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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF BUPIVACAINE IN HUMAN SERUM

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SUMMARY

A selective high-performance liquid chromatographic method for the determination of bupivacaine in human serum is described. The technique is based on a single extraction of the drug from alkalinized serum with a mixture of hexane—isopropanol—chloroform. Desmethyldoxepin is used as internal standard. The chromatographic system consists of a home-packed Nucleosil C_8 (10 μm) column; the mobile phase is acetonitrile—0.05 *M* potassium phosphate buffer (pH 3.3) (28:72, v/v).

The method can accurately measure serum bupivacaine concentrations down to 20 μg/l using 500 μl of sample. The coefficient of variation for intra-assay variability of bupivacaine is 2.1% (n = 13) and for inter-assay variability of bupivacaine 5.7% (n = 11) at 1.00 mg/l. The calibration graph is linear over the range 0.02—5.00 mg/l and the extraction efficiency is 91.8 ± 3.8% (± S.D., n = 7).

The method is accurate and sensitive for both clinical and pharmacokinetic studies on bupivacaine in man. The method is applied to the analysis of serum samples obtained from orthopaedic patients during both spinal and epidural analgesia.

INTRODUCTION

Local anaesthetic agents are an important part of modern anaesthesiology. Most of the systemic toxic reactions from the local anaesthetics are correlated with high concentrations of these agents in the circulation [1]. Determination of serum concentrations of local anaesthetics can therefore make their use safer for the patients undergoing various anaesthetic procedures.

Bupivacaine hydrochloride (Marcain®) is a new amide-type local anaesthetic agent of long duration of action [2] which is gaining more and more popularity, especially in obstetrics, orthopaedics and post-operative analgesia [3—5].

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Several gas chromatographic methods for the determination of bupivacaine have been reported [6—12]. However, these methods involve various extraction steps and are rather laborious and time-consuming. Gas chromatography—mass spectrometry has also been used in the analysis of local anaesthetic agents [13].

No high-performance liquid chromatographic (HPLC) methods for the determination of bupivacaine in human serum have been published. We describe here an accurate, rapid and sensitive procedure for the detection of bupivacaine using HPLC. The method allows a more rapid measurement of therapeutic and toxic concentrations of bupivacaine in serum. Because of the high sensitivity this method is also very suitable for pharmacokinetic studies on bupivacaine.

We applied the procedure described herein for monitoring bupivacaine serum concentrations during spinal and epidural analgesia in various orthopaedic operations.

EXPERIMENTAL

Reagents and chemicals

The following reagents and chemicals were used: bupivacaine hydrochloride (Marcain, A.B. Bofors Nobel Pharma, Bofors, Sweden) and desmethyldoxepin hydrochloride (Pharmaceutical Div., Pennwalt, Rochester, NY, U.S.A.); *n*-hexane, isopropanol, chloroform and acetonitrile were of analytical reagent grade (E. Merck, Darmstadt, F.R.G.).

Internal standard. A solution of desmethyl doxepin (100 mg/l) was prepared by dissolving 11.4 mg of desmethyldoxepin hydrochloride in 100 ml of distilled water. A second solution containing desmethyldoxepin (5 mg/l) was prepared by dilution with distilled water.

Drug standards. Working drug solution contained 50 mg of bupivacaine in distilled water. Drug serum standards were prepared by spiking blank control serum with appropriate microlitre volumes of working drug solution to obtain seven serum standards with the following concentrations of bupivacaine: 0.1, 0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg/l.

Extraction procedure

To a 0.5-ml serum sample, 0.1 ml of desmethyldoxepin solution (5 mg/l) was added. The serum was made alkaline by adding 0.5 ml of sodium hydroxide solution (0.1 mol/l). Bupivacaine and the internal standard were extracted with 5.0 ml of a mixture of hexane—isopropanol—chloroform (30:60:10, v/v/v) by shaking for 20 min. The organic phase was separated by centrifugation and transferred to a clean test-tube. The organic mixture was evaporated at 50°C under a gentle stream of air. The residue was reconstituted with 100 µl of phosphoric acid (0.05 mol/l) and 50 µl were injected into the chromatograph. The concentration of bupivacaine in the serum sample was determined from a calibration curve of peak height ratio (drug/internal standard) versus drug concentration in serum standards carried through this procedure.

High-performance liquid chromatography

The analysis was carried out on a Model SP 740 HPLC system (Spectra-

Physics, Santa Clara, CA, U.S.A.) using an HP 1030B ultraviolet detector (Hewlett-Packard, Waldbronn, F.R.G.) set at 210 nm. An SP 4100 computing integrator (Spectra-Physics) was used to calculate peak heights. The mobile phase consisted of acetonitrile—0.05 *M* potassium phosphate buffer (28:72, v/v) (pH 3.3) and the flow-rate was 2.0 ml/min. The reversed-phase column was home-packed with 10-μm Nucleosil C_8 (Macherey-Nagel, Düren, F.R.G.) and measured 30 cm × 3.9 mm I.D.

Application of the method

To five patients suffering from rheumatic arthritis (four women and one man, age 26—61 years, height 158—177 cm, weight 72—87.5 kg) 150 mg of bupivacaine hydrochloride were given for epidural analgesia before various orthopaedic operations. For spinal analgesia 22.5 mg of bupivacaine hydrochloride were given to three women (age 65—71 years, height 153—175 cm, weight 52—71.5 kg). All the patients abstained from eating and drinking for at least 6 h before and 4 h after the operations. They were also without their usual antirheumatic and other drugs.

Nine blood samples were drawn from cubital venous cannulas until 3 h after administration of the bupivacaine. The blood samples were kept in a refrigerator at 4° C and centrifuged 3—9 h after drawing. After centrifugation the sera were deep-frozen and kept at −20° C until analysed.

RESULTS AND DISCUSSION

Chromatograms of extracts from blank serum, blank serum spiked with 1.0 mg/l bupivacaine and the serum sample of a patient during spinal analgesia are illustrated in Figs. 1A—C, respectively. Bupivacaine and the internal standard, desmethyldoxepin, were well separated and no interference was noted. The retention times of bupivacaine and the internal standard were 4.15 and 5.70 min, respectively, which means that the total chromatographic run time was only about 7 min. Thus it is possible to determine 30 serum samples in duplicate during an 8-h working day.

The calibration graph was linear for samples over the concentration range studied here, 0.02—5.00 mg/l. The least-squares linear regression line which represents the best fit of the bupivacaine data had an equation of $Y = 0.55X + 0.10$ (Y = peak height ratio, drug/internal standard, and X = bupivacaine concentration). The correlation coefficient was > 0.999.

The precision was assessed by multiple analyses of seven standard serum pools in the concentration range 0.10—5.00 mg/l. Inter-assay variability was determined over a period of three months. The coefficients of variation for intra-assay and inter-assay variability of bupivacaine are given in Table I. The values for intra-assay variability vary from 1.0% to 5.1% and for inter-assay variability from 5.7% to 10.5% in the range studied. The results demonstrate the high accuracy and reproducibility of the method.

From a comparison of bupivacaine peak heights obtained from direct injection of aqueous solutions and from samples carried through the assay procedure, the extraction efficiency was estimated as 91.8 ± 3.8% (± S.D., n = 7) (Table II). The coefficient of variation for recovery was 4.2% (n = 7) over the concentration range 0.10—5.00 mg/l.

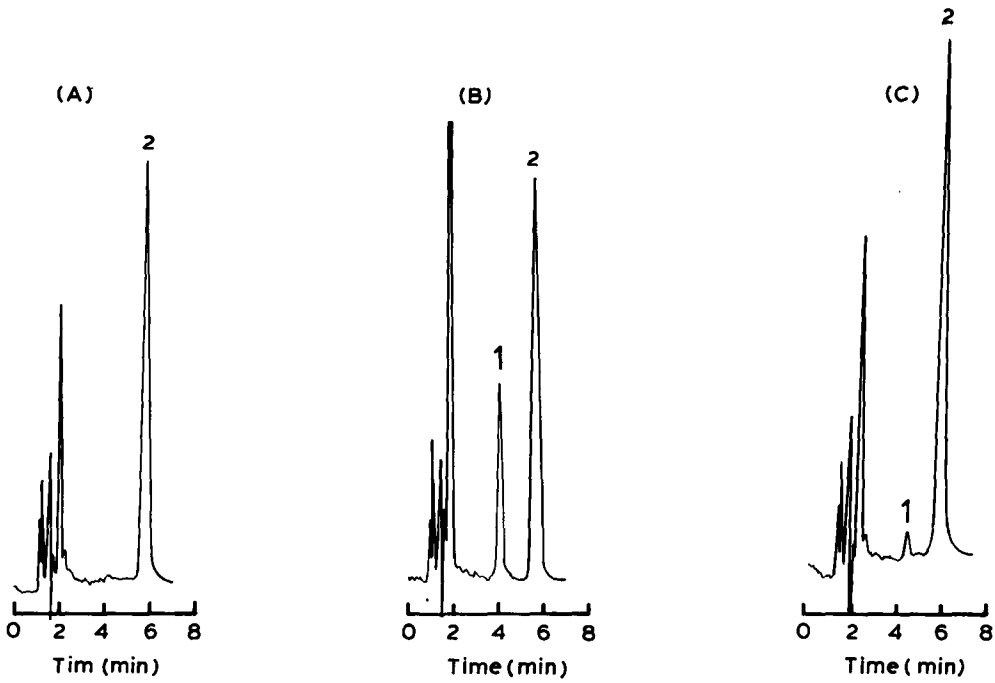

Fig. 1. Liquid chromatograms of the extracts from blank serum (A), blank serum spiked with 1.0 mg/l bupivacaine (B), and from a serum sample of a patient during spinal analgesia 30 min after the beginning of the spinal block (C). The retention time of bupivacaine (1) is 4.15 min and that of internal standard, desmethyldoxepin (2), 5.70 min. The concentration of bupivacaine in the patient's serum (C) is 0.087 mg/l. For chromatographic conditions, see text.

TABLE I

INTRA- AND INTER-ASSAY REPRODUCIBILITY OF HPLC FOR THE DETERMINATION OF BUPIVACAINE IN HUMAN SERUM

Concentration (mg/l)	Coefficient of variation (%)	
	Intra-assay (n = 13)	Inter-assay (n = 11)
0.10	2.4	8.1
0.25	5.1	10.5
0.50	4.0	9.5
0.75	4.1	6.6
1.00	2.1	5.7
2.50	2.5	7.3
5.00	1.0	6.2

If the limit of sensitivity is defined as that signal which is three times higher than the background signal, this method can be used to determine serum containing 20 μg of bupivacaine per litre. Greater sensitivity may be possible by increasing the volume of serum used. Berlin et al. [8] reported the accuracy of the gas chromatographic method as 15 μg/l and Reynolds and Beckett [6] as 40 μg/l. A detection limit of 1 μg/l using gas chromatography has been

TABLE II

BUPIVACAINE DETERMINATION IN HUMAN SERUM

Bupivacaine concentration (mg/l)	No. of parallel determinations	Recovery (mean) (%)	Standard deviation (%)
0.10	13	89.7	3.5
0.25	13	91.4	4.0
0.50	13	98.4	4.1
0.75	13	94.2	4.0
1.00	13	86.1	5.8
2.50	13	92.2	3.4
5.00	13	90.5	4.4
Range 0.10—5.00	7	91.8	3.8

published [12], but the coefficient of variation for that measurement was 15%, so the value is very dubious.

Because our orthopaedic patients were suffering from rheumatic arthritis and using many drugs, we tested a lot of different drugs for possible interference by injecting stock solutions of these compounds into the chromatograph. Diclofenac, indomethacin, cortisol, fentanyl, salicylate, furosemide, amiloride and hydrochlorthiazide did not interfere.

Fig. 2 shows the serum concentrations of bupivacaine in our rheumatic patients. The peak plasma concentration after the extradural administration of 150 mg of bupivacaine hydrochloride has been shown to be at the level 1.0—1.2 mg/l [14], which is identical with our results.

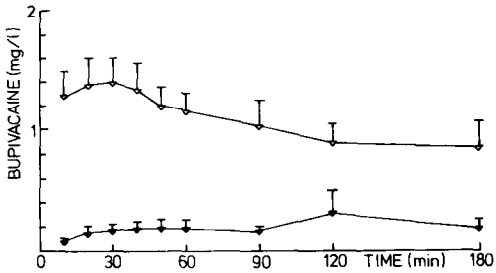

Fig. 2. Mean (+ S.E.M.) serum concentrations of bupivacaine during spinal (▼) (n = 3) and epidural (▽) (n = 5) analgesia.

After spinal analgesia with 15 mg of bupivacaine hydrochloride, Dennhardt and Konder [15] have shown the blood peak concentration of bupivacaine to be 0.30 ± 0.13 mg/l. Tucker and Mather [16] have reported the blood/plasma concentration coefficient for bupivacaine to be 0.73 ± 0.08 in normal adult males with normal blood pH values. Our patients were not suffering from chronic pulmonary or renal diseases, so we can suppose they had quite normal blood pH values. Because they did not have abnormally low serum protein values either, we can use the concentration coefficient of 0.73 when comparing our serum concentrations with whole blood concentrations of bupivacaine. Our results are at the same level as the values obtained by Dennhardt and Konder [15].

A small increase in our mean values at 120 min following spinal analgesia could be explained by the occurrence that one of our patients was transferred from the operating table to his bed some minutes before drawing the 120-min sample. This move might have caused an increase in the pressure of the intradural space thus augmenting the absorption of bupivacaine from the cerebrospinal fluid.

In conclusion, the HPLC method for bupivacaine in human serum that has been developed has been demonstrated to be accurate, selective, simple and rapid for the analysis of bupivacaine. The method has also been successfully applied in studies of spinal and epidural analgesia of orthopaedic patients.

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REFERENCES

1 G.T. Tucker and L.E. Mather, Clin. Pharmacokin., 4 (1979) 241.
2 B.G. Covino, Anesthesiology, 35 (1971) 158.
3 H. Pedersen, H.O. Morishima and M. Finster, Int. Anesthesiol. Clin., 16 (1978) 73.
4 J.A.W. Wildsmith, G.T. Tucker, S. Cooper, D.B. Scott and B.G. Covino, Brit. J. Anaesth., 49 (1977) 461.
5 R.A. Ross, J.E. Clarke and E.N. Armitage, Anaesthesia, 35 (1980) 663.
6 F. Reynolds and A.H. Beckett, J. Pharm. Pharmacol., 20 (1968) 704.
7 G.T. Tucker, Anesthesiology, 32 (1970) 255.
8 A. Berlin, B.-A. Persson and P. Belfrage, J. Pharm. Pharmacol., 25 (1973) 466.
9 E. Zylber-Katz, L. Granit and M. Levy, Clin. Chem., 24 (1978) 1573.
10 P.E. Verheesen, P.J. Brombacher and H.M.H.G. Cremers, J. Clin. Chem. Clin. Biochem., 18 (1980) 351.
11 L.J. Lesko, J. Ericson, G. Ostheimer and A. Marion, J. Chromatogr., 182 (1980) 226.
12 A.G.L. Burm, J.W. van Kleef and A.G. de Boer, Anesthesiology, 57 (1982) 527.
13 P.M. Kuhnert, B.R. Kuhnert, J.M. Stitts and T.L. Gross, Anesthesiology, 55 (1981) 611.
14 P.C. Lund, J.C. Cwik and R.T. Pagdanganan, Anest. Analg., 52 (1973) 482.
15 R. Dennhardt and H. Konder, Reg. Anaesth., 6 (1983) 72.
16 G.T. Tucker and L.E. Mather, Brit. J. Anaesth., 47 (1975) 213.