose concentration is measured spectrophotometrically. Extensive investigations (1, 3, 4, 7–16) of the reaction conditions established that the analytical procedure is subject to many variables, which are minimized by concurrently analyzing blank, standard, and sample.

Certain kinetic aspects of the blue tetrazolium reaction were reported, *e.g.*, the corticosteroid reaction is first order (17). Significant variations in the reaction rates of corticosteroids of closely related structures also were noted (1, 9, 14, 15, 18, 19). The reaction rate of corticosteroids with I was inversely related to the dielectric constant of the