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Micromethod for the determination of bupivacaine in maternal and foetal blood during obstetric analgesia

ANITA BERLIN, BENGT-ARNE PERSSON AND PATRICK BELFRAGE‡

Central Military Pharmacy, S-104 01 Stockholm 60, Sweden and ‡ Department of Obstetrics and Gynecology, Karolinska Hospital, S-104 01 Stockholm 60, Sweden

A method for the determination of bupivacaine in maternal and foetal blood during obstetric analgesia is described. The drug and the internal standard, 1-pentyl-2-(2,6-xylylcarbamoyl)-piperidine, are initially extracted into methylene chloride. Perchloric acid is added to retain bupivacaine and the standard as perchlorates in the organic phase accomplishing a selective separation from less hydrophobic amines. Bupivacaine and the standard are then re-extracted into sulphuric acid, followed by a purification with methylene chloride. The aqueous extract is finally made alkaline and the compounds extracted into 10 μl methylene chloride. This extract is analysed by gas chromatography using a 3% OV-17 column. The standard deviation of the method at therapeutic concentrations is about 10% and the lowest level which can be determined with reasonable accuracy is 15 ng ml^{-1}.

Bupivacaine (Marcain, AB Bofors Nobel Pharma, Sweden) is a local anaesthetic agent of the anilide type used in obstetric analgesia. Some gas chromatographic methods have been published for the determination of bupivacaine (Asling, Shnider & others, 1969; Moore, Bridenbaugh & others, 1970; Pratt, Warrington & Grego, 1967; Reynolds & Beckett, 1968; Thomas, Climie & Mather, 1969; Tucker, 1970 and Wilkinson & Lund, 1970). These methods require at least 1 ml of plasma or whole blood for accurate determination. The use of gas chromatography—mass spectrometry has also been used in the analysis of local anaesthetic agents (Strong & Atkinson, 1972).

An evaluation of a possible relation between the foetal blood concentrations and the side-effects sometimes reported to occur in the foetus during labour (e.g. bradycardia) as well as studies on the foeto-maternal distribution after different types of blocks, require a method where 200 μl blood can be used (foetal scalp blood obtained by the Saling sampling technique). The method also has to be highly sensitive because of the low concentrations expected in the foetus and child (Thomas & others, 1969).

We have developed a method for bupivacaine, based on selective extraction and gas chromatography, that has a sensitivity about ten times higher than previous methods and fulfils the requirements mentioned.

MATERIALS AND METHODS

Reagents

Tetrabutylammoniumhydrogensulphate ($TBAHSO_4$), AB Hässle, Mölndal. Bupivacaine and the internal standard, 1-pentyl-2-(2,6-xylylcarbamoyl)-piperidine were received from AB Bofors Nobel Pharma, Mölndal.

Internal standard solution: 40 ng ml^{-1} in HCl 0·01 M.

Chromatography

A Varian 1400 Gas Chromatograph equipped with a flame ionization detector was used. The column was a 6 ft, 3 mm i.d. silanized glass column packed with OV-17 (3% on Gas-Chrom Q 80/100 mesh). Temperatures were 222° in the column, 248° in the injector and 265° in the detector. The nitrogen flow rate was 30 ml min^{-1} and the hydrogen and oxygen flow rates 20 and 250 ml min^{-1} respectively.

The retention times were 6·5 min for bupivacaine and 8·1 min for the internal standard (Fig. 1).

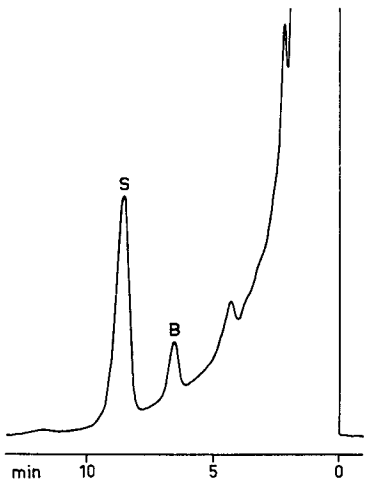

FIG. 1. Gas chromatogram from a patient given bupivacaine. Blood concentration of bupivacaine (B) = 28 ng ml^{-1}. S = Internal standard.

Partition coefficients and extraction constants

Partition coefficients and extractions constants for bupivacaine were determined according to Persson & Schill (1966). In the ultraviolet, bupivacaine in organic phase was measured in base form (B) at a plateau in the absorbance spectrum since there is no maximum. Due to the low concentration in organic phase after the extraction of bupivacaine as sulphate ion pair, the amine was determined as picrate. Picrate 5×10^{-2} M in phosphate buffer pH 6·0 was used. Compensation had to be made for incomplete extraction (88%). Constants for bupivacaine are in Table 1.

Table 1. *Base partitioning and extraction constants for bupivacaine.* Aqueous phase: $\mu = 0{\cdot}1$; $\log \epsilon_{282{\cdot}5} = 2{\cdot}59$ (HB^+). Organic phase: CH_2Cl_2; $\log \epsilon_{282{\cdot}5} = 2{\cdot}65$ (B).

Extracted form in organic phase	pH	C_X^-	$C_B^\circ \times 10^3$	log E_{HBX}	log K_{2HBX}	log $k_{d(B)} \times K'_{HB^+}$
B	3·9—4·2		10·8			—3·60
$HB^+ClO_4^-$	1	0·01—0·02	0·6—10·3	2·03	1·96	
$HB^+HSO_4^-$	1	0·1	3·9—7·8	—1·5		

C_X^- = concentration of anion in aqueous phase
$C_B^\circ \times 10^3$ = initial total concentration of B
Log E_{HBX} = log extraction constant of HBX
Log $K_{2\,HBX}$ = log dimerization constant of HAX
Log $k_{d(B)}$ = log partition coefficient of B
K'_{HB^+} = acid dissociation constant of HB^+

From the base partition experiments log $k_{d(B)} = 4{\cdot}5$ in methylene chloride could be evaluated assuming a pK'_{HB^+} of 8·1 (AB Bofors Nobel Pharma, pers. comm.).

Studies on the extraction of bupivacaine perchlorate indicated some side reaction. A dimerization constant was evaluated. Sulphuric acid 0·1 M was used as the aqueous phase in the studies on the extraction of bupivacaine as sulphate ion pair. The amine was assumed to be extracted as hydrogensulphate ($HBHSO_4$) which best satisfied the results obtained.

For the internal standard with one methylene group more in the alkyl substituent at the amino-group, the log $k_{d(B)}$ as well as the log E value are assumed to be 0·6 units higher than for bupivacaine according to studies on homologous compounds by Gustavii (1967).

Blood sampling

Maternal blood samples were collected by venous puncture in tubes containing heparin. The volume needed for the analysis was dispensed in 3 ml extraction glass tubes and stored at −18° until analysis. Foetal blood was collected according to Saling (1966) and dispensed and stored as described above.

Extraction

Bupivacaine and standard are extracted as bases into methylene chloride, extracted with perchloric acid to form the ion pair $HB^+ClO_4^-$ and re-extracted to an acidic aqueous phase. This phase is purified once with methylene chloride, and bupivacaine and standard are extracted to a small organic phase after being made alkaline.

Procedure. To blood (200 μl) with internal standard (1·00 ml) and NaOH (0·1 ml; 1M) is added CH_2Cl_2 (1·00 ml). After shaking (15 min) the organic phase is separated, $HClO_4$ (0·2 ml; 2M) is added and after shaking (15 min) the organic phase is transferred to a tapered glass tube and 0·1 ml of a mixture of $TBAHSO_4$ (0·1M) and H_2SO_4 (0·1M) added. After shaking (15 min) the aqueous phase is extracted once with CH_2Cl_2 (0·5 ml). To the aqueous phase remaining is added Na_2CO_3 (0·1 ml; 0·5M). After extraction with CH_2Cl_2 (10 μl), 2 μl is taken for gas chromatography.

Standard curve. A standard curve was prepared by subjecting blood containing known amounts of bupivacaine to the procedure. The ratio between the peak heights of bupivacaine and internal standard was plotted against the concentration of bupivacaine.

Standard deviation. Double determinations were made in the concentration ranges normally obtained after obstetric analgesia to measure the standard deviation of the method. The standard deviation in per cent was related to the interval mean value. The results are shown in Table 2.

RESULTS AND DISCUSSION

The size of the partition coefficient indicates that bupivacaine is extracted quantitatively as base into methylene chloride. A theoretical recovery of more than 99·9% is obtained. The extraction is made from an aqueous phase of NaOH (0·1M) to remove acid components.

The transfer of bupivacaine in the methylene chloride extract from base to perchlorate form is made so that a little more than 99% remains in the organic phase. By this procedure, less hydrophobic amines are largely removed.

Table 2. *Standard deviation of bupivacaine analyses at different concentrations.* (Number of pairs in each concentration range = 10.)

Concentration range ng ml^{-1}	s.d. ng ml^{-1}	s.d. %
10–25	3	17
26–50	4	11
51–75	3	5
76–100	7	8
101–150	9	7
151–200	8	5
201–400	8	3

Re-extraction of bupivacaine into sulphuric acid is made to remove lipophilic non-protolytic compounds. Tetrabutylammonium hydrogen sulphate is present to displace bupivacaine in the perchlorate ion pairs. The aqueous phase is then purified once with methylene chloride. Due to unfavourable phase volume ratios chosen to give a tenfold concentration, a little bupivacaine will be lost. Theoretically about 95% will remain in the aqueous phase, the rest being wasted as sulphate in the organic phase. For the internal standard the theoretical recovery is about 85%. The extraction with 10 μl methylene chloride is made after making the solution alkaline with carbonate. No further losses are made here. The final volume of extract is about 5 μl.

The cleaning up procedure although tedious is necessary to attain sensitivity. Successive phase volume reductions avoids disturbances by solvent impurities.

No endogenous compounds or other drugs administered have been found to interfere with the bupivacaine or standard peaks. The purification by shaking with 0·5 ml methylene chloride was included to eliminate disturbances from a peak with a retention time of about 50 min, thereby making injections with 20 min intervals possible.

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