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Determination of mivacurium in plasma by high-performance liquid chromatography

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Abstract

An assay has been developed and validated for the routine monitoring of mivacurium in plasma. It consists of liquid–liquid extraction with dichloromethane and high-performance liquid chromatography with fluorometric detection (excitation and emission wavelengths 220 nm and 320 nm, respectively). A Spherisorb C_1 5 μm column and a mobile phase containing acetonitrile, KH_2PO_4 and methanol are used. At a flow-rate of 1 ml/min, a concentration gradient is applied. The detection limit is approximately 1 ng/ml in plasma. For the separation of stereoisomeres, the Spherisorb SCX 10 μm column and acetonitrile–Na_2SO_4 as a mobile phase can be used. The assay shows good linearity over the range 1–1000 ng/ml. The accuracy and precision allows the utilisation in clinical pharmacokinetic studies.

Keywords: Mivacurium

1. Introduction

Mivacurium chloride, (*E*)-(1*R*,1′*R*)-2,2′-[4-octenedioyl-bis (oxytrimethylene)] bis[1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxybenzyl)isoquinolinium] dichloride (Fig. 1), is a non-depolarizing neuromuscular blocker of short duration of action with minimal haemodynamic effects and apparent lack of histamine releasing properties [1]. In humans it undergoes extremely rapid decomposition by plasma cholinesterase. The metabolites of mivacurium are quaternary alcohol and quaternary ester. These metabolites are potentially harmless and do not produce any neuromuscular effects [1].

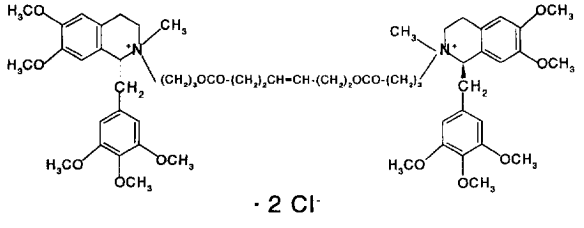

Fig. 1. Chemical formula of mivacurium chloride.

Several assays for the analysis of mivacurium in plasma by means of high-performance liquid chromatography (HPLC) have been suggested [2–4]. These include the use of solid phase extraction and reversed-phase HPLC with UV [3] or fluorometric [2,4] detection. Also a stereoselective HPLC assay with a solid-phase extraction, reversed-phase separation and fluorometric detection has been developed

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by Burroughs Wellcome [5], but has not been published yet.

Accurate determination of muscle relaxants in biological fluids is a prerequisite for extensive studies of pharmacokinetics and clinical dose-response relationships of neuromuscular blocking agents. Therefore, any analytical method used should be precise, sensitive, rapid and cost efficient. The resolution and sensitivity of the published procedures [2–4], however, proved to be inadequate, at least in the low concentration range, to support pharmacokinetic studies of mivacurium. In addition, as the analytical technique described by Brown et al. [2] could not be reproduced in our hands, we have developed a more sensitive reversed-phase chromatographic procedure with liquid–liquid sample extraction.

2. Experimental

2.1. Reagents and chemicals

Mivacurium chloride was supplied by Glaxo Wellcome Pharma (Vienna, Austria). Dichloromethane (Fluka Chemie, Buchs, Switzerland), acetonitrile (Promochem, Wesel, Germany) and methanol (Sigma, St. Louis, MO, USA) were HPLC grade; potassium iodide, monobasic potassium phosphate, phosphoric acid and sodium sulphate (E. Merck, Darmstadt, Germany) were analytical grade. Laudanosine and D-tubocurarine, used as internal standards, were also supplied by Sigma.

2.2. Collection of plasma samples

Na–EDTA was used as an anticoagulant. Immediately after collection, blood was mixed with a solution of echothiopate iodide (1 mg/ml) to inhibit plasma cholinesterase and centrifuged at 1500 *g* for 10 min. Plasma samples were stored at −80°C until assayed.

2.3. Liquid–liquid extraction

A 100-μl volume of KI solution (5%), 20 μl internal standard solution (0.1 mg/ml of D-tubocurarine (0.01 mg/ml for concentration level <100 ng/ml) for method I or 0.01 mg/ml of laudanosine for method II, respectively) and 6.0 ml dichloromethane were added to 0.5–2.0 ml plasma and stirred for 30 s on a vortex mixer. After centrifugation (5 min, 1500 *g*) the upper layer was removed by aspiration. The bottom (organic) layer was poured into another test tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 300 μl HPLC eluent. An aliquot of 100 μl was introduced onto the HPLC column using an autosampler.

2.4. Chromatographic apparatus and conditions

A Model 126 System Gold HPLC pump, a System Gold 406 analog interface (Beckman Instruments, San Ramon, CA, USA), an FP-920 fluorescence detector (220 nm excitation and 320 nm emission wavelength, gain 10 or 100, respectively) and an AS 950 autosampler (Jasco, Tokyo, Japan) were used. The HPLC system was operated and the chromatograms were recorded and processed using System Gold chromatography software (Beckman Instruments).

2.4.1. Method I: determination of total concentration of mivacurium

The column was a Spherisorb C_1 5 μm particle size, 150×4.6 mm I.D. (SRD HPLC, F. Pannosch, Vienna, Austria). Mobile phases were (A) 70:30 acetonitrile–0.01 *M* KH_2PO_4 (pH 3) containing 10% methanol and (B) 70:30 0.05 *M* KH_2PO_4 (pH 3) containing 10% methanol. At a flow-rate of 1 ml/min, the following concentration gradient was applied: 0–3 min 100% A, 3–5 min 0–100% B, 5–9 min 100% B, 9–11 min 0–100% A, 11–13 min 100% A.

2.4.2. Method II: separation of stereoisomers

A Spherisorb SCX 10 μm particle size (250×4.6 mm I.D.) HPLC column and a mobile phase of 60:40 acetonitrile and 0.07 *M* Na_2SO_4 in 0.005 *M* H_2SO_4 were used in an isocratic system at 1 ml/min.

2.5. Preparation of standards

Analytical stock solutions of standards (1 mg/ml) were prepared by dissolving mivacurium chloride or the internal standards (D-tubocurarine or laudanosine) in HPLC mobile phase A. They were stable for

several months at −20°C and were diluted with the same solvent to the specific concentration as needed.

2.6. Test for interferences with other drugs

In pilot studies 500 ng of morphine, sufentanil, ketamine, vecuronium, propofol or midazolam were added to separate tubes with 1 ml of plasma containing 1000 ng of mivacurium. These plasma samples were assayed as outlined above.

2.7. Analysis of human plasma

After induction of general anaesthesia with fentanyl and propofol, blood samples were drawn from a female patient before and 6 min after an intravenous bolus injection of mivacurium (0.3 mg/kg).

3. Results

3.1. Method I

Representative chromatograms of extracted control human plasma and extracted human plasma spiked with 250 ng/ml mivacurium and 2000 ng/ml internal standard (D-tubocurarine) are shown in Fig. 2a,b. The retention times were 8.6 min for D-tubocurarine and 9.7 min for mivacurium and they were insensitive to changes in chromatographic conditions and small changes in mobile phase composition. The chromatogram of blank plasma did not show any interfering peaks from endogenous compounds, even at highest sensitivity.

The recovery rates of mivacurium from plasma, estimated by comparing peak areas obtained from unextracted standards and standards extracted from plasma, were about 50 to 60%.

Plasma measurements of mivacurium (analyte/internal standard peak-area ratio in extracted plasma samples versus calculated value) were linear (r^2= 0.99237 for concentrations ranging from 1 to 1000 ng/ml, r^2=0.99856 for concentrations ranging from 1 to 50 ng/ml). The within-day and between-day precision and accuracy data of the assay are shown in Table 1. The within-day and between-day precision are determined as the coefficient of variation (in percent) of five plasma samples per day with the same concentration of mivacurium determined on the same day and on four different days. The accuracy is expressed as a relative error (mean concentration found/concentration added in percent).

Fig. 2c shows the chromatogram of a plasma sample taken from the patient 6 min after a bolus dose of 0.3 mg/kg mivacurium. The estimated concentration of mivacurium was 57 ng/ml plasma, demonstrating the clinical feasibility of the assay.

For routine analysis 1 ml of plasma was processed. As 2 ml can be processed, the detection limit was as low as 1 ng/ml plasma. A chromatogram of

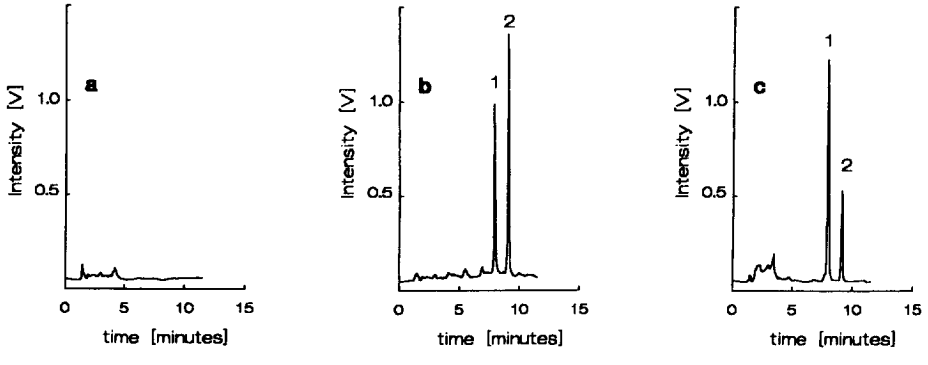

Fig. 2. Representative chromatograms of (a) extracted control human plasma, (b) extracted human plasma spiked with 250 ng/ml mivacurium and 2000 ng/ml internal standard (D-tubocurarine) and (c) plasma sample taken from the patient 6 min after a bolus dose of 0.3 mg/kg mivacurium. Conditions: Spherisorb C_1 5 μm particle size, 150×4.6 mm I.D. column, mobile phases (A) 70:30 acetonitrile–0.01 *M* KH_2PO_4 (pH 3) containing 10% methanol and (B) 70:30 0.05 *M* KH_2PO_4 (pH 3) containing 10% methanol, flow-rate 1 ml/min. Gradient: 0–3 min 100% A, 3–5 min 0–100% B, 5–9 min 100% B, 9–11 min 0–100% A, 11–13 min 100% A. Detector gain 10. Peaks 1 and 2 represent D-tubocurarine and mivacurium, respectively. The concentration of mivacurium in the patient's plasma (57 ng/ml) was calculated using linear calibration curve (analyte/internal standard peak-area ratio in HPLC buffer A).

Table 1
Precision and accuracy of the determination of mivacurium in human plasma

Concentration added (ng/ml)	Mean concentration found (ng/ml)	Coefficient of variation (%)	Relative error (%)
Within-day (n=5)			
1	0.99	10.13	−1.0
10	10.15	9.55	1.5
300	299.2	3.47	−0.3
1000	989.8	3.14	−1.0
Between-day (n=20)			
1	0.95	17.30	−5.0
10	9.82	10.23	−1.8
300	299.4	2.91	−0.2
1000	1004	4.24	0.4

extracted human plasma spiked with 1 ng/ml mivacurium and 200 ng/ml internal standard is shown in Fig. 3.

3.2. Method II

Fig. 4 shows a chromatogram of plasma spiked with 1000 ng/ml total concentration of mivacurium when the stereoisomers have been eluted separately. The retention times of *cis–cis*, *cis–trans* and *trans–trans* isomers were 6.5, 6.8, and 7.0 min, respectively.

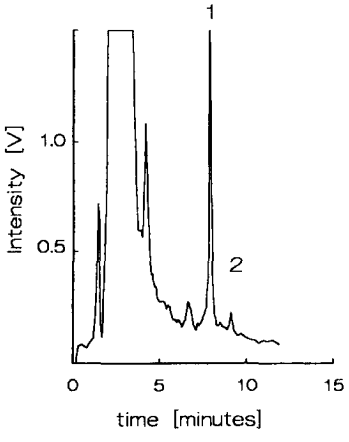

Fig. 3. Chromatogram of extracted human plasma spiked with 1 ng/ml mivacurium and 200 ng/ml internal standard (D-tubocurarine). Conditions similar to those in Fig. 2, but detector gain increased to 100. Peaks: 1=D-tubocurarine; 2=mivacurium, respectively.

4. Discussion

The procedure presented for the analysis of mivacurium is rapid, sensitive, selective and especially suited for the quantification of the drug in plasma. Using liquid–liquid extraction of the samples, the costs for determination could be reduced significantly. By the addition of KI the solubility of mivacurium in organic solvents was increased [6], however pH adjustment did not improve the recovery rate. The obtained recovery of 50–60% shows good correspondence with the values described in the

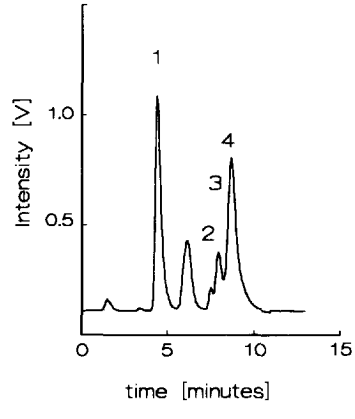

Fig. 4. Chromatogram of extracted human plasma spiked with 1000 ng/ml total concentration of mivacurium and 200 ng/ml laudanosine when the stereoisomers are eluted separately. Conditions: Spherisorb SCX 10 μm particle size (250×4.6 mm I.D.) column; mobile phase: 60:40 acetonitrile and 0.07 *M* Na_2SO_4 in 0.005 *M* H_2SO_4, an isocratic system at 1 ml/min flow-rate. Detector gain 10. Peaks: 1=laudanosine, 2–4=*cis–cis*, *cis–trans* and *trans–trans* isomers of mivacurium, respectively.

literature [2]. Moreover, only a 13-min interval between repeated injections is adequate to re-establish the required HPLC conditions. Due to the concentration gradient, a good separation between mivacurium, the internal standard and endogenous substances is guaranteed.

For quantitation, a fluorometric detection was chosen (220 nm excitation and 320 mn emission wavelength). We preferred to use a longer excitation wavelength compared to Brown et al. [2] and Lacroix et al. [4], in order to get a more stable baseline. Even though at 220 nm the signal response is somewhat lower than at 210 nm, the sensitivity (at 1 ng/ml) surpassed that reported previously [2,4]. With a C.V.-value of 10% within-day (or 17% between-day), quantification of extremely low concentrations in plasma shows good reproducibility.

Our analytical technique offers several advantages over the two analytical procedures which have been described for the determination of mivacurium [2,4]. In both assays, solid-phase extraction cartridges were used for sample preparation. This extraction method is rather expensive. The liquid–liquid extraction procedure used in our study is cheaper and as fast as the liquid–solid technique. Both methods allow the determination of the stereoisomers, but their value for the measurement of clinical samples can be questioned. In terms of neuromuscular blocking activity, the *cis–trans* and *trans–trans* isomers are equipotent whereas the *cis–cis* isomer shows only 10% activity compared with the former [2]. The amount of *cis–cis* isomer found in commercially available ampoules is only 3.8 to 8.2% [2]. Therefore, the determination of the isomers is clinically irrelevant and is of interest only when pharmacodynamic data are to be correlated with respective plasma levels of a neuromuscular blocker.

As we could demonstrate (Fig. 4), the separation of the isomers is possible with our analytical approach (II). For quantification of the stereoisomers, isolated isomers are required. As these purified substances were not available to us, statistical evaluation of this analytical procedure (II) was not possible.

In our assay, the addition of a cholinesterase inhibitor is necessary because mivacurium could be enzymatically hydrolysed even after the collection of a blood sample. In contrast to Brown et al. [2], who suggested the addition of only 0.33 mg/ml of echothiopate iodide to plasma immediately before extraction, we found it necessary to add a higher concentration (1 mg/ml blood), and to add the inhibitor immediately after blood collection.

The selectivity of our analytical procedure was verified in the presence of other drugs used in anaesthetic clinical practice (morphine, sufentanil, ketamine, vecuronium, propofol and midazolam). No peaks interfering with mivacurium or internal standard could be detected in the chromatograms. In addition, drug-free human plasma samples (both haemolytic and non-haemolytic), were analysed, and no interfering peaks could be observed from endogenous substances or metabolites.

In contrast to the previously published procedures, D-tubocurarine or laudanosine were chosen as the internal standard because they are stable, have extraction characteristics and detector response similar to those of mivacurium, but show different HPLC retention times. A further advantage of using these compounds as internal standard is their free availability from various distributors.

In conclusion, our method of measurement of mivacurium in plasma proved reproducible, highly sensitive, isomer selective and cost effective.

Acknowledgments

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