DETERMINATION OF THIOPENTAL IN POSTMORTEM TISSUES AND BLOOD OF RATS BY GC/MS

[1] Fatma Ismail, [2] Ghada Fouad, [3] Yvette Issac*

This work focuses on toxicological issues associated with the analysis of postmortem specimens that are traditionally used and those not so commonly used; detailed analytical features and artifacts associated with the analysis of postmortem specimens and finally provides monograph on thiopental. Our aim was to focus on the relative advantages of specimens that can be collected and the factors that affect the drug concentration in different tissues by using GC/MS. Results in this study showed that brain, liver and kidney tissues are the tissues of choice for the postmortem toxicological analysis of thiopental followed by the femoral muscle tissue especially in the lower administrated dose.

Introduction

General anesthesia depresses the central nervous system (CNS) and causes loss of consciousness associated with an inability to perceive pain. An ideal anesthetic would produce unconsciousness analgesia, and muscle relaxation suitable for all surgical procedures and would be metabolically inert and rapidly eliminated. No anesthetic fulfils all there requirements in safe concentrations and it is customary to employ a number of drugs to achieve the required conditions, while minimizing the risk of toxicity [1].

Although all types of anesthesia involve some risk major side effects, complications occur; the role of forensic toxicologist to detect and determine the drugs used in the anesthesia and quantifies it for the malpractice claims cases [2].

Among anesthesia drugs thiopental was chosen for this study as an example of drugs used in general anesthesia. Thiopental is famously associated with number of anesthetic deaths. It is used in some places as truth serum to weaken the resolve of the subject and

* (1),(2) Forensic Toxicologist, Central Chemical Laboratories, Medico legal Administration, Ministry of Justice. (3) Prof. of Organic Chemistry, Faculty of Science, Banha University.

make them more compliant to pressure. In 2009, the state of Ohio USA became the first to use a single dose of sodium thiopental for its capital execution instead of the three drugs cocktail during recent executions.

During the past two decades, in addition to classical drugs such as morphine, cocaine and marijuana, chemically synthesized drugs, have become spread [3]. Although thiopental abuse is rare, it is abused by the specialists like the nurses and doctors. The need of applying sensitive, selective and reliable methods, applicable to both detection and determination of thiopental in solid tissues such as liver, brain, muscle, kidney, hair, spleen and biological fluids such as blood. A quantitative determination of thiopental by means of a gas chromatography equipped with mass spectrometer was attempted.

Materials AND Methods

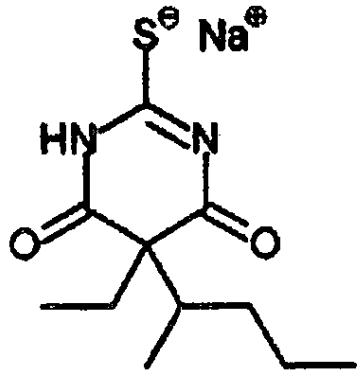
(1) Experimental Animals

Male albino rats were used in the present investigation. Their body weights ranged from 120-180 g. Animals was provided by Laboratory Animal House in The National Research Center. During the experimental periods, rats were maintained on standard pellets and allowed free access to water.

(2) **Drug**

Common name: Thiopental sodium.
Molecular Weight: 264.32 g/mol
Empirical formula: $C_{11}H_{17}N_2NaO_2S$
CAS Number: 71-73-8 (sodium salt) 76-75-5 (free acid)
Chemical name: (RS)- [5- ethyl- 4,6- dioxo- 5- (pentan-2-yl)-1,4,5,6-tetrahydropyrimidin-2-yl] sulfanide sodium.

Structure of thiopental:



Methods

1 - Dosage and Experimental Design

For estimation of the postmortem drug concentration in different tissues of rat, two doses were used for the drug according to the LD50 value of sodium thiopental (120 mg/kg) according to Material safety data sheet [4] as follow:

1- 1/10 LD_{50} dose of sodium thiopental (12mg/kg body weight).

2- 1/5 LD_{50} dose of sodium thiopental (24 mg/kg body weight).

Control animals (control group) were injected a physiological saline solution instead of the drug. Sodium thiopental was injected intraperitoneally to rats, which were sacrificed after three hours of administration. Animals were dissected as soon as possible and samples from blood, liver, kidney, brain, femoral muscle, spleen and hair were achieved.

2 - Tissue Extraction Method

Extraction of the drug, ammonium sulfate method was used according to Nickolls(1956) [5], for deproteinization to tissue samples followed by liquid-liquid extraction by dichloromethane.

3 - Hair Extraction Method

Hair is decontaminated and then cut into 3 mm segments. About 5-10mg of it is incubated over night in 0.01 N NaOH, then the homogenate is neutralized with HCl. The drug is extracted with dichloromethane samples, and sonicated for 1 hour at room temperature. After sonication, the samples were decanted, evaporated and analyzed by GC/MS[6].

(GC/MS) Conditions

The analysis were carried on Hewlett-Packard 6890 series gas chromatograph equipped with an autosampler and a 5973 series MSD in electron impact mod (EI) (70 eV) for determination of sodium thiopental. A 5µl aliquot of the sample was introduced in a splittless way onto a DB 17 column with a nominal length of 25 m, an internal diameter of 0.25 mm and a film thickness of 0.25 µm. A constant high purity helium flow of 1 ml/ min was applied through the column. The GC separation was obtained using a program with an initial oven temperature of 60 °C held for 1 min. and was increased at a rate of 15 °C/ min to a final temperature of 300 °C. The oven was held at a final temperature for an additional 5 min., the injector temperature was maintained at 250 °C. The mass source was held at 280°C. The mass selective (ms) quadrepole temperature was held at 100°c. The transfer line was held at 280 °C. The mass selective detection was operated in the scan monitoring mode[7].

Quantitative Analysis

A sample solution 100 µl were pipetted into vial containing 10µl internal standard (IS) in methanol, then the mixture was evaporated.

Calibration curves were prepared by the IS method; adding known concentrations of IS (Phenobarbital) in methanol and different concentrations of sodium thiopental in methanol to accurately weighed control rat specimen. The samples were sonicated for 1 hour, decanted and used for analysis. A five point standard curve was prepared by linear least- square regression analysis of the ratio of the peak area of the IS.

To determine the analytical recoveries, one set of drug – free samples was spiked with known amounts of sodium thiopental without IS. Another set of standards containing the same corresponding amount of sodium thiopental in methanol was also prepared. After extraction with dichloromethane, elutes in both sets were evaporated to dryness at room temperature. 50 ml of the IS (20ug/ml) was added to each extract. The analytes and the IS in the residue were then analysed by GC. The average recovery of sodium thiopental were determined by comparing the ratio of the peak area of sodium thiopental to the peak area of the IS from extracted specimens spiked with sodium thiopental and extracted methanol solution. The limit of quantification (LOQ) for sodium thiopental was established as the lowest concentration that the method can detect with a consistent response to the actual solution concentration [8], and the peak ratio of a test specimen is applied to the calibration curve to obtain its drug concentration.

Results and Discussion:

The isolation of the substances from postmortem matrices is in general more difficult than that from clinical specimens, primary owing to the range of specimens encountered and inferior quality of many specimens resulting from putrefaction. Isolation techniques in postmortem toxicological tend to favor liquid extraction[9].

Several investigators previously used different methods for the extraction of drugs in blood and tissues by using liquid-liquid extraction after deproteinization[10].

Wherever possible blood is the preferred specimen and allows concentration to be compared with clinical and pharmacokinetic data. However, in order to properly interpret blood concentration in a postmortem specimen it is preferable to quantify the substances in other tissue specimens [11].

Although, thiopental administration intraperitoenally with single two doses and animals sacrificed after three hours and showed nearly the same tissue organs descending order in both doses (1/5 and 1/10 LD_{50} (Table 1&2).

Table (3) brain, kidney and liver showed higher concentrations of thiopental than muscle, spleen; blood and hair with no dose dependency[12], see Figure (1&2).

It should be of no surprise that the uptake of drugs into tissues is not uniform. The distribution of drugs into tissues depends on various factors, e.g. blood flow to and within the tissues ionization characteristics of the drugs, affinity of the drug for the tissue and the degree of protein binding. While tissue concentrations depend on the relative affinity of the drug to blood, the time required to reach some forms of steady state may be quite long (many hours to days)[13]. Also, who reported that all drugs no matter how they are introduced, will be distributed throughout the body into its various tissues. At some point these substances are excreted through one or more processes.

Our study revealed that brain tissue has the highest thiopental concentration. This finding agreed with Yasuda et al.(1993)[14] who stated that, among ten tissues examined by HPLC the brain and thymus showed the highest level of the drug in an individual who had died due to intravenous injection of a clinical thiopental. Also, Leikin and Watson (2003)[15] stated that significant increases in thiopental and pentobarbital concentrations have been demonstrated in the first four hours postmortem.

The present study revealed that kidney tissue showed a relatively high degree of abundance of the studied drug, that is due to the specific structure of the kidney which favors the accumulation of chemicals in its tissue[16].

Moreover, Nguyen et al. (1996)[17] illustrated that thiopental is a drug of relatively low almost entirely metabolized by hepatic metabolism. Accordingly; detection of thiopental will depend on the activity of the hepatic drug metabolizing enzymes not on its poor water solubility.

The present work finding demonstrate that the liver has rather high concentration of thiopental, liver is a highly valuable specimen since many substances are present in higher concentrations in the liver and thus more easily detected than in femoral blood[18]. Also, this could be explained as the drug-metabolizing enzymes which are predominantly located in the liver, enabling efficient first-pass

metabolism of foreign entities and thus constituting an important factor in the bioavailability of drugs[19].

Thiopental concentration was rather medium in skeletal muscle as the present work revealed. Drummer (2004)[20] stated that that skeletal muscle has been used by toxicologists in special types of analysis. Muscle will often represent the greatest single mass of drug in a body and will therefore represent a greater body burden of drug than any other tissue mass. This applies particularly to drugs of abuse with high volumes of distribution. However, muscle is a difficult tissue to work with it and care is required to ensure complete drug extraction.

In addition, the present work demonstrates that spleen and blood had a rather low thiopental concentration; this may be explained by the rout of administration factor[21]. Martindale (2005)[22] stated that thiopental rapidly disappears from plasma because of its metabolism and secondary distribution in poorly vascularized tissues. Hair sample has the lowest thiopental and its major metabolite pentobarbital concentrations. Bomba et al.,(2006)[23] mentioned that it could be stated that hair is an oriented polymeric network, partially crystalline containing different functional chemical groups (e.g. acidic, basic etc.) which have the potential to bind small molecules. The capillary blood supply around the follicle provides nutrients and delivers any extraneous substances that might be in the blood stream such as trace metals, drug etc.

Our study showed that brain, liver and kidney tissues are the tissues of choice for the postmortem toxicological analysis of thiopental followed by femoral muscle tissue especially in the lower administrated dose.

Table (1)
Thiopental concentrations (µg/ g tissue) in samples obtained from rats administrated a dose of 12 mg/kg b.wt. sacrificed after 3 hours of treatment.

Animal no	Brain	Kidney	Liver	Femoral muscle	Spleen	Blood	Hair
1	5.23	3.11	2.93	2.43	2.07	0.31	0.026
2	5.58	3.40	3.21	2.91	2.21	0.46	0.029
3	3.51	1.93	1.76	0.86	0.69	0.08	-
4	4.09	2.22	1.98	0.95	0.77	0.16	0.009
5	5.09	2.49	2.27	1.05	0.92	0.19	O.010
Mean	4.70	2.63	2.43	1. 64	1.33	O.24	0.015
Standard deviation	± 0.865	± 0.612	± 0.619	± 0.957	± 0.743	± 0.148	± 0.010

Table (2)
Thiopental concentrations (µg /g tissue) in samples obtained from rat administered a dose of 24mg/kg b.wt., sacrificed after 3 hours of treatment.

Animal no.	Brain	Kidney	Liver	Muscle	Spleen	Blood	Hair
1	4.60	3.57	4.13	1.76	2.01	0.35	0.009
2	6.61	6.90	7.62	3.01	3.43	0.73	0.028
3	6.34	4.98	5.47	1.97	2.222	0.44	0.011
4	7.11	5.73	6.18	2.64	2.79	0.61	0.019
5	4.49	2.82	3.70	1.47	1.8	0.27	0.003
Mean	5.83	4.80	5.42	2.17	2.45	0.48	0.014
Standard deviation	± 1.20	± 1.63	±1.58	± 0.637	± 0.660	± 0.188	± 0.009

Table (3)
Degree of thiopental abundance in different tissues of rats after 3 hours of treatment

Dose	Descending order of thiopental concentration						
	1	2	3	4	5	6	7
12 mg / Kg b.wt.	Brain	Kidney	Liver	Femoral muscle	Spleen	Blood	Hair
24 mg / Kg b.wt.	Brain	Liver	Kidney	Spleen	Femoral muscle	Blood	Hair

Fig. (1)
Descending order of thiopental concentration at dose of 12mg/kg body weight.

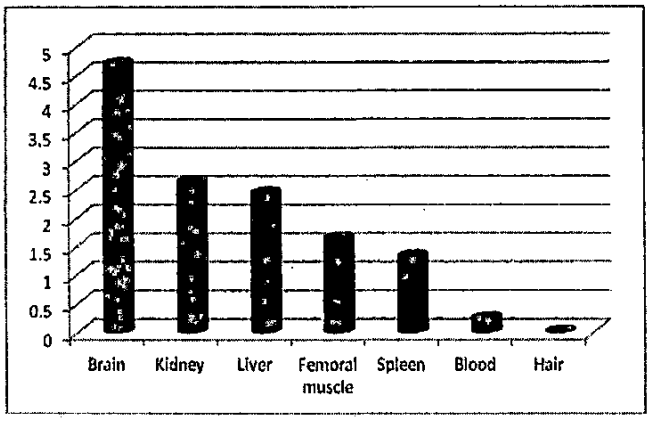
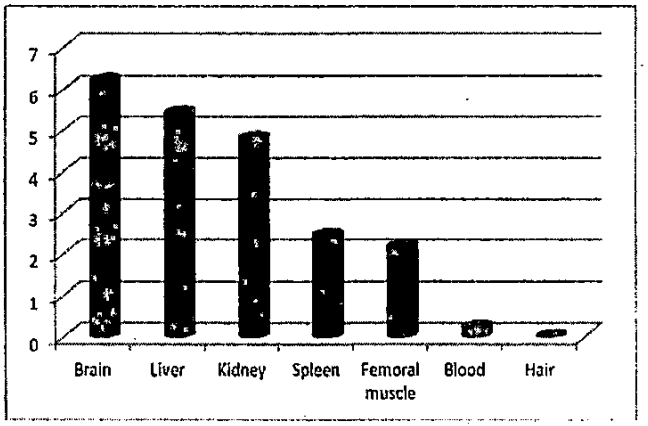


Fig. (2)
Descending order of thiopental concentration at dose of 24mg/kg body weight.



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تحديد كمية الثيوبنتال فى دم وأنسجة الجرذان بعد الوفاة باستخدام جهاز الكروماتوجرافيا الغازية/ مطياف الكتلة (GC/BS)

فاطمة إسماعيل، غادة فؤاد، وإيفيت إسحاق

يناقش هذا البحث المسائل المتعلقة بالسموم، والمصاحبة لتحليل العينات التشريحية المعتاد استخدامها بعد الوفاة، وتلك التى لا تستخدم بصورة شائعة. كما يناقش أيضًا الخصائص التحليلية المفصلة والعوامل المصاحبة لتحليل هذه العينات، وأخيرًا، يقدم دراسة عن عقار الثيوبنتال،

ويهدف البحث إلى التركيز على المميزات المرتبطة بالعينات التى يمكن تجميعها، والعوامل التى تؤثر على تركيز العقار فى الأنسجة المختلفة باستخدام جهاز الكروماتوجرافيا الغازية/ مطياف الكتلة.

وأظهرت النتائج أن أنسجة المخ والكبد، والكلى هى الأنسجة المختارة لتحليل السمية فى العينات التشريحية بعد الوفاة لعقار الثيوبنتال، ثم يليها نسيج عضلة الفخذ، وخاصة فى الجرعات الصغيرة.