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Case reports

Postmortem concentrations of thiopental in tissues: a sudden death case

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Summary. High-performance liquid chromatography was employed to determine the concentration of thiopental in body fluids and tissues in an individual who had died due to intravenous injection of the clinical dose. The blood concentration of thiopental was 0.6 mg/l. Among the 10 tissues examined, the brain and thymus showed the highest level of the drug; 11.9 mg/kg and 7.66 mg/kg, respectively. The results are discussed in the light of the relevant literature.

Key words: Thiopental – Tissue concentration – Anesthetic – HPLC

Zusammenfassung. Zur Bestimmung von Thiopental in Körperflüssigkeiten und Geweben einer Person, welche aufgrund einer intravenösen Injektion einer klinischen Dosis verstarb, wurde mit Hilfe der Hochdruckflüssigkeitschromatographie bestimmt. Die Blutkonzentration von Thiopental war 0,6 mg/l. Unter den 10 Geweben, welche untersucht wurden, zeigten Hirn und Thymus die höchsten Spiegel des Medikaments, nämlich 11,9 mg/kg bzw. 7,66 mg/kg. Die Resultate werden unter Berücksichtigung der Literatur diskutiert.

Schlüsselwörter: Thiopental – Gewebskonzentration – Anäesthetikum – HPLC

Introduction

Thiopental (5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid) is an ultrashort-acting thiobarbiturate which, like thiamylal, is used for general anesthesia. Respiratory depression caused by an overdose of thiopental may lead to death if mechanical ventilation is not available. The average blood concentration in suicides due solely to thiopental administration is reported to be 273 mg/l in 3 cases [1], but in rare instances intravenous administration of this drug in clinical doses is known to be fatal [2–4]. Although such cases can be forensically problematic in determining whether the death is due to anaphylaxia or malpractice, little information is available on the distribution of the drug in various autopsy samples in cases of sudden death due to thiobarbiturates.

The present report describes a case of sudden death due to thiopental administration, and the distribution of the drug in body fluids and tissues. The quantitation of thiopental was performed using high-performance liquid chromatography (HPLC) as previously described [5–7].

Case history and autopsy findings

Case history. A 43-year-old Japanese housewife underwent a peroral endoscopical examination of the stomach. The patient requested an anesthetic medication in order to avoid any discomfort or anxiety during the examination. According to the clinical record and the doctor's testimony, the patient was given an intravenous injection of approx. 250 mg of thiopental sodium, before insertion of the endoscope. During this procedure, the patient suddenly went into shock with signs of severe cyanosis. The patient's respiration ceased about 5 min after administration of the drug. An autopsy was performed to clarify the cause of death, including possible malpractice or a causal relationship to the administration of thiopental.

Autopsy findings. The deceased was 151 cm in height, 52 kg in weight, and of average build. External examination revealed one needle puncture wound in the back of the right hand, which was the route of thiopental injection. There were 6 other needle puncture wounds, 3 on the outer aspect of the left arm and 3 on the left breast, all of which were the result of emergency first-aid treatment. The sclerae and palpebral conjunctivae showed several petechial hemorrhages. The internal examination revealed no distinct injury or disease, but gross congestion was evident in the brain and lung tissues. The pleural surfaces showed scattered petechial hemorrhages, and the lungs showed non-infective edema.

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The thymus (weight 27 g) was slightly heavier than average for a Japanese woman of corresponding age, and histologically parenchyma were found.

Toxicological analysis

Materials. Authentic thiopental sodium was kindly provided by Tanabe Pharmaceutical Ltd. (Osaka, Japan). The autopsy materials were kept at −80°C until analysis. In addition, human tissue samples from other autopsy cases were obtained and kept at −80°C for thiopental recovery experiments.

Extraction of thiopental. Approximately 10 g of each tissue was minced, acidified with tartaric acid and homogenized in 50 ml of ethanol. After centrifugation, the resulting supernatant was evaporated to dryness *in vacuo*. The residue was dissolved in 0.1% sulfuric acid and then extracted three times with ether. The combined organic phase was applied to a column packed with 0.5 g Florisil (Nakarai tesque, Kyoto, Japan) [8], and the eluate was evaporated to dryness *in vacuo*. One milliliter of whole blood or urine was mixed with 10 ml of methanol and centrifuged. The resulting supernatant was applied to the Florosil column, and the eluate was evaporated to dryness *in vacuo*. These residues were dissolved in a small volume of methanol for further analysis.

Identification of thiopental. The identification of thiopental was performed using gas chromatography/mass spectrometry (GC/MS) and thin-layer chromatography (TLC). GC/MS analysis was performed on a JMS-DX 300 instrument (JEOL, Tokyo, Japan) equipped with a methylsilicone capillary column (25 m × 0.3 mm) in the chemical ionization mode using methane as the reactant gas. The temperature of the injector and separator was 280°C, and the column temperature was programmed to increase by 4°C/min from 150°C to 220°C. The voltage and current of ionization were 70 eV and 300 μA, respectively. TLC analysis was carried out according to the recommended standard procedure [9] on 0.25 mm thick silica gel GF_{254} plates (Merck, Darmstadt, Germany); the solvent system used for development was a mixture of chloroform (saturated with ammonia) and methanol (9:1 by volume). After development, the plate was sprayed with N-2,6-trichloro-*p*-benzoquinone imine.

Quantitation of thiopental. Quantitation of thiopental in autopsy samples was carried out by HPLC using a model LC-5A instrument (Shimadzu Co., Kyoto, Japan) equipped with a CLC-ODS column (150 mm × 6 mm, Shimadzu) at 40°C. The mobile phase was methanol/0.067 *M* phosphate buffer, pH 6.8, (6:4 by volume) with a flow rate of 0.5 ml/min. Detection of thiopental was carried out by measuring the absorbance at 254 nm with a model C-R3A detector (Shimadzu). Aliquots of the sample (10 μl) were applied to the HPLC, and under these conditions thiopental was eluted at a retention time of 17.6 min.

In order to evaluate the recovery of thiopental from the autopsy samples, approx. 10 g of each minced tissue was prepared with the addition of 50 μg of authentic thiopental sodium, and the drug was quantified according to the method described above.

Results and discussion

In order to confirm the therapeutic dose administered to the patient, thiopental in blood and liver was identified using both GC/MS and TLC. On GC/MS analysis, the typical peak was detected with both a retention time and mass spectrum (or characteristic fragment ion) corresponding to those of the authentic thiopental. Each extract of blood and liver gave a spot, blue-purple in color after development, with a Rf value corresponding to that of the authentic substance in TLC. Furthermore, the corresponding peak was detected by HPLC in the extracts of blood and liver at the same retention time as that of the authentic substance.

Table 1. Concentrations of thiopental (mg/l or mg/kg) in postmortem biological samples. The values were corrected by reference to the recovery by drug extraction from each tissue

Sample	Concentration (mg/l or mg/kg)
Whole blood	0.60
Urine	N.D.[a]
Adrenal gland	1.71>[b]
Brain	11.90
Greater omentum	2.13
Heart	3.05
Kidney	6.59
Liver	6.08
Lung	1.87
Pancreas	2.21
Spleen	2.39
Thymus	7.66>[b]

[a] N.D., Not detected
[b] The control experiment for evaluation of drug recovery from the tissue could not be performed. Therefore, the values were not corrected

On the basis of these data, thiopental concentrations in the body fluids and tissues obtained at autopsy were determined by HPLC, and the results are shown in Table 1. Although the drug levels were very low in all samples, the highest levels were found in the brain and thymus tissues; no thiopental was detectable in the urine. From these results and the weights of each tissue, we were able to estimate the total amount of thiopental to be approx. 100 mg.

The therapeutic level of thiopental in plasma has been reported to be 4.2–134 μg/ml, and the dose of thiopental used for general anesthesia is about 50–100 mg [9]. The minimum lethal dose of thiopental is estimated to be 1 g [1]. Costantino et al. [1] reported that the average concentration of the drug in suicides in which thiopental administration was the sole cause of death was 273 mg/l blood, 318 mg/kg liver and 128 mg/kg kidney. Moreover, in one case reported by Fernando [10], the blood thiopental level in a case of suicide by infusion of the drug was 153 mg/l. On the other hand, blood concentrations responsible for deaths in a clinical setting, which occurred either during infusion of the drug or a few minutes after administration, were estimated to be 14.6 mg/l blood, 29.6 mg/kg liver and 10.4 mg/kg kidney [1]. The blood and tissue concentrations in the present case deviated markedly not only from the range of fatal thiopental concentrations, but were also below those of anaesthesia deaths shortly after administration as reported previously. In the light of the autopsy findings in the lung and thymus, it was estimated that the patient had been administered a therapeutic dose of thiopental and that death could have been due to anaphylactic shock. Therefore, we were able to rule out both malpractice and poisoning (overdose) in the present case.

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