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Note

High-performance liquid chromatographic method for the simultaneous determination of lidocaine and its N-dealkylated metabolites in plasma

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Lidocaine (I) has been used for many years in coronary care units for the prevention and treatment of cardiac arrhythmias. Therapeutic and toxic responses to I appear to be related to its plasma concentration [1]. Therefore, it may be desirable that plasma levels of I be monitored in those patients who receive the drug; this would be particularly true if therapy is protracted, toxicity or failure of therapy is suspected, or if the patient has concurrent pathology which is known to alter the disposition of the drug [2]. However, the two dealkylated metabolites of I, namely monoethylglycinexylidide (II), and glycinexylidide (III), possess some pharmacological and toxicological activity [3, 4], and these compounds should therefore be included in the design of analytical methods which are intended to be used for plasma level monitoring as an aid to patient care [5]. Lidocaine and its metabolite (II) have been used as markers in pharmacokinetic studies [6, 7].

A large number of gas chromatographic and gas chromatographic—mass spectrometric methods have been described for the quantitation in plasma of I alone [8—14], and of I together with one (II) [15, 16], or both [17—19] of its dealkylated metabolites. A high-performance liquid chromatographic (HPLC) assay for I in plasma has been reported [20]. In that method I was extracted from the plasma, together with added procaine which served as an internal standard, by a charcoal adsorption technique. The chromatographic separation was subsequently carried out on an octadecyl reversed-phase column, and detection was by ultraviolet absorption. The lower limit of quantitation was 0.1 μg/ml using a 1-ml plasma sample. The method did not

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include quantitation of II and III, and the report of the method [20] did not state whether or not these metabolites of I were separated from the parent drug.

The purpose of the present paper is to describe a rapid, sensitive and specific HPLC method for the simultaneous quantitation of I, II, and III in a small volume of plasma. The method may be useful for routine clinical monitoring, or for pharmacokinetic studies in animals and humans.

EXPERIMENTAL

Reagents

Lidocaine (I), monoethylglycinexylidide (II), glycinexylidide (III), and ethylmethylglycinexylidide, all as their hydrochloride salts, were donated by Astra (Worcester, Mass., U.S.A.). Amounts and concentrations of these compounds are expressed as the salts, unless otherwise noted. Glass-distilled ethyl acetate and acetonitrile (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.), phosphoric acid, sulfuric acid (Fisher Scientific, Fair Lawn, N.J., U.S.A.), and sodium hydroxide (J.T. Baker, Phillipsburg, N.J., U.S.A.) were obtained commercially. A 0.006% solution of phosphoric acid in distilled water was prepared (final pH 2.8), and passed through a 0.45-μm membrane filter (Millipore, Bedford, Mass., U.S.A.) for subsequent use in the preparation of the HPLC mobile phase. An aqueous solution with a pH of 2.2 was prepared by dilution of sulfuric acid with distilled water.

HPLC system

The HPLC system consisted of a Model M-6000A pump, a Model U6K sample injector, a μBondapak alkyl phenyl reversed-phase column (30 cm × 3.9 mm I.D., 10-μm particle size) obtained from Waters Assoc., (Milford, Mass., U.S.A.), and a Model LC55 variable wavelength HPLC ultraviolet detector (Perkin-Elmer, Oakbrook, Ill., U.S.A.). The output from the detector was connected, via an attenuator, to a 25.4 cm recorder which had 1 and 10 mV calibrated positions (Linear Instruments, Irvine, Calif., U.S.A.).

The mobile phase for the chromatographic separation was prepared by mixing 30 parts of acetonitrile with 70 parts of 0.006% phosphoric acid solution. This mobile phase was pumped through the HPLC system at 2 ml/min, which resulted in a pump pressure of approximately 1800 p.s.i.g. The separation was carried out at ambient temperature (approximately 24°). The HPLC detector was operated at a wavelength of 200 nm, and the recorder chart speed was 0.33 cm/min.

Procedure

Preparation of plasma samples. To a 500-μl aliquot of plasma contained in a culture tube (13 × 100 mm) were added 2 μg of internal standard in an aqueous solution (ethylmethylglycinexylidide hydrochloride; 20 μg/ml), 100 μl of 1 *M* NaOH, and 3 ml of ethyl acetate. The tube was closed with a screw cap and vortexed for 1 min to promote mixing of the immiscible phases. After centrifugation (800 *g*; 2 min), most of the ethyl acetate was transferred by disposable pipet to a 12-ml tube which had a tapered base, and which con-

tained 0.1 ml of dilute sulfuric acid solution (pH 2.2). This tube was capped, and the contents were vortex-mixed for 1 min followed by brief centrifugation as described above. Approximately 20—30 μl of the lower aqueous phase were injected into the chromatograph.

Plasma assays were also performed using 100-μl aliquots of plasma together with 0.5 μg of internal standard (1 μg/ml aqueous solution) in the first extraction; all other conditions were as described above for the analysis of 500-μl aliquots of plasma.

Standard curves were prepared by spiking blank human plasma with I, II, and III, followed by extraction and chromatography as described above. The ratios of the peak heights of I, II, and III, to that of the internal standard, were plotted against the concentrations of I, II, and III, respectively.

For reproducibility studies one 5-ml aliquot of blank human plasma was spiked with I, II, and III at 0.5 μg/ml and another aliquot handled correspondingly at 2.0 μg/ml, with replicates (500 μl only) analyzed for I, II, and III at each concentration.

RESULTS AND DISCUSSION

Chromatograms resulting from the HPLC analysis of 500-μl aliquots of plasma which had been spiked with I, II, and III, together with a chromatogram of a similarly analyzed blank plasma sample, are shown in Fig. 1. The retention times of I, II, and III were 10.0, 7.4, and 5.9 min, respectively, and the internal standard had a retention time of 8.3 min. The drug, its dealkylated metabolites, and the internal standard were eluted as symmetrical peaks which were satisfactorily resolved from each other and from endogenous components in the plasma; no interfering peaks were observed when the blank plasma was analyzed. The total analysis time of a single plasma sample was approximately 18 min.

The standard curve for (I) in the concentration range from 0.1 to 10 μg/ml in plasma, using 500-μl aliquots of plasma, is shown in Fig. 2. Standard curves for (II) and (III) were prepared over the same concentration range but are not shown. Each standard curve was apparently linear at low concentrations but downward curvature occurred with increasing concentration. Such an effect could conceivably result from a non-linearity in the extraction steps during sample preparation, or in chromatographic separation. Injection into the HPLC of given amounts (0.025 to 0.3 μg) of I, II, and III in 20-μl aliquots of aqueous solution resulted in similarly curved standard curves when the peak height of each compound was plotted against amount injected. Since the injection volume was constant in this experiment, and no extractions were performed, the observed curvature of these standard curves may have resulted from a non-linearity of the distribution coefficients of the compounds during the HPLC separation. A close inspection of chromatograms (Fig. 1), indicates a small but obvious broadening of the peaks of I, II, and III, as the concentration of these compounds in plasma increased. The peak broadening, with resultant curvature of standard curves based on peak height ratio, was also observed when a different column, of the same type, was tested. The experimental data were well described by quadratic equations (Fig. 2) with r^2 values of 0.9968,

Fig. 1. Chromatograms resulting from the analysis of 500-μl aliquots of blank human plasma (A), and plasma which had been spiked with I, II, and III, each at a concentration of 1 μg/ml (B), and 10 μg/ml (C).

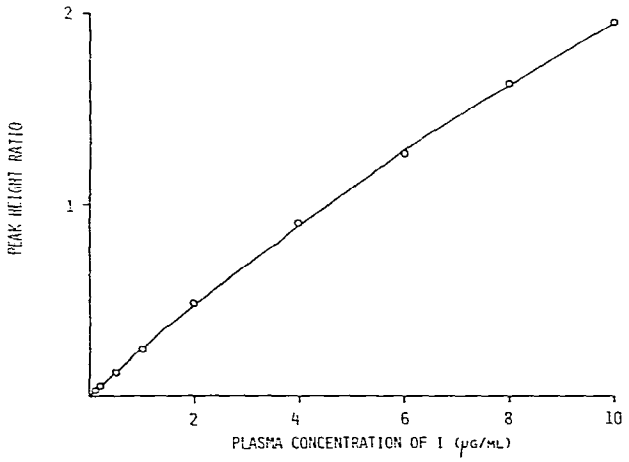

Fig. 2. Plot of the ratio of peak height of lidocaine (I) to that of the internal standard versus concentration of I in plasma, using 500-μl aliquots of plasma. $y = (-0.00428)x^2 + (0.2378)x + (0.00515)$, $r^2 = 0.9968$.

0.9998, and 0.9996 for the standard curves of I, II, and III, respectively. Linear calibration curves may result if peak areas are used to prepare standard curves.

Standard curves for I, II, and III using 100-μl aliquots of plasma were prepared over the concentration range from 0.5 to 10.0 μg/ml (Table I). These standard curves were apparently linear probably because smaller amounts of compounds were injected onto the HPLC column.

Based on a signal-to-noise ratio of 3:1, the lower limit of quantitation of I, II, and III using 500 μl of plasma was approximately 0.02 μg/ml; for 100 μl of plasma the corresponding value was 0.1 μg/ml. The reproducibility of the method at concentrations of 0.5 μg/ml and 2.0 μg/ml of each compound in plasma, using 500-μl aliquots, is summarized in Table II.

The extraction work-up procedures that are used to prepare plasma samples for HPLC analysis are relatively simple and rapid. The extraction of basified plasma with ethyl acetate followed by back extraction of the organic layer with a small volume of aqueous acid serves to provide a degree of sample clean-up. In addition, no evaporation steps are necessary because sufficient concentration of the samples can be obtained by using a small volume of acid for the back extraction step. The recoveries of I, II, and III were determined by comparing the chromatographic peak height of each compound from extracted 500-μl plasma aliquots with the corresponding peak height from an aqueous solution

TABLE I

STANDARD CURVES FOR I, II, AND III IN PLASMA USING 100-μl ALIQUOTS OF PLASMA FOR ANALYSIS

Results obtained are for single determination. Linear regression equations: I $y = 0.1778x - 0.00326$, $r^2 = 0.9989$; II $y = 0.2717x - 0.0178$, $r^2 = 0.9999$; III $y = 0.3100x - 0.0304$, $r^2 = 0.9996$. PHR = ratio of peak height of the compound to that of the internal standard; response factor = peak height ratio divided by the concentration of the compound in the sample.

Concentration in plasma (μg/ml)	I		II		III	
	PHR	Response factor	PHR	Response factor	PHR	Response factor
0.5	0.078	0.156	0.119	0.238	0.134	0.268
1.0	0.172	0.172	0.259	0.259	0.293	0.293
2.0	0.360	0.180	0.519	0.260	0.580	0.290
5.0	0.912	0.182	1.33	0.266	1.48	0.296
7.5	1.29	0.172	2.04	0.272	2.32	0.309
10.0	1.79	0.179	2.69	0.269	3.07	0.307

TABLE II

REPRODUCIBILITY DATA USING 500-μl ALIQUOTS OF PLASMA

PHR = peak height ratio; S.D. = standard deviation; C.V. = coefficient of variation.

Compound	0.5 μg/ml ($n = 7$)			2.0 μg/ml ($n = 8$)		
	Mean PHR	S.D.	C.V.	Mean PHR	S.D.	C.V.
I	0.117	0.0059	5.0	0.474	0.0085	1.8
II	0.174	0.012	6.9	0.688	0.012	1.7
III	0.185	0.014	7.6	0.744	0.029	3.9

of the compounds which had not been extracted; correction was made for volume changes. The mean (± S.D.) percentage recoveries (n = 5) at 6 μg/ml of each compound in plasma were 87.4 ± 2.4%, 86.4 ± 1.6%, and 62.3 ± 0.8% for I, II, and III, respectively.

Chromatograms resulting from the analysis of plasma samples collected from patients who were receiving lidocaine infusions are shown in Fig. 3. The initiation of lidocaine therapy had occurred 6, 30, and 24 h prior to collection of these plasma samples for patients A, B and C, respectively. Lidocaine infusion rates were 1—3 mg/min. The other medications being taken by these patients were procainamide, propranolol, potassium, diazepam, and morphine. The concentrations of I, II, and III in the plasma from these patients (Fig. 3) are of the same order as those previously reported in patients receiving lidocaine infusions [18].

Plasma samples collected from a number of patients who were receiving a variety of other drugs, but not lidocaine, were analyzed. The drugs known to have been taken by these patients included ampicillin, chlorthalidone, digoxin, furosemide, hydrocortisone, metaproterenol, methyldopa, penicillin, prednisone, terbutaline, and theophylline. No interfering peaks in the HPLC chromatograms were observed from the analysis of any of these plasma samples. Patients in clinical settings may concurrently receive lidocaine and another antiarrhythmic agent. Therefore, propranolol, 4-hydroxypropranolol,

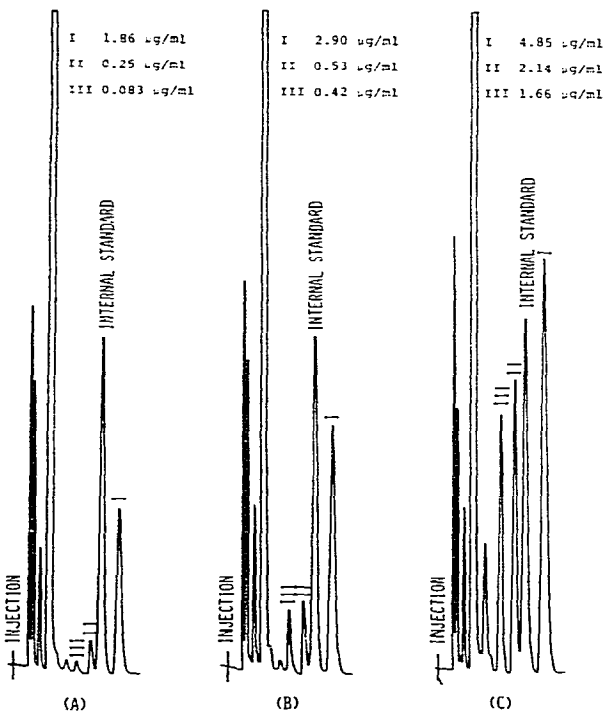

Fig. 3. Chromatograms resulting from the analysis of 500-μl aliquots of plasma samples collected from patients who were receiving lidocaine by infusion. The concentrations of I, II, and III (expressed as the base) in each sample are also shown. Detector sensitivity was 0.04 a.u.f.s.

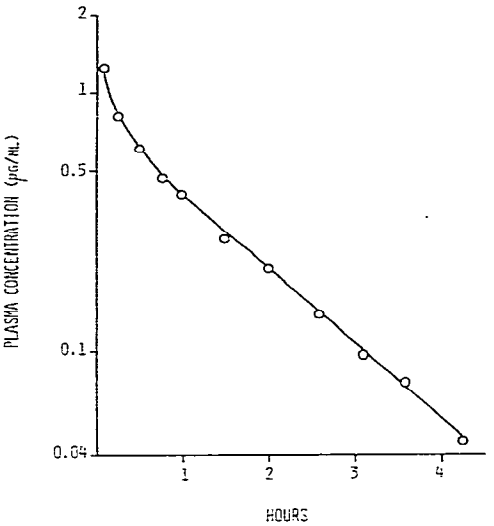

Fig. 4. Semilog plot of plasma concentration of lidocaine (I) versus time in a 4-kg albino rabbit which had been administered 8 mg of I during 30 sec by the intravenous route.

procainamide, N-acetylprocainamide, quinidine, and verapamil were tested for potential interference of the HPLC method by injecting stock solutions of these compounds into the chromatograph; however, no interference was observed.

The time course of plasma concentration of I in a rabbit following intravenous administration is shown in Fig. 4. In agreement with similar studies in man [21] plasma concentrations of II and III were too low to quantitate in this experiment which involved administration of a single dose of I. However, plasma concentrations of II and III do accumulate during chronic administration of I to cardiac patients (see Fig. 3), and probably contribute to the clinical responses elicited in such individuals [18].

The HPLC method reported here for simultaneous determination of lidocaine and its N-dealkylated metabolites may be useful for plasma level monitoring in patients, and for pharmacokinetic studies in animals and man.

REFERENCES

1 R. Gianelly, J.O. Van der Groben, A.P. Spivak and D.C. Harrison, N. Engl. J. Med., 277 (1967) 1215.
2 P.D. Thomson, K.L. Melmon, J.A. Richardson, K. Cohn, W. Steinbrunn, R. Cudihee and M. Rowland, Ann. Intern. Med., 78 (1973) 499.
3 J. Blumer, J.M. Strong and A.J. Atkinson, J. Pharmacol. Exp. Ther., 186 (1973) 31.
4 R.G. Burney, C.A. DiFazio, M.J. Peach, K.A. Petrie and M.J. Silvester, Amer. Heart J., 88 (1974) 765.
5 S. Riegelman and W. Sadee, Clinical Pharmacokinetics: A Symposium, American Pharmaceutical Association, Academy of Pharmaceutical Sciences, Washington, D.C., 1974, p. 169.
6 K.S. Pang and M. Rowland, J. Pharmacokin. Biopharm., 5 (1977) 655.
7 K.S. Pang and M. Rowland, J. Pharmacokin. Biopharm., 5 (1977) 681.

8 G. Svinhufvud, B. Ortengren and S.E. Jacobson, Scand. J. Clin. Lab. Invest., 17 (1965) 162.
9 J.B. Keenaghan, Anesthesiology, 29 (1968) 110.
10 F. Reynolds and A.H. Beckett, J. Pharm. Pharmacol., 20 (1968) 704.
11 M. Rowland, P.D. Thomson, A. Guichard and K.L. Melmon, Ann. N.Y. Acad. Sci., 179 (1971) 383.
12 N. Benowitz and M. Rowland, Anesthesiology, 39 (1973) 639.
13 H.B. Hucker and S.C. Stauffer, J. Pharm. Sci., 65 (1976) 926.
14 G. Caille, J. Lelorier, Y. Latour and J.G. Besner, J. Pharm. Sci., 66 (1977) 1383.
15 J.M. Strong and A.J. Atkinson, Anal. Chem., 44 (1972) 2287.
16 R.L. Nation, E.J. Triggs and M. Selig, J. Chromatogr., 116 (1976) 188.
17 C.A. DiFazio and R.E. Brown, Anesthesiology, 34 (1971) 86.
18 J.M. Strong, M. Parker and A.J. Atkinson, Clin. Pharmacol. Ther., 14 (1973) 67.
19 K.K. Adjepon-Yamoah and L.F. Prescott, J. Pharm. Pharmacol., 26 (1974) 889.
20 R.F. Adams, F.L. Vandemark and G. Schmidt, Clin. Chim. Acta, 69 (1976) 515.
21 R.L. Nation, Ph.D. Thesis, University of Sydney, 1977.