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Simultaneous analysis of lignocaine and bupivacaine enantiomers in plasma by high-performance liquid chromatography

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Abstract

A sensitive analytical procedure is described for the simultaneous determination of lignocaine and the enantiomers of bupivacaine in biological fluids using diazepam as an internal standard. After solvent extraction into hexane, the local anaesthetics were separated using an α_1-acid glycoprotein (AGP) column and detected at 214 nm. Calibration curves were linear ($r^2 > 0.99$) in the concentration range of 5 to 500 ng/ml for the enantiomers of bupivacaine and 12.5 to 1000 ng/ml for lignocaine. The corresponding limits of detection were 4 ng/ml and 10 ng/ml, respectively. The method was applied to the analysis of plasma from a healthy woman undergoing tubal ligation. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Lignocaine; Bupivacaine

1. Introduction

Local anaesthetic drugs are widely used in the management of major pain, and are administered into either the CNS (spinal and epidural) or the periphery [1]. The drugs are increasingly being used to spare parenteral narcotic analgesics, which lead to more frequent and severe adverse effects, such as respiratory depression [2]. They are also very important in facilitating operations carried out in day surgery such as tubal ligation and extraction of impacted wisdom teeth.

Lignocaine and bupivacaine are amide type local anaesthetics. Whereas lignocaine is achiral, bupivacaine has one chiral centre and is marketed as a racemic mixture of *R*-(+) and *S*-(−) enantiomers. Although the enantiomers are equipotent nerve blockers in vitro [3], *R*-(+)-bupivacaine is more toxic than its antipode [4]. Following subcutaneous or intravenous administration *S*-(−)-bupivacaine is characterised by a longer duration of anaesthesia [3]. These differences are probably the result of enantioselectivity in binding and disposition [5] and have provided an incentive for the development of a single enantiomer formulation.

The current practice in tubal ligation and for lengthy dental and oral surgical procedures is to administer a local anaesthetic as pre-emptive analgesic prior to surgery and then use opiates or minor analgesics post-operatively [6]. An assay which can simultaneously determine the concentrations of lignocaine and the enantiomers of bupivacaine is therefore potentially useful to study the pharmacokinetics of these drugs when used in combination.

Several non-chiral [7,8] and chiral assays [9,10]

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have been described for analysis of bupivacaine in plasma. This paper describes a sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous analysis of lignocaine and the enantiomers of bupivacaine. The assay was applied to a study of drug disposition in a healthy female undergoing tubal ligation.

2. Experimental

2.1. Chemicals

Lignocaine, (*RS*)-bupivacaine hydrochloride and diazepam for assay development were purchased from Sigma (St. Louis, MO, USA). *R*-(+)- and *S*-(−)-bupivacaine were donated by Chiroscience (Cambridge, UK). 3′-Hydroxybupivacaine, 4′-hydroxybupivacaine and desbutylbupivacaine were gifted by Astra Pain Control (Sodertalje, Sweden). All other reagents were of analytical grade. Plasma for assay development was obtained from the Blood Bank, Dunedin Hospital. Blood samples for the clinical study were collected in heparinized Vacutainer tubes (6 ml).

2.2. Apparatus

The HPLC system consisted of a Shimadzu solvent delivery system (Model LC-AS10), a Rheodyne injector (Model 7725) with a 100 μl capacity loop, a Shimadzu UV detector (Model SPD-10 A) set at 214 nm and a SRI Model 8600-2000 peak simple II data system as integrator. The analytical column (Chrom Tech, Hagersten, Sweden; AGP: 50.4) was of stainless-steel packed with AGP bonded to a silica support (150×4 mm, 5 μm). A guard column (10× 3.0 mm) of the same material was included in the system. The mobile phase consisted of 8 m*M* sodium dihydrogen phosphate and 0.1 *M* sodium chloride containing 4% (v/v) 2-propanol and 0.6% (v/v) diethylamine. The pH of the mobile phase was adjusted to 7.05 with 50% phosphoric acid. The mobile phase was delivered isocratically at 0.9 ml/min.

2.3. Assay procedure

Stock solutions (0.1%, w/v) of (*RS*)-bupivacaine hydrochloride, lignocaine and diazepam were prepared in methanol and stored at 4°C. Plasma (1 ml) was spiked with 0.2 ml of diazepam solution (2 μg/ml) followed by the addition of 2 ml of water. Acetonitrile (2 ml) was added and the mixture was vortexed gently, set aside for 3 min and centrifuged at 2200 *g* for 20 min. The clear supernatant was separated, made alkaline by addition of 0.5 ml of 0.2 *M* sodium hydroxide and extracted with 6 ml of *n*-hexane by vortexing for 2 min. After extraction, the tube was centrifuged (2200 *g*) for 15 min and the organic phase (5 ml) transferred by pipette to a clean tube and evaporated to dryness with the aid of a Savant Speed Vac Model SVC-200H concentrator equipped with a two-stage Savant pump. Extracts were reconstituted in 120 μl of mobile phase and 100-μl aliquots analysed by HPLC.

2.4. Assay validation

Standard solutions of (*RS*)-bupivacaine and lignocaine (5–1000 ng/ml) were prepared in water. Plasma standards were prepared daily by spiking 1 ml aliquots of drug free plasma with 1 ml each of the standards of bupivacaine and lignocaine and 0.2 ml of internal standard solution. Curves of peak height ratio (PHR) of drug to internal standard versus the spiked concentration were constructed. The precision and accuracy of the technique [11] was determined by the analysis of four samples of each of three concentrations of bupivacaine and lignocaine over the concentration range 50–500 ng/ml in plasma. Inter-day precision was determined using plasma spiked with low, medium and high concentrations on three separate days over a period of two weeks.

Recoveries from spiked plasma of each of the compounds over the range 20–1000 ng/ml were determined. Plasma extracts and aqueous samples of the same known concentrations were prepared and internal standard was added prior to extraction and injection onto the column. Recoveries were calculated by comparing peak height ratios of extracted samples with those obtained from aqueous injections at two concentrations.

2.5. Clinical application

A pilot study of local anaesthetic disposition in a single patient undergoing tubal ligation was approved by the Southern Regional Health Authority Ethics Committee (Otago, New Zealand). Anaesthesia was induced and maintained with a bolus dose of propofol (80 mg), droperidol (1 mg) and fentanyl (100 μg). Lignocaine (1%) was administered by subcutaneous injection into the umbilicus (2 ml) and pubis (2 ml) prior to incision. 7.34 min after administration of lignocaine, *RS*-bupivacaine (0.5%) was administered (2 ml) by infiltration into each of the fallopian tubes prior to ligation. Venous blood samples (6 ml) were taken immediately before anaesthetic administration and at 1, 2.5, 5, 7.5, 10, 12.5, 15, 30, 45, 60 and 120 min after lignocaine administration into heparinized vacutainers. After separation by centrifugation at 2200 *g*, the plasma was frozen and stored at −84°C until assayed.

3. Results and discussion

3.1. Chromatography

A typical chromatogram of a plasma extract containing internal standard only is shown in Fig. 1A and one containing lignocaine, diazepam and the enantiomers of bupivacaine is shown in Fig. 1B. The peaks were symmetric with complete resolution. The retention times of the analytes, when extracted from plasma, were: lignocaine 7.5±0.55 min; diazepam 19.21±0.17 min; *R*-(+)-bupivacaine 29.35±0.11 min; and *S*-(−)-bupivacaine 38.25±0.21 min (n= 11). The order of elution of *R*-(+)-bupivacaine and *S*-(−)-bupivacaine agreed with that previously reported by Hermansson [12]. There was no interference from endogenous plasma components nor from 3′-hydroxybupivacaine, 4′-hydroxybupivacaine and desbutylbupivacaine, the three principal metabolites of bupivacaine [13]. In addition propofol, droperidol and fentanyl did not interfere with the peaks of interest.

Retention time was highly dependent on mobile phase pH. Adjusting the pH of the mobile phase to 7.1 caused the retention times to increase to 9.5, 32.5 and 43.5 min, respectively for lignocaine, *R*-(+)-bupivacaine and *S*-(−)-bupivacaine. Decreasing the pH to 7.0 or increasing the concentration of the organic modifier above 4% resulted in reducing the retention times of the analytes, causing lignocaine to elute with the endogenous material.

To extend the life span of the AGP column, it was washed after each assay day with 10% 2-propanol in HPLC water for 40 min at a flow-rate of 0.2 ml/min.

3.2. Validation

The calibration curves for lignocaine, *R*-(+)-bupivacaine and *S*-(−)-bupivacaine were linear in the concentration ranges 5–500 ng/ml for bupivacaine and 12.5–1000 ng/ml for lignocaine. The intercepts were not significantly different from zero.

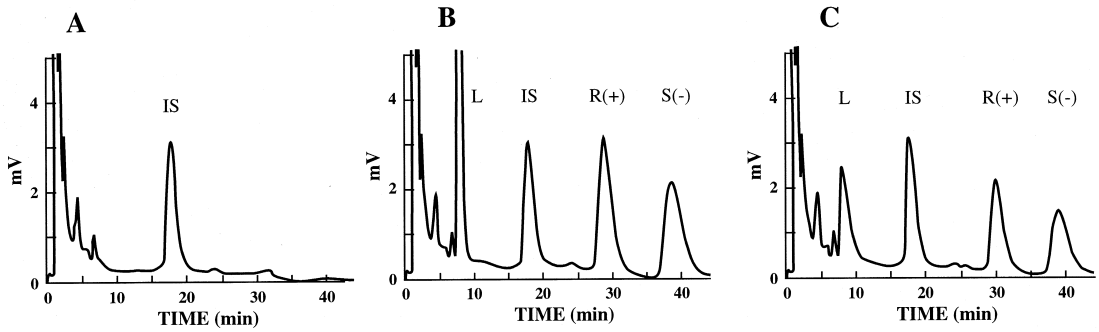

Fig. 1. HPLC elution profiles of plasma extracts: (A) blank plasma with diazepam as internal standard (I.S.), (B) plasma spiked with lignocaine (L, 500 ng/ml) and *RS*-bupivacaine (750 ng/ml), (C) plasma obtained from a healthy female 45 min after subcutaneous injection of 1% lignocaine and 37.6 min after fallopian infiltration of 0.5% *RS*-bupivacaine.

Table 1
Intra-day precision and accuracy ($n=4$)

Spiked concentration (ng/ml)	Plasma mean concentration found (ng/ml)	C.V. (%)	Accuracy (%)
Lignocaine			
50	46.2	2.8	7.7
100	95.3	3.1	4.7
500	464.2	1.9	7.1
R-(+)-Bupivacaine			
50	47.2	3.1	4.2
100	94.8	1.7	5.1
500	491.2	1.2	1.8
S-(−)-Bupivacaine			
50	45.5	7.8	9.1
100	95.1	3.7	4.8
500	475.9	1.5	4.8

The lower limits of detection (signal-to-noise ratio of 3:1) were found to be 4 ng/ml for each of the enantiomers of bupivacaine and 10 ng/ml for lignocaine when 1 ml samples of plasma were used.

Coefficients of variation for intra- and inter-day precision (Tables 1 and 2) were all less than 8%. As regards accuracy, the mean concentrations agreed well with the spiked concentrations for each of the compounds (Table 1). Recoveries from spiked plasma of each of the compounds over the range 20–1000 ng/ml were greater than 95%.

Table 2
Inter-day precision and accuracy ($n=6$)

Spiked concentration (ng/ml)	Plamsa mean concentration found (ng/ml)	C.V. (%)	Accuracy (%)
Lignocaine			
12.5	11.4	7.0	9.1
100	94.6	3.1	5.4
1000	952.0	3.2	4.8
R-(+)-Bupivacaine			
25	23.6	3.9	5.5
100	94.7	2.1	5.3
500	488.5	5.4	2.3
S-(−)-Bupivacaine			
25	22.6	6.5	9.6
100	94.5	5.1	5.5
500	474.5	2.1	5.1

3.3. Clinical study

The uptake of local anaesthetics into the general circulation is of concern because of their potential to elicit systemic side effects and toxicity. Although it is known that toxic concentrations of lignocaine and bupivacaine are not reached in the systemic circulation after subcutaneous injection of an anaesthetic dose [14], nothing is known about blood levels after infiltration into fallopian tubes [15]. Further, there is no published information about the relative absorption profiles of these drugs from these sites.

A chromatogram obtained 45 min after injection of lignocaine and 37.6 min after infiltration of bupivacaine is shown in Fig. 1C. The concentration vs. time curves for the single female patient are shown in Fig. 2. In Fig. 2B, the time zero is 7.4 min after administration of lignocaine. The absorption of *R*-(+)-bupivacaine and *S*-(−)-bupivacaine was rapid and peak concentrations of both enantiomers were observed 5.4 min after infiltration. The t_{max} for lignocaine was considerably longer at 15 min. The plasma concentrations of *S*-(−)-bupivacaine were higher than those of *R*-(+)-bupivacaine with C_{max} values of 108 ng/ml for lignocaine, 144 ng/ml for *R*-(+)-bupivacaine and 212 ng/ml for *S*-(−)-bupivacaine. The areas under the curve extrapolated to infinity, $AUC_{0-\infty}$, were 134 ng/ml h for lignocaine, 104 ng/ml h for *R*-(+)-bupivacaine and 157 ng/ml h for *S*-(−)-bupivacaine.

The plasma concentrations of *S*-(−)-bupivacaine were consistently higher than those of *R*-(+)-bupivacaine as shown in Fig. 2. It is possible that this is due to higher binding of *S*-(−)-bupivacaine to plasma proteins, which in the case of bupivacaine is predominantly to AGP [16]. This is consistent with the observation that on an AGP column, *S*-(−)-bupivacaine is retained longer than *R*-(+)-bupivacaine. A recent study of the pharmacokinetics of the enantiomers of bupivacaine by Burm et al. [3] in ten non-smoking males following intravenous administration of the racemate showed the plasma clearance of *R*-(+)-bupivacaine was greater than that of *S*-(−)-

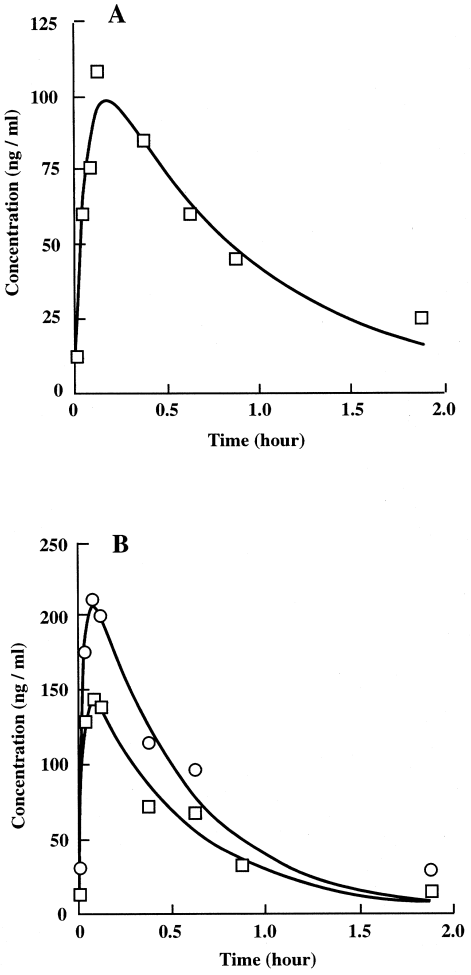

Fig. 2. Plasma drug concentration vs. time curve of (A) lignocaine (□), in a female after administration of 1% (4 ml) lignocaine during tubal ligation and (B) *R*-(+)-bupivacaine (□) and *S*-(−)-bupivacaine (○), after infiltration of 0.5% (4 ml) racemic bupivacaine to the same patient.

bupivacaine probably due to enantioselective plasma binding [17].

The rate of absorption of local anaesthetic agents from different anatomical sites varies to a great extent [18]. It is likely that higher plasma concentration of bupivacaine would occur following infiltration into the fallopian tubes as compared with subcutaneous injection of the same anaesthetic into the umbilicus due to the greater vascularity of this area. There are obvious clinical implications in this relationship of administration site to rate of absorption since the same dose of a local anaesthetic agent may be more toxic at one site than another. Estimated plasma threshold concentrations, associated with systemic toxicity are 5 to 10 mg/ml for lignocaine and 2 to 4 mg/ml for bupivacaine [6]. This is many orders of magnitude higher than the C_{max} values observed in this study. A detailed report comparing absorption of lignocaine and bupivacaine in tubal ligation will be published elsewhere.

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References

[1] G. Levinson, in J.L. Benumof (Editor), Clinical Procedures in Anesthesia and Intensive Care, J.B. Lippincott, Philadelphia, PA, 1992, Ch. 31, p. 645.
[2] J.E.F. Reynolds (Editor), Martindale, Pharmaceutical Press, London, XIIIth ed., 1993, p. 995.
[3] A.G.L. Burm, A.D. van der Meer, J.W. van Kleef, P.W.M. Zeijlmans, K. Groen, Br. J. Clin. Pharmacol. 38 (1994) 125.
[4] G. Aberg, Acta Pharmacol. Toxicol. 31 (1972) 273.
[5] E.J. Ariens, E.W. Wuis, E.J. Veringa, Biochem. Pharmacol. 37 (1988) 9.
[6] C.J. Bacsik, J.Q. Swift, K.M. Hargreaves, Oral Surg. Oral Med. Oral Pathol. 79 (1995) 18.
[7] J.X. Mazoit, C. Lambert, A. Berdeaux, J.L. Gerard, R. Froideveaux, Anesth. Analg. 67 (1988) 961.
[8] S.A. Schweitzer, D.J. Morgan, Anaesth. Intens. Care 15 (1987) 425.
[9] E.J.D. Lee, S.B. Ang, T.L. Lee, J. Chromatogr. 420 (1987) 203.
[10] A.J. Rutten, L.E. Mather, C.F. Mclean, Br. J. Anaesth. 67 (1991) 247.
[11] R.H. Eggers, J. Bircher, Eur. J. Clin. Pharmacol. 34 (1988) 319.
[12] J. Hermansson, J. Chromatogr. 298 (1984) 67.
[13] H. Kastrissios, M.F. Hung, E.J. Triggs, J. Chromatogr. 577 (1992) 103.

[14] R.M. Giasi, E. D'Agostino, B.G. Covino, Anesth. Analg. 58 (1979) 360.
[15] R. McKenzie, P. Phitayakorn, N.T.L. Uy, J. Chalasani, B.M. Melnick, R.L. Kennedy, A.F. Vicinie, Can. J. Anaesth. 36 (1989) 510.
[16] D. Denson, D. Coyle, G. Thompson, J. Myers, Clin. Pharmacol. Ther. 35 (1984) 409.
[17] R.H. Levy, A.V. Boddy, Pharm. Res. 8 (1991) 551.
[18] D.P. Braid, D.B. Scott, Br. J. Anaesth. 37 (1965) 394.