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Note

Measurement of thiamylal in human plasma using reversed-phase high-performance liquid chromatography

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Thiamylal (Fig. 1), which is an ultra-short-acting barbiturate, is one of the most popular acting barbiturates. Although it is used principally to induce an anaesthetic state in conjunction with nitrous oxide or other inhalation anaesthetics [1,2], responses to a given dose of it are reported to vary with the individual [3]. This variability might be due to the differences in plasma thiamylal concentration.

Several methods to measure plasma thiamylal concentration have been reported. Gas chromatography (GC) with electron-capture or flame photometric detection has been used [4]. However, this method has many problems: thermal decomposition, a need for derivatization, a limited detector linearity and lifetime, a poor reproducibility and specificity, a reversible or irreversible adsorption on the packing materials, peak tailing, etc. Hiba et al. [5] measured the thiamylal concentration in plasma by a high-performance liquid chromatographic (HPLC) method, in which *p*-hydroxypropyl benzoate was used as an internal standard. However, this substance is known to be insoluble in water and is poorly dissolved

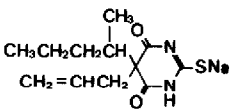

Fig 1. Structural formula of thiamylal.

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in plasma. Moreover, these authors did not measure the potential interference by other drugs.

This paper describes a simple, sensitive and highly selective HPLC method to measure the plasma thiamylal concentration. Rapid analysis by this method appears to be of particular interest for therapeutic drug monitoring and/or pharmacokinetic studies of this drug.

EXPERIMENTAL

Chemicals

Thiamylal, in the form of thiamylal sodium salt (Isozol®), was obtained from Yoshitomi Pharmaceutical (Osaka, Japan). HPLC-grade acetonitrile was purchased from Wako (Osaka, Japan). Purified water was obtained from a Milli-Q reagent water system equipped with ion-exchange, organic and carbon filters (Japan Millipore, Tokyo, Japan).

Apparatus

The Shimadzu chromatographic system (Kyoto, Japan) consisted of a Model LC-6A solvent-delivery system and a Model SPD-6A variable-wavelength UV detector connected to a C-R4A integrator. A Rheodyne 7125 injection valve with a 20-μl injection loop was used.

Chromatography

The analytical column used was a 150 mm × 6.0 mm I.D. Shimadzu Shim-pack CLC-ODS (5 μm particle size). The mobile phase, acetonitrile–water (55:45, v/v), was filtered through a Millipore 0.5-μm filter (type FH) and degassed ultrasonically before use. The flow-rate was 1.2 ml/min and chromatography was performed at ambient temperature (22–25 °C). The detection wavelength was set at 288 nm with the sensitivity at 0.02 a.u.f.s.

Standard solutions

The standard stock solution was made by dissolving 5.0 g of the thiamylal sodium salt in 100 ml of water. This stock solution was dissolved to a concentration of 0.05–100 μg/ml to make a calibration curve for human drug-free plasma. Quantification was done by comparing the peak area of the sample with a standard calibration curve.

Sample preparation

Acetonitrile (0.1 ml) was added to 0.1 ml of plasma in a 1.5-ml plastic microcentrifuge tube. The tube was vortexed for 10 s and after 10 min equilibration it was vortexed again for 10 s. After centrifugation (2 min, 12 000 *g*) the supernatant (20 μl) was injected into the HPLC system.

Kinetic studies

For the pharmacokinetic verification of the method, blood samples were drawn serially from a patient who had received an induction dose of thiamylal (5.0 mg/

kg). The blood samples were collected in heparinized tubes and centrifuged to obtain plasma. The plasma samples were then stored at −20 °C until analysis.

RESULTS

Chromatography and selectivity

Fig. 2 shows typical chromatograms obtained from drug-free plasma (A), drug-free plasma supplemented with thiamylal (B) and plasma from a patient who had received intravenous thiamylal (C). Thiamylal was well separated from the other endogenous compounds of human plasma by isocratic elution, the retention time at a flow-rate of 1.2 ml/min being 4.9 min. Table I shows the drugs tested for interferences. The only drug with a clearly defined peak was thiopental. As shown in Fig. 2D, the thiopental peak had a retention time of 4.5 min and was easily distinguishable from the thiamylal peak. All the other drugs eluted much

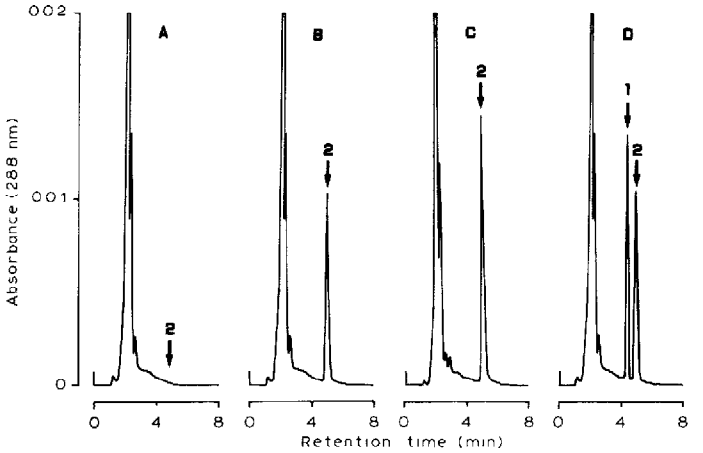

Fig 2. Chromatograms of (A) drug-free plasma, (B) drug-free plasma with 5 μg/ml thiamylal added, (C) a plasma sample from a patient (thiamylal concentration, 6.8 μg/ml), (D) drug-free plasma with 5 μg/ml thiopental and thiamylal added. Peaks: 1 = thiopental; 2 = thiamylal.

TABLE I

DRUGS NOT INTERFERING WITH DETECTION OF THIAMYLAL BY HPLC

Anesthetics	Antibiotics	Antineoplastics	Other Drugs
Amobarbital	Amphotericin B	Adriamycin	Acetaminophen
Barbital	Amikacin	Allopurinol	Aspirin
Droperidol	Ampicillin	Cisplatin	Caffeine
Hexobarbital	Carbenicillin	Cyclophosphamide	Chlorpromazine
Ketamine	Chloramphenicol	Cytarabine	Cimetidine
Metharbital	5-Fluorocytosine	Dactinomycin	Cyclosporin A
Pentobarbital	Gentamicin	5-Fluorouracil	Ethosuximide
Phenobarbital	Ketoconazole	6-Mercaptopurine	Furosemide
Secobarbital	Miconazole	Methotrexate	Mizoribine
Thiopental	Vancomycin	Tegafur	Procainamide

earlier than thiopental and thiamylal under the chromatographic conditions, and did not interfere with the thiamylal peak.

Linearity and recovery

Different concentrations of thiamylal in the concentration range of clinical interest were added to the same drug-free plasma. They were determined from the peak area between 0.05 and 100 μg/ml. The equation describing the standard curve determined using linear least-squares regression analysis was $y = 1.942x + 0.004$ (y = thiamylal concentration in μg/ml, x = peak area). The corresponding correlation coefficient (r) was 0.999. The detection limit was 0.01 μg/ml, at a signal-to-noise ratio of 3:1.

Known amounts of thiamylal were added to five samples of plasma. After extraction and chromatography they were performed in six replicates, the peak area was determined, and a standard curve was used to calculate the concentration of thiamylal in plasma. Recovery was calculated as (amount determined after ex-

TABLE II

RECOVERY OF THIAMYLAL FROM PLASMA BY THE HPLC METHOD

Thiamylal added (μg/ml)	Concentration found (mean ± S.D. $n=6$) (μg/ml)	Recovery (mean ± S.D.) (%)
5.0	4.93 ± 0.15	98.6 ± 3.0
10.0	9.87 ± 0.22	98.7 ± 2.2
20 0	20.14 ± 0.29	100.7 ± 1.5
40.0	39.02 ± 0.38	97.6 ± 1.0
60.0	61.05 ± 0.57	101.8 ± 1.0
		Mean 99.5 ± 1.7

TABLE III

PRECISION AND ACCURACY OF THE HPLC METHOD FOR DETERMINING THIAMYLAL IN PLASMA

Actual concentration (μg/ml)	Concentration found (mean ± S.D.) (μg/ml)	C.V (%)
Within-day ($n=10$)		
1.0	1.08 ± 0.04	3.704
10.0	10.16 ± 0.19	1.870
50.0	49.25 ± 0.44	0.893
Between-day ($n=5$)		
1.0	1.03 ± 0.05	4 850
10.0	9.87 ± 0.34	3.445
50.0	50.63 ± 0 68	1.343

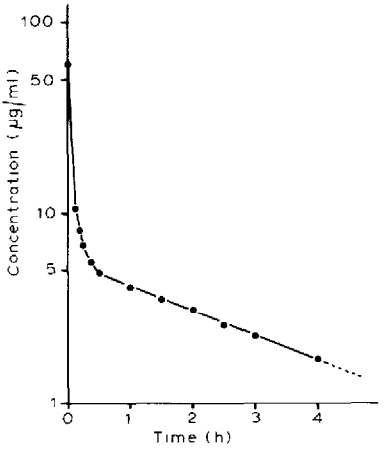

Fig. 3. Time course of thiamylal concentration in a patient's plasma. The drug (300 mg) was given intravenously over 1 min. The apparent volume of distribution (0.63 l/kg) and half-life (2.31 h) were calculated from the regression line.

traction/amount determined after direct injection of standard solution) $\times 100$. Table II shows that the range of recovery from plasma was 98.6–101.8%.

Precision and accuracy

The precision and accuracy of the HPLC method were calculated for four different concentrations of thiamylal in the plasma (Table III). Within-day coefficients of variation (C.V.) ranged from 0.893 to 3.704% and the between-day C.V. values were slightly higher, ranging from 1.343 to 4.850%.

Pharmacokinetic verification of the method

Fig. 3 shows a representative time course curve for the plasma thiamylal concentration after intravenous administration.

DISCUSSION

To study the pharmacokinetics of thiamylal, we developed a new analytical method which is sensitive and selective. To date, a few methods have been used to measure the plasma thiamylal concentration. However, the low extraction efficiency [4] and time-consuming procedure [5] have left problems to be solved. The most sensitive method described so far appears to be the GC method [4], which offers a sensitivity of 1 ng/ml. Although our method is less sensitive than this method, it has the advantage of not requiring extraction or derivatization. The sample need only be protein-precipitated before it is injected into the HPLC column. We have investigated the interfering effects of commonly encountered drugs. None of these drugs interfered with the thiamylal determination. The ability to assay thiamylal in human plasma easily and reproducibility will facilitate studies of the pharmacokinetics of this drug.

In summary, we have described an accurate, rapid, selective and reproducible

HPLC method for determining the concentrations of thiamylal in the patient's plasma. The simplicity of the extraction procedure and rapid analysis time of 6 min make this assay ideal for use in the operating room or when rapid turnaround time for results is necessary.

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