Journal of Chromatography, 311 (1984) 291—299
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2263

SIMULTANEOUS DETERMINATION OF LIDOCAINE AND ITS METABOLITES IN PLASMA AND MYOCARDIUM

FRANK A. LUZZI, THOMAS L. WENGER*, JAYNE K. KLINGER, AARON BARCHOWSKY and HAROLD C. STRAUSS

Departments of Medicine and Pharmacology, Duke University Medical Center, Box 3845, Durham, NC 27710 (U.S.A.)

(First received March 27th, 1984; revised manuscript received July 19th, 1984)

SUMMARY

No validated method exists for measuring lidocaine and its metabolites in myocardial tissue. We modified a previously described high-performance liquid chromatographic assay and applied it to plasma and to homogenized myocardial samples obtained from dogs that had received lidocaine by a double-infusion technique. Recovery of lidocaine, monoethylglycylxylidide and glycylxylidide after homogenization and extraction is reported. Assay variability, sensitivity and linearity over a wide range of sample sizes are also described. The results obtained with high-performance liquid chromatographic analysis are compared to quantitation of ^{14}C-labeled lidocaine plus metabolites measured by an oxidation—scintillation technique. Myocardium to plasma partition coefficients for lidocaine, monoethylglycylxylidide and glycylxylidide were 2.16, 4.27, and 2.91, respectively.

INTRODUCTION

Lidocaine, a widely used local anesthetic and antiarrhythmic agent, is deethylated in the liver to monoethylglycylxylidide (MEGX) and glycylxylidide (GX) [1, 2]. Extensive data already exist regarding plasma concentration of lidocaine and its relationship to clinical effects. These data call attention to lidocaine's narrow toxic-to-therapeutic ratio, which often requires plasma concentration monitoring in patients treated with the drug. Even when one ensures that plasma concentrations of lidocaine fall within the usual therapeutic range toxic reactions still have been reported to occur [3—5]. It is possible that alterations in lidocaine metabolism or metabolite excretion may contribute to variability in the toxic-to-therapeutic ratio. Specifically, MEGX and GX have both been proposed to contribute to the antiarrhythmic and toxic effects of the parent compound [6—9]. Until recently, however, it has not been possible

0378-4347/84/$03.00 © 1984 Elsevier Science Publishers B.V.

to measure these metabolites and, as such, the nature of their contributions has not been rigorously established. The effectiveness of the intravenously administered compound and the rapidity with which lidocaine is de-ethylated after administration, provide incentives for making such determinations.

Although assays of plasma MEGX and GX are now available [10—12] little work has been done to clarify the concentration—response relationships of these compounds and how their effects may relate to myocardial levels. As yet no method has been described for simultaneously measuring lidocaine, MEGX and GX in tissue. Previous efforts have sought only to measure tissue lidocaine concentration [13—16]. The marked variability in myocardium-to-plasma lidocaine concentration ratios reported in these studies suggests the possibility of inadequate assay methodology. A better understanding of tissue uptake of this drug and its metabolites may also help explain variations in lidocaine's actions.

A previous report from our laboratory describes lidocaine and metabolite levels in canine plasma and myocardium [17] using a modification of the plasma assay described by Nation et al. [11]. In the present paper we describe in detail our methodology, which permits accurate simultaneous quantification of lidocaine, MEGX and GX in canine myocardium via homogenization followed by extraction and subsequent high-performance liquid chromatographic (HPLC) analysis.

EXPERIMENTAL

Surgical procedure and drug infusion

Four mongrel dogs of either sex weighing 10—15 kg were anesthetized with sodium pentobarbital, 30 mg/kg intravenously. An additional 60-mg dose was given just prior to sacrifice. The dogs were intubated and ventilated at 20 cycles per min with a tidal volume of 13 ml room air per kg body weight plus dead space using a Harvard Apparatus Model 607 respirator. The right femoral artery and vein were exposed and catheterized. Blood samples for lidocaine plasma concentration determinations were obtained via the arterial catheter just before sacrifice and centrifuged at 1000 *g* for 15 min. Plasma was subsequently pipetted into screw cap tubes and stored at −10° C.

Using a Harvard Apparatus Model 906 infusion pump, lidocaine was administered intravenously by a two-stage procedure at 0.4 mg/kg/min for the first 5 min, and at 0.08 mg/kg/min for the remainder of the 90-min study. ^{14}C-Radiolabeled lidocaine (>97% pure, labeled at the carbonyl carbon, New England Nuclear) was infused along with the cold drug at a ratio of 40 μCi per 150 mg cold lidocaine.

A left thoracotomy was performed and the heart exposed 15 min prior to the study's conclusion. Sacifice of the dog was carried out at 90 min by excision of the heart, which in turn was blotted and stored at −10° C until time for assay.

Preparation of tissue samples

Triplicate transmural samples of frozen left ventricular (LV) tissue from each

dog (n = 4) were isolated, weighed (0.9–1.4 g) on a Mettler H51 balance and placed into ice cold siliconized test tubes each containing 2 ml of distilled deionized water.

Homogenization was carried out using a Brinkman Instruments ST10 polytron. Of each homogenate 0.5 ml was dispensed into a 15-ml siliconized tube and covered with a PTFE-lined cap (American Scientific Products) for extraction.

Drug-free tissue samples for standard curve generation were treated in the same fashion as the above except that the distilled deionized water used was supplemented with known amounts of lidocaine, MEGX and GX.

HPLC system

Reagents. All reagents were of HPLC grade unless otherwise specified. Lidocaine, ethylmethylglycylxylidide (the internal standard), MEGX and GX were supplied by Astra Pharmaceuticals.

Extraction. The extraction procedure used here is similar to those described previously for extraction of lidocaine, MEGX and GX from plasma [11, 17]. To each of the 0.5-ml tissue homogenates or to 0.5-ml samples of plasma 100 μl of 10 μg/ml internal standard, 100 μl of 1 mol/l NaOH and 4 ml of ethyl acetate were added. Extraction into the organic phase was completed by vortex mixing for 1 min followed by centrifugation at 1000 *g* for 5 min. Back-extraction from the organic phase was accomplished, after careful removal of the ethyl acetate layer to a separate tube, by vortex mixing with 100 μl of 0.005 mol/l sulfuric acid. Portions of 20 μl from the acid–water pellet were injected onto the HPLC column using a micro syringe. All glassware was siliconized with Aquasil (Pierce).

Apparatus. Analyses were carried out with an HPLC system employing a Spectromonitor III variable-wavelength ultraviolet detector (Laboratory Data Control) set at 200 nm and recorded on a Linear Products recorder. The mobile phase consisted of 0.04 mol/l sodium phosphate buffer pH 3.0–acetonitrile–triethylamine (Aldrich, 99% pure) (87:12:1). Separation was accomplished with a Zorbax ODS column from Dupont (5–6 μm column particle size, 25 cm × 4.6 mm I.D.). A constametric pump (Laboratory Data Control) maintained a flow-rate of 1.5 ml/min.

Data analysis. Linear regressions (standard curves) were constructed by plotting the peak height ratios (drug-to-internal standard peak height ratio obtained from the HPLC chromatograms) against known (standard) concentrations of drug contained in tissue homogenate or plasma samples. For tissue homogenates, standard curves were constructed for lidocaine over a range of 1.25–10.0 μg/g, and for MEGX and GX over a range of 0.9–5.0 μg/g. Standard curves for plasma samples were constructed for lidocaine from 0.5 to 4.0 μg/ml, and for MEGX and GX from 0.25 to 2.0 μg/ml. Lidocaine, MEGX and GX levels in experimental myocardium and plasma were determined using each sample's HPLC-derived peak height ratio and the appropriate standard curve.

Oxidation–scintillation analysis

Samples to be analyzed were dispensed into individual combustocones and

left to dry overnight. A Packard Instruments oxidizer was used to combust the samples into $^{14}CO_2$, which was subsequently trapped by 7 ml of Carbosorb per sample. Permafluor (12 ml) was added to each sample; radioactive content was then measured using a Beckman LS 100C spectrometer. All instruments and solutions used, with the exception of the scintillation counter, were Packard products.

Assay evaluation procedures

Drug recovery after homogenization. Four 1-g samples of experimental myocardium containing ^{14}C-labeled lidocaine and metabolites were homogenized, dispensed in triplicate volumes of 0.5 ml, and subjected to oxidation—scintillation analysis. These results were compared to those obtained by direct oxidation—scintillation analysis of non-homogenized tissue obtained from contiguous sites of myocardium.

Extraction efficiency. Extractions from homogenized myocardial tissue were carried out for lidocaine (at 2.7, 4.5 and 6.3 μg/g), ethylmethylglycylxylidide (at 6.0 μg/g), MEGX and GX (at 0.9, 1.8 and 2.7 μg/g). Extraction recoveries were determined by comparing the peak heights of these samples to the peak heights generated by direct injection onto the column of non-extracted drug of the same concentrations.

Coefficient of variation. The coefficient of variation (C.V.) of the assay was determined by dividing the standard deviation obtained from chromatogram ethylmethylglycylxylide peak heights (n = 14 for myocardium and n = 12 for plasma) by each set's mean value.

Sensitivity of the assay. Samples containing variable amounts of lidocaine, MEGX and GX, 2 ml of distilled deionized water, and 1 g of LV tissue were homogenized and extracted. The peak heights generated by injection of 20 μl of back-extracts onto the column were evaluated for their signal-to-noise ratios.

Tissue size and assay linearity. To determine the accuracy of our method for analyzing tissue samples of varying sizes, pieces of LV tissue (from dog No. 2) ranging from 0.165 to 1.98 g (n = 16) were assayed for uniformity of drug concentration.

Comparison of HPLC and oxidation data. Of the back-extraction phase, prepared as described in *HPLC system, Extraction*, 40 μl were subjected to oxidation—scintillation analysis to measure the content of ^{14}C-labeled drug. By determining the hot-to-cold drug ratio of the infusate, as well as the extraction efficiency and molecular-weight differences amongst the three compounds of interest, the drug concentrations from HPLC samples were converted to dpm/g and compared to drug concentrations obtained from oxidation—scintillation analyses.

RESULTS

At a flow-rate of 1.5 ml/min the chromatographic peaks resolved clearly with retention times of 4, 5, 6 and 8 min for GX, MEGX, ethylmethylglycylxylidide and lidocaine, respectively (Fig. 1). Recovery of ^{14}C-labeled drug from the homogenization step was 91%. The C.V. derived from internal standard peak heights was 12.0% for myocardial samples and 6.1% for plasma samples. Linear

regression coefficients for all standard curves were > 0.999. We chose a signal-to-noise ratio of 3:1 as the minimum clearly measurable limit for peak heights. Using this definition and a standard 20-μl injection of back-extract, the assay sensitivity for each drug was found to be 1.5 ng per 20 μl injection of back-extract. This corresponds to 0.045 μg/g of myocardium.

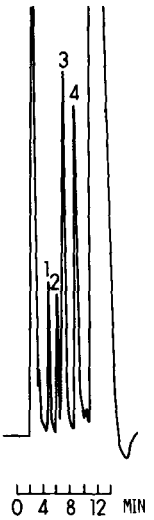

Fig. 1. Typical chromatogram of myocardial sample with peaks at 4, 5, 6 and 8 min for GX (1), MEGX (2), ethylmethylglycylxylidide (3) and lidocaine (4). a.u.f.s. setting = 0.05.

Extraction efficiencies for MEGX and GX at concentrations of 0.9, 1.8, and 2.7 μg/g were ⩾ 88% and ⩾ 55%, respectively (Table I). Extraction efficiency for lidocaine at concentrations of 2.7, 4.5 and 6.3 μg/g was ⩾ 95%. Each of these drugs had better extraction efficiency at the lower concentrations. Extraction was found to be 95% efficient for the one concentration of ethylmethylglycylxylidide used in this study.

Drug concentrations from LV tissue for each dog (n = 4) are shown in

TABLE I

EXTRACTION EFFICIENCIES FOR LIDOCAINE, MEGX AND GX AT NOTED CONCENTRATIONS

Compound	Concentration (μg/g)	Extraction efficiency (%)
Lidocaine	2.7	98
	4.5	99
	6.3	95
MEGX	0.9	98
	1.8	90
	2.7	88
GX	0.9	67
	1.8	57
	2.7	55

Table II. The average lidocaine concentration was 3.37 μg/g, which is a little more than the combined value of its metabolites. Although the mean values of MEGX (1.51 μg/g) and GX (1.40 μg/g) are similar, the ratio MEGX:GX shows considerable variability, ranging from 1.74 for dog 2 to 0.56 for dog 4. Myocardial concentrations at the time of sacrifice appear to correlate with the respective plasma concentrations, including those values noted above for MEGX and GX in dogs 2 and 4.

TABLE II

LV TISSUE AND 90-min PLASMA CONCENTRATIONS ALONG WITH TISSUE-TO-PLASMA PARTITION RATIOS

Compound	Concentration (mean ± S.D.)		Ratio
	Tissue (μg/g)	Plasma (μg/ml)	
Dog No. 1			
Lidocaine	3.40 ± 0.21	1.30 ± 0.01	2.62
MEGX	1.62 ± 0.16	0.33 ± 0.01	4.91
GX	1.18 ± 0.21	0.36 ± 0.01	3.28
Dog No. 2			
Lidocaine	3.64 ± 0.19	1.64 ± 0.01	2.22
MEGX	1.69 ± 0.08	0.43 ± 0.01	3.93
GX	0.97 ± 0.07	0.30 ± 0.01	3.23
Dog No. 3			
Lidocaine	3.91 ± 0.50	1.67 ± 0.02	2.34
MEGX	1.74 ± 0.12	0.40 ± 0.01	4.35
GX	1.63 ± 0.15	0.62 ± 0.01	2.63
Dog No. 4			
Lidocaine	2.51 ± 0.15	1.61 ± 0.01	1.56
MEGX	1.01 ± 0.05	0.26 ± 0.01	3.88
GX	1.81 ± 0.05	0.73 ± 0.01	2.48
Mean			
Lidocaine	3.37 ± 0.60	1.56 ± 0.17	2.16 ± 0.45
MEGX	1.51 ± 0.32	0.36 ± 0.08	4.27 ± 0.48
GX	1.40 ± 0.37	0.50 ± 0.21	2.91 ± 0.41

The relationships between tissue and plasma concentrations are also shown in Table II. Both MEGX and GX are found in higher proportion in myocardium relative to plasma than is lidocaine. The partition coefficient of MEGX (4.27) is almost double that of lidocaine (2.16), whereas the partition coefficient for GX (2.91) lies in between these two values. As can be seen from the standard deviations, the partition ratios vary little amongst experiments. Thus, differences in myocardial metabolite concentration are caused predominantly by variation in hepatic metabolism rather than variation in myocardial uptake.

Fig. 2 displays the linearity between lidocaine, MEGX and GX concentration, and myocardial sample size for dog 2. Tissue weights range from 0.165 to 1.980 g. Drug concentrations (n = 16) show little variation over this range of tissue weights. The slopes for MEGX (—0.094) and GX (0.054) are nearly parallel to the X-axis. The lidocaine slope (—0.362) is accentuated by one outlier at the smallest sample size.

To evaluate the concordance between HPLC and oxidation—scintillation methods, portions from experimental tissue sample back-extractions (n = 12) were assayed by each technique. The results using HPLC give total drug values that are 88 ± 23% (mean ± S.D.) of those obtained with the oxidation—scintillation method.

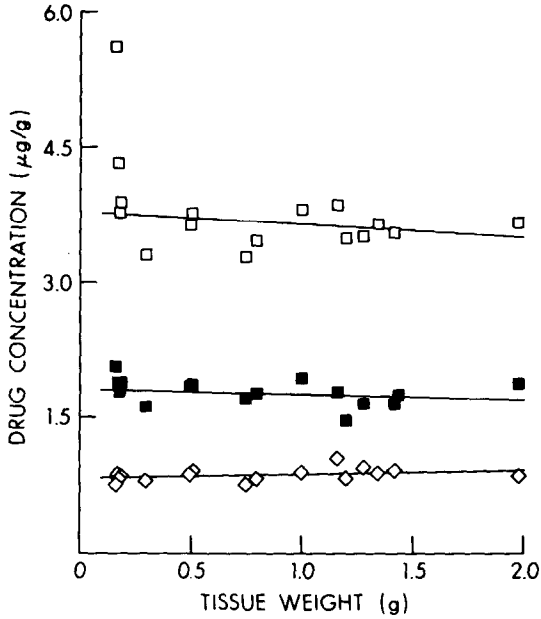

Fig. 2. Myocardial drug concentrations over a range of sample sizes obtained from dog 2. □, Lidocaine; ■, MEGX; ◇, GX.

DISCUSSION

There has been recurrent interest in the measurement of lidocaine and its metabolites in plasma and myocardium. Previous studies using gas—liquid chromatography report a wide range of myocardium-to-plasma partitioning of lidocaine and do not provide data on MEGX and GX [13—16, 18]. Because this range may reflect differences in technique, we set out to describe and validate a method for measuring plasma and tissue concentrations of lidocaine and its de-ethylated metabolites using HPLC.

We evaluated our method for sensitivity (1.5 ng per injection), reproducibility (C.V. of 12.0% for tissue and 6.1% for plasma), homogenization recovery (91%) and extraction efficiency ($\geqslant$ 95% for lidocaine, $\geqslant$ 88% for MEGX, and $\geqslant$ 55% for GX). The sensitivity of the assay can be improved by injecting larger samples of the back-extraction onto the column. In addition, the tissue concentration was shown to be independent of tissue sample size for values ranging between 0.165 and 1.98 g. Myocardium-to-plasma partition coefficients between myocardium and plasma for lidocaine, MEGX and GX were measured to be 2.16, 4.27 and 2.91, respectively, at a sampling time known to be

adequate for equilibrium between plasma and myocardium for these drugs [17].

Other reports of lidocaine myocardium-to-plasma partition ratio vary considerably. Ahmad and Medzihradsky [13] determined lidocaine tissue-to-plasma partitioning in dogs to be 0.45. This method requires large pieces of tissue for homogenization, as well as deproteination and evaporation steps for extraction. Using a simpler extraction procedure, Benowitz and co-workers [14, 15] found lidocaine to partition into myocardium with a ratio of 0.96 relative to plasma in monkeys. Although noted later to be sensitive and reproducible [19], this method does not report extraction efficiencies for tissue lidocaine, and as with the methodology of Ahmad and Medziradsky [13], requires large samples of tissue for homogenization. Naito et al. [18] presented a technique which enabled examination of smaller pieces of tissue (0.3 g) by pulverizing the sample prior to homogenization. No drug plasma levels were reported however, and the assay's sensitivity was 0.5 μg/g. Holt et al. [16] were able to determine lidocaine in pieces of myocardium as small as 0.1 g by digesting samples for 15 h with a bacterial proteinase. The partition ratio was determined to be 4.76 at a sensitivity of 1.0 μg/g. Variations in time of sampling are unlikely to account for much of these discrepancies, since lidocaine equilibrates rapidly between blood and myocardium [17].

In addition to the failure of the different methodologies noted above to yield consistent values for tissue-to-plasma partition ratios, none of these methods measured myocardial content of lidocaine metabolites. Much of the interest in quantifying these metabolites stems from reports of their toxic and pharmacologic activities, in particular their antiarrhythmic potential. Smith and co-workers [8, 20] noted that the peak antiarrhythmic action of lidocaine occurred after peak blood levels were attained following oral administration of the drug to dogs. This observation, it was suggested, may have been due to contributions of metabolites to the parent compound's activity. Similarly, Boyes et al. [5] found that plasma levels of lidocaine required for antiarrhythmic protection following oral administration were less than those needed after intravenous dosing of the drug. In this regard, Smith and Duce [8] showed MEGX to have one-third to one-half the ventricular antiarrhythmic potency of lidocaine in mice and dogs. Also, Burney et al. [7] demonstrated that MEGX was 83% as effective as lidocaine in protecting against ouabain-induced arrhythmias in guinea pig atrial tissue. By administering MEGX and GX for protection against chloroform-induced arrhythmias in mice, Strong et al. [9] determined potencies for the metabolites to be 99% and 26%, respectively, compared to that of lidocaine. Most recently, Broughton et al. [21] found that MEGX and GX each decrease the upstroke velocity of early premature beats and delay the recovery of action potential upstroke velocity following repolarization in guinea pig papillary muscle, confirming that both MEGX and GX have blocking effects on the sodium channel.

These metabolites have been shown to contribute a variety of toxic effects as well. They provoke convulsions [8], impair mental concentration [9] and may cause dizziness [5]. In addition, they have been reported to elicit emesis [8], frontal headaches and possibly hallucinations [9].

Extraction and subsequent HPLC analysis of lidocaine, MEGX and GX

removes the possibility, which may exist with procedures measuring only lidocaine, of non-resolved metabolites contributing to the lidocaine peak on the chromatogram. The extraction efficiencies obtained in our study for these three compounds are similar to those determined using the same procedure in plasma [11]. The lower extraction efficiencies registered at higher concentrations of drug (Table I) may be due to a decreasing acid-to-drug ratio in the extraction phase. This phenomenon may require further investigation if the analysis of higher drug concentrations is desirable. The accuracy with which drug content is measured over a range of tissue sizes adds credence and flexibility to this technique and may allow extension to animal models other than the dog.

ACKNOWLEDGEMENTS

We wish to thank Astra Pharmaceuticals for their generous supply of lidocaine and metabolites. We also wish to thank Mrs. T. LeMay for her secretarial expertise. Funding for this project was provided in part by U.S. Public Health Service Grants HL-17670 and 19216, National Institute of Arthritis, Diabetes, and Kidney Diseases, AM07386 NIADDK, and by a research grant from Burroughs Wellcome Co.

REFERENCES

1 A.H. Beckett, R.N. Boyes and P.J. Appleton, J. Pharm. Pharmacol., 18 (Suppl.) (1966) 76.
2 J.B. Keenaghan and R.N. Boyes, J. Pharmacol. Exp. Ther., 180 (1982) 454.
3 H. Halkin, P. Meffin, K.L. Melmon and M. Rowland, Clin. Pharmacol. Ther., 17 (1975) 669.
4 J.M. Strong, M. Parker and A.J. Atkinson, Jr., Clin. Pharmacol. Ther., 14 (1973) 67.
5 R.N. Boyes, D.B. Scott, P.J. Jebson, M.J. Godman and D.G. Julian, Clin. Pharmacol. Ther., 12 (1971) 105.
6 J. Blumer, J.M. Strong and A.J. Atkinson, Jr., J. Pharmacol. Exp. Ther., 186 (1973) 31.
7 R.G. Burney, C.A. DiFazio, M.J. Peach, K.A. Petrie and M.J. Silvester, Amer. Heart J., 88 (1974) 765.
8 E.R. Smith and B.R. Duce, J. Pharmacol. Exp. Ther., 179 (1971) 580.
9 J.M. Strong, D.E. Mayfield, A.J. Atkinson, Jr., B.C. Burris, F. Raymon and L.T. Webster, Jr., Clin. Pharmacol. Ther., 17 (1975) 184.
10 C.E. Hignite, C. Tschanz, J. Steiner, D.H. Huffman and D.L. Azarnoff, J. Chromatogr., 161 (1978) 243.
11 R.L. Nation, G.W. Peng and W.L. Chiou, J. Chromatogr., 162 (1979) 466.
12 J. Hill, A. Roussin, J. Lelorier and G. Caille, J. Pharm. Sci., 69 (1980) 1341.
13 K. Ahmad and F. Medzihradsky, Res. Commun. Chem. Pathol. Pharmacol., 2 (1971) 813.
14 N. Benowitz and M. Rowland, Anesthesiology, 39 (1973) 639.
15 N. Benowitz, R.P. Forsyth, K.L. Melmon and M. Rowland, Clin. Pharmacol., Ther., 16 (1974) 87.
16 D.W. Holt, M. Loiozou and R.K.H. Wyse, J. Clin. Pathol., 32 (1979) 225.
17 F. Handel, F.A. Luzzi, T.L. Wenger, A. Barchowsky, D.G. Shand and H.C. Strauss, J. Cardiovasc. Pharmacol., 5 (1983) 44.
18 E. Naito, M. Matsuki and K. Shimoji, Anesthesiology, 47 (1977) 466.
19 R.A. Zito, V.J. Caride, T. Holford and B.L. Zaret, Amer. J. Cardiol., 50 (1982) 497.
20 E.R. Smith, B.R. Duce and R.N. Boyes, Amer. Heart J., 83 (1972) 365.
21 A. Broughton, A.O. Grant, J.K. Klinger and H.C. Strauss, Circulation, 68 (1983) III-295.