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QUANTITATION OF LIDOCAINE AND ITS DEETHYLATED METABOLITES IN PLASMA AND URINE BY GAS CHROMATOGRAPHY–MASS FRAGMENTOGRAPHY

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SUMMARY

A sensitive, precise and accurate method for simultaneous quantitation of lidocaine and its deethylated metabolites by gas chromatography–mass fragmentography has been developed. Propyl derivatives of the deethylated metabolites are formed directly in either plasma or urine by treatment with propionaldehyde and sodium cyanoborohydride. The propyl derivatives and unchanged lidocaine are extracted, separated by gas chromatography and quantitated by mass fragmentography using mepivacaine as the internal standard. Quantitation of these compounds to levels as low as 50 ng/ml body fluid has been achieved with coefficients of variation less than 10%.

INTRODUCTION

Lidocaine is used extensively to treat ventricular cardiac arrhythmias, especially in patients who have had cardiac surgery or sustained an acute myocardial infarction. This drug is extensively biotransformed (Fig. 1) with less than 10% being excreted unchanged[1].

Two of its metabolites, monoethylglycinexylidide (MEGX) and glycinexylidide (GX) may be of major importance in lidocaine therapy since they possess antiarrhythmic activity which adds to that of lidocaine[2–4] and may be partly responsible for the adverse effects associated with the use of lidocaine[5,6]. MEGX has been associated with convulsions in man when plasma concentrations reach 22 μg/ml (ref. 7) and when the combined concentrations of lidocaine and MEGX rise above 20 μg/ml (ref. 8). GX causes headache and impaired central nervous system (CNS) performance at plasma concentrations of 1 μg/ml, but no severe toxic effects have

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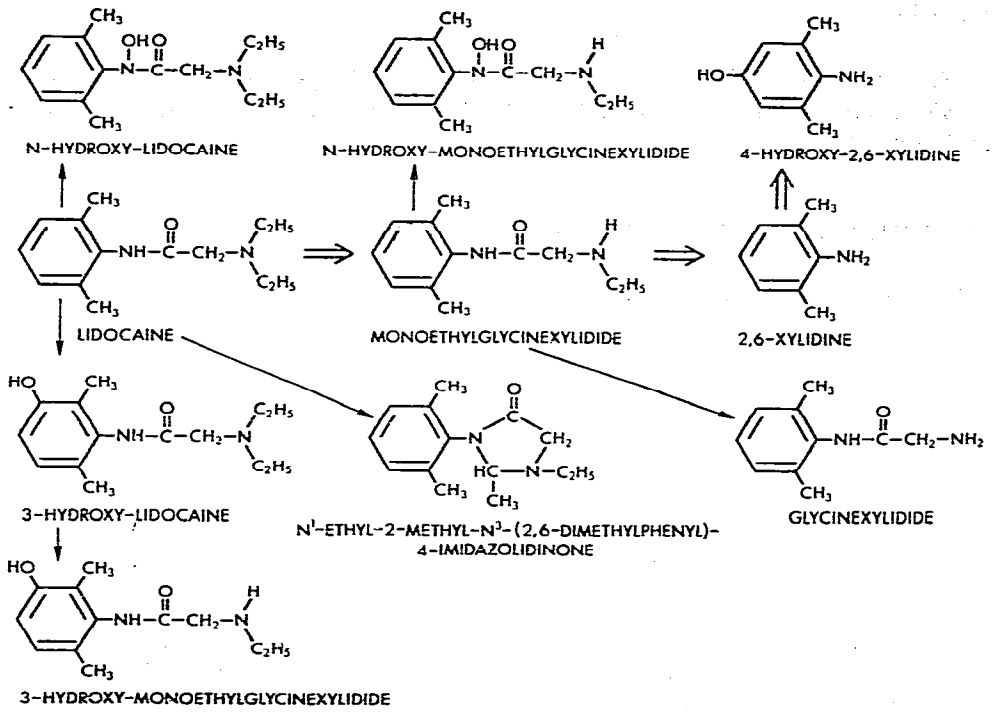


Fig. 1. Metabolic transformation of lidocaine.

been observed up to concentrations of 9 μg/ml. The mean plasma elimination half-life ($t_{1/2}$) of GX is 10 h (ref. 9), approximately 5 times longer than the 2-h $t_{1/2}$ of both lidocaine and MEGX[6,10–12]. Therefore, GX in particular may accumulate to significant plasma levels during chronic infusions of lidocaine. However, when lidocaine is given orally MEGX and GX reach higher plasma concentrations than after intravenous administration due to the "first pass effect". The resulting high levels of these metabolites could be at least partially responsible for the difficulties in obtaining a therapeutic without an adverse effect when lidocaine is administered orally[13]. Patients with typical signs of CNS toxicity when receiving lidocaine were frequently observed to have plasma lidocaine levels within the accepted therapeutic range but concentrations of MEGX and GX which were higher than in non-toxic patients[14]. An accurate method for quantitation of GX and MEGX would therefore be useful in determining more accurately their role in lidocaine therapy.

We have therefore developed a method which allows quantitation of these two metabolites simultaneously with lidocaine to levels as low as 50 ng/ml in both plasma and urine. The method involves preparation of the tertiary derivatives of MEGX (I) and GX (II) directly in aqueous solution (plasma or urine) using propionaldehyde and sodium cyanoborohydride according to the scheme shown in Fig. 2. After extraction, lidocaine and I and II were quantitated by gas chromatography (GC)–mass fragmentography using mepivacaine as an internal standard.

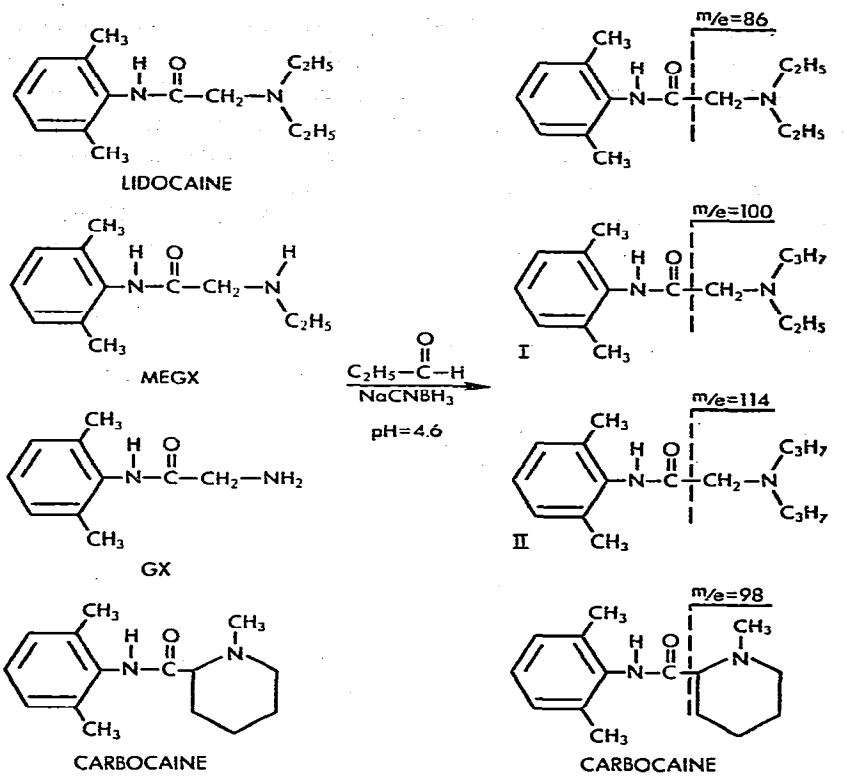


Fig. 2. Synthetic pathway for the preparation of derivatives I and II from MEGX and GX and the ions used for GC–mass fragmentography quantitation.

EXPERIMENTAL

Materials

Lidocaine (Elkins-Sinn, Cherry Hill, N.J., U.S.A.) and mepivacaine (Winthrop Labs., New York, N.Y., U.S.A.) were used as the parenteral dosage forms. MEGX and GX were kind gifts from Astra Pharmaceuticals (Worcester, Mass., U.S.A.). Propionaldehyde and sodium cyanoborohydride were purchased from Aldrich (Milwaukee, Wisc., U.S.A.). The GC column was packed with 3% OV-1 on 100–200 mesh Gas-Chrom Q (Supelco, Bellefonte, Pa., U.S.A.). Mass fragmentography results were obtained with a Finnigan 3300 gas chromatograph–mass spectrometer operated on line with a Finnigan 6000 data system (Finnigan, Sunnyvale, Calif., U.S.A.).

Quantitation of lidocaine, MEGX and GX

A 1-ml volume urine spiked with 2 μg mepivacaine (as its hydrochloride salt in water) or a 0.5-ml sample of plasma spiked with 0.2 μg mepivacaine was pipetted into a 15-ml PTFE-capped glass tube and carried through the following procedure in an efficient hood along with appropriate standards and blanks. A 10-μl volume of propionaldehyde and 1.0 ml 1.0 *M* acetate buffer, pH 4.6, were added and the mixture vortexed 5 sec. From a freshly prepared aqueous solution containing sodium

cyanoborohydride (100 mg/ml) was added 0.1 ml and this mixture was vortexed 5 sec. The tubes were capped loosely and then incubated in a 60° water bath for 1 h. A 1-ml volume of 5 *N* KOH and then 5 ml diethyl ether were added to each tube and the resulting mixtures were shaken vigorously for 5 min. An aliquot (about 4.7 ml) of the ether layer was transferred to a conical tube and evaporated to dryness. The sides of the tube were washed with a small volume of ether and evaporated to dryness to concentrate the residue in the bottom of the tube. Methanol (30 μl) was added, the tube was vortexed and 2–5-μl aliquots of the resulting solution were used for the analyses.

Analyses were carried out by GC–mass fragmentography (70 eV electron impact). The GC column was glass 6 ft. × 1/8 in I.D. and packed with 3% OV-1 on Gas-Chrom Q (100–200 mesh). The temperature of the column was adjusted so that the retention time of lidocaine was 1–1.5 min. All four compounds eluted from the column in less than 5 min. Areas under the appropriate peaks in the mass fragmentograms of $m/e = 86$ for lidocaine, $m/e = 100$ for I, $m/e = 114$ for II and $m/e = 98$ for the internal standard, mepivacaine, were used for quantitation. The origin of these ions is shown in Fig. 2.

Standard curves were generated by adding to a series of blank urine or serum samples 0.05–50 μg each of lidocaine, MEGX and GX. These samples were carried through the above procedure for quantitation. The ratios of the areas of the peaks in the mass fragmentograms of the appropriate ions of lidocaine, I and II to that of the internal standard were plotted against the concentration of each compound.

RESULTS

The lower portions of the standard curves for quantitation of lidocaine, MEGX and GX in plasma are shown in Fig. 3. The curves were linear from at least 0.05 to 50 μg/ml. Plasma concentrations of lidocaine and MEGX obtained in a patient who received 200 mg lidocaine orally are shown in Fig. 4. Concentrations of GX did not rise above 0.1 μg/ml and are not shown. The elimination rates of lidocaine, MEGX and GX in the urine of a patient who received 100 mg lidocaine intravenously are recorded in Fig. 5.

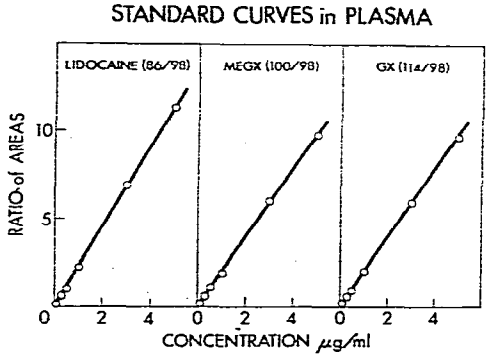
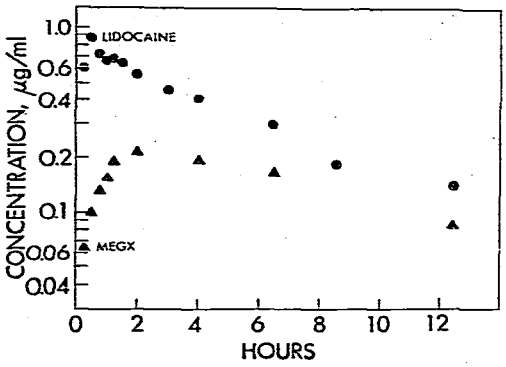


Fig. 3. Standard curves obtained in plasma for lidocaine, MEGX and GX using the ratios of ions indicated. Mepivacaine was the internal standard. Additional data points not shown established the linearity of the curves between 0.05 and 50 μg/ml.



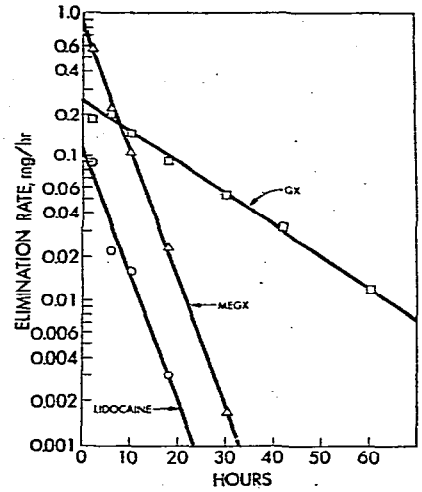


Fig. 4. Plasma concentrations of lidocaine and MEGX in a patient who received 200 mg lidocaine orally. Concentrations of GX did not rise above 0.1 μg/ml and are not shown.

Fig. 5. The rates of elimination of lidocaine, MEGX, and GX in a patient who received 100 mg lidocaine intravenously.

The precision of the assay procedure was determined by measuring five different plasma samples spiked with 0.2 μg each of lidocaine, MEGX and GX. The coefficients of variation for these assays were 10.1, 5.5 and 3.5%, respectively. Analyses of five different urine samples spiked with 2 μg of each compound gave coefficients of variation of 6.4% for lidocaine, 9.9% for MEGX and 8.1% for GX.

DISCUSSION

The method we describe for the quantitation of lidocaine, MEGX and GX in plasma and urine samples offers advantages over previous methods. Heptafluorobutyryl derivatives of the metabolites of lidocaine were prepared and analyzed by GC in one study[5], but an internal standard was not used. It was therefore necessary to measure precisely the volumes of solvents for extraction of samples for dissolving the sample extracts. This method also requires reproducible chromatographic injections of microliter quantities of samples. Accurate quantitation under these circumstances is tedious and difficult. Another GC method[15] failed to resolve adequately the underivatized metabolites from interfering substances making quantitation at low levels impossible. A third GC method[16] employed acetyl derivatives of the metabolites but was very time consuming since the volatility of the acetyl derivatives differed substantially from lidocaine and one another and temperature programming was necessary for each sample.

The most specific methods[17,18] reported utilize GC–mass spectrometry. However, in one study the GC peaks of MEGX and GX, which are polar secondary and primary amines, respectively, were asymmetrical and the lower limit of analytical sensitivity for both metabolites was about 0.5 μg/ml (ref. 17). The other mass spectral method[18], based on earlier work using stable isotope-labeling and chemical-ionization

procedures[19], involves the use of deuterated lidocaine and its metabolites as internal standards. Samples containing these standards are extracted and then analyzed by direct probe introduction and chemical-ionization mass spectrometry. This method may be the best presently available but the lower limits of detection are not given and GX cannot be accurately quantitated in urine due to interfering substances.

The procedure we described overcomes all the difficulties of the other methods discussed above by employing an internal standard, mepivacaine, which obviates the tedious quantitative transfers of small volumes of solvents. The propyl derivatives of MEGX and GX we use have volatilities similar to lidocaine and mepivacaine such that isothermal GC conditions can be used to minimize analysis time. In addition to improved extraction properties the propyl derivatives also produce sharp, symmetrical GC peaks. Since only very low level interferences have identical retention times to the target compounds, accurate quantitation to low (0.05 μg/ml) levels is possible. The mass spectra (not shown) of the propyl derivatives I and II, each exhibit one major ion which can be used for quantitation and therefore are ideal since the greater the percentage of the total ion current residing in the ion used for quantitation, the greater the sensitivity for the compound.

Sodium cyanoborohydride was used as the reducing agent in the reductive alkylation of MEGX and GX because the reaction is simple and the reagent is a very mild and selective reducing agent. Neither lidocaine nor mepivacaine is affected by it. Among the metal hydrides used for the preparation of amines from either aldehydes or ketones and precursor amines, lithium or sodium cyanoborohydride have been found to be ideal. The chemistry and conditions of reductions effected by this reagent have been reviewed[20]. The reagent is toxic and like most laboratory chemicals should be used in a well ventilated hood.

We have used the above method to analyze lidocaine, MEGX and GX in the plasma and urine of 15 patients receiving single doses of lidocaine p.o. (200 mg) one day and then i.v. (100 mg) at least one week later. We have encountered no difficulties with the method in these analyses. Full details of the study are described elsewhere[21].

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