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Journal of Chromatography B, 805 (2004) 1–5
JOURNAL OF CHROMATOGRAPHY B
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Liquid chromatographic method for the determination of lidocaine and monoethylglycine xylidide in human serum containing various concentrations of bilirubin for the assessment of liver function

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Received 28 April 2003; received in revised form 11 November 2003; accepted 16 January 2004

Abstract

A high-performance liquid chromatographic method is described for determination of lidocaine (2-(dietyloamino)-*N*-(2,6-dimetylofenylo) acetamid) and its metabolite, monoethylglycine xylidide (MEGX), in human serum containing various concentration of bilirubin. Lidocaine and its metabolite were extracted from human serum using dichloromethane. After separation of the layers and freezing at −32 °C, the organic layer was decanted and evaporated under a stream of nitrogen. The sample was dissolved in the mobile phase (12% acetonitrile in 15 mM potassium dihydrogen orthophosphate, pH 3.0), and after separation on a Supelcosil LC-8-DB column, the analytes were measured by ultraviolet detection at 205 nm. Trimethoprim (TMP) was used as the internal standard. The recovery of the examined analytes ranged from 95.7 to 97.9% for lidocaine and from 98.0 to 99.9% for MEGX. The lower limit of quantification (LLOQ) was established at 200 μg/l for lidocaine and at 10 μg/l for MEGX. The choice of suitable conditions for chromatographic separation of lidocaine and its metabolite MEGX allowed the elimination of the influence of endogenous bilirubin on the result of analysis.

Keywords: Lidocaine; Monoethylglycine xylidide; Bilirubin

1. Introduction

Conventional serum biochemical tests such as: bilirubin concentration, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities have been widely used to assess liver function. In recent years, tests based on metabolism of different xenobiotics (lorazepam, indocyanine green, antipyrine and caffeine) have been used to evaluate the functional status of the liver. The rate of elimination of these drugs depends on liver function and varies in different liver diseases [1]. The tests require technically difficult analyses, long study time and collection of a large number of blood samples [2].

Promising results were obtained by performing a dynamic test based on quantification of lidocaine and its metabolites in venous blood [3,4]. Lidocaine is a drug of high hepatic extraction ratio. This ratio describes liver's activity on the basis of the dependence between the metabolism of lidocaine and the functional condition of liver. Lidocaine is highly sensitive indicator of disfunction of this organ. It is metabolized in the human liver to monoethylglycine xylidide (MEGX) and glycine xylidide (GX) [5] by the cytochrome P-450 3A4-dependent mono-oxygenase system. The FPIA method of determining MEGX concentration has been reported by many authors as the most convenient one [3,6–8]. This method is characterized as very sensitive and requiring short time of analysis: 20 min, but the MEGX measurements are influenced by high blood concentration of bilirubin, triglycerides and cholesterol [4,9]. Test methods using high-performance liquid chromatographic method to determine lidocaine and its metabolite are widely covered in the literature [10–14]. However, information on whether endogenous substances present in blood of patients with liver disease, such as bilirubin, cholesterol and triglycerides, affect the results of the MEGX measurement, is still lacking.

The purpose of this work is to establish, by means of HPLC, an MEGX test, to choose appropriate conditions of chromatographic separation and to compare the correlation

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doi:10.1016/j.jchromb.2004.01.030

between the results of the test and values of bilirubin concentration, which is the standard biochemical index of liver function.

2. Experimental

2.1. Materials

MEGX and lidocaine were obtained from Astra-Zeneca (Sodertalje, Sweden). MEGX and lidocaine had purities no less than 98.0%. Trimethoprim (TMP), used as the internal standard (IS) and bilirubin, were supplied by Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, dichlormethane, chloroform and potassium dihydrogen orthophosphate were purchased from J.T. Baker (Deventer, Holland) and used as analytical-grade reagents. Disodium tetraborate decahydrate and phosphoric acid (85%) were obtained from POCH (Gliwice, Poland).

2.1.1. Preparation of solution

The standard solutions of MEGX (1.5 mg/l) and lidocaine (5 mg/l) were made in water and stored at 4 °C. Standard solution of TMP (1.5 mg/l) was prepared by dissolving TMP in mobile phase (1.5 mg/1 ml) and making it up with water to the volume of 1000 ml. The bilirubin standard solution (1 mg/1 ml) was made in chloroform.

2.2. HPLC system

The chromatographic separation was carried out on a Shimadzu liquid chromatograph equipped with LC-10 AS pump and SPD-10 AV detector in combination with a Chromatopac CR-6A integrator. A Supelcosil LC-8-DB Column (150 mm × 4.6 mm, 5 μm) and precolumn, Supelquard LC-8 DB 2 cm replacement were used.

2.3. Assay conditions

Acetonitrile (12%) in 15 mM potassium dihydrogen orthophosphate was used as the mobile phase. The pH was adjusted to 3.0 with 85% phosphoric acid. The flow rate was 0.8 ml/min and the UV detector was set at 205 nm.

2.4. Sample preparation

In the extraction procedure, 0.5 ml of serum, 100 μl of the internal standard (TMP), 0.5 ml of saturated solution of disodium tetraborate decahydrate and 3 ml of dichlormethane were shaken for 1 min. After separation of the layers and freezing at −32 °C, the organic layer was decanted and evaporated under a stream of nitrogen. The sample was dissolved in 250 μl of the mobile phase and 100 μl was injected onto the HPLC system.

2.5. Calibration curves

The first calibration curve was prepared from serum containing MEGX in concentrations of: 10, 25, 50, 100, 250 and 500 μg/l. The second calibration curve was prepared from serum containing lidocaine in concentrations of: 200, 500, 1000, 2500, 3500 and 5000 μg/l. All samples contained 0.15 μg of the internal standard.

2.6. Method validation

The precision and accuracy of the method were determined by replicate analyses ($n = 10$) of serum samples containing different concentrations (low, medium and high) of MEGX or lidocaine. The repeatability was determined by performing analyses of the concentrations of these analyses during 1 day under the same conditions by one operator. The intermediate precision was determined by analyzing low, medium and high concentrations of MEGX and lidocaine in serum ($n = 21$) on different days by two operators n (each of them analyzed one sample of each concentration on 21 different days). The recovery was calculated by comparing analyses of concentrations obtained from water solutions ($n = 3$) and from serum samples. The calibration curve was calculated from six standards, in six repetition.

2.7. Influence of bilirubin concentration

To establish the influence of bilirubin on the results of lidocaine and MEGX determination we added bilirubin, lidocaine and MEGX to 0.5 ml of serum of a healthy volunteer; the final concentration of bilirubin was 5, 10, 50, 100, 250 and 500 μmol/l, of MEGX 10–500 μg/l and of lidocaine 200–5000 μg/l. Analyses were extracted according to the method described in Section 2.4.

3. Results

3.1. Chromatography

Separation of the mixture of lidocaine and its metabolite was performed and optimum retention times were determined: for lidocaine (14.3 min), for MEGX (9.4 min) and for TMP (internal standard) (10.2 min). Fig. 1 presents the chromatograms obtained from blank human serum of a healthy volunteer after extraction 1A— blank human serum, 2A—human serum spiked with 0.15 μg internal standard, 0.5 μg lidocaine, 5 ng MEGX, 5 μmol bilirubin.

3.2. Calibration curves

The response was linear within the studied range 200–5000 μg/l for lidocaine and 10–500 μg/l for MEGX. Calculation of the calibration curve was by linear regression

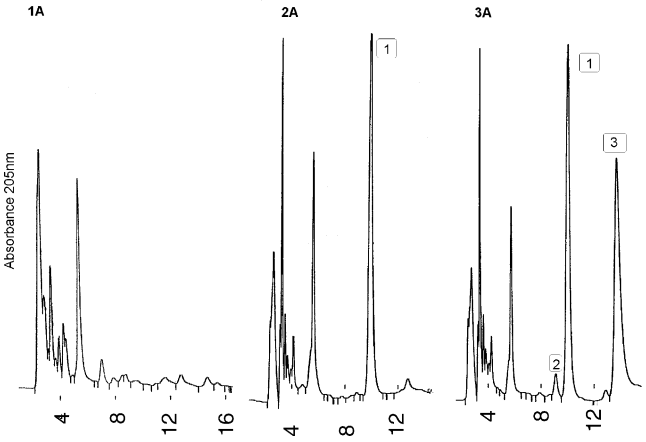

Fig. 1. Chromatograms obtained from blank human serum of a healthy volunteer after extraction: 1A, blank human serum; 2A, human serum spiked with 0.15 μg internal standard (1) (t_R = 10.2 min); 3A, human serum spiked with 0.5 μg lidocaine (3) (t_R = 14.3 min), 5 ng MEGX (2) (t_R = 9.4 min), 5 μmol bilirubin and IS (1) (t_R = 10.2 min).

($y = mx + b$). The linear regression parameters, calculated from peak areas/peak IS. Curve parameters for lidocaine: slope 2.3607 ± 3.6101, *y*-intercept 0.3650 ± 4.4706, R^2 = 0.9987 and for MEGX: slope 0.0012 ± 0.4511, *y*-intercept 0.0075 ± 199.6747, R^2 = 0.9997. Both the slope and the *y*-intercept are expressed as the mean of six separate assays, n = 12. The lower limit of quantification (LLOQ) was established at 200 ± 0.0121 μg/l (CV = 6.25%) for lidocaine and at 10 ± 1.2263 μg/l (CV = 11.94%) for MEGX.

3.3. Precision and accuracy

Repeatability of the HPLC method for quantification of lidocaine and its metabolites was checked (Table 1) and the following coefficients of variation (%CV) were obtained: 2.1, 3.8 and 4.26% for lidocaine, for 1000, 2500 and 5000 μg/l, respectively, and 1.62, 1.71 and 1.50% for MEGX for concentrations of 10, 100 and 500 μg/l, respectively.

Table 2
Recovery of lidocaine and MEGX

Compound	Concentration	Recovery (% ± S.D.)
LID (μg/l)	1000	95.70 ± 4.00
	2500	97.50 ± 4.49
	5000	97.90 ± 3.41
MEGX (μg/l)	10	98.00 ± 3.59
	100	99.25 ± 1.70
	500	99.90 ± 0.36

To establish the recovery of lidocaine and its metabolite, 100 μl of standard solutions were added to plasma samples (1000, 2500 and 5000 μg/l for lidocaine and 10, 100 and 500 μg/l for MEGX, respectively). The extraction procedure was carried out as described. The extraction was performed in duplicates and the ratio of extraction was calculated.

The recovery of the examined analyses ranged from 95.7 to 97.9% for lidocaine and from 98.0 to 99.9% for MEGX (Table 2). The high recovery of lidocaine and its metabolite suggests that the extraction conditions were appropriate for these compounds. The temperature of freezing of the sample seems not to affect the degree of purity of serum. The same chromatographic separation was obtained independently of the fact whether the samples were frozen at −32 or −80 °C. Adding bilirubin (5–500 μmol/l) to plasma samples did not have any affect on the recovery of the method. The presence of bilirubin at concentration between 5 and 500 μmol/l did not affect the determination of lidocaine and MEGX in the concentration ranges of 200–2500 and 10–500 μg/l, respectively.

Table 3 presents cross-validation comparing of parameters validation of our HPLC method with that of Chen and Potter [9].

Table 1
Precision and accuracy of determination of lidocaine and MEGX

Compound	Reference values (μg/l)	Concentration observed (mean value ± S.D.)	R.S.D. (%)	Confidence interval for mean values (P = 95%)	Accuracy (%)
LID (within day (n = 21))	1000	950 ± 20	2.10	950 ± 15	−5.0
	2500	2470 ± 90	3.80	2470 ± 60	−1.2
	5000	4890 ± 200	4.26	4890 ± 140	−2.2
MEGX	10	9.85 ± 0.10	1.62	9.85 ± 0.07	−1.5
	100	99.15 ± 1.30	1.71	99.15 ± 0.99	−0.9
	500	499.50 ± 7.49	1.500	499.50 ± 5.51	−0.1
LID (between day (n = 21))	1000	980 ± 20	2.04	980 ± 8	−2.0
	2500	2460 ± 90	3.66	2460 ± 60	−1.6
	5000	4700 ± 150	3.19	4700 ± 110	−6.0
MEGX	10	9.81 ± 0.02	3.04	9.81 ± 0.11	−1.9
	100	98.14 ± 2.33	2.40	98.14 ± 0.93	−1.9
	500	497.40 ± 7.60	1.53	487.40 ± 3.00	−0.5

Table 3
Cross-validation

Validation parameters	Determination of MEGX		
	Our HPLC method	HPLC method [9]	FPIA method [9]
Linearity by linear regression with a calibration/range (μg/l)	10–500	10–250	25–250
LOD (μg/l)	<10	<8	<10
Inter- and intra-assay coefficients of variation (%)	<2.3	<9.5	
Recovery (%)	98.0–99.9	>85	

3.4. Measurement of MEGX and lidocaine in serum of patients with various liver diseases and with known concentration of bilirubin

MEGX and lidocaine concentrations in venous blood were measured at time 0 (prior to injection) and 15 min after intravenous administration of lidocaine at a dose of 1 mg/kg of body weight. Serum was extracted according to the method described in Section 2.4. Lidocaine test was conducted in 34 patients with various liver diseases. The results are shown in Table 4.

Fig. 2 presents chromatograms of human serum from patients with different liver disfunction (1B and 1C) at time 0 (before the injection of the drug) and (2B and 2C) 15 min after intravenous administration of lidocaine (1 mg/1 kg of body weight) with high (2B: 416.8 μmol/l) and low (2C: 8.56 μmol/l) concentration of bilirubin.

Table 4
Lidocaine and MEGX concentrations 15 min after lidocaine administration in serum and bilirubin concentrations, from patient with different liver disfunctions

Sample	Bilirubin (μmol/l)	Lidocaine (μg/l)	MEGX (μg/l)
1	8.21	800	78
2	8.56	730	20
3	9.58	1050	40
4	10.27	480	442.4
5	11.12	200	90
6	11.29	600	154
7	13.35	920	375
8	13.52	350	10
9	13.52	975	5
10	13.69	1875	45
11	15.23	300	0
12	16.43	800	170
13	17.28	1620	40
14	18.14	440	338
15	18.65	1850	60
16	19.68	1400	285
17	19.68	25	90
18	22.24	1100	0
19	25.32	375	10
20	27.38	300	340
21	32.34	2350	45
22	39.70	780	256
23	41.92	0	25
24	53.39	1160	182
25	64.68	1800	70
26	83.84	370	90
27	118.92	1400	40
28	145.44	360	128
29	169.23	150	30
30	177.96	440	200
31	217.31	380	0
32	244.69	1100	0
33	347.36	360	0
34	416.80	600	0

4. Discussion

The determination of lidocaine and MEGX is useful to test liver function as the criterion determining the efficiency of this organ in donors and recipients. The lidocaine test requires only two blood samples collected at time 0 (prior to injection of the drug) and 15 min after intravenous administration of lidocaine [15,16]. Lidocaine is given in subtherapeutic doses and does not cause undesirable effects. Therefore, HPLC methods for quantification of MEGX are now recommended for the lidocaine test [10–14,17].

Chen and Potter [15] compared the FPIA and HPLC methods, performing the MEGX test in donors and recipients of liver transplants, with high concentrations of bilirubin, triglycerides and cholesterol. MEGX concentration measured by the FPIA method were significantly higher than the values obtained by the HPLC method. Therefore, these authors suggest the exercise of caution while using the determination of MEGX when the concentration of bilirubin is high.

Schutz et al. [18] obtained satisfactory results by modification of the FPIA procedure (precipitation of serum with the Abbott Digoxin II), as an alternative HPLC.

The HPLC method of determination of MEGX and lidocaine have already been described [13,17]. In order to improve sensitivity, the authors suggested to modify this method using solid phase extraction in conjunction with electrospray mass spectrometry. Another publication [14] shows high sensitivity of the lidocaine method (0.2–30 ng/ml).

The authors of all of the above-mentioned publications have found an influence of bilirubin on MEGX concentration.

The determination of MEGX using HPLC, which we have described, is short and simple to use. The high bilirubin concentration has no influence on the result of the lidocaine test. High recovery of lidocaine, MEGX and TMP from human serum indicates the choice of appropriate conditions of extraction of these compounds.

For precision of the method of MEGX and lidocaine determination the variability coefficient (CV) amounted for MEGX 5.8% and for lidocaine 6.26%.

It would be useful to determine the value of the test in evaluating the function of the liver during its various

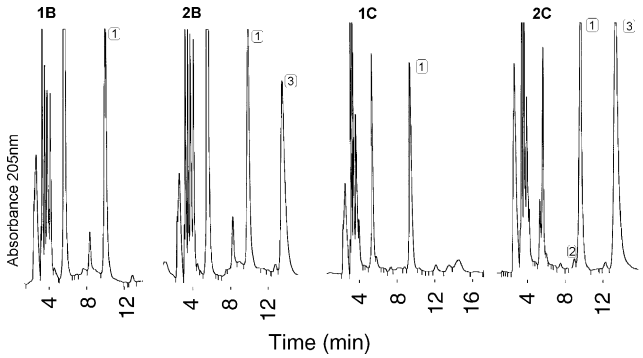

Fig. 2. Chromatograms of human serum from patients with different liver disfunction (1B and 1C) at time 0 before the injection of the drug and (2B and 2C) 15 min after intravenous administration of lidocaine (1 mg/1 kg of body weight). The concentration of the analytes were—2B: 600 μg/l for lidocaine (3), 0 μg/l for MEGX and 416.8 μmol/l for bilirubin and 2C: 730 μg/l for lidocaine (3), 20 μg/l for MEGX (2) and 8.56 μmol/l for bilirubin. Peaks (1): IS.

diseases, for the hitherto existing researches [19–21] are mainly concentrated on describing, how the test can be used to evaluate the usefulness of the organ for transplantation and to forecast its functions.

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