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Short communication

Capillary gas chromatographic method for the measurement of small concentrations of monoethylglycinexylidide and lidocaine in plasma

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Abstract

The major metabolite of lidocaine, monoethylglycinexylidide (MEGX) is currently used as a dynamic marker of liver function. It has been proven, in recent advances, that the determination of MEGX formation after intravenous injection of lidocaine was an effective means of assessing liver dysfunction in critically ill patients. An accurate and sensitive gas chromatographic method has been developed for the determination of small quantities of MEGX formed in such cases. The procedure involves a solid-phase extraction and injection of the extract (splitless mode) into a gas chromatograph equipped with a capillary column and nitrogen–phosphorus detector. The limit of detection is 1 ng/ml and the limit of quantification is 2.5 ng/ml. The response is linear up to 50 ng/ml. The inter- and intra-assay coefficients of variation for MEGX and lidocaine are between 5 and 9%. This method can be used for the determination of small concentrations of MEGX in plasma and could be applied to analysis of small amounts of many other nitrogenous molecules. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Monoethylglycinexylidide; Lidocaine

1. Introduction

Lidocaine is commonly used as a local anaesthetic and antiarythmia agent. It is converted by the liver into several metabolites including monoethylglycinexylidide (MEGX), which is produced by an oxidative *N*-deethylation catalysed by cytochrome P4503A4. The measurement of MEGX concentration after a standard intravenous dose of lidocaine has been developed as a test of liver function, particularly in liver donors for transplantation. It is also a diagnostic test to distinguish patients with cirrhosis of the liver from normal subjects [1–4]. It has been proven, in recent advances, that the determination of MEGX production after intravenous injection of lidocaine was also an effective mean of assessing liver dysfunction due to a splanchnic ischemia in critically ill patients. In the latter case, MEGX concentrations of the order of less than 10 ng/ml must be detected in patients with a poor prognosis of survival [5].

The most currently used analytical method for the measurement of MEGX is a fluorescence polarization immunoassay (Tdx, Abbot Division Diagnostic,

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USA). Several high-performance liquid chromatographic (HPLC) using ultraviolet (UV) detection and gas chromatographic (GC) assays with ionization or nitrogen–phosphorus detection are also reported [6–11], but all these techniques have a limit of quantification higher than 10 ng/ml.

This work describes a new capillary gas chromatographic method using nitrogen–phosphorus detector. This method includes an efficient solid-phase cartridge extraction and is characterized by a high sensitivity with a limit of detection of 1 ng/ml.

2. Experimental

2.1. Instrumentation

The gas chromatograph was a Hewlett Packard model 5890 series II equipped with a nitrogen–phosphorus detector and a split–splitless injector. Samples were injected in splitless mode, using an automatic sampler HP 6890. Data were recorded and analysed with HP Chemstation Software (version A.03.04 for MS-DOS). The column was a capillary column HP-5 (5% diphenyl/95% dimethylsiloxane, 30 m×0.32 mm; Hewlett Packard).

2.2. Chromatographic conditions

The injector temperature was 200°C and the detector temperature was 300°C. The oven temperature program started at 80°C, was held constant for 1 min, then the temperature was raised by 40°C/min up to 210°C and held constant for 3.5 min.

The carrier gas was nitrogen, quality 5.0, at a flow-rate of 5 ml/min. Hydrogen was produced by a hydrogen generator Packard 9200 and air consisting of 80% nitrogen and 20% oxygen, quality K. Hydrogen and air flow-rates in the detector were 4 and 70 ml/min, respectively. The head pressure was 20 psi and the total flow was 100 ml/min.

2.3. Chemicals and reagents

Lidocaine hydrochloride monohydrate, MEGX hydrochloride and mepivacaine hydrochloride (internal standard) were kind gifts from Astra, Sweden.

The reagents used for extraction were acetic acid HPLC grade (Prolabo), ammonium acetate P.A. (Osi), methanol (Carlo Erba) and Milli Q water quality (Millipore).

2.4. Standards

Stock solutions at concentrations of 200 μg/ml were prepared by dissolving lidocaine, MEGX and mepivacaine in methanol. These solutions were stored at −20°C.

A standard solution of mepivacaine (I.S.) at a concentration of 250 ng/ml was obtained from the stock solution by dilution in water, and stored at +4°C.

Calibration samples were obtained from stock solutions by dilution, first in water, then in drug-free human plasma at the following concentrations for MEGX and lidocaine: 2.5, 5, 10, 25 and 50 ng/ml. Aliquots of 1.2 ml were frozen at −20°C.

2.5. Sample preparation

Solid-phase sample extraction was performed, using vacuum-assisted Bond-Elut cyanopropyl (CN) cartridges (1 cc/100 mg, Varian). The cartridges were first conditioned with 1 ml of methanol, followed by 1 ml of water and 1 ml of acetate buffer (0.05 *M*, pH=4.5). One ml of sample and 100 μl of I.S. solution (250 ng/ml) were applied to the cartridge. The cartridges were then rinsed with 1 ml of water and dried under vacuum. MEGX, lidocaine and I.S. were eluted with 3×200 μl of methanol. The eluate phase was evaporated to dryness at 40°C under nitrogen and the residue was finally reconstituted with 100 μl of methanol. One microliter was injected into the column.

The area under the peaks of MEGX and lidocaine were measured by HP Chemstation, and the area ratio MEGX/I.S. and lidocaine/I.S. were used to calculate the concentrations of MEGX and lidocaine.

3. Results

3.1. Chromatographic parameters

Fig. 1 shows chromatograms of an extracted blank plasma (a), an extracted plasma spiked with 25

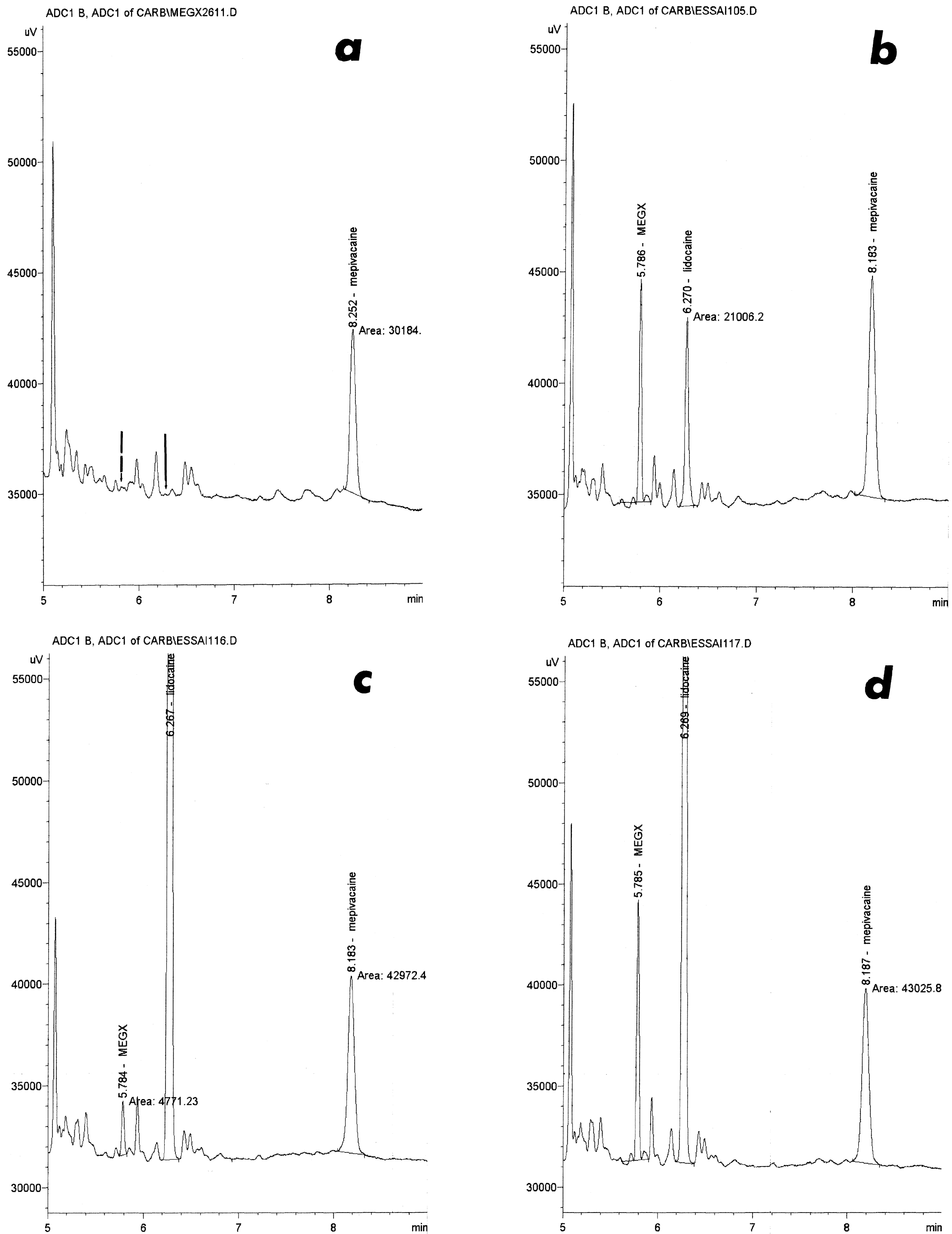

Fig. 1. Chromatograms obtained from an extracted blank plasma (a), from an extracted plasma spiked with MEGX, lidocaine and mepivacaine (I.S.) at 25 ng/ml (b), from extracted plasma samples of a patient with liver cirrhosis, collected 15 min after 1 mg/kg of lidocaine bolus injection (c), and 24 h after injection (d).

ng/ml of MEGX and lidocaine (b), the extracted plasma of a cirrhotic patient 15 min after intravenous injection of 1 mg/kg of lidocaine hydrochloride (c) and 24 h after injection (d).

The chromatographic parameters for MEGX, lidocaine and internal standard are summarised in Table 1.

3.2. Extraction recovery

The extraction recoveries were calculated for MEGX and lidocaine at three levels of concentrations (10, 25 and 50 ng/ml) by comparing the peak area obtained after extraction to that obtained after direct injection ($n=3$). The results are presented in Table 2.

Table 2
Extraction recoveries for MEGX and lidocaine ($n=3$)

Concentrations	Extraction recoveries (%)	
	MEGX	Lidocaine
10 ng/ml	74.6	82.4
25 ng/ml	72.5	72.6
50 ng/ml	71.6	68.4

For chromatographic conditions and sample preparation, see Section 2.

3.3. Linearity

The minimum detectable concentration and the lowest quantifiable level were determined for MEGX and lidocaine according to the method of Knoll [12]. The limit of detection (LOD) is 1 ng/ml and the limit of quantification (LOQ) is 2.5 ng/ml, value for which the relative standard deviation (measure of precision) and accuracy are to be less than 20% (Table 4).

The detector response is linear in the concentration range of 2.5–50 ng/ml for MEGX and lidocaine. The mean linear regression equation for MEGX obtained unweighted from six calibration curves, three intraseries and three interseries, is: $y=(52.87\pm2.54)\times+0.042\pm0.96$, where y=peak area ratio MEGX/I.S. and×=MEGX concentration in plasma; the mean correlation coefficient is 0.9992.

For lidocaine, the mean equation obtained in the same conditions is: $y=(45.92\pm4.14)\times+0.198\pm1.45$ and the mean correlation coefficient is 0.9995.

Above 50 ng/ml, the detector response is not linear any more. Therefore, the samples need to be diluted before extraction when MEGX concentration is higher than 50 ng/ml.

3.4. Reproducibility, precision and accuracy

The reproducibility was calculated for MEGX and lidocaine at 4 levels of concentrations: 1, 10, 25 and 50 ng/ml ($n=10$). The results are presented in Table 3.

From the six calibration curves obtained in the conditions described in the previous paragraph, the precision was evaluated by the coefficient of variation of the recalculated concentrations, and the accuracy by the deviation mean (difference between calculated and theoretical concentrations/theoretical concentration). The precision and accuracy of MEGX and lidocaine calibration are presented in Table 4.

Quality controls at two levels of concentration (4 and 20 ng/ml) were measured twice in three different runs. Table 5 shows precision and accuracy of quality controls for MEGX and lidocaine.

Table 1
Chromatographic parameters for MEGX, lidocaine and mepivacaine (I.S.)

	MEGX	Lidocaine	Mepivacaine
Retention time (min)	5.8	6.3	8.2
Relative retention time	0.7	0.76	
Width (min)	0.027	0.034	0.065
Number of plates	248 350	188 970	89 160
Resolution	16		38
Symmetric factor	0.89	0.9	0.96

Chromatographic conditions are described in Section 2.2.

Table 3
Reproducibility for MEGX and lidocaine calculated from area ratio MEGX/I.S. and lidocaine/I.S. (n=10)

	1 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml
MEGX				
Mean	0.047	0.261	0.674	1.378
S.D.	0.004	0.018	0.037	0.068
C.V. (%)	8.28	6.96	5.45	4.95
Lidocaine				
Mean	0.039	0.352	0.8995	2.065
S.D.	0.003	0.024	0.048	0.077
C.V. (%)	7.44	6.79	5.34	3.74

For chromatographic conditions and sample preparation, see Section 2.

3.5. Interferences

Most nitrogenous molecules that can interfere were studied. They can be divided into three groups: drugs that have a shorter retention time than MEGX: most of barbiturics (amobarbital, butobarbital, secobarbital, vinbarbital...), meprobamate and acetaminophen, drugs that have a longer retention time than lidocaine (antidepressants, benzodiazepines and opiates) and drugs that have a retention time between that of MEGX and lidocaine (caffeine, 1–7 dimethyl xanthine and thiopental). Caffeine and its metabolite 1–7 DMX could interfere with MEGX if they were present in large amounts. They are eliminated during the solid-phase extraction, so that they do not interfere with MEGX measurement.

The most-often used drugs in the intensive care unit were particularly studied: adrenaline, noradrenaline, dopamine, dobutamine, midazolam and sulfentamyl. None of these drugs interfered with MEGX or lidocaine measurement.

Table 5
Precision and accuracy data for quality controls of MEGX and lidocaine

	QC 4		QC 20	
	MEGX	Lidocaine	MEGX	Lidocaine
Mean	4.12	4.24	19.49	20.04
S.D.	0.28	0.30	0.58	0.74
C.V. (%)	6.82	7.07	2.99	3.69
Deviation (%)	3.04	5.96	−2.52	0.20

Chromatographic conditions and sample preparation are the same as in Table 3.

3.6. Analytical applications

This technique can be applied to determine the MEGX production after intravenous injection of 1 mg/kg of lidocaine in evaluating the liver function. The population can be divided into three groups: a first group of normal subjects which has a concentration of MEGX formed around 50 ng/ml (on average), a second group of patients with liver impairment which has a concentration of MEGX formed between 10 and 50 ng/ml and a third group of critically ill patients with significant hepatic dysfunction, which has a MEGX production inferior to 10 ng/ml.

This method has been used to measure MEGX production in two subjects, one with normal hepatic function and in the other with cirrhosis of the liver. The blood samples were collected before, 15 min

Table 4
Precision and accuracy data for MEGX and lidocaine calibration

	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml
MEGX					
Mean	2.61	5.10	10.21	24.37	50.20
S.D.	0.24	0.47	0.69	1.21	0.45
C.V. (%)	9.06	9.21	6.79	4.96	0.90
Deviation (%)	4.27	2.04	2.14	−2.46	0.41
Lidocaine					
Mean	2.93	5.15	9.82	24.60	50.18
S.D.	0.53	0.31	0.59	0.66	0.29
C.V. (%)	18.01	5.93	6.03	2.67	0.59
Deviation (%)	10.36	2.93	−1.86	−1.59	0.36

Chromatographic conditions and sample preparation are described in Section 2.

after and 24 h after lidocaine hydrochloride bolus injection (1 mg/kg). The concentrations obtained 15 min after injection were 38 ng/ml for the normal subject and 6 ng/ml for the cirrhotic subject. At 24 h after injection, the concentrations were respectively 2.5 ng/ml and 33 ng/ml. Concerning the lidocaine, the concentrations were much higher than the limit of linearity: the therapeutic concentrations are between 2 and 5 μg/ml. They can be determined by using the chromatograph in split mode.

4. Discussion

The greatest difficulty with this work has been to develop an analytical method both sensitive and specific. All stages of this method have been studied to yield the best possible compromise.

Concerning the extraction, we tested first the liquid–liquid extraction. MEGX and lidocaine are extracted by ter-butyl-methyl-ether (TBME) or dichloromethane with an alkaline buffer (pH=10), but this extraction is not selective enough: it extracts also caffeine and its metabolite 1–7 DMX, that have retention times comparable to that of MEGX. As reported by Lorec and all. [9], these two substances may interfere with MEGX measurement if they are present in large quantities in plasma, considering that the resolution factor between MEGX and caffeine is too small. This resolution could be improved if nitrogen (carrier gas) was substituted by helium or hydrogen.

As the liquid–liquid extraction does not have enough selectivity, we tested the solid-phase extraction, less often used for lidocaine and MEGX [6,10]. We tested different kinds of cartridges (C_2, C_8, C_{18}, CH, PH and CN). The extraction on CN cartridge has been chosen for its high selectivity: it allows to eliminate caffeine, 1–7 DMX and plasmatic components. The extraction recovery has been optimized in conditioning the cartridge with acetate buffer (pH=4.5).

We chose to reconstitute the evaporated extract with methanol. Because of its polarity, this solvent may reactivate the insert in the injector, which leads to a fixation of injected molecules and therefore to a fall in sensitivity. We tested some other less polar solvents (ethyl acetate, hexane, heptane) and other solvents belonging to the same class on Snyder's triangle (ethanol, isopropanol). In the two cases, we observed a fall in sensitivity, so we decided to keep the methanol as solvent for reconstitution.

Concerning the chromatographic conditions, we first tested injections in split mode. The oven temperature was 200°C, the injector and detector temperatures were 300°C and the head pressure was 25 psi. Under these conditions, the limit of detection cannot be reduced to less than 10 ng/ml.

By using the injector in splitless mode and modifying the previous chromatographic conditions, we achieved the limit of detection to 1 ng/ml. The maximum increase of sensitivity is obtained only if the injected extract is not vaporized and goes entirely in liquid state into the column. Therefore, the injector and oven temperatures must not be too high during the injection: the injector temperature has been reduced to 200°C and the oven temperature has been programmed (from 80°C at the injection to 210°C). The head pressure has also been reduced to 20 psi to increase the resolution of the peaks.

The good chromatographic running and the sensitivity of analysis depend on many parameters. The most important are the state of the insert in the injector, the performances of the column and the sensitivity of detector.

This method is reproducible and quite rapid despite the oven program: it needs only 10 min per analysis.

The results described in Section 3.6 confirm the conclusions of Oellerich and all. [4]: MEGX formation and elimination in subjects with cirrhosis of the liver are slower than in normal subjects. In this case, the determination of MEGX production is therefore an extremely effective test to distinguish cirrhotic subjects from normal subjects.

This technique appears suitable for assessing liver dysfunction in critically ill patients [5].

In summary, the use of the injector in splitless mode is an effective process to obtain a significant increase of sensitivity and then to measure with precision and accuracy MEGX concentrations less than 10 ng/ml.

Furthermore, this method could be applied to the analysis of small amounts of many other nitrogenous molecules.

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