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Short communication

High-performance liquid chromatographic assay of mepivacaine enantiomers in human plasma in the nanogram per milliliter range

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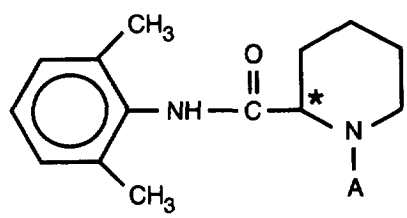
Abstract

A method enabling quantification of *R*-(−)- and *S*-(+)-mepivacaine in human plasma in the low nanogram per milliliter range is described. The procedure involves extraction from plasma with diethyl ether, centrifugation, back-extraction into an acidified aqueous solution, washing with a mixture of pentane and isoamylalcohol, alkalinisation, followed by extraction with a mixture of *n*-pentane and isoamylalcohol. After evaporation of the organic phase, the residue is redissolved in the mobile phase used for the HPLC analysis, which consists of a 6.8:93.2 (v/v) isopropanol–sodium hydrogenphosphate buffer solution with the pH adjusted to 6.8 using phosphoric acid. The HPLC method has been described previously. Separation of the enantiomers is achieved with an α_1-AGP column and the UV detection wavelength is 210 nm. The minimal detectable concentration is ca. 3 ng/ml and the lower limit of quantification is 5 ng/ml for each enantiomer. For both enantiomers r is >0.9995 over the plasma enantiomeric concentration range of 10.5–1053 ng/ml.

Keywords: Enantiomer separation; Mepivacaine

1. Introduction

Mepivacaine is an amino–amide type local anaesthetic possessing a chiral carbon atom (Fig. 1). Clinically it is used as the racemate. Studies in animals indicated that the enantiomers have quantitatively different pharmacological and toxicological properties [1,2], including different pharmacokinetics [3]. The latter study also showed that no conversion of the enantiomers occurs in vivo. In humans, plasma concentrations of the enantiomers following administration of the racemate for combined psoas compartment/sciatic nerve block, differ approximately



Mepivacaine: A = CH_3
Ropivacaine: A = C_3H_7
Bupivacaine: A = C_4H_9

Fig. 1. Chemical structures of mepivacaine, ropivacaine and bupivacaine. * Indicates the chiral carbon atom. Whereas mepivacaine and bupivacaine are racemic mixtures, ropivacaine is the pure *S*-(−)-enantiomer.

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twofold [4], indicating a difference in the total plasma clearance of the enantiomers. In addition, differences may exist in the volumes of distribution, as has been observed with the enantiomers of bupivacaine, a related local anaesthetic agent [3,5]. However, volumes of distribution cannot be estimated accurately following extravascular administration of local anaesthetic agents [6].

To delineate the pharmacokinetics of the enantiomers of mepivacaine, we have performed a study involving intravenous administration of racemic mepivacaine in volunteers. We anticipated that to allow accurate estimation of the major pharmacokinetic variables, a sampling period of 8 h would be necessary and that a quantitation limit in the order of 5 ng/ml would be required for each enantiomer. Since the detection limit of the previously reported method for the assay of mepivacaine enantiomers in human plasma was 400 ng/ml [4], we have modified that method to obtain a lower detection limit.

2. Experimental

2.1. Instrumentation

A modular HPLC system was used, consisting of an SF400 pump (S.A.I., Hendrik Ido Ambacht, Netherlands), a Promis II auto-injector, an SpH99 column thermostat (Spark Holland, Emmen, Netherlands), a stainless steel guard column 10×4.0 mm prepacked with 40 μm α_1-AGP, a stainless-steel analytical column 100×4.0 mm prepacked with 5 μm α_1-AGP (J.T. Baker, Deventer, Netherlands) and a SF 757 absorbance detector equipped with a 12-μl flow cell (S.A.I.).

2.2. Chemicals

Racemic mepivacaine-HCL and ropivacaine-HCL were obtained from Astra Pain Control (Södertälje, Sweden). HPLC-grade *n*-pentane and isopropanol were obtained from J.T. Baker. Analytical grade ethanol, diethyl ether, isoamyl alcohol, phosphoric acid (85%), HCl, NaOH and $Na_2HPO_4 \cdot 12H_2O$ were purchased from Merck (Darmstadt, Germany).

2.3. Calibration standards

A stock solution was made by dissolving 40 mg racemic mepivacaine-HCL in 100 ml ethanol. Working solutions were prepared by diluting the stock solution with ethanol to obtain 1053 ng, 527 ng, 263 ng, 132 ng, 52.7 ng, 26.3 ng and 10.5 ng of the free base of each enantiomer/25 μl. The internal standard solution (ropivacaine) was made by dissolving 40 mg ropivacaine-HCL in 100 ml ethanol. A working solution of 440 ng ropivacaine base/25 μl was obtained by diluting it with ethanol. All solutions were stored at 4°C.

2.4. Sample preparation

A 25-μl volume of a standard solution of mepivacaine (for calibration), or ethanol (samples), and 25 μl of the internal standard solution of ropivacaine were added to 1 ml of human plasma and mixed in the centrifuge tube. Samples were extracted with 5 ml diethyl ether on a Vortex whirlmixer for 3 min and then centrifuged for 10 min at 2000 *g* and 4°C. The organic phase was transferred to another centrifuge tube and 0.5 ml 0.1 *M* HCl was added. The drugs were then back extracted for 3 min into the aqueous phase. The organic phase was discarded and 5 ml *n*-pentane containing 0.1 ml isoamyl alcohol was added. The aqueous phase was washed for 3 min and again the organic phase was discarded. A 50-μl volume of 2 *M* NaOH was added, and the aqueous phase was extracted for 3 min with 5 ml *n*-pentane containing 0.1 ml isoamyl alcohol and then centrifuged for 10 min at 2000 *g* and 4°C. The organic phase was transferred to an evaporation tube and evaporated to dryness at 40°C under a gentle stream of pure nitrogen. The residue was redissolved in 100 μl mobile phase, and 30 μl were injected onto the HPLC system.

2.5. Operating conditions

Chromatography was performed in the reversed-phase mode. The column temperature was 30°C. The absorption wavelength of the detector was set at 210 nm with a detector time-constant of 0.5 s. The mobile phase consisted of 6.8:93.2 (v/v) isopropanol–buffer containing 3.6 g $Na_2HPO_4 \cdot 12H_2O$/liter

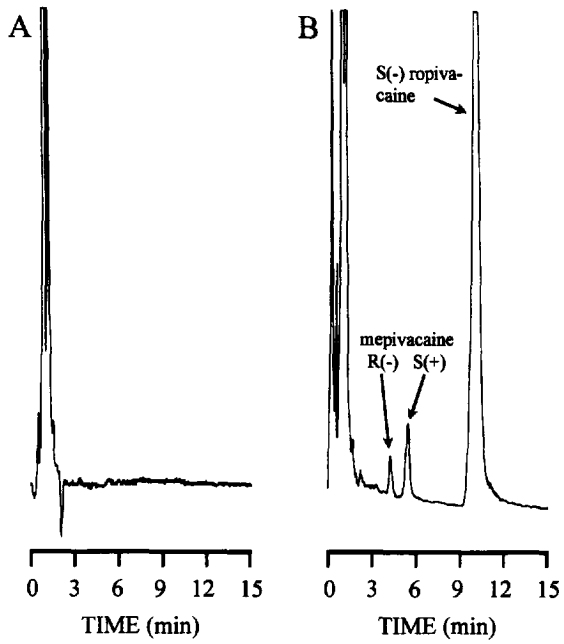

Fig. 2. Chromatograms of (A) blank human plasma, and (B) a plasma sample taken 420 min after intravenous administration of 52.8 mg racemic mepivacaine. Measured concentrations are 10.3 ng/ml and 20.8 ng/ml for *R*-(−)- and *S*-(+)-mepivacaine, respectively.

and adjusted to pH 6.8 with phosphoric acid. The flow-rate of the mobile phase was 1.1 ml/min.

3. Results

Typical chromatograms are shown in Fig. 2. The retention times were 4.6 min, 5.8 min and 10.5 min for *R*-(−)-, *S*-(+) mepivacaine and ropivacaine, respectively, assuming that the order of elution for the enantiomers was the same as that described previously [7], because no pure enantiomer of mepivacaine was available for testing. Neither endogenous components nor enantiomers of bupivacaine interfered with the assay. However, lidocaine, as well as (enantiomers of) prilocaine did interfere. The detection limit, defined as a *S/N* of 3, was 3 ng/ml plasma per enantiomer. The limit of quantification, corresponding with a coefficient of variation of 15% was 5 ng/ml for each enantiomer.

Calibration curves were obtained by weighted least-squares linear regression analysis (weight factor $1/y^2$) of the enantiomer/ropivacaine peak-height ratio versus the concentration of that enantiomer. The calibration lines for both enantiomers were

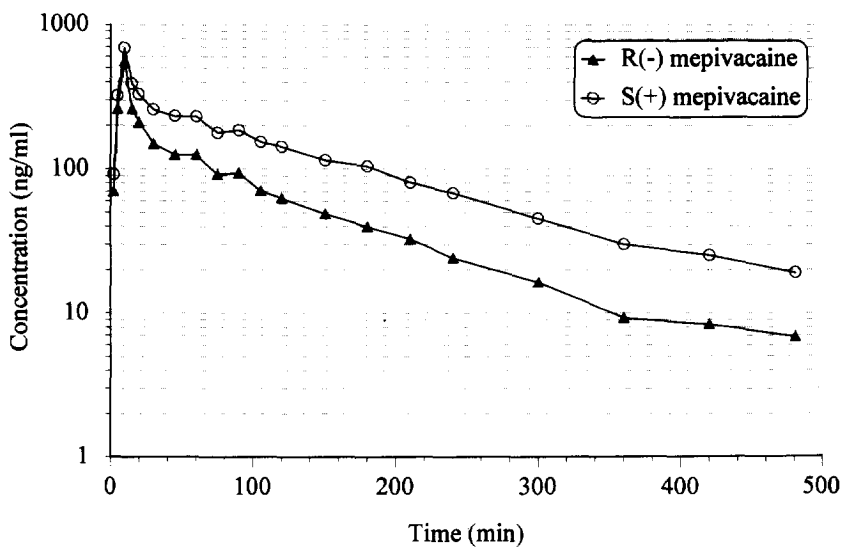

Fig. 3. Plasma concentration versus time curves of *R*-(−)- and *S*-(+)- mepivacaine in a volunteer who received an intravenous infusion of 52.8 mg racemic mepivacaine over 10 min.

Table 1
Inter-day variabilities

Concentration of	Coefficient of variation (%)	
R-(−)- or *S*-(+)-mepivacaine (ng/ml)	*R*-(−)-mepivacaine	*S*-(+)-mepivacaine
10.5	8.7	9.5
26.3	6.0	5.3
52.7	5.4	3.2
132	7.9	4.9
263	9.1	7.9
527	5.9	5.4
1053	5.4	5.0

$n=8$ for all samples.

linear in the investigated range (10.5–1053 ng/ml per enantiomer, 7 calibration points) with a correlation coefficient (r) varying from 0.9996 to 0.9999 and 0.9997 to 0.9999 for *R*-(−)- and *S*-(+)-mepivacaine, respectively ($n=8$).

The inter-day variability over eight days with plasma of male human volunteers showed coefficients of variation (C.V.) of the peak-height ratio of the enantiomers versus ropivacaine that were ≤9.1% for *R*-(−)-mepivacaine and ≤9.5% for *S*-(+)-mepivacaine (Table 1). The intra-day variability was 5.0% ($n=9$) for both enantiomers at a concentration of 263 ng/ml per enantiomer.

The recovery at 26.3 ng/ml per enantiomer was 61.5±1.6% ($n=3$) and 63.6±2.7% ($n=3$) for *R*-(−)- and *S*-(+)-mepivacaine, respectively, and at 263 ng/ml, it was 65.8±1.2% ($n=3$) and 66.4±2.1 ($n=3$), respectively. The recovery for ropivacaine was 72.7±2.7% ($n=3$) at a concentration of 440 ng/ml.

4. Discussion

It has been shown that the enantiomers of mepivacaine can be separated using an α_1-AGP column [3,7] and that concentrations of the enantiomers in human plasma can be quantified using ropivacaine as internal standard [4]. The latter method involved a simple extraction procedure using *tert.*-butyl ether and had a quantitation limit of 400 ng/ml, based on a *S/N* of 3. Modification of the extraction technique enabled us to bring the quantification limit down to 5 ng/ml, i.e., below the limit that is necessary to determine the pharmacokinetics after intravenous administration. This is illustrated in Fig. 3.

So far, the method described here has been used to determine plasma concentrations of both *R*-(−)- and *S*-(+)-mepivacaine in more than 400 human plasma samples, obtained after intravenous and epidural administration of racemic mepivacaine, with little or no column deterioration. In addition, the method has been used to measure concentrations in dialysate, obtained after equilibrium dialysis to determine the degree of plasma protein binding using a method described previously for bupivacaine [8]. This may be of importance as it has been shown that the volume of distribution and total plasma clearance of the enantiomers of bupivacaine based on total (bound+unbound) plasma concentrations differ markedly, whereas the volume of distribution and clearance based on unbound plasma concentrations are very similar for both enantiomers [5].

Since analysis of plasma samples from clinical studies showed interference when lidocaine or prilocaine were used for skin infiltration, the possible interference of other commonly used local anesthetic agents was examined. These examinations confirmed that lidocaine and (enantiomers of) prilocaine interfered with the assay of mepivacaine enantiomers, whereas the enantiomers of bupivacaine did not.

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