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Note

Determination of bupivacaine in human plasma by high-performance liquid chromatography

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The efficacy and safety of regional anaesthesia with local anaesthetics have improved considerably during recent years. However, the undesirable effects of these drugs, especially those of central nervous system and cardiac toxicity, can still cause severe problems [1]. Since these effects are directly related to the concentrations of local anaesthetics in the systemic circulation, their determination in plasma is of paramount importance. Due to its favourable properties, bupivacaine is one of the most frequently used local anaesthetics in many regional anaesthetic techniques, especially in obstetric anaesthesia [2]. Until now, it has been assayed by gas chromatography [3—6]. In the present communication, an assay for bupivacaine using high-performance liquid chromatography (HPLC) with a simple one-step extraction procedure is described. This assay permits the determination of bupivacaine concentrations in plasma following the administration of this drug by various regional anaesthetic techniques.

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EXPERIMENTAL

Reagents and materials

All chemicals and solvents were of analytical or HPLC grade (E. Merck, Darmstadt, F.R.G.) and were used without further purification. Bupivacaine·HCl and the internal standard 1-pentyl-2-(2′,6′-xylylcarbamoyl)piperidine (PXP) (Fig. 1) were supplied by Astra Läkemedel (Södertälje, Sweden). All glassware used for sample preparation was acid-washed and rinsed extensively with double-distilled water. Silanization of the glassware is not necessary, although it is recommended so as to decrease the variation of the results. The PTFE linings of the screw caps of culture tubes were ultrasonicated in methanol and then in double-distilled water.

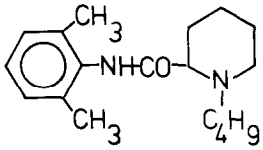

Fig. 1. Chemical structure of bupivacaine. The internal standard (PXP) carries a pentyl group on the piperidine nitrogen.

Sample preparation

The internal standard solution (4 μl), containing 3.2 μg of PXP (1.8 μg for lower concentrations of bupivacaine) was added to 1-ml plasma samples in screw-capped (lined with PTFE) culture tubes using a Hamilton Microlab M automatic pipette. The samples were alkalinized with 100 μl of sodium hydroxide solution (2 mol/l) and extracted into 7 ml of hexane by rotating slowly for 20 min. The hexane phase was separated from the aqueous phase by centrifuging at 1000 *g* for 10 min; it was transferred to a conical glass tube and evaporated to dryness under a gentle nitrogen stream. The residues were redissolved in 50 μl of methanol and then further diluted with 50 μl of water. An 85-μl aliquot of this final solution was injected onto the chromatograph.

Chromatography

A model 6000A high-pressure solvent delivery system (Waters Assoc., Milford, MA, U.S.A.) coupled with an automatic injector (WISP 710 B, Waters Assoc.), a μBondapak C_{18} reversed-phase column (30 cm × 3.9 mm I.D., particle size 10 μm, Waters Assoc.) and a filterphotometer (Model 160, Beckman Instruments, Berkeley, CA, U.S.A.) was used. The mobile phase, consisting of 60 parts of methanol and 40 parts of 50 m*M* sodium phosphate buffer (adjusted to pH 5.0 with phosphoric acid), was passed through a 0.45-μm filter (RC 55, Schleicher and Schüll, Dassel, F.R.G.) before use. The flow-rate was 1.0 ml/min. The effluent was monitored either at 0.003 or 0.005 a.u.f.s. at 254 nm and recorded at 10 mV with a chart speed of 1 mm/min. All chromatography was performed at ambient temperature.

Calibration

Calibration curves were constructed by adding known amounts of bupivacaine·HCl (50, 100, 200, 400, 600, 1000, 1400, 2000, 2400, 2800, 3200 ng)

to 1-ml aliquots of pooled human plasma. The peak height ratios of bupivacaine·HCl to the internal standard were plotted against the concentrations of bupivacaine·HCl. The least-squares linear regression line was fitted through the data points. The bupivacaine concentrations of the unknown samples were determined by using the regression equation of the calibration curve which was assayed concurrently with the unknown samples.

Application of the method

In order to test the method under clinical conditions, plasma concentrations of two patients, who received either 187.5 or 250 mg of bupivacaine·HCl for brachial plexus block, were determined. Blood samples were taken frequently following the block from an antebrachial vein using silanized plastic syringes with heparin (approximately 10 I.U./ml of blood) as anticoagulant. The plasma was separated by centrifugation and stored at −20°C until assayed.

RESULTS AND DISCUSSION

The resolution of the chromatographic system was checked daily by the injection of 20 μl of a mixture containing bupivacaine and the internal standard PXP. Retention times were 8.5 and 12.5 min for bupivacaine and internal standard, respectively (Fig. 2). Using the extraction method described, endogenous plasma components did not interfere with either bupivacaine or the internal standard (Fig. 2). The detection limit for bupivacaine from plasma samples, using a signal-to-noise ratio of 4, was approximately 50 ng/ml.

The linearity of the detector response was assessed by injecting 100 μl of aqueous bupivacaine solutions with concentrations ranging from 50 to 5000

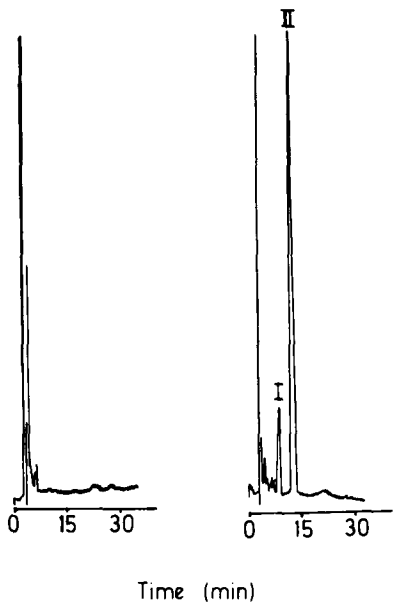

Fig. 2. Chromatograms of blank plasma (left) and plasma spiked with 300 ng/ml bupivacaine·HCl (I) and 3200 ng/ml internal standard (II) (right).

ng/ml. The absolute peak heights of bupivacaine were plotted against the corresponding concentrations. The relationship was linear (r = 0.999) with a y-intercept close to zero (0.00214). The regression line of the plasma calibration curve also showed excellent linearity (r = 0.998) with a small intercept on the y-axis (0.00843).

The precision of the assay was evaluated in a blind study in the concentration range of approximately 70—3000 ng/ml. The experimentally determined concentrations agreed well with the theoretical concentrations (Table I). The day-to-day variation of the assay stayed in an acceptable range. The slope of the standard curve showed a coefficient of variation of 5.9% (n = 12) within a time period of two months.

TABLE I

PRECISION OF THE ASSAY

Concentration of bupivacaine·HCl (ng/ml)		Accuracy* (%)
Theoretical	Experimental	
72.3	77.4 ± 5.0**	6.9
239.3	237.6 ± 8.45	0.7
939.0	944.3 ± 11.1	0.6
1671.6	1652.1 ± 42.8	1.2
2498.6	2466.4 ± 54.6	1.3
3156.0	3157.8 ± 41.4	0.06

*Calculated according to ref. 7.
**Mean ± S.D. (n = 5).

The recovery of the extraction procedure was estimated by comparing the bupivacaine peak height ratio in a plasma extract with that in an aqueous solution of the same concentration. The recovery of bupivacaine in the concentration range 70—4000 ng/ml was 94.5 ± 4.44% (n = 14).

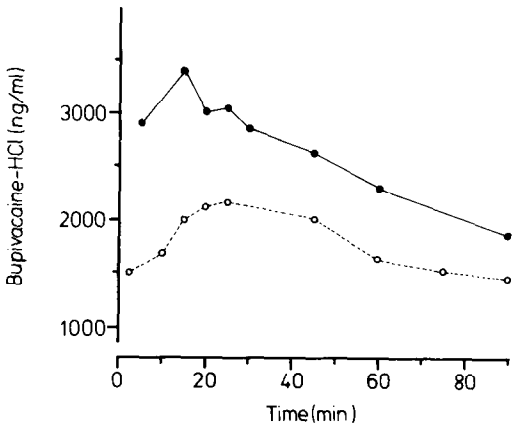

Fig. 3. Plasma concentrations of bupivacaine in two patients who received the drug for brachial plexus block: 187.5 mg (○), or 250 mg (●).

The application of the assay is demonstrated for two patients who received a brachial plexus block with a dose of either 187.5 or 250 mg of bupivacaine (Fig. 3). The plasma concentration—time profile for bupivacaine showed that this method is sensitive and specific enough to determine concentrations of bupivacaine in the systemic circulation following such an anaesthetic technique.

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