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Note

Determination of thiopental measured in human blood by reversed-phase high-performance liquid chromatography

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Thiopental is injected intravenously for the induction of general anaesthesia. It is the most widely used barbiturate in obstetrics for the induction of general anaesthesia for Caesarian section and is employed as the standard against which newer agents are assessed [1]. It is also used to reduce intracranial pressure [2] in the prevention and treatment of brain ischemia [3,4]. Since 1934, when the drug was introduced into clinical practice, piecemeal information has accumulated about its disposition in different physiological conditions, in animals and humans [5,6].

Because of the compound's physiochemical and kinetic characteristics [5,7–9], routine clinical monitoring of its levels in blood does not seem useful for individual patient management. However, the determination of its levels in different body matrices may be useful as a guide for rational dosage adjustments in different critical clinical states [6,10–12], as well as for better defining the drug disposition [13–15].

Previous methods of thiopental measurement by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection required large samples [16], lacked specificity [17,18], called for extensive sample preparation

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[16,20], or a high temperature in elution [18], or expensive materials [17,19], or else could be used only for plasma and/or serum and/or tissue homogenates [16,18].

As part of a wider project on better thiopental utilization in different clinical states (prematurity, Caesarian section, resistant seizures) [21,22], we have set up thiopental assays in different biological specimens, in particular the whole blood, to minimize the variability associated with sample handling for serum/plasma. The method described here is reliable, sensitive, and rapid and needs only small samples. Since pentobarbital is an active metabolite of thiopental [23] and is also used as a therapeutic agent [24,25], particular attention was paid to simultaneous analysis of both barbiturates.

EXPERIMENTAL

Chemicals and reagents

Thiopental (ethyl-5-(1-methylbutyl)-2-thiobarbiturate), pentobarbital (ethyl-5-(1-methylbutyl)barbituric acid) and secobarbital (allyl-5-(1-methylbutyl)barbituric acid, used as internal standard) were purchased from Abbott (Latina, Italy). Ethanol, diethyl ether, *n*-hexane, and acetonitrile (Li-Chrosolv, Merck, Darmstadt, F.R.G.) were UV grade. Sodium phosphate was obtained from Farmitalia-Carlo Erba (Milan, Italy).

Standards

Stock solutions of compounds (100 μg/ml) in 75% ethanol were prepared weekly and stored in dark bottles at 4°C.

Two standard concentrations of thiopental and pentobarbital (2 and 10 μg/ml) were prepared by serial dilution from a pool of blood to provide the material for quality control. Each pool was divided into 1-ml samples and frozen at −20°C until analysis. The two blocks of samples were analysed over two months to obtain twenty replicates for each block.

Extraction procedure

A 0.2–0.5-ml sample of heparinized human blood was vortex-mixed in a test-tube for 1 min with 1 ml of phosphate buffer (0.1 *M*, pH 7.5), 7 ml of *n*-hexane–diethyl ether (50:50, v/v), and 0.05 ml of internal standard (5 μg). The tube was shaken for 15 min and centrifuged for 15 min at 2000 *g* at 4°C. The organic layer was transferred to another test-tube and evaporated to dryness under a gentle stream of nitrogen at 37°C. The tube was removed promptly from the bath and capped until analysis. The residue was dissolved in 0.1 ml of mobile phase and vortex-mixed, and a 5–20-μl sample was injected into the chromatograph. A calibration curve (0.2–20 μg/ml thiopental and pentobarbital) was plotted for each series of samples, prepared by adding increasing amounts of compounds to blank samples.

Chromatographic conditions

A Beckman (San Ramon, CA, U.S.A.) System Gold liquid chromatograph equipped with a PC-NEC 8300 controller, a Model 160 detector, a Beckman 210A sample injector valve, and a reversed-phase column (Nucleosil C_{18}, 5-μm, 100×3 mm I.D., LiChromopack, Middelburg, The Netherlands) was used. The column was eluted isocratically at room temperature with acetonitrile–water (32:68, v/v). The flow-rate was 0.3 ml/min, and the detector was set to 254 nm.

Calculations

Concentrations of thiopental and pentobarbital were calculated from the peak-area or peak-height ratios of compounds to secobarbital using a Shimadzu C-R6A (Kyoto, Japan) integrator. Results were expressed as the mean, S.D. and coefficient of variation (C.V.). Regression lines were obtained by the least-squares method [26].

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms from drug-free blood, the same sample spiked with 0.016 μg of thiopental, 0.083 μg of pentobarbital and secobarbital (10 μg/ml standard solution), and a patient's blood sample. No interfering peaks were found at retention times close to those of the two compounds measured (capacity factors $k' = 4.4$ and $k' = 2.1$, respectively) or of secobarbital ($k' = 3.1$) under the conditions used. In order to identify the peaks potentially corresponding to the compounds after injection into the column, elution fractions were collected, using the UV absorption trace on the recorder as reference, and analysed by mass spectrometry (data not shown). The spectra were identical with those from authentic compounds.

Calibration curves of thiopental and pentobarbital passed through the origin and were linear at least up to 20 μg/ml for both compounds ($y = 2.5x - 0.03$ and $y = 0.32x + 0.03$, respectively; $r = 0.999$). Analytical recovery averaged 94 and 77% for thiopental and pentobarbital, respectively, over the range 0.2–20 μg/ml. The detection limit was 0.01 μg/ml for both compounds at a signal-to-noise ratio of 3:1. Reproducibilities, expressed as the C.V. values of the slopes of the blood standard curves ($n = 10$) used in routine analysis of compounds, were 3.2 and 6.1% for thiopental and pentobarbital, respectively. Day-to-day ($n = 20$) precision of the assay was assessed over a two-month period by determining the C.V. values at 2 (3.8 and 3.7%) and 10 μg/ml (3.8 and 3.5%) for the two compounds. Accuracy was calculated as the percentage error from the true value on processing twenty samples containing different amounts of the two compounds; the average was 2.6%. Using the procedures described, the lifespan of the analytical column averaged 1000 h.

The data detailed here were obtained from blood specimens, but similar re-

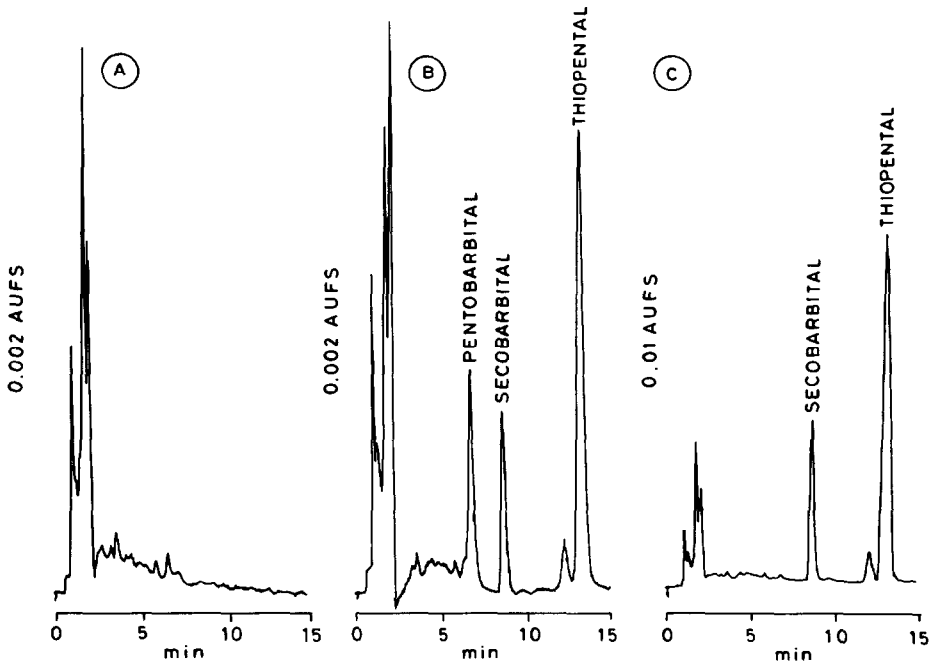

Fig. 1. Chromatograms showing the resolution of thiopental, pentobarbital and secobarbital (internal standard). (A) Extract of 0.5 ml of blank human blood; (B) extract of 0.5 ml of blank human blood spiked with 16 ng of thiopental, 83 ng of pentobarbital and secobarbital; (C) extract of 0.5 ml of patient's blood with 2 μg/ml thiopental.

sults were obtained from other sample matrices, such as plasma, serum, red blood cells, and tissue homogenates [27]. This is an essential requirement for a method suitable for clinical use.

To assess the potential clinical usefulness of the present method, we followed the blood concentrations of thiopental of ten pregnant women (and their babies) given the drug (Pentothal®, Abbott) for the induction of general anaesthesia for Caesarian section (Fig. 2A) [21]. Similarly, we determined the blood concentrations in nine severely asphyxiated newborns with seizures resistant to common therapy, given intravenous injections of 10 mg/kg thiopental (Fig. 2B) [22]. The concentrations and observed disposition profile are in good accord with previous findings [28,29].

Since there is general agreement that the monitoring of blood levels of pharmacological agents used in critical patients (such as premature infants) or in a few clinical states (such as pregnancy and delivery) may be a basis for rational drug utilization [30], an easy and reliable analytical method is mandatory for clinical investigations. The method described, routinely used in our laboratory, overcomes the limitations of previously reported HPLC assays. It is simple and rapid and requires only common instrumentation; it is efficient

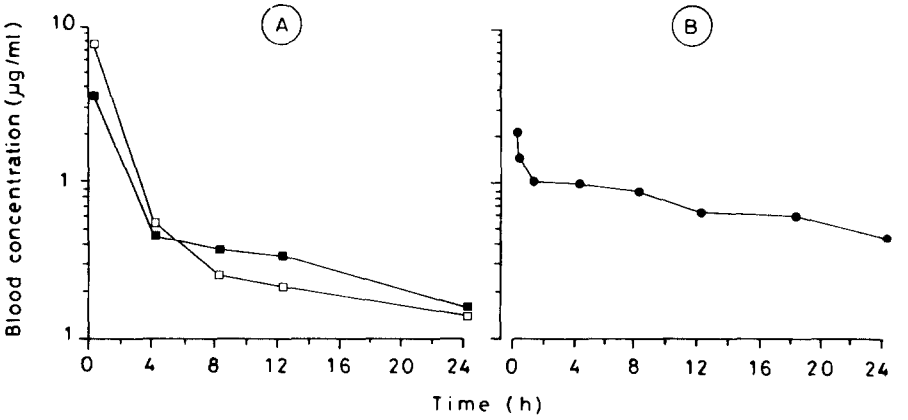

Fig. 2. Semilogarithmic plot of blood thiopental concentrations vs. time (A) in one typical pregnant woman (□) given thiopental (4 mg/kg) as a bolus followed by an infusion of 1.7 mg/kg/h, and her baby (■), and (B) in one typical asphyxiated newborn given 10 mg/kg i.v. of thiopental.

and involves only limited time and costs for sample analysis. Furthermore, small samples are sufficient and different matrices can be processed with the same performance to determine thiopental and pentobarbital. Any laboratory with basic HPLC equipment can routinely determine thiopental and pentobarbital. All the findings reported here are well within the performance limits of an analytical technique required for medical management [31], particularly of critical patients.

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