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Short communication

Easy and fast LC–MS/MS determination of lidocaine and MEGX in plasma for therapeutic drug monitoring in neonates with seizures

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ABSTRACT

A fast liquid chromatography–tandem mass spectrometry with electrospray ionization method was developed and validated for simultaneous quantification of lidocaine and its active metabolite MEGX in 10 μL of plasma of neonates with seizures. The sample preparation consists of an easy protein precipitation sample pre-treatment with methanol. Chromatographic separation was achieved on a Alltima HP C18-EPS 150 mm × 2.1 mm column with an isocratic mobile phase of 0.1% (v/v) ammonium acetate in purified water–0.1% (v/v) formic acid in acetonitrile (70:30, v/v). The analytes were detected with a Thermo Scientific triple quadrupole Quantum Access with positive ionization. Ions monitored in the selected reaction monitoring (SRM) mode were m/z 235.2 → 86.6 for lidocaine (at 3.35 min), m/z 207.1 → 58.8 for MEGX (at 2.75 min) and 280.1 → 86.7 for 3-nitrolidocaine (internal standard, at 3.20 min). The method was validated over a linear range of 0.2–18.0 mg/L for lidocaine and MEGX, using 3-nitrolidocaine as the internal standard. The lower limit of quantification (LLQ) was 0.2 mg/L for lidocaine and MEGX. The within-run and between-run CV (%) were lower than 6.9% for both lidocaine and MEGX. Recoveries were in the range of 99.4% to103.6%. Observed LC–MS/MS matrix effects were −6.2% for MEGX (ion suppression) and were negligible for lidocaine and the internal standard (i.e. <0.1%). Compared to other bioanalytical articles published in medical literature (PubMed) during the last 15 years that described LC–MS/MS methods for quantification of lidocaine in human plasma, our method uses less plasma, has a shorter and more simple sample pre-treatment and has a short run time.

1. Introduction

Lidocaine (syn. Lignocaine), an amine derivative of cocaine, is widely used worldwide for different indications. It has anesthetic, sedative, antiarrhythmic and also anticonvulsant properties. It was introduced as a local anesthetic in 1948 and as an antiarrhythmic agent in the 1960s. The application of lidocaine as an anticonvulsant in neonates originated in Scandinavia with the first report appearing in the medical literature in 1970 [1].

Therapeutic Drug Monitoring is indicated because of its extensive interpatient variability and of its narrow therapeutic index. In cardiology, lidocaine plasma concentrations above 1.5 mg/L are required for primary ventricular fibrillation prophylaxis after acute myocardial infarction. Objective signs of neurological adverse events occur at concentrations from 6 to 8 mg/L, but serious signs of neurological toxicity occur at plasma concentrations above 8 mg/L in adults [2,3]. In contrast, in neonates, where lidocaine is used for its neurological property instead of its antiarrhythmic property, cardiac effects are undesired. Fortunately, in this population the desired neurological effects occur at lower plasma concentrations than cardiological adverse effects. Therefore, lidocaine plasma concentrations in neonates can exceed 8 mg/L without undesired cardiac consequences [4,5].

Lidocaine is metabolized into primarily monoethylglycinexylidide (MEGX) by the cytochrome P450 system. After a subsequent N-desethylation, glycinexylidide (GX) is formed, but to a lesser extent than MEGX. MEGX has been shown to have 80% to 90% of the antiarrhythmic potency of lidocaine in animal models of arrhythmia [6]. Its anticonvulsant potency is unknown. For prevention of cardiac adverse events monitoring of both lidocaine and MEGX plasma concentrations is necessary. Because the observed half-live for MEGX (8.9–28.4 h) in newborns is longer than the half-live of lidocaine (5.2–5.4 h), MEGX can accumulate and can contribute to clinical toxicity [7,8]. Although its contribution to lidocaine toxicity has not been fully confirmed, there have been reports of lidocaine toxicity in patient with lidocaine plasma concentrations within the therapeutic range, probably because of metabolite accumulation. Our goal was to develop a sensitive and fast bioanalytical assay

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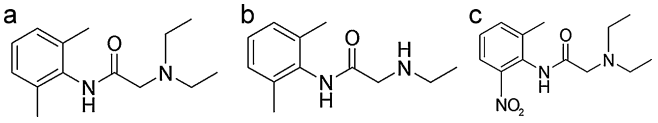

Fig. 1. Chemical structures of lidocaine (a), MEGX (b) and internal standard 3-nitro lidocaine (c).

which can be used for therapeutic drug monitoring and pharmacokinetic studies of lidocaine and MEGX in small sample volumes in newborns with neonatal seizures.

2. Experimental

2.1. Reagents

Lidocaine (Fig. 1a) was purchased from Bufa (IJsselstein, The Netherlands). MEGX (Fig. 1b) was kindly donated by AstraZeneca (Sweden) and internal standard 3-nitro lidocaine, a synthetic derivative of lidocaine, (Fig. 1c) was purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada).

Formic acid was purchased from Merck (Darmstadt, Germany), 0.1% (v/v) ammonium acetate in water from Riedel-de Haën (Seelze, Germany). Methanol and 0.1% (v/v) formic acid in acetonitrile were purchased from Biosolve (Valkenswaard, The Netherlands).

2.2. Standard solutions and QC samples

Stock solutions of lidocaine, MEGX and 3-nitro lidocaine were prepared in methanol with a concentration of 0.5 mg/mL for lidocaine and MEGX and 1.0 mg/mL for the internal standard 3-nitro lidocaine. 3-Nitro lidocaine was chosen as the internal standard because it is not a metabolite of lidocaine but a synthetic derivative. Stock solutions of lidocaine and MEGX were diluted with methanol to a solution with a concentration of 10 mg/L. From this solution standards were prepared in newborn calf serum at the concentrations of 1.0, 4.0, 8.0 and 12.0 mg/L. The quality control was prepared from a second stock solution lidocaine and MEGX. The quality controls were prepared in newborn calf serum with a concentration of 0.2 mg/L (LLQ), 0.5 mg/L (LOW, lowest control), 3.0 (MED, medium control) and 10.0 mg/L (HIGH, highest control).

The internal standard stock solution was diluted with methanol to a final concentration of 50 μg/L.

2.3. Sample preparation

A plasma aliquot of 10 μL was diluted to 100 μL with newborn calf serum into a 2.0 mL Eppendorf tube. Subsequently 250 μL internal standard was added. The vials were vortexed for 1 min and centrifuged at 10,900 rpm for 3 min. 200 μL of the clear upper layer of the supernatant was transferred into a vial and 250 μL formic acid 2% was added and vortexed for 5 s. 5 μL was injected into the LC–MS/MS system.

2.4. Instrumentation and chromatographic conditions

All experiments were performed on a Thermo Fisher Scientific (Waltham, MA) triple quadrupole Quantum Access LC–MS/MS system with a Surveyor MS pump and a surveyor Plus autosampler with an integrated column oven. The Quantum Access mass selective detector was operated in electrospray-positive ionization mode and performed selected reaction monitoring. Data acquisition and data processing were performed using Xcalibur software version 2.10.

The analytical column was an Alltima HP C18-EPS 150 mm × 2.1 mm column with 3 μm particle size (GRACE Davison, Lokeren, Belgium). The Surveyor Plus autosampler temperature was set at 15 °C, and the column temperature was kept at 30 °C. An isocratic run of 5.0 min was performed using a mixture of 0.1% (v/v) ammonium acetate in purified water and 0.1% (v/v) formic acid in acetonitrile (70/30, v/v) at a flow rate of 200 μL/min.

The retention times of lidocaine, MEGX and 3-nitro lidocaine were, respectively, 3.35, 2.75 and 3.20 min (Fig. 2).

Analytes were detected by MS/MS with electrospray ionization (ESI)-interface in selected reaction monitoring (SRM)-mode. The transitions used were m/z 235.2 → 86.6 for lidocaine, m/z 207.1 → 58.8 for MEGX and 280.1 → 86.7 for 3-nitrolidocaine (internal standard).

High purity nitrogen was used for the sheath gas and auxiliary gas, and argon was used as collision gas. The transition, scan time, optimum tube lens and collision energy (CE) values were optimized and listed in Table 1. Scan time was set on 0.1 s for all compounds.

The optimum capillary temperature is 375 °C; the ion source spray voltage was set at 4000 V, the sheath gas pressure at 90 AU, and the auxiliary gas pressure at 4 AU.

2.5. Validation procedures

Validation of the method in human plasma included the following parameters according to the FDA guidelines for bio-analytical method validation [9].

2.5.1. Linearity, accuracy and precision

Eight calibration points in the range of 0.2–18.0 mg/L including a zero sample were used to determine linearity on three separate days using linear regression.

Plasma concentrations were calculated by linear regression using the calculated ratios of sample/internal standard by area.

For determination of accuracy and precision all concentrations (LLQ, LOW, MED and HIGH) were prepared and analyzed in five-fold in three separate runs on three different days.

Within run, between-run and overall CVs were calculated using one-way ANOVA.

The accuracy and precision have to be within the maximum tolerated bias and CV (20% for LLQ, 15% for the other concentrations).

2.5.2. Recovery and matrix effect

The recovery was determined by comparing the chromatographic ratios of a standard, containing both lidocaine and MEGX, after sample preparation and a spiked standard of both compounds.

Matrix effect is the direct or indirect interference with the signal due to presence of unintended analytes, such as interfering substances or co-administered drugs in the sample.

Matrix-effects were assessed by post-extraction addition experiments [10]. Sample extracts of 10 blank patient samples were spiked with lidocaine, MEGX and the internal standard after sample preparation and were compared with a neat solution prepared in the same solvent composition as the reconstitution solution used for the post-extracted spiked samples. Both the solutions were prepared at a concentration of 4.0 mg/L for lidocaine and MEGX and 50 μg/L for 3-nitro lidocaine. Matrix effects less than 15% were accepted.

2.5.3. Stability

Stability of lidocaine and MEGX during the analytical process was assessed in the autosampler at 15 °C for 30 h and during three repetitive freeze–thaw cycles at −20 °C. Total compound degradation should be less than 10% during the whole simulation.

2.5.4. Selectivity

Selectivity is the ability to differentiate and quantify the analyte from other compounds in the sample. For selectivity, analyses of

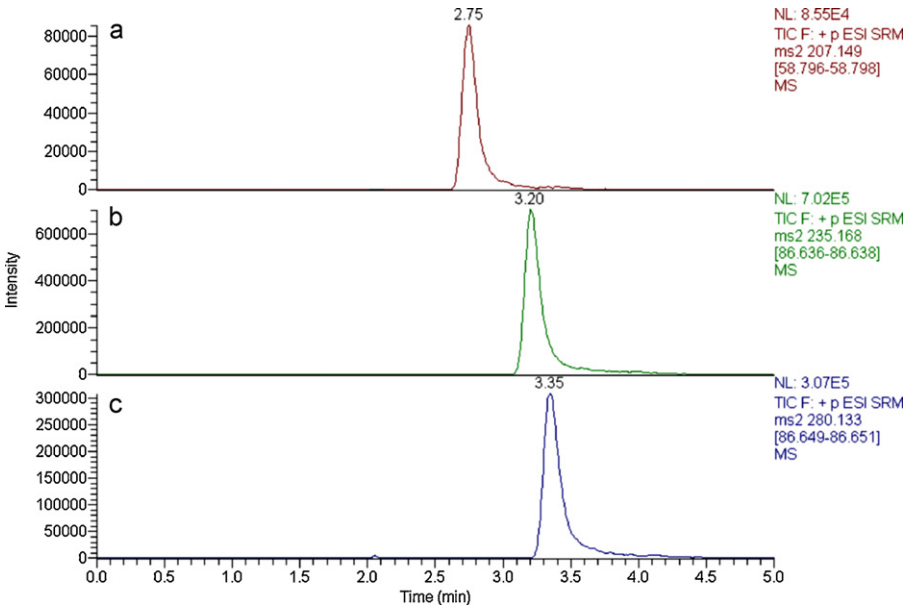

Fig. 2. Representative chromatogram of a patient sample with MEGX (a, 1.94 mg/L), lidocaine (b, 2.54 mg/L) and 3-nitro lidocaine (c, 50 μg/L).

six blank serum samples (from different pediatric patients that did not receive lidocaine) were performed.

Interference of frequently co-administered drugs, and some of their clinical relevant metabolites, on Neonatal Intensive Care Units were tested by spiking a serum sample with these drugs. Investigated co-administered drugs were dopamine, ceftazidime, cefazoline, amoxicillin, sildenafil, midazolam and morphine. For midazolam and morphine their respective main active metabolites (1-hydroxymidazolam, morphine-β-3-glucuronide and morphine-β-6-glucuronide) were also tested.

3. Results

3.1. Linearity, accuracy and precision

Linearity was determined without the use of a weighting factor. Regression coefficients (R^2) are 0.994 for lidocaine and 0.999 for MEGX.

The validation results for accuracy and precision are within the maximum tolerated bias and CV (20% for LLQ, 15% for the other concentrations) as shown in Table 2.

Overall CV is less than 9% for both LLQ's and less than 8% for the other concentrations.

The LLQ was set at 0.2 mg/L based on pharmacokinetic relevance. Given the fact that LLQ's may have maximum tolerated CV's of 20%, the actual analytical LLQ will be lower than 0.2 mg/L.

3.2. Recovery and matrix effect

Recovery is shown in Table 3. Recovery was determined using a calibration point with a concentration of 8.0 mg/L for lidocaine as well as MEGX. Recoveries were 100.5% for lidocaine, 99.4% for MEGX and 103.6% for the internal standard.

Matrix effects were calculated by comparing the average areas of the analytes in the neat solution to the average areas of the analytes spiked into a blank matrix sample after sample preparation. No matrix effects were observed for lidocaine and the internal standard whereas the MEGX signal showed 6.2% ion suppression.

3.3. Stability

Samples were found to be stable during processing in the autosampler at 15 °C for at least 30 h. Freeze–thaw stability was determined during three repetitive cycles and no instability (i.e. <10% degradation) was observed for both components. Data are shown in Table 3.

These results show that no significant degradation occurred during sample preparation and chromatography.

3.4. Selectivity

In none of the blank patient samples a significant (interfering) signal was detected at the retention times of lidocaine, MEGX and the internal standard. In the spiked sample with frequently co-administered drugs no interfering signals were detected at the relevant retention times. This is due to the high selectivity using a triple quadrupole system.

4. Analytical advantages and clinical implications

The developed method has several advantages over immunoassay methods based on fluorescence polarization technique; there is

Table 1
Settings of tandem quadrupole mass spectrometer in ESI-mode.

Compound	Parent ion (*m*/*z*)	Product ion (*m*/*z*)	CE (V)	Tube lens (V)
Lidocaine	235.2	86.6	18	88
MEGX	207.1	58.8	14	83
3-Nitro lidocaine (IS)	280.1	86.7	20	59

Abbreviations: CE = collision energy; IS = internal standard.

Table 2
Validation results of lidocaine and MEGX.

	Regression coefficient (linear range)	Concentration (mg/L)	Within-run CV (%)	Between-run CV (%)	Overall CV (%)	Overall bias (%)
Lidocaine	0.994 (2.0–18.0 mg/L)	LLQ (0.2)	5.4	4.4	7.0	3.0
		LOW (0.5)	3.0	3.0	4.3	1.1
		MED (3.0)	1.6	2.9	3.3	−3.0
		HIGH (10.0)	2.3	4.7	5.3	−5.6
MEGX	0.999 (2.0–18.0 mg/L)	LLQ (0.2)	6.9	5.0	8.6	−4.6
		LOW (0.5)	2.9	6.6	7.2	0.9
		MED (3.0)	2.1	6.2	6.5	−0.5
		HIGH (10.0)	3.2	7.3	8.0	−3.2

Abbreviations: LLQ = lower limit of quantification; CV = coefficient of variation.

Table 3
Stability results of lidocaine and MEGX.

		Concentration (mg/L)	Within-run CV (%)	Between-run CV (%)	Overall CV (%)	Overall bias (%)
Lidocaine	Freeze–thaw (n = 5)	MED (3.0)	1.8	5.4	5.7	−3.4
		HIGH (10.0)	3.1	5.4	6.2	−5.0
	Autosampler stability	MED (3.0)	4.0	NA	NA	4.8
MEGX	Freeze–thaw (n = 5)	MED (3.0)	1.4	6.4	6.6	0.2
		HIGH (10.0)	1.3	3.7	4.3	−2.0
	Autosampler stability	MED (3.0)	4.8	NA	NA	1.5

Abbreviations: CV = coefficient of variation; NA = not applicable.

no cross reactivity with structure-relates substances and the precision and accuracy are higher using LC–MS/MS. Moreover, MEGX is determined in the same run as lidocaine. With fluorescence polarization immunoassay two separate methods (i.e. kits) would be required. Because nowadays MEGX kits are not available anymore for a lot of immunoassays of different manufacturers, only plasma concentrations of lidocaine are determined with FPIA in clinical settings.

In contrast, a disadvantage of our method is a slightly longer sample pre-treatment time compared with an immunoassay method which takes about 5–10 min more.

Compared to other bioanalytical articles published in medical literature (PubMed) during the last 15 years that described LC–MS/MS methods for quantification of lidocaine in human plasma [11,12], our method uses less plasma, has a shorter and more simple sample pre-treatment and has a short run time. Although the LLQ in the other methods is lower than our developed method, this is not applicable for our goal. Moreover, the other methods are not able to determine therapeutic and supratherapeutic (>9 mg/L) lidocaine plasma concentrations in neonates because of their low range of quantification.

For therapeutic drug monitoring and pharmacokinetic studies in (preterm) neonates it is important to limit the amount of blood that is required for quantitative analysis of the drug under investigation. Too much blood withdrawal in small (preterm) newborns can compromise basic hemodynamic parameters, hemoglobine and hematocrite values, blood component transfusions or fluid requirements [13]. With our method a volume of (not more than) 10 μL of serum was required for the quantitative analysis of both lidocaine and its active main metabolite.

For neonates experiencing cardio- and/or neurotoxicity or for neonates with (persisting) seizures the time between the moment of blood withdrawal and the result of the analysis should be as short as possible to improve clinical outcome because lidocaine toxicity is potentially life threatening. In our case lidocaine, MEGX and the internal standard eluted from the column in less than 3.5 min.

5. Conclusions

An easy and fast HPLC–MS/MS method for the determination of lidocaine and its active main metabolite MEGX in plasma of neonates has been developed and validated. Lidocaine and MEGX were extracted from human plasma by a fast and simple protein precipitation sample pre-treatment method with methanol.

The assay requires 10 μL of human plasma, uses minimal sample preparation and can be used for therapeutic drug monitoring and pharmacokinetic studies in neonates in a clinical setting.

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