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Letter to the Editor

Determination of bupivacaine in human plasma by capillary gas chromatography with nitrogen-selective detection

Sir,

Bupivacaine (Marcaine) is a long-acting local anaesthetic that is used for regional anaesthesia and in pain treatment to provide analgesia [1]. As cardiotoxic effects have been reported [2, 3], the low toxic-to-therapeutic ratio indicates a need to reevaluate the pharmacokinetics of bupivacaine in these different indications.

Several methods have been reported, using gas chromatography (GC) with [4] or without [5—10] mass spectrometry, or high-performance liquid chromatography (HPLC) [11—13].

We have developed an assay using a single-step extraction followed by open-tubular fused-silica capillary column GC with nitrogen-selective detection. This method combines high sensitivity, selectivity and simplicity. It was used to determine plasma levels in patients receiving bupivacaine in local anaesthesia.

A Delsi Model 300 gas chromatograph equipped with a solid injector and a nitrogen-selective detector (rubidium type) was used. The column was a thin-film fused-silica capillary column coated with OV-1 (30 m × 0.32 mm I.D., 0.20 μm film thickness; Spiral, Dijon, France). The temperatures were as follows: oven, 240°C; injector, 270°C; detector, 260°C. The carrier gas (helium) flow-rate was 2 ml/min. A make-up of helium was used to obtain a 30 ml/min flow-rate through the detector.

To a 1-ml aliquot of plasma was added 1 μg of internal standard (etidocaine). The plasma was made alkaline with 0.1 *M* sodium hydroxide and extracted with diethyl ether. After evaporation to dryness of the organic layer, an aliquot was injected into the chromatograph. Blood samples were collected in heparinized tubes. After immediate centrifugation, plasma was frozen and stored at —18°C until analysed.

Typical chromatograms obtained by the above procedure are shown in Fig. 1. The retention times of bupivacaine and etidocaine were 8.5 and 5.4 min, respectively. We chose etidocaine as internal standard, as Ha et al. did for HPLC [11], because of the similarity of its structure and liposolubility.

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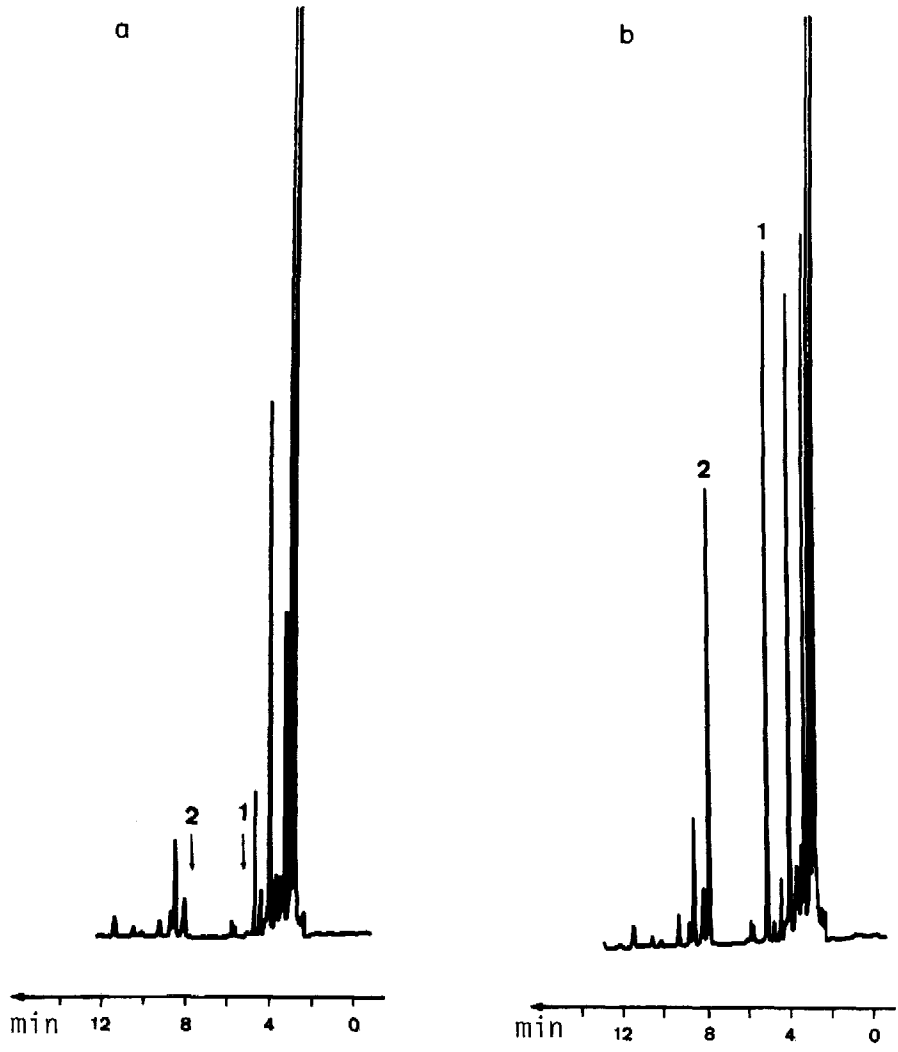

Fig. 1. Chromatograms obtained from: (a) blank human plasma (collected before bupivacaine administration); (b) plasma of patient after injection of 100 mg of bupivacaine. Chromatographic conditions were as described in Experimental. Peaks: 1 = etidocaine, the internal standard (1 μg); 2 = bupivacaine.

Linearity was evaluated in the range 0.125—4 μg/ml by plotting peak-height ratio (bupivacaine to etidocaine) versus bupivacaine concentrations obtained by spiking blank plasma. The relation was: $y = 0.456x - 0.002$ ($r = 0.999$). Samples with concentrations higher than 4 μg/ml needed dilution prior to analysis. Calibration was carried out each day by spiking blank plasma with bupivacaine and analysing these standards by the described procedure.

Intra-assay precision was checked by dosing with three different concentrations (0.5, 1 and 2 μg/ml). The results were respectively 0.51 ± 0.03, 1.03 ± 0.06 and 1.98 ± 0.11 μg/ml ($n = 5$). For inter-assay precision evaluation, blank plasma was spiked (1.0 μg/ml) and analysed every day for ten days. The result was 1.04 ± 0.08 μg/ml.

With the described procedure, the limit of detection was 6 ng/ml of plasma

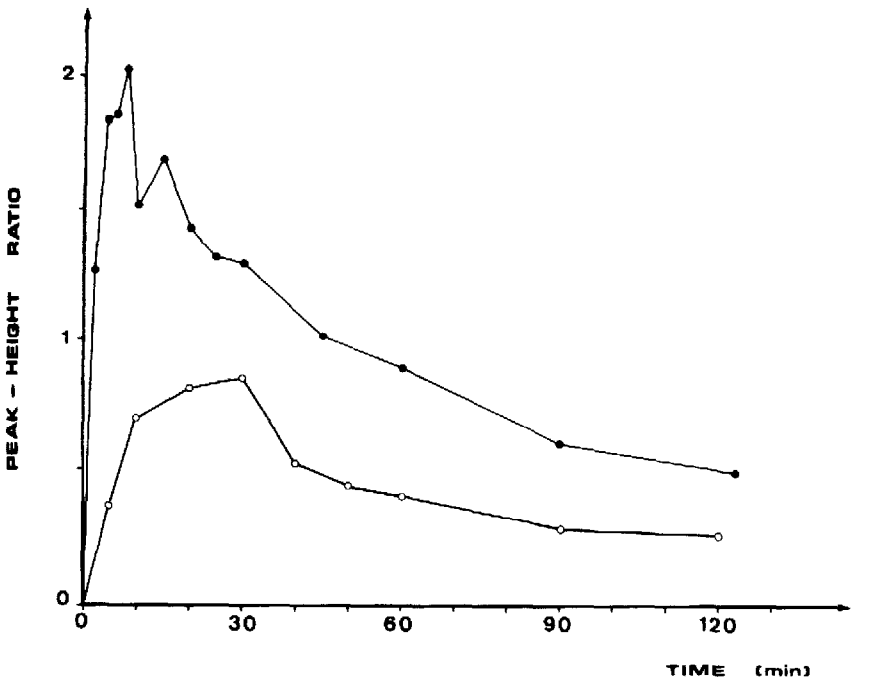

Fig. 2. Plasma concentration versus time profile for bupivacaine. (•) Intercostal nerve block (100 mg) in an adult patient; (○) femoral nerve block (30 mg) in a child.

(0.4 ng injected into the chromatograph). When pure bupivacaine was injected, the minimum detectable amount was 0.25 ng. These limits are lower than currently available values except for those of Burm et al. [5], whose method reached 1 ng/ml. On the other hand, the latter assay is linear in the range 0.025–2 μg/ml [4]. In contrast, our calibration curve was linear from 0.125 to 4 μg/ml, which allows a precise determination in the range of toxic plasma concentrations.

From blank plasma obtained before bupivacaine administration, we checked the absence of interference of endogenous compounds and of numerous drugs used before and during anaesthesia. Our assay was cross-validated with a published HPLC procedure [11]. We obtained a good correlation ($y = 1.106x + 0.019$, $n = 100$, $r = 0.969$).

We used the above method to measure plasma bupivacaine for pharmacokinetic studies. Typical concentration–time curves are presented in Fig. 2. For patient A (intercostal nerve block) the maximum plasma level was 2.03 μg/ml, obtained 8 min after bupivacaine administration. The slope of the terminal phase was 0.011 min^{-1} corresponding to a half-life of 62.6 min. For patient B (femoral nerve block), these values were 0.85 μg/ml, 30 min and 112.6 min, respectively.

In our laboratory, thirty determinations can be easily performed in a working day; this permits an application of this assay to pharmacokinetic studies. Moreover, the short extraction step and the rapidity of the chromatography allow an emergency determination within 1 h.

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