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Note

Stereoselective high-performance liquid chromatographic assay for bupivacaine enantiomers

EDMUND J.D. LEE* and S.B. ANG

Department of Pharmacology, National University of Singapore, Kent Ridge Crescent, Singapore 0511 (Singapore)

and

T.L. LEE

Department of Anaesthesia, National University Hospital, Singapore (Singapore)

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Bupivacaine (B) is a local anaesthetic with a chiral carbon centre. It has been used clinically as the racemate. Although the enantiomers are equipotent as in vitro nerve blockers, $R(+)$-bupivacaine (RB) is more toxic than the $S(-)$-enantiomer (SB) following subcutaneous or intravenous administration [1]. However, SB is characterized by a longer duration of anaesthesia [1]. These differences have been thought to be a consequence of enantioselective differences in the binding and disposition of the two enantiomers.

Racemic mixtures may be resolved by means of precolumn formation of an appropriate diastereomer, chiral mobile phases or chiral stationary phases. Hermansson [2] demonstrated that a number of basic drugs, including B, could be resolved using α_1-acid glycoprotein as the chiral stationary phase. We report here a method for the assay of B enantiomers that uses the α_1-acid glycoprotein column, and the application of the assay to describe the disposition of the enantiomers following intrapleural administration of B.

EXPERIMENTAL

Apparatus

The liquid chromatograph comprised a Varian 5560 pump, a Varian UV200 variable-wavelength detector and a Varian Vista 402 data system. The column was a commercially available Enantiopak (LKB, Bromma, Sweden) α_1-acid gly-

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coprotein column (100×4 mm I.D.), maintained at 30°C by means of a Waters TCM column oven.

Chemicals

Pure bupivacaine and Marcaine® were gifts from Astra, Sweden. Diazepam was purchased from Sigma. 2-Propanol and hexane were commercially available HPLC-grade solvents from Merck and J.T. Baker, respectively.

Extraction and chromatography

An aliquot (1 ml) of serum containing B was spiked with diazepam (1 μg). The serum was alkalinized with 100 μl of 2 *M* sodium hydroxide and extracted with 7 ml of *n*-hexane. The organic phase was evaporated to dryness and reconstituted in 100 μl of mobile phase. A 20-μl aliquot was injected onto the column. The mobile phase consisted of 9% 2-propanol in 0.008 *M* sodium phosphate buffer with 0.1 *M* sodium chloride and was pumped at 0.3 ml/min. At this flow-rate the column pressure was not recordable. The detector was set at 215 nm.

Calibration curves

Serum standards of B at concentrations of 0.5–5 μg/ml were prepared and assayed as described above. The variability and accuracy of the assay were estimated by assaying four serum standards of B (2 μg/ml) using a calibration standard of RB and SB at concentrations of 0.5, 1 and 2.5 μg/ml.

Intrapleural bupivacaine

Venous blood samples were withdrawn at 5, 10, 15, 20, 30, 60, 90 and 120 min from a patient who had been given an unilateral intrapleural injection of bupivacaine (15 ml of 0.5% Marcaine). After centrifugation, the sera were assayed as described.

RESULTS

Typical chromatograms are shown in Fig. 1. The retention times for diazepam, RB and SB were 15.7, 21.0 and 28.3 min, respectively. There was no interference from serum components. The calibration curves for RB and SB were linear for the concentration range studied ($y_{RB}=1.0x+0.062$, $r^2=0.999$; $y_{SB}=0.90x+0.058$, $r^2=0.999$). The accuracy and within-day variability of the assay are shown in Table I. Recoveries of SB and RB from serum are listed in Table I, and were 72 and 68%, respectively, at a serum concentration of 1 μg/ml.

The absorption of RB and SB following intrapleural administration was rapid (Fig. 2). Peak concentrations were observed within 15 min. Serum concentrations of SB were ca. 15% higher than those of RB.

DISCUSSION

Diazepam, RB and SB were successfully resolved by the α_1-acid glycoprotein column. We were unable to assign the correct enantiomers to the bupivacaine

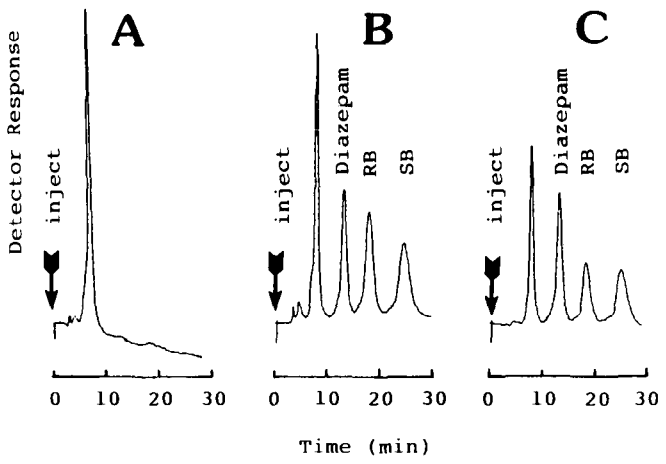

Fig. 1. Typical chromatograms of (A) blank serum, (B) serum spiked with 1 μg/ml diazepam and 2 μg/ml racemic bupivacaine and (C) serum sample following intrapleural administration of 75 mg of Marcaine. Analyses were performed at a wavelength of 215 nm, 0.005 a.u.f.s.

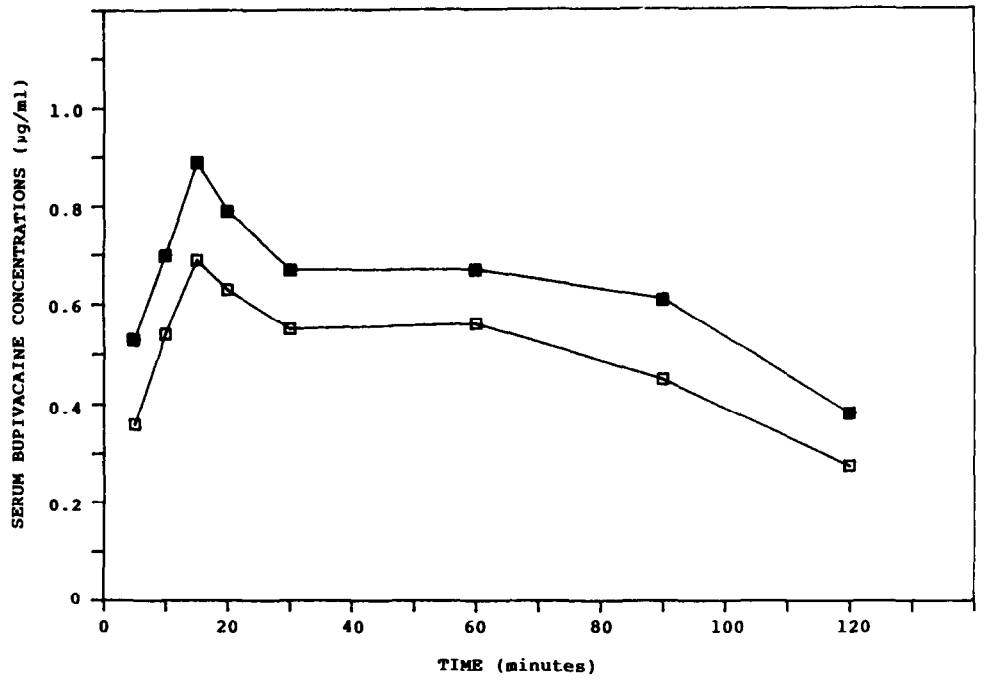

Fig. 2. Serum concentrations of $R(+)$-bupivacaine (□) and $S(-)$-bupivacaine (■) following intrapleural administration of 75 mg of Marcaine.

TABLE I

VARIABILITY AND ACCURACY OF THE ASSAY ($n=4$)

Compound	Concentration added (μg/ml)	Concentration measured (μg/ml)	Coefficient of variation (%)	Recovery (%)
RB	0.5	0.508	1.96	64.6
	1.0	0.98	3.10	68.0
	2.5	2.53	4.07	77.2
SB	0.5	0.549	9.2	64.4
	1.0	1.01	6.1	72.0
	2.5	2.48	4.47	78.6

peaks because the pure enantiomers were not available to us. However, Hermansson [2] has characterized the resolution of bupivacaine and has described the order of elution as being RB followed by SB.

We therefore report a relatively simple and reproducible stereoselective assay for B enantiomers. The assay has been used to examine the systemic absorption of the enantiomers following intrapleural administration of B. Previous reports have suggested that the in vivo differences in the efficacy and toxicity of RB and SB were due to dispositional factors since no enantioselective differences were noted in the in vitro studies [1]. In the one patient that we have studied, serum concentrations of SB were slightly higher than those of RB. It is likely that this is due to a higher binding of SB to serum proteins, which in the case of B is largely α_1-acid glycoprotein [3]. This is supported by the observation that on an α_1-acid glycoprotein column, SB elutes after RB.

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