Journal of Chromatography, 383 (1986) 357–364
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3355

SIMULTANEOUS DETERMINATION OF BUPIVACAINE AND ITS TWO METABOLITES, DESBUTYL- AND 4′-HYDROXYBUPIVACAINE, IN HUMAN SERUM AND URINE

R.L.P. LINDBERG*

Department of Pharmacology, Institute of Biomedicine, University of Turku, Turku (Finland)

J.H. KANTO

Departments of Clinical Pharmacology and Anaesthesiology, Turku University Central Hospital, Turku (Finland)

and

K.K. PIHLAJAMÄKI

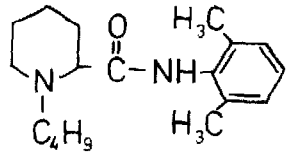
Department of Pharmacology, Institute of Biomedicine, University of Turku, Turku (Finland)

(First received June 11th, 1986; revised manuscript received July 17th, 1986)

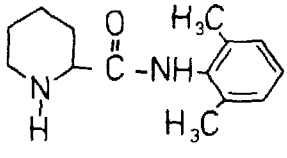
SUMMARY

A sensitive and selective high-performance liquid chromatographic (HPLC) method for the determination of bupivacaine and its two metabolites, desbutyl- and 4′-hydroxybupivacaine, in human serum and urine is decribed. Bupivacaine, both metabolites and the internal standard, etidocaine, are extracted with diethyl ether and then back-extracted into an acidic aqueous phase. After subsequent extraction into diethyl ether, evaporation and reconstitution in the mobile phase, bupivacaine and the metabolites are determined by HPLC using a reversed-phase C_8 column with tetrahydrofuran–potassium phosphate buffer (8:92, v/v, pH 2.4) as the mobile phase. The sensitivity of the method is 10 μg/l for bupivacaine and both metabolites and the extraction efficiencies are 95, 54 and 92% for bupivacaine and desbutyl- and 4′-hydroxybupivacaine, respectively. The reproducibility of the method is good, the coefficients of variation varying between 1.8 and 7.4% in the concentration range 0.10–2.00 mg/l. The procedure was applied to human serum and urine samples from two elderly women who had been operated on under epidural analgesia (plain bupivacaine, 1.5 mg/kg) because of uterine prolapse.

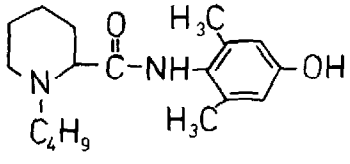
0378-4347/86/$03.50



Bupivacaine



Desbutylbupivacaine



4′-Hydroxybupivacaine

Fig. 1. Structures of bupivacaine and its two metabolites.

INTRODUCTION

Bupivacaine hydrochloride (Marcain) is a local anaesthetic of the anilide type [1] used for different kinds of regional analgesia with predictable kinetic properties [2,3]. Desbutylbupivacaine (DBB), which is formed in the liver by oxidative dealkylation of the parent drug, has been considered to be the main metabolite of bupivacaine. Bupivacaine is also metabolized to 4′-hydroxybupivacaine (4-OH-B) [4] (Fig. 1). Bupivacaine is a highly lipid-soluble amide-type local anaesthetic agent which can cross the biological membranes fairly easily. It is important to be able to measure bupivacaine and its metabolites in human blood and urine and other biological fluids in order to evaluate the toxicity and disposition kinetics of bupivacaine.

A number of methods for the determination of bupivacaine have been published [5–11], including gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) techniques. However, these methods measure only the parent drug. Only two GC assays for bupivacaine and its main metabolite, desbutylbupivacaine, have been published [12,13]. No method for the determination of 4′-hydroxybupivacaine has been published.

This paper describes a selective, sensitive and accurate HPLC procedure for the simultaneous determination of bupivacaine and its two metabolites in human serum and urine. The procedure was used to monitor serum and urine concentrations of bupivacaine and the metabolites in two elderly women operated on under epidural analgesia with bupivacaine.

EXPERIMENTAL

Reagents and chemicals

Bupivacaine hydrochloride (Marcain) was obtained from Bofors Nobel Pharma (Bofors, Sweden). Desbutyl- and 4′-hydroxybupivacaine were donated by Ster-

ling-Winthrop Research Institute (New York, NY , U.S.A.). Etidocaine hydrochloride (Duranest) was purchased from Astra (Lund, Sweden). Diethyl ether and tetrahydrofuran were of analytical-reagent grade (E. Merck, Darmstadt, F.R.G.). A solution of etidocaine in distilled water (2 mg/l) was used as the internal standard.

Working bupivacaine and metabolite solutions contained 10 or 100 mg/l in distilled water. Drug serum standards were prepared by spiking blank control serum with appropriate microlitre volumes of each working bupivacaine and metabolite solution to obtain seven serum standards with concentrations of all three compounds of 0.05, 0.10, 0.25, 0.50, 0.75, 1.00 and 2.00 mg/l.

Instruments and chromatographic conditions

The determinations were carried out using a chromatographic system consisting of an LKB 2210 pump (LKB, Bromma, Sweden), a Rheodyne injector with a 50-μl sample loop (Rheodyne, Berkeley, CA, U.S.A.) and an SF 773 variable-wavelength UV detector set at 210 nm (Kratos, Ramsey, NJ , U.S.A.). The reversed-phase column was laboratory-packed with 10-μm Nucleosil C_8 (Macherey, Nagel & Co., Düren, F.R.G.) (30 cm$\times$3.9 mm I.D.). The temperature was ambient. The solvent used was tetrahydrofuran–0.01 *M* potassium phosphate buffer (pH 2.4) (8:92, v/v) and the flow-rate was 1.6 ml/min. Chromatograms were recorded with a laboratory potentiometric recorder.

Procedure and application

To a 0.5-ml serum sample, 200 μl of etidocaine solution (2 mg/l) were added and the serum was made alkaline by adding 1 ml of 0.1 *M* sodium tetraborate buffer (pH 9.0). Bupivacaine, the two metabolites and the internal standard were extracted with 5 ml of diethyl ether by shaking for 20 min. After centrifugation at 1200 *g* the diethyl ether layer was transferred into a clean tube containing 0.5 ml of 0.2 *M* hydrochloric acid and the mixture was shaken for 20 min. The diethyl ether phase was separated by centrifugation at 1200 *g* and aspirated. The acidic layer was made alkaline with 1 ml of 0.1 *M* sodium tetraborate buffer (pH 9.0). Bupivacaine, the metabolites and the internal standard were extracted with 5 ml of diethyl ether by shaking for 20 min and the organic phase was evaporated at 50°C under a gentle stream of air. The residue was reconstituted in 100 μl of the mobile phase and 50 μl were injected into the chromatograph. The concentrations of both bupivacaine and the metabolites in the serum samples were determined from a calibration graph of peak-height ratio (bupivacaine or metabolite to internal standard) versus bupivacaine or metabolite concentration in serum standards carried through the procedure. The urine samples were treated, extracted and analysed similarly to the serum samples.

Two elderly women, T.I. and A.T., both aged 67 years, weights 94 and 57 kg, heights 158 and 164 cm) were operated on under epidural analgesia (plain bupivacaine, 1.5 mg/kg) because of uterine prolapse. The concentrations of bupivacaine and its two metabolites in serum and urine were determined. Blood samples were drawn at time points indicated in Table III and urine was collected as fractions as indicated in Table IV.

RESULTS AND DISCUSSION

Chromatograms of extracts from blank serum and the blank serum spiked with 0.5 mg/l of bupivacaine and both metabolites and the urine samples collected during 3–6 h after epidural analgesia with bupivacaine are illustrated in Fig. 2A, B and C, respectively. Bupivacaine, the metabolites and the internal standard were well separated with retention times of 4.15, 5.80, 7.85 and 10.60 min. The method showed a linear increase in response over the concentration range 0.05–2.00 mg/l in serum for both bupivacaine and the metabolites (Fig. 3). A plot of peak-height ratio against bupivacaine or metabolite concentration gave linear calibration graphs, the equations of which are shown in Fig. 3. When defined as a signal level exceeding three times the background noise, the detection limit of the method is 10 μg/l for all three substances. The method of Lesko et al. [12] had a sensitivity of 100 μg/l for bupivacaine and desbutylbupivacaine when using 0.1 ml of serum. The mass spectrometric technique published earlier [13] is slightly more sensitive than our method, but it requires special apparatus that is available in only a few laboratories.

Several eluents were investigated before the final selection of the chromatographic conditions was made. Methanol, tetrahydrofuran and acetonitrile were evaluated as organic components of the eluent. When using methanol or acetonitrile some endogenous compounds coeluted with the metabolites, interfering with their quantitation. The best separation was achieved with pure tetrahydrofuran.

The precision was assessed by multiple analyses of five standard serum pools in the concentration range 0.10–2.00 mg/l for both bupivacaine and the metabolites. The coefficients of variation for the intra-assay variability of all three substances are given in Table I. The results demonstrate the high reproducibility of the method. The accuracy was confirmed with spiking low and high concentrations of bupivacaine and the metabolites in serum samples in which the bupivacaine and metabolite levels were known and analysing the samples.

To extract bupivacaine and the metabolites several different extraction solvents were tested. The highest recovery was found with a mixture of *n*-hexane, chloroform and isopropanol for bupivacaine and desbutylbupivacaine and with diethyl ether for 4′-hydroxybupivacaine. To develop a method for the simultaneous determination of bupivacaine and both metabolites we made a compromise and chose diethyl ether as the extraction solvent.

The extraction efficiencies were tested by determining admixtures of known amounts of bupivacaine, desbutyl- and 4′-hydroxybupivacaine in serum in the range 0.10–2.00 mg/l. Comparison of the peak heights for bupivacaine and the metabolites from serum samples with those obtained after direct injection of the corresponding aqueous solutions into the chromatograph indicated that the recoveries were 95.0 ± 1.7, 54.2 ± 7.1 and $91.7 \pm 3.4\%$ for bupivacaine and desbutyl- and 4′-hydroxybupivacaine, respectively (Table II).

The back-extraction into an acidic aqueous phase was necessary in order to purify the extract from endogenous compounds coeluting with the metabolites and the internal standard. Etidocaine was chosen as the internal standard because

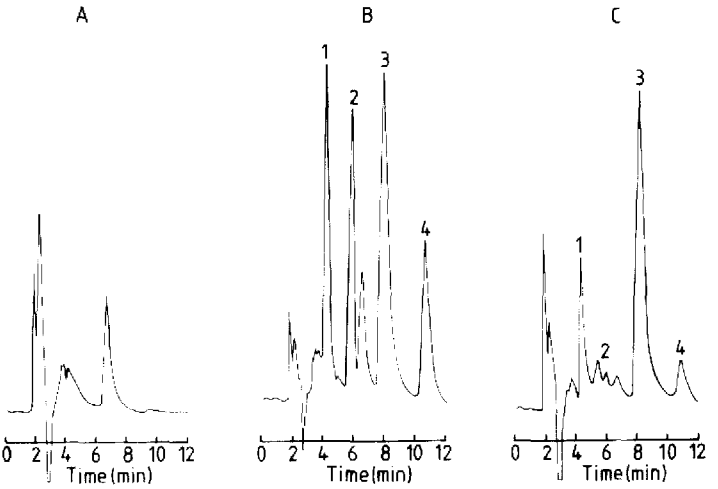


Fig. 2. Chromatograms of the extracts from blank serum (A), from blank serum spiked with 0.5 mg/l bupivacaine and both metabolites (B) and from a urine sample from a patient collected 3–6 h after epidural analgesia performed with bupivacaine, 1.5 mg/kg (C). Peaks: 1 = DBB; 2 = 4-OH-B; 3 = internal standard (etidocaine); 4 = bupivacaine.

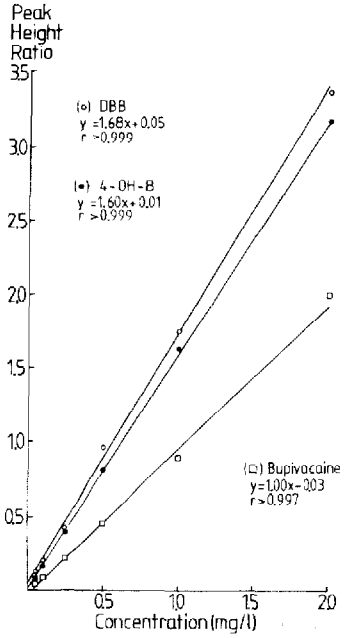


Fig. 3. Calibration graphs for bupivacaine and its two metabolites. Peak-height ratios of bupivacaine or the metabolites to those of the internal standard are plotted against bupivacaine or metabolite concentrations in serum.

of its structural similarity to bupivacaine. Etidocaine is never administered together with bupivacaine, so no interference is expected in patient samples. Etidocaine was extracted well in the procedure used here and eluted just after desbutyl- and 4′-hydroxybupivacaine.

TABLE I

INTRA-ASSAY REPRODUCIBILITY ($n=12$) OF HPLC FOR THE DETERMINATION OF BUPIVACAINE AND ITS TWO METABOLITES IN HUMAN SERUM

Concentration (mg/l)	Coefficient of variation (%)		
	Bupivacaine	DBB	4-OH-B
0.10	4.1	7.4	4.8
0.25	2.3	5.4	2.3
0.50	2.1	5.6	2.9
1.00	5.1	4.6	4.2
2.00	2.4	2.9	1.8

The parent drug, bupivacaine, eluted after the metabolites and the internal standard with a retention time of 10.60 min. The method is sensitive and accurate for the analysis of bupivacaine also. However, a simpler and faster assay for the quantitation of bupivacaine published earlier [11] is recommended when only the parent drug is of interest.

The peak concentrations of bupivacaine in systemic circulation were reached in 15 and 30 min in the two patients after epidural analgesia (Table III). Only very low levels of the two metabolites were detected in the plasma samples. The urinary excretion of bupivacaine and its metabolites is shown in Table IV. About 0.2% of the bupivacaine dose was excreted unchanged, 0.9–1.6% as DBB and 0.1% as 4-OH-B during 24 h after epidural analgesia. Friedman et al. [14] found 0.7% and 4.8% of the bupivacaine dose unchanged and as DBB, respectively, in the urine during 72 h after intravenous bupivacaine infusion. The mean urinary pH was 6.1 ± 0.9. The higher pH values in our patients (range 5.8–7.0) caused by alkalinizing i.v. infusions, may have decreased the excretion. The effect of urine pH on the excretion of similar amide local anaesthetics such as lidocaine and prilocaine has been studied by Eriksson and Granberg [15], and they found an increased excretion of those substances in urine of lower pH.

In conclusion, the HPLC method described here has been demonstrated to be accurate and selective, and therefore offers the possibility of obtaining more

TABLE II

DETERMINATION OF BUPIVACAINE AND ITS TWO METABOLITES IN HUMAN SERUM ($n=12$)

Concentration (mg/l)	Extraction efficiency (mean ± S.D.) (%)		
	Bupivacaine	DBB	4-OH-B
0.10	93.5 ± 3.9	57.0 ± 4.2	96.1 ± 4.6
0.25	94.7 ± 2.2	44.4 ± 2.4	89.4 ± 2.1
0.50	97.8 ± 2.0	63.0 ± 3.5	94.4 ± 2.7
1.00	94.1 ± 4.8	56.1 ± 2.6	88.2 ± 3.7
2.00	94.7 ± 2.2	50.3 ± 1.5	90.3 ± 1.6
Mean	95.0 ± 1.7	54.2 ± 7.1	91.7 ± 3.4

TABLE III

SERUM CONCENTRATIONS OF BUPIVACAINE AND ITS TWO METABOLITES IN TWO WOMEN AFTER EPIDURAL ANALGESIA WITH PLAIN BUPIVACAINE (1.5 mg/kg)

Compound	Patient	Time after analgesia (min)								Time after analgesia (h)				
		0	5	10	15	20	30	45	60	2	3	6	12	24
Bupivacaine	T.I.	—	0.37	0.60	1.00	1.31	1.45	1.02	0.80	0.54	0.45	0.32	0.22	0.17
	A.T.	—	0.15	0.21	0.46	0.43	0.35	0.37	0.35	0.24	0.18	0.12	0.08	0.07
DBB	T.I.	—	—	—	—	—	—	0.01	0.01	0.03	0.03	0.03	0.03	0.03
	A.T.	—	—	—	—	—	—	<0.01	0.01	<0.01	<0.01	<0.01	—	—
4-OH-B	T.I.	—	—	—	—	—	—	0.01	0.01	0.01	0.01	0.01	0.01	0.03
	A.T.	—	—	—	—	—	—	—	—	0.02	0.02	0.02	—	—

TABLE IV

RENAL EXCRETION OF BUPIVACAINE AND ITS TWO METABOLITES IN TWO WOMEN AFTER EPIDURAL ANALGESIA WITH PLAIN BUPIVACAINE (1.5 mg/kg)

Compound	Patient	Bupivacaine or metabolite (μg)					Total (0-24 h) (mg)	Dose (0-24 h) (%)
		0-1 h	1-3 h	3-6 h	6-12 h	12-24 h		
Bupivacaine	T.I.	8.2	20.0	18.2	49.6	220.0	0.316	0.22
	A.T.	15.0	11.0	10.5	28.5	84.0	0.149	0.17
DBB	T.I.	—	27.5	33.8	268.8	1850.0	2.180	1.55
	A.T.	—	2.2	42.0	57.0	644.0	0.745	0.87
4-OH-B	T.I.	—	2.5	3.9	20.8	125.0	0.152	0.11
	A.T.	—	6.6	21.0	28.5	—	0.056 (0-12 h)	0.07
Urine pH	T.I.	7.0	6.7	6.7	6.8	6.2		
	A.T.	6.4	5.8	5.8	5.8	6.2		

knowledge about the metabolic properties of bupivacaine and of the possible role of the two metabolites, DBB and 4-OH-B, in systemic toxicity caused by bupivacaine administration. Even with the present method, however, only minor components of the apparently many metabolites of bupivacaine in urine can be determinated.

ACKNOWLEDGEMENTS

The authors thank Mrs. Helena Hakala and Mrs. Ulla Uoti for valuable technical assistance, and Dr. A.E. Soria of Sterling-Winthrop Research Institute for the generous gift of reference substances.

REFERENCES

1 P.C. Lund, J.C. Cwik and F. Vallersteros, Anaesth. Analg., 49 (1970) 103.
2 M. Concepcion and B.G. Covino, Drugs, 27 (1984) 256.
3 G.T. Tucker and L.E. Mather, Clin. Pharmacokinet., 4 (1979) 241.
4 R.N. Boyes, Br. J. Anaesth., 47 (1975) 225.
5 F. Reynolds and A.H. Beckett, J. Pharm. Pharmacol., 20 (1968) 704.
6 G.T. Tucker, Anesthesiology, 32 (1970) 255.
7 E. Zylber-Katz, L. Granit and M. Levy, Clin. Chem., 24 (1978) 1573.

8 P.E. Verheesen, P.J. Brombacher and H.M.H.G. Gremers, J. Clin. Chem. Clin. Biochem., 18 (1980) 351.
9 D.E. Coyle and D.D. Denson, Ther. Drug. Monit., 8 (1986) 98.
10 U.-W. Wiegand, R.C. Chou, E. Lanz and E. Jännchen, J. Chromatogr., 311 (1984) 218.
11 R.L.P. Lindberg and K.K. Pihlajamäki, J. Chromatogr., 309 (1984) 369.
12 L.J. Lesko, J. Ericson, G. Ostheimer and A. Marion, J. Chromatogr., 182 (1980) 226.
13 P.M. Kuhnert, B.R. Kuhnert, J.M. Stitts and T.L. Gross, Anesthesiology, 55 (1981) 611.
14 G.A. Friedman, J.C. Rowlingson, C.A. DiFazio and M.F. Donegan, Anesth. Analg., 61 (1982) 23.
15 E. Eriksson and P. Granberg, Acta Anaesthesiol. Scand., Suppl., 16 (1965) 79.