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Note

Gas chromatographic method for the quantitative determination of lidocaine and its metabolite monoethylglycinexylidide in plasma

R. L. NATION and E. J. TRIGGS
Department of Pharmacy, University of Sydney, Sydney, N.S.W. 2006 (Australia)
and
M. SELIG
Intensive Care Unit, Lidcombe Hospital, Lidcombe, N.S.W. 2141 (Australia)
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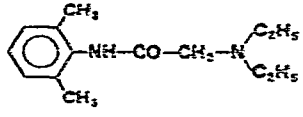
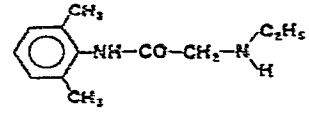
Lidocaine has been used extensively for treatment of cardiac arrhythmias accompanying acute myocardial infarction. Therapeutic[1–3] and toxic[3] plasma levels of lidocaine have been reported. Monoethylglycinexylidide (MEGX), a dealkylated metabolite formed after the administration of lidocaine, has been reported to be approximately 80% as potent an anti-arrhythmic agent as the parent drug while the didesethylated metabolite glycinexylidide (GX) had only about 10% the potency[4]. Further, MEGX has been suggested as a contributing factor in the occurrence of adverse reactions that sometimes arise after the administration of lidocaine[5]. Therefore, the monitoring of plasma levels of lidocaine and MEGX in postmyocardial infarction patients receiving lidocaine may be of benefit in the control of antiarrhythmic therapy.



LIDOCAINE



MEGX

A number of gas chromatographic (GC) methods have been reported for the determination of lidocaine alone[6–10], and of lidocaine and its dealkylated metabolites[5,11–14] in biological material. The latter procedures, however, appear to suffer from some disadvantages. Di Fazio and Brown[11] described a 10% UCW 98 on Chromosorb W system but the lidocaine, MEGX and GX peaks were not completely resolved and no mention was made of the accuracy of the method. Keenaghan and Boyes[5] reported the quantitative chromatography, without the use of an internal standard, of heptafluorobutyryl derivatives of lidocaine and its metabolites. Mass fragmentography was the detection method used for the GC estimation of lidocaine, MEGX and GX as described by Strong and Atkinson[12] and Strong *et al.*[13]. The stationary phase employed was 3% SE-30–OV-17 (6:1) coated on a Chromosorb support.

However, the peak shapes of the primary and secondary amine metabolites were asymmetrical and the calibration curve for MEGX was non-linear below about 0.5 μg/ml. Adjepon-Yamoah and Prescott[14] used a single-column temperature-programming technique to determine lidocaine and its metabolites after they had been converted to their acetyl derivatives. This paper describes a quantitative method for the isothermal GC of underivatised lidocaine and MEGX following extraction from plasma samples.

MATERIALS AND METHODS

Materials

Lidocaine hydrochloride, MEGX hydrochloride and GX were supplied by Astra Chemicals (Sydney, Australia). Benzhexol hydrochloride (Cyanamid Australia, Melbourne, Australia) was used as an internal standard. All reagents and solvents were analytical reagent grade except diethyl ether, which was anaesthetic grade (BP). The diethyl ether was distilled immediately before use. Ucon 75-H-90,000 (Union Carbide Australia, Sydney, Australia) was the stationary phase employed.

Gas chromatography

A Hewlett-Packard Model 5710 A gas chromatograph equipped with a flame ionization detector was used. The column was glass (2.5 ft. × ¼ in. O.D.) packed with 2% Ucon 75-H-90,000–2% KOH on 80–100 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.). A pan coating method[15] using methanol as the solvent was the procedure used to coat the KOH and Ucon onto the solid support. The packed column was conditioned for 3 h at 195° (no nitrogen flow) followed by 18 h at 185° (50 ml/min nitrogen flow). The operating conditions were: injection port temperature, 250°; column temperature, 175°; detector temperature, 250°; carrier gas (nitrogen) flow-rate, 50 ml/min; hydrogen flow-rate, 60 ml/min; air flow-rate, 240 ml/min.

Extraction procedure and sample preparation

Plasma (1 ml) and aqueous internal standard solution (5.0 μg/0.1 ml) were placed in a 15-ml glass-stoppered centrifuge tube. After addition of 0.5 ml of 10 *M* NaOH and 5 ml diethyl ether the tube was mixed for 2 min (vortex mixer) and centrifuged for 5 min at 1500 *g*. The tube was cooled in a dry ice–acetone bath for 1 min and the ether layer decanted into a second 15-ml glass-stoppered centrifuge tube containing 1 ml of a 1 *M* HCl solution. The ether and HCl mixture was mixed (1 min) and centrifuged as described above and the ether phase discarded. Following the addition of 0.5 ml 10 *M* NaOH the aqueous phase was extracted with 5 ml diethyl ether by mixing for 2 min. After centrifugation, the separated ether phase was transferred to a 15-ml tube with a 100-μl elongated bubble at the base, a silica boiling chip was added and the ether was evaporated by immersion of the tube in a water-bath at 40°. When no liquid ether was left the tube was stoppered and plunged into ice to condense the remaining ether vapour and wash down the sides of the tube. This evaporation and condensation procedure was repeated until approximately 10 μl of ether solution remained and 5–10 μl were injected into the gas chromatograph.

Calibration curves

Following chromatography, the heights of the peak corresponding to lidocaine MEGX and benzhexol were measured. Calibration curves of peak height ratio against amount of lidocaine hydrochloride or MEGX hydrochloride were established simultaneously by assaying drug-free plasma to which known amounts of the compounds had been added.

RESULTS

Performance of analytical procedure

Symmetrical chromatographic peaks were obtained for all compounds (Fig. 1) and the retention times of lidocaine, MEGX and benzhexol were 4.8, 7.6 and 10.0 min, respectively. The peak shape of GX was also good and with a retention time of 11.9 mn it was resolved from the other three peaks. However, due to its greater polarity the primary amine metabolite could not be quantitatively extracted with diethyl ether.

Calibration curves for lidocaine hydrochloride and MEGX hydrochloride in plasma were constructed over the range 0.05–25 μg and 0.05–5 μg, respectively, and are shown in Figs. 2 and 3. In each case the plot was linear and passed through the ori-

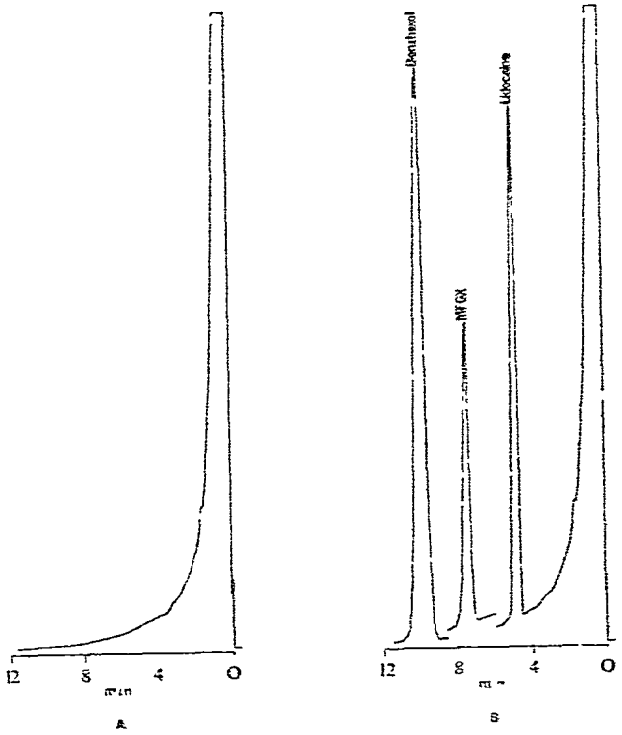


Fig. 1. Gas chromatograms of (A) blank plasma extract and (B) extract of plasma taken from a patient receiving lidocaine by intravenous infusion. The attenuation setting was ×160 for lidocaine and benzhexol and ×80 for MEGX.

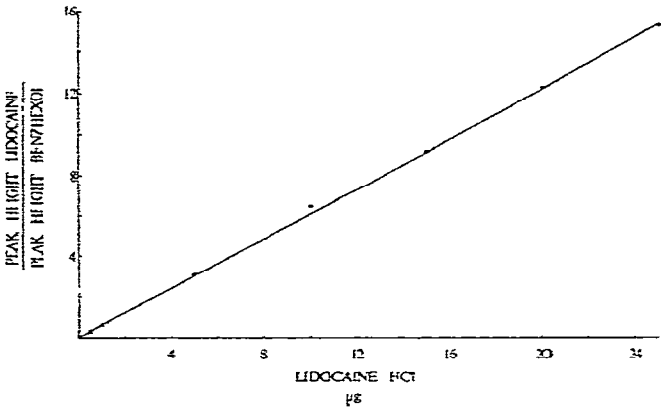


Fig. 2. Calibration curve for lidocaine hydrochloride in human plasma.

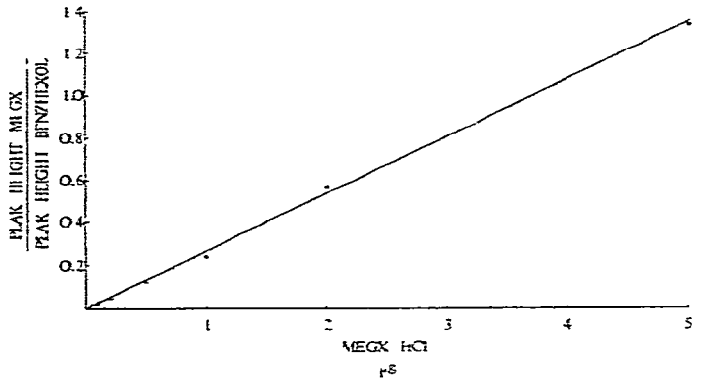


Fig. 3. Calibration curve for MEGX hydrochloride in human plasma.

gin. The reproducibility of replicate analyses of plasma samples containing different concentrations of the two compounds is recorded in Table I.

Application of the GC assay to biological samples

The assay procedure outlined has been used in a clinical setting to study the

TABLE I

REPRODUCIBILITY OF REPLICATE SIMULTANEOUS ANALYSES (MEANS FROM FIVE SAMPLES AT EACH CONCENTRATION) OF LIDOCAINE HYDROCHLORIDE AND MEGX HYDROCHLORIDE ADDED TO HUMAN PLASMA

Compound	*Plasma concentration (μg/ml)*	*Standard deviation (%)*
Lidocaine hydrochloride	0.25	3.09
	1.5	0.82
MEGX hydrochloride	0.1	5.21
	1.0	7.03

plasma levels of lidocaine and MEGX in patients who were being treated for cardiac arrhythmias with intravenous lidocaine infusions. Venous blood samples were collected in tubes containing 100 units of ammonium heparin together with blood separation granules. Plasma was separated by centrifugation as soon after sampling as possible and was stored at −20° until assayed. In some cases blood samples were collected from the patients during the lidocaine infusion. More commonly, however, blood samples were collected just prior to cessation of the infusion and for a number of hours subsequent to this. The plasma levels of lidocaine and MEGX in one patient in the latter category are illustrated in Fig. 4. No interfering chromatographic peaks were noted in plasma samples obtained before commencement of lidocaine therapy.

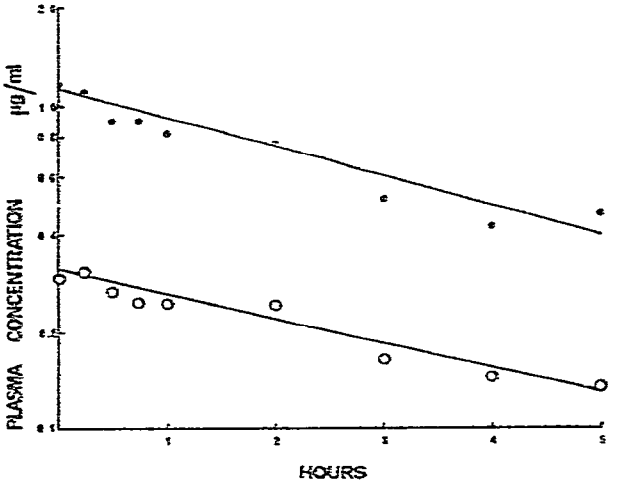


Fig. 4. Plasma levels of lidocaine hydrochloride (●) and MEGX hydrochloride (○) for 5 h after the cessation of the lidocaine infusion.

DISCUSSION

The method is sensitive and accurate for the determination of lidocaine and MEGX in plasma, and has been found to be satisfactory for clinical application. At this stage the technique has not been used to quantitate GX in plasma since, as stated earlier, this metabolite has much less pharmacological activity. However, if estimation of GX was required the use of a more polar organic solvent for extraction should be the only modification necessary. The use of the KOH-treated solid support in the GC system minimised adsorption of the amines and obviated the need for derivatisation procedures to ensure symmetrical peak shapes. The method has also been used to quantitate lidocaine and MEGX in urine.

ACKNOWLEDGEMENTS

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