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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF LIDOCAINE AND NINE OF ITS METABOLITES IN HUMAN PLASMA AND URINE

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SUMMARY

A simple high-performance liquid chromatographic procedure for quantifying lidocaine and nine of its metabolites in human plasma and urine is described. This method involves aqueous acetylation of the metabolites with acetic anhydride under mild basic conditions (pH ~ 8.5). Lidocaine and the derivatized metabolites are extracted under basic conditions with ethyl acetate. Calibration curves constructed for these compounds are linear in the concentration range studied (0.2–500 μg/ml). The relevance of this method is shown by measuring the plasma and urine profiles of two patients with suspected myocardial infarction.

INTRODUCTION

Lidocaine (LID) is a commonly used anti-arrhythmic agent. One of the most common modes of administration of this drug to cardiac patients is by intravenous infusion. The pharmacokinetics of LID during prolonged infusion is poorly understood. A steady-state condition is not commonly reached when the duration of infusion exceeds seven half-lives [1–3]. A number of postulations, which include enzyme saturation and deactivation, end-product inhibition, changes in binding and hepatic blood flow, have been put forward to explain this so-called time-dependent phenomenon of LID kinetics [2,4–7]. Recent evidence suggests

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that deactivation of the enzymes responsible for the deethylation process in the rat could be one of the major reasons for the time effects of LID metabolism [8].

Preliminary results in our laboratory suggest that the major metabolite of LID, 4-hydroxy-2,6-xylidine (4-OH-XY), found in human, is formed from a number of probable precursors such as 4-hydroxy-LID (4-OH-LID), 4-hydroxymonoethylglycinexylidide (4-OH-MEGX) and 4-hydroxyglycinexylidide (4-OH-GX) in addition to xylidine (XY). This observation has revived an interest in studying LID metabolism in detail because *p*-hydroxylation is the most dominant process for the elimination of LID and its metabolites in man. The relationship between *p*-hydroxylation and time-dependent elimination of LID is not known.

In order to study LID metabolism in man and animals, it is imperative to have an assay which is capable of measuring LID and its known metabolites. A search in the literature showed that most assays provide measurements of LID and its two active metabolites MEGX and glycinexylidide (GX) [8–16]. Tam and coworkers [8,17] have separated five and quantitated four of the LID metabolites (MEGX, GX, 3-OH-LID and 3-OH-MEGX) isolated from perfused rat liver samples. Kawai et al. [18] reported a similar separation of the same metabolites. There is no assay available to separate and measure LID and its known metabolites, especially the *p*-hydroxylated precursors of 4-OH-XY, in man. In this study we describe a simple high-performance liquid chromatographic (HPLC) method which is used to measure LID and nine of its metabolites identified in man.

EXPERIMENTAL

Reagents

The hydrochloride salts of LID and its metabolites, MEGX, GX, the *m*-hydroxylated forms of LID (3-OH-LID and 3-OH-MEGX), XY and 4-OH-XY, were kindly supplied by Astra Pharmaceuticals (Mississauga, Canada). 4-Hydroxylated products of LID (4-OH-LID, 4-OH-MGEX and 4-OH-GX) were synthesized in our laboratory from N-chloroacetyl-4-hydroxy-2,6-xylidine and structures were confirmed by combined gas chromatography–mass spectrometry (GC–MS) [19]. The method of synthesis will be published elsewhere. Procaine hydrochloride, purchased from K & K Labs. (Plainview, NY, U.S.A.), was used as the internal standard. Acetonitrile (HPLC grade), ethyl acetate (BDH analytical reagent), acetic anhydride, phosphoric acid and triethylamine (TEA) were purchased from Fisher Scientific (Fairlawn, NJ, U.S.A.). The other chemicals used were reagent grade.

Chromatography

The HPLC system (Waters Assoc.) consisted of two M-45 pumps, an M-480 variable-wavelength UV detector (set at 200 nm), an automatic sampler (WISP, model 721) and data processing station (Model 840). Separation of LID and the derivatized metabolites was achieved using a 5-μm C_{18} reversed-phase Novopak cartridge (11.5 cm $\times$ 8 mm I.D.). The aqueous mobile phase (pH 3) consisted of 12% acetonitrile, 0.1% phosphoric acid and 0.15% TEA, and was pumped at a flow-rate of 2 ml/min.

Sample treatment

Plasma. All labware used was made of glass. The caps of tubes were either made of or lined with Teflon. A stock solution containing the hydrochloride salts of LID and its metabolites (except 4-OH-XY) was prepared by dissolving these compounds in blank human plasma. This preparation was stable for at least a week. Adequate concentrations were obtained by spiking appropriate quantities of 4-OH-XY (in the form of aqueous solution) and making to volume with plasma. 4-OH-XY solution had to be prepared daily because it was unstable. The concentration range of the standards studied was between 0.2 and 5.0 μg/ml for all the components except 4-OH-XY (highest concentration is 20 μg/ml). To 0.5 ml of plasma standard or patient plasma was added 0.5 ml of 6 *M* hydrochloric acid. To hydrolyze conjugates, the mixture, in a tightly capped glass tube, was incubated at 100°C for 45 min. After cooling to room temperature, 0.5 ml of 6 *M* sodium hydroxide, 1 ml of 0.15 *M* sodium phosphate buffer (pH 6.8) and 0.1 ml of internal standard (20 μg/ml in water) were added. the pH of the resultant mixture was approximately 8.5.

Urine. The preparation of urine standards was similar to that of the plasma standards except the concentration range of 4-OH-XY was extended to 500 μg/ml. To 0.1 ml of urine was added 0.15 ml of 6 *M* hydrochloric acid. The rest of the acid hydrolysis procedures were the same as described for the plasma samples except 0.15 ml of 6 *M* sodium hydroxide was used to neutralize the acid after incubation.

Aqueous acetylation

The method of Coutts et al. [19] was used. Approximately 1.5 g of potassium bicarbonate were added to the acid-hydrolyzed plasma or urine samples. To the mixture were added 300 μl of acetic anhydride and when effervescence stopped, the solution was vortexed (IKA-VIBRAX-VXR, Terrochem, setting at 1000) with 6 ml of ethyl acetate for 10 min. After centrifugation (1000 *g*) for 10 min, 5 ml of the organic layer were removed and dried under a gentle stream of nitrogen. The residue was reconstituted with 300 μl of the mobile phase, and 50–200 μl were injected into the HPLC system. Because it lacks free amino or phenolic groups, LID was not acetylated during the derivatization procedure.

Human studies

Patients with suspected myocardial infarction were recruited into the LID trial conducted at the Foothills Hospital. The sub-protocol published by Rademaker et al. [20] for LID infusion and blood sampling was used with slight modifications. Briefly, the patient was given two 100-mg LID·HCl loading doses 30 min apart. Infusion (3 mg/min) began immediately after the first loading dose for a period up to 48 h. Additional blood samples were withdrawn from the patient up to 10 h post-infusion. Total urine was collected from the beginning of LID infusion until 72 h post-infusion. Since most of the plasma samples collected in this trial were analyzed prior to the development of this method, the post-infusion profile of only one patient is reported to demonstrate the applicability of this assay. Urine samples from another patient was also analyzed.

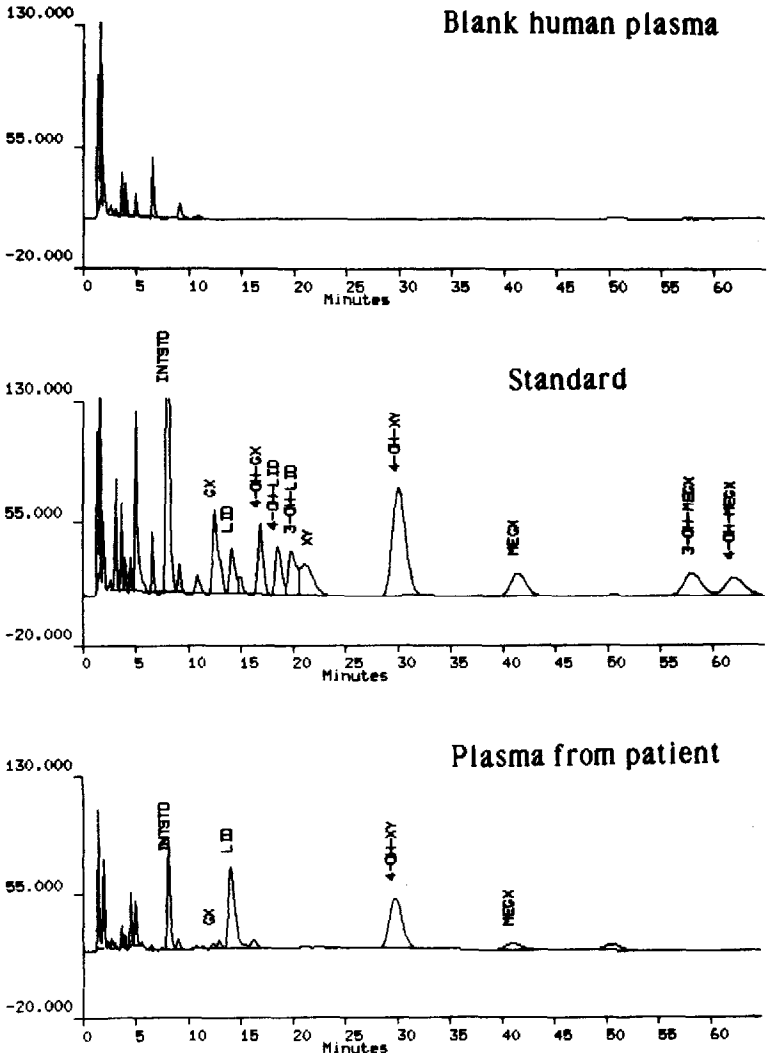

Fig. 1. Chromatograms obtained from blank, spiked and patient plasma samples. The concentrations of the spiked components were 5 μg/ml, except 4-OH-XY (20 μg/ml). The signals were measured in mV.

RESULTS

Figs. 1 and 2 are the HPLC traces of human plasma and urine, respectively. The blank samples showed the absence of endogenous interference. LID and the acetylated derivatives of the metabolites were well separated to allow accurate quantification. Calibration curves prepared for the standards were linear ($r^2 > 0.99$) in the concentration ranges studied (0.2–500 μg/ml for 4-OH-XY and 0.2–5 μg/ml for the rest). Each calibration curve consisted of at least five points and each point was determined in triplicate. The coefficients of variation

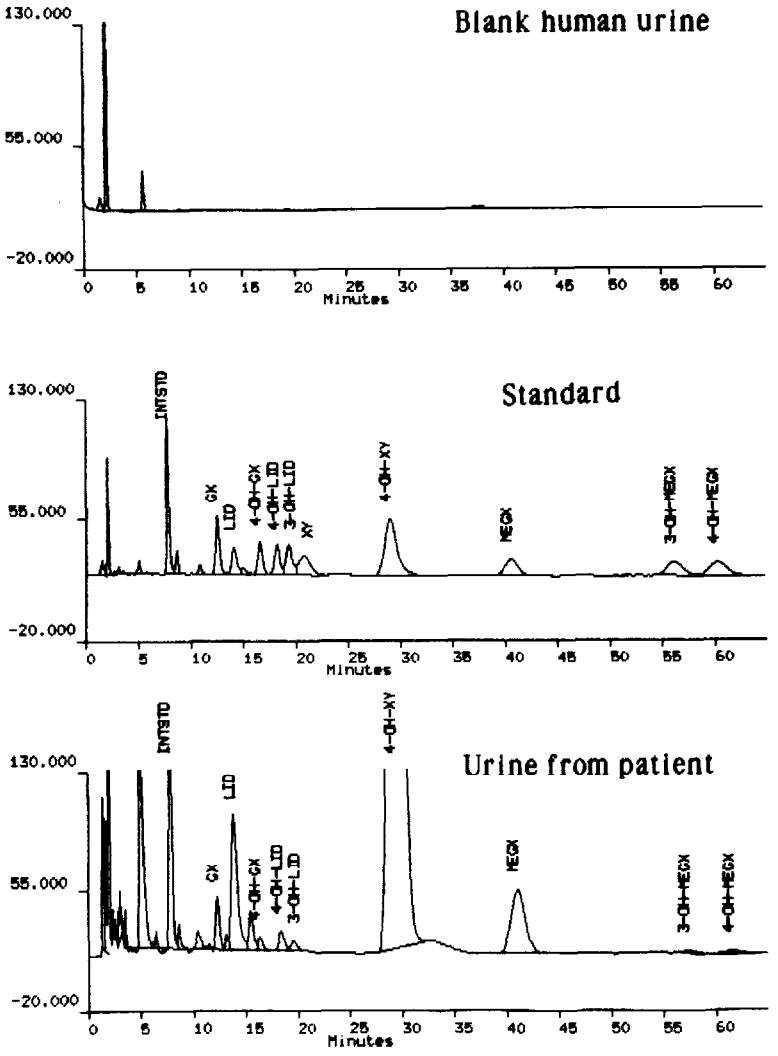

Fig. 2. Chromatograms obtained from blank, spiked and patient urine samples. The concentrations of the spiked components were 3 μg/ml, except 4-OH-XY (12 μg/ml). The signals were measured in mV.

(mean ± S.D.) were 4.68 ± 3.88% for plasma and 3.1 ± 3.43% for urine. The ranges were 0.2–13.5 and 0.13–17.4%, respectively.

4-OH-XY was the major metabolite present in plasma (Fig. 1). Its concentration was higher than that of LID. The level of MEGX and GX was approximately an order of magnitude lower than that of 4-OH-XY. The *m*-hydroxylated metabolites of LID and MEGX and *p*-hydroxylated metabolites of LID, MEGX and GX were absent in plasma. XY, the reported precursor of 4-OH-XY [21], was not detectable. The post-infusion profiles of LID, MEGX, GX and 4-OH-XY are shown in Fig. 3. The time courses of LID, MEGX and 4-OH-XY were parallel.

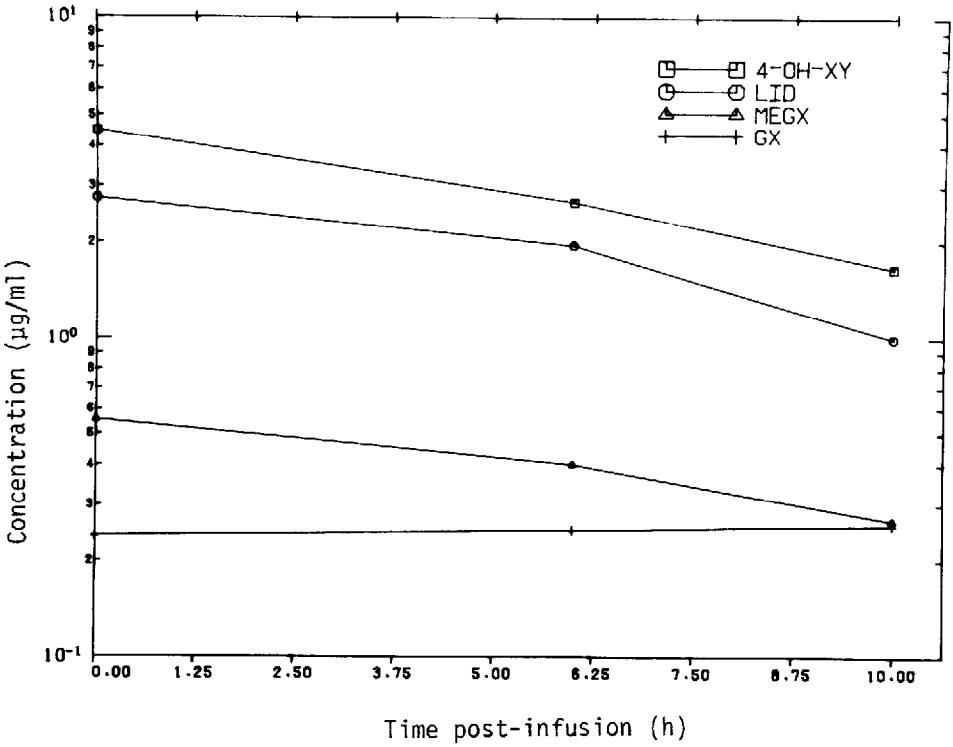

Fig. 3. Time course of LID, MEGX, GX and 4-OH-XY during the post-infusion phase.

Although the decline of LID concentration was apparently non-linear, it cannot be established at this time whether the elimination of LID after prolonged infusion is non-linear. More studies are required.

Similar to the plasma profiles, 4-OH-XY was the most abundant metabolite in urine (Table I). It accounted for 80% of the administered dose. The rest of the

TABLE I

RECOVERY OF LID AND ITS METABOLITES AFTER A COURSE OF LID INFUSION

Compound	Recovery (% of dose)
LID	2.1
MEGX	1.7
GX	0.55
4-OH-LID	0.28
4-OH-MEGX	0.06
4-OH-GX	0.24
4-OH-XY	80.1
3-OH-LID	0.13
3-OH-MEGX	0.04
Total	85.2

metabolites consisted of approximately 5% of the dose. In this particular patient, both the *m*- and *p*-hydroxylated metabolites of LID and its deethylated metabolites were present.

In addition to the *p*-hydroxylated precursors of 4-OH-XY, the levels of the other metabolites were similar to those reported in the literature [21]. It is interesting to note that XY was not detected in plasma and urine.

DISCUSSION

Although LID metabolism has been a major subject of research in the past [21,22], no information is available to permit elucidation of the time-dependent kinetics of LID. A working hypothesis in our laboratory is that LID and/or its deethylated metabolites (MEGX and GX) could be significantly *p*-hydroxylated prior to amide hydrolysis which leads to the formation of 4-OH-XY. This hypothesis is supported by the detection of 4-OH-LID in infants [23]. Subsequently, we have isolated and identified these metabolites in human (Table I) and animal urine [24]. This observation suggests that LID metabolism is more complicated than previously anticipated. In order to characterize the metabolism of LID in humans and animals, the *p*-hydroxylated precursors of 4-OH-XY were synthesized in our laboratory and used as standards for this assay The purity and identity of these compounds and their acetylated derivatives have been confirmed by GC–MS [19].

The addition of TEA to the mobile phase is known to facilitate the separation of LID and its metabolites [17]. We found that the concentration of TEA had to be adjusted from column to column in order to obtain maximum separation. The concentration of TEA used ranged from 0.1 to 0.25%.

The separation of XY and 3-OH-LID was less than optimal, but the accuracy of measuring these compounds was not adversely affected. It was determined that the level of XY was very low in our patient population and, in many cases, it was undetectable. Furthermore, 3-OH-LID was not always detected in our subjects either.

Metabolites of LID are known to be poorly extracted [18]. Using the aqueous acetylation method which was developed by Coutts et al. [19], it was found that the efficiency of acetylation and extraction was maximal and the derivatives formed were stable. This conclusion was drawn when the HPLC trace of a second ethyl acetate extract was devoid of any metabolites and derivatives.

The acetylation procedure also enhanced the separation of LID and its metabolites. It was found, for example, that underivatized *m*- and *p*-hydroxylated analogues failed to be separated when analyzed by HPLC, whereas separation was complete after acetylation (see Figs. 1 and 2).

A number of β-blockers, such as propranolol, nadolol, pindolol, acebutolol and metoprolol, and calcium channel blockers, such as verapamil and diltiazem, which might be given to the patients along with LID, have been found not to interfere with the assay.

The sensitivity of this assay is comparable to available methods in that the limit of detection is 0.1 μg/ml when 0.5 ml of plasma or 0.1 ml of urine is used.

In conclusion, we have demonstrated that this newly developed HPLC assay can be used to study LID pharmacokinetics in human.

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