Gas Chromatographic Determination of Bupivacaine in Plasma Using a Support Coated Open Tubular Column and a Nitrogen-selective Detector

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A capillary gas chromatographic method for the quantitative determination of bupivacaine in plasma is described. Bupivacaine and an added internal standard were extracted from plasma with n-pentane. The extracted products were introduced into the gas chromatograph via a solid injection system. The combination of a support coated open tubular column and a nitrogen-selective detector insure a good selectivity and a high sensitivity. The coefficient of variation at concentrations exceeding 3 ng bupivacaine/ml plasma is less than 6%. The detection limit is about 1 ng/ml. (Key words: Anesthetics, local: bupivacaine. Measurement techniques: bupivacaine; gas chromatography.)

Bupivacaine in biologic fluids usually is assayed using gas chromatography. Conventional methods include the use of a packed column and a flame ionization detector. Some investigators use a nitrogen-selective detector, or a gas chromatography mass spectrometry technique. Compared with a flame ionization detector, a nitrogen detector is more selective and more sensitive. Selectivity is an important feature because interference by endogenous compounds and other drugs or metabolites cannot always be precluded, unless time-consuming clean up procedures are used. A high sensitivity is essential because plasma or blood levels after a local or regional anesthetic procedure are usually very low, e.g., peak plasma levels after spinal anesthesia with bupivacaine generally do not exceed 0.1 μg/ml. Both the selectivity and the sensitivity can be improved further by using a capillary column instead of a packed column. The combination of a support coated open tubular (SCOT) column and a nitrogen-selective detector has been applied successfully for the assay of lidocaine in human blood and plasma and in rat blood by De Boer et al. Their method includes solid injection, thus avoiding the injection of relatively large amounts of solvent. The method, described in this paper also includes the use of a SCOT column, a nitrogen detector and a solid injection system and was developed for the measurement of bupivacaine concentrations after administration for epidural and spinal anesthesia.

Materials and Methods

Reagents

Bupivacaine and the internal standard 1-pentyl-2,6-piperidoxylidide hydrochloride were supplied by Astra Pharmaceutica (Rijswijk, The Netherlands). The n-pentane (Merck, Darmstadt, G.F.R.) used for extraction was either analytical grade or reagent grade; the latter was first redistilled. Ethanol (Merck) was analytical grade. Internal standard (is) solutions, containing 4 or 32 μg is/ml ethanol were prepared monthly from a stock solution (400 μg/ml).

Extraction Procedure

A 1.0-ml plasma sample was placed into a centrifuge tube. After addition of 25 μl of the internal standard solution the mixture was homogenized. Five milliliters n-pentane were added and extraction was carried out for 30 s on a Whirlmix. After centrifugation for 5 min at 2,500 g the supernatant organic layer was transferred into a conical centrifuge tube. To the residue, another 5 ml of n-pentane were added and the extraction and centrifugation procedures were repeated. The supernatant phase was added to that of the first extraction. The solvent then was evaporated in a stream of dry nitrogen on a water bath at 40° C and the residue was taken up in 50 or 100 μl absolute ethanol.

The ethanol solution, 1–6 μl, was placed onto the needle of the solid injection system, and after allowing

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ml plasma samples spiked with known amounts of bupivacaine. After the addition of the internal standard solution, the plasma samples were extracted and chromatographed as described above. The ratios of the peak heights of bupivacaine and the internal standard were determined from the obtained chromatograms and were plotted against the corresponding bupivacaine concentrations.

The recovery of bupivacaine from human plasma was determined in a similar way, but without addition of the internal standard. However, 0.8 μg of the pentyl homologue of bupivacaine was added as external standard after evaporation of pentane from the extracts. The peak height ratios of bupivacaine and the external standard were determined and compared with the peak height ratios obtained from standard solutions in ethanol.

Results

Typical chromatograms obtained in the analysis of plasma samples are shown in figure 1. These illustrate the absence of a significant solvent peak. The relatively small recorder deflection shortly after the injection is due to a disturbance related to the solid injection technique used, rather than to the elution of solvent. The retention times of the bupivacaine and internal standard peaks are 3.2 min and 4.2 min, respectively. These peaks are well-separated, facilitating the determination of a wide range of bupivacaine concentrations with a fixed amount of internal standard. Another peak, which is often observed, resulted from caffeine and has a retention time of 2.3 min. Interference of endogenous compounds is negligible, as shown by the chromatogram of the blank sample.

Calibration lines show minimal day-to-day variation, and are linear in the ranges tested (3–100 ng/ml and 0.025–2 μg/ml). The correlation coefficients, obtained by linear regression analysis of a composition of different lines, determined on four different occasions, exceeded 0.999 for both ranges. The variation coefficient, reflecting both within a day and day-to-day variations, was 10% at a bupivacaine concentration of 3 ng/ml plasma and did not exceed 6% at higher concentrations. The minimum concentration of bupivacaine that can be determined with reasonable precision is about 1 ng/ml (variation coefficient ±15%). The recovery of bupivacaine in the range of concentrations investigated (0.025–1.6 μg/ml) is 73%, while the recovery of the internal standard, estimated from the standard solutions and the calibration line, is 71%.

Discussion

The described method combines the advantages of a SCOT column, a nitrogen detector, and a solid injection technique, that have been discussed extensively.
Fig. 2. Plasma concentration curve of bupivacaine (semi-logarithmic scale) obtained after administration of 15 mg bupivacaine hydrochloride (isotonic 0.5% solution) for spinal anesthesia.

elsewhere. The result is a relatively easy, fast, and sensitive technique. The high selectivity minimizes possible interferences from endogenous compounds and from other drugs or metabolites. No disturbing peaks were seen in the chromatograms of plasma samples of patients premedicated with diazepam or lorazepam. The retention times of diazepam and lorazepam are 20.7 min and 19.5 min, respectively. The method has been used for the analysis of plasma samples, collected after epidural and spinal anesthesia with bupivacaine. A typical plasma concentration curve obtained after spinal administration is shown in figure 2. This illustrates the validity of the method, where other techniques might fail to demonstrate the very low plasma levels after spinal anesthesia.

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