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Note

Analysis of thiopentone in human plasma by high-performance liquid chromatography

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As part of a study of perinatal drug transfer, we are investigating the placental transfer of drugs administered just prior to delivery, especially in caesarian section. The drug of choice for induction of anaesthesia is thiopentone [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid], an ultra short-acting barbiturate. Numerous methods of analysis for thiopentone have been presented in the literature [1—5]. Oroszlan and Maengwyn-Davies [1] reported a spectrophotometric assay involving three different wavelengths and having a sensitivity of 1 μg/ml. Scoppa [2] described a spectrofluorimetric determination with a sensitivity of 0.5 μg/ml. Neither method is specific for thiopentone. Bogan and Smith [3] used extraction followed by gas—liquid chromatography to separate and quantitate thiopentone specifically. Sensitivity was improved by both Sennello and Kohn [4] and Schepens and Heyndricks [5] by the use of a nitrogen-sensitive detector. Concentrations of 0.1 μg/ml were quantitated by this method.

The gas chromatographic determination of thiopentone is preferable to other previously reported analytical methods owing to its accurate quantitation and high specificity for low levels of drugs. It does, however, require time-consuming extraction procedures followed by lenghthy derivatization to enable the sample to be chromatographed at a low enough temperature to stop desulphuration.

Accordingly, a high-performance liquid chromatographic (HPLC) assay for plasma levels of free thiopentone was developed since the extraction and derivatization procedures could be eliminated, leading to the rapid and accurate assay of a large number of plasma samples. To simplify preparation of the sample prior to chromatographing a precolumn was inserted between the injection port and the main analytical column to remove proteins and other particulate matter from the plasma samples. Using this method, pretreatment of the sample involved a single dilution of plasma with internal standard to produce a sample suitable for chromatographing.

EXPERIMENTAL

Instrumentation

Analysis of samples was performed on a Perkin-Elmer 1220 liquid chromatograph using a reversed-phase 25 cm × 4.6 mm I.D. Partisil 10/25 ODS column (Whatman, Maidstone, Great Britain) protected by a 3 cm × 2.8 mm I.D. precolumn separated from the main column by a 2-μm stainless-steel frit.

The mobile phase [methanol—0.1% sodium citrate buffer (pH 6.5), 45:55] was eluted at 0.5 ml/min. The detector was a Perkin-Elmer LC-55 operated at 290 nm and its output led through a Perkin-Elmer M-2 calculating integrator which gave peak area ratios for thiopentone to internal standard.

Sample preparation

At 1-min intervals after the induction of anaesthesia with intravenous thiopentone, 2-ml samples of whole blood were collected from the contralateral median cubital vein of women undergoing delivery by ceasarian section. The blood was stored in sequestrene (EDTA) tubes at 4° until analysed. Just prior to analysis the tubes were thoroughly mixed on a Vortex mixer for 30 sec and a 0.5-ml portion was centrifuged at 1600 *g* for 5 min. A 95-μl portion of the supernatant was withdrawn and mixed with 5 μl of internal standard (0.02% quinoline) in glass vials. Aliquots (10 μl) were withdrawn for analysis.

RESULTS AND DISCUSSION

Specificity

A representative chromatogram is shown in Fig. 1. Analysis of samples from a number of patients has shown that a variety of drugs used pre-operatively, postdelivery, and as premedication did not interfere. These include folic acid, penicillin V, hyoscine, nitrazepam, morphine, ergometrine, phenobarbitone, pethidine and chlorpromazine.

A typical plasma profile from a patient who received intravenously 250 mg of thiopentone is shown in Fig. 2. The well-known rapid decrease in thiopentone plasma levels is evident.

Precision

A standard curve was constructed with points ranging from 0 to 20 μg/ml of thiopentone and the appropriate amount of internal standard. A plot of the

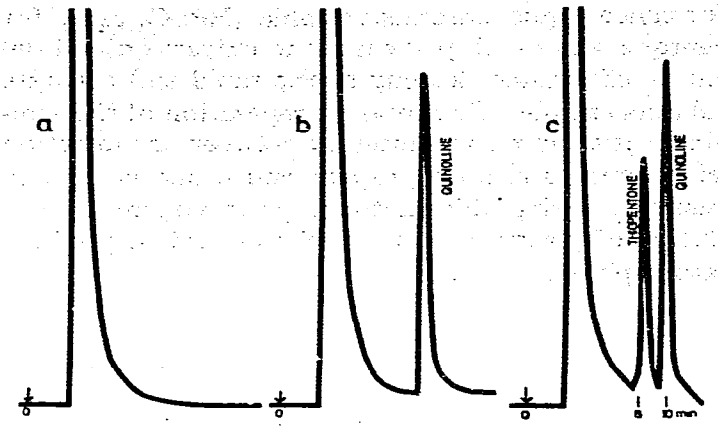

Fig. 1. Liquid chromatograms of human plasma following direct injection. (a) Drug-free plasma; (b) plasma with internal standard; (c) drug-containing plasma with internal standard.

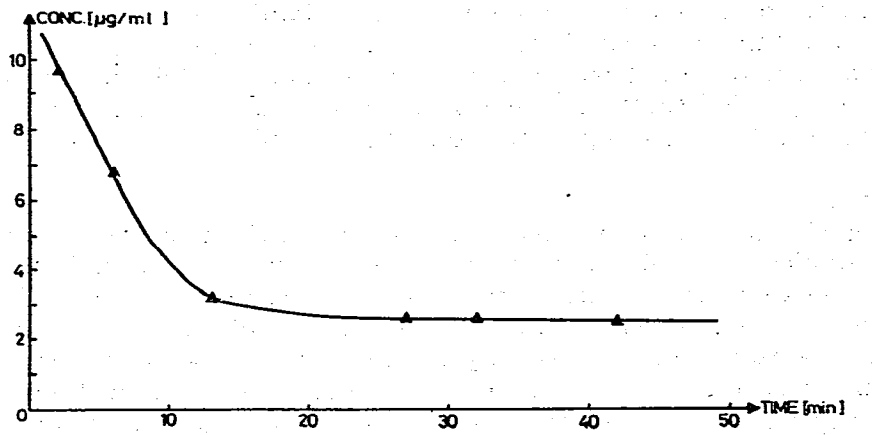

Fig. 2. Maternal plasma concentrations of thiopentone as a function of time.

ratio of peak areas (y) versus concentration of thiopentone (x) gave a line which fitted the equation

$$y = 0.0349\,x - 0.0060$$

with a coefficient of correlation of 0.9993. The lower limit of detection (signal-to-noise ratio 10:1) was 0.5 µg/ml for a 10-µl injection and the curve was linear over the range 0.5 to 20 µg/ml.

Precolumn

In developing this assay such pretreatments as extraction and ultrafiltration were considered in addition to the use of a precolumn. Extraction was found to be time-consuming while ultrafiltration was inappropriate owing to both extensive binding of thiopentone to the membrane and the filtration time required to obtain a suitable sample. The precolumn offered the only viable alternative.

The column was constructed of 3 cm × 2.8 mm I.D. (¼ in. O.D.) 316 stainless steel and was packed with 10-μm Partisil 10/25 ODS packing. Plasma samples were then injected through the septum directly on to the precolumn. The backpressure of the system was monitored and when this pressure rose above ~2500 p.s.i.g., the precolumn was replaced.

Approximately 500 μl of plasma (fifty 10-μl injections) could be filtered by the one precolumn before it required replacement.

CONCLUSION

A simple HPLC method for the determination of plasma levels of thiopentone has been described. Sample pretreatment is minimized by the use of a precolumn inserted between the injection port and the analytical column. In a 10-μl injection the minimum detectable concentration of free thiopentone in plasma is 0.5 μg/ml.

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