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Release of Tissue Paraquat into Formalin Solution During Fixation

REFERENCE: Kuo, T.-L., **"Release of Tissue Paraquat into Formalin Solution During Fixation,"** *Journal of Forensic Sciences*, JFSCA, Vol. 35, No. 3, May 1990, pp. 668–674.

ABSTRACT: Formalin-fixed tissues and formalin solutions are among the most frequently found materials in pathology and forensic science laboratories. However, these materials are seldom used for the identification of poisons for forensic toxicology purposes. In this study, the possibility that paraquat may be released from formalin-fixed tissues during the fixation process was investigated. However, because of the interference of formaldehyde on the reduction of paraquat with dithionite reagent, paraquat in formalin solutions was treated with ion-pair column chromatography and then determined by measuring the derivative spectrum of reduced paraquat. The results show that the interference of formalin on paraquat determination has been eliminated by the proposed method. Furthermore, a study on the formalin solutions of fixed organs in cases with suspected paraquat intoxication revealed that portions of tissue paraquat had been released into formalin during the fixation process. Moreover, the paraquat levels in formalin increased with increased storage time. Therefore, these data suggest that the combined concentrations of paraquat in the formalin-fixed tissues and formalin solutions might reflect more reliably the total paraquat in the postmortem tissues. This investigation could be of value to the forensic toxicologist, especially in cases in which no fresh tissue samples are available for analysis.

KEYWORDS: toxicology, paraquat, tissues (biology), formalin-fixed tissue, formalin

Among the herbicides, paraquat is the agent most frequently involved in episodes of acute intoxication by accident or intention. The diagnosis and treatment of such intoxication is usually established on the basis of the history taken, the symptoms appearing, and the confirmation of paraquat in blood or urine samples [*1–7*]. However, in cases of poisoning by ingesting small but potentially fatal amounts of paraquat, this information might be not available [*6–8*]. Therefore, the diagnosis of paraquat intoxication might sometimes be overlooked by physicians and even by pathologists. Consequently, the autopsy specimens would be preserved conventionally in formalin solution.

On the other hand, it is not uncommon in medicolegal practice for drug intoxication to be suspected some time after autopsy. In this situation, the autopsy specimens might already have been preserved in formalin. When fresh tissues are not available, the formalin-fixed tissues and the preserved formalin are all that remains to be analyzed. However, because of its reactivity, formaldehyde can oxidize, condense, or detroy many drugs [*9*]. Furthermore, the recoveries of drugs from formalin-fixed tissues are very low. There-

This study was supported by a grant from the National Science Council of the Republic of China (NSC77-0412-B002-40). Received for publication 24 April 1989; accepted for publication 19 June 1989.

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fore, the identification of poisons or drugs from formalin-fixed tissues, formalin solutions, or both, is one of the most formidable tasks a forensic toxicologist might face.

A method for the determination of paraquat in formalin-fixed tissues has already been developed at the author's laboratory [*10*]. It certainly will provide valuable information for medicolegal practice. However, during the course of study, it was observed that paraquat levels in formalin-fixed tissues were lower than those in fresh tissues. It was thought that tissue paraquat might be released into formalin solution during the fixation process. If this were the case, the identification of paraquat in formalin solution might provide additional evidence for forensic toxicology purposes. However, the interpretation of analytical results from formalin-fixed tissues might require further studies.

Up to the present, there has been no report concerning the identification of paraquat in formalin. The aim of this study was to find out whether and to what extent paraquat is released into formalin solutions from tissues. Therefore, an ion-pair column chromatographic method [*11*] was used to quantify paraquat in formalin. In addition, some specimens from cases of paraquat intoxication were used for this investigation, which included determinations of paraquat in fresh, formalin-fixed tissues and formalin solutions.

Materials and Methods

Instrumentation

A Model 240 (Shimazu, Kyoto, Japan) double-beam ultraviolet-visible recording spectrophotometer equipped with a Model OP 1-2 option program for zero-order and second-derivative measurements was used. The second-derivative spectra were obtained with a 2-nm bandwidth, scan speed of 180 nm/min, and a 0.2 absorbance full scale (a.f.s.) for 0.1 to 1.0 mg/L of paraquat or 0.02 a.f.s. for 0.01 to 0.1 mg/L.

Reagents

Amberite XAD-2 resin, 20-50 mesh (Sigma Chemical Co., St. Louis, Missouri), was purified according to the method of Stajic et al. [*12*] and stored refrigerated. Standard solutions of paraquat (calculated as the ion) were prepared by dissolving appropriate amounts of paraquat (methyl viologen, Sigma), dried at 105°C to a constant weight before use, in distilled water and 4% (w/v) formaldehyde (Merck, Darmstadt, Federal Republic of Germany), respectively. The solutions were kept in plastic bottles at 4°C. Sodium dodecyl sulfate solution was prepared by dissolving 2.0 g of sodium dodecyl sulfate (SDS) (Sigma) in 100 mL of distilled water. Solvent mixtures were prepared by mixing equal volumes of methyl isobutyl ketone and isobutanol (Merck), then adding 2 g of SDS per litre, and saturating the solvent mixtures with distilled water (about 75 mL/L). Alkaline sodium dithionite solution was freshly prepared by dissolving 0.1 g of sodium dithionite (Merck) in 10 mL of 1 mol/L sodium hydroxide (NaOH) (Merck).

Procedure

An appropriate amount (1 to 10 mL) of formalin solution obtained from fixed tissues was mixed with one tenth its volume of 20 g/L of SDS in a test tube and this solution was passed through a syringe packed with 2 mL of purified XAD-2 resin at a flow rate about 1 mL/min. The column was washed with 20 mL of distilled water. Paraquat was eluted, extracted, and reduced by using solvent mixtures, sodium chloride (NaCl) solution, and dithionite reagent, respectively, as in a method previously reported by the author [*13*]. The zero-order or second-derivative spectrum of reduced paraquat was recorded from 450 to 380 nm in a 1-cm quartz semimicrocuvete against a freshly prepared

reagent blank [*14*]. Standard curves were prepared by treating standard samples in the same way as the test samples and calculating the sample concentrations by reference to these curves.

The paraquat concentrations in fresh tissues and formalin-fixed tissues were determined according to methods reported previously [*10,14*].

Case Histories

Case 1

A 23-year-old male with abdominal pain was admitted to a regional hospital. During the next few days, he developed oliguria, dyspnea, and jaundice. No information concerning drug intoxication had been obtained and he was treated symptomatically. The patient expired nine days after admission. All the organs were preserved in formalin after autopsy. Two days later, it was suspected that this was an intoxication case, and some formalin-fixed organs, together with the formalin solution, were sent to the author's laboratory.

Case 2

A woman confessed that she had mixed paraquat in food to murder her husband. One month later, toxicological confirmation was requested by the court, but only formalin-fixed organs and the fixed formalin solution were available.

Case 3

In a suicide attempt, a male drank an unknown quantity of paraquat. Approximately 20 h after ingestion of this substance, the patient was taken to a hospital. At admission, the plasma and urine paraquat concentrations were 1.45 and 52.85 mg/L, respectively. The patient died on the eighth day after ingestion of the paraquat. Portions of fresh organs were homogenized for quantification of paraquat. The remaining tissue samples were preserved with formalin solution. An appropriate amount (1 to 10 mL) of the formalin solution was removed after various storage times to determine the paraquat concentrations. Two months after preservation, the paraquat concentrations of the fixed organs were determined.

Results

Figure 1*a* shows the second-derivative spectrum of paraquat (0.05 mg/L) in formalin determined directly by the dithionite reagent. The absorption peak at 392 nm was completely masked. Figure 1*b* shows the result for the same formalin sample by the proposed method using ion-pair column chromatographic pretreatment [*11*]; the absorption peak at 392 nm was clearly observed. It revealed that the interference of formalin on the quantification of paraquat could be eliminated by using the proposed method.

Table 1 shows that the mean recoveries in water and formalin solution of paraquat spike in the range of 0.02 to 2.0 mg/L were 86.1 and 85.6%, respectively. It demonstrates that the recoveries of paraquat from formalin and water were not significantly different from those obtained by the proposed method.

The paraquat concentrations of formalin-fixed tissues and their preserved formalin solutions in Cases 1 and 2 are shown in Table 2. The specimens of Cases 1 and 2 were preserved in formalin solutions for 2 and 30 days, respectively; however, paraquat could be detected in both the formalin-fixed tissues and formalin solutions.

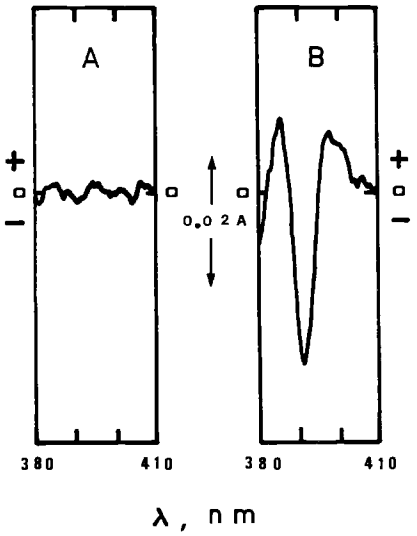

FIG. 1—*Second derivative spectra of paraquat in formalin solution: paraquat (0.05 mg/L) reduced* (a) *with dithionite directly;* (b) *by the proposed method.*

Table 3 reveals that the paraquat levels of formalin-fixed tissues in Case 3 were lower than those of fresh tissues. The formalin fixation period was two months. It is thought that portions of paraquat might have been released from tissues into formalin solution.

Figure 2 indicates the time course of paraquat concentrations in the formalin solutions of Case 3. Formalin samples were determined from one day to two months after the

TABLE 1—*Recovery of paraquat from formalin solution.*

Paraquat Concentration, mg/L	Analytical Recovery, %[a]	
	In Water	In Formalin
0.02	86.2 ± 2.0	85.7 ± 2.1
0.05	85.9 ± 1.9	85.5 ± 2.0
0.50	86.1 ± 1.6	85.3 ± 1.5
2.00	86.3 ± 1.8	85.8 ± 1.4
Overall	86.1 ± 1.8	85.6 ± 1.8

[a]Each value is the mean of six determinations with standard deviation.

TABLE 2—*Paraquat concentrations in the formalin solutions and formalin-fixed organs of suspected poison cases.*

Case	Preserved Time, day	Paraquat Concentrations, mg/kg			
		Liver	Kidney	Lung	Formalin
1	2	0.65	1.33	0.80	0.75
2	30	0.88	1.01	...[a]	0.65

[a]Not determined.

TABLE 3—*Paraquat concentrations in tissues of Case 3.*

Tissues	Paraquat Level, mg/kg	
	Fresh	Formalin-Fixed[a]
Liver	0.54	0.29
Kidney	1.18	0.51
Lung	0.35	0.19

[a]The fixation time was two months.

fixation. The figure shows that paraquat concentrations in the formalin solutions increased with increasing fixation periods. However, a rapid increase was found in the first four days after fixation.

Discussion

Formalin solutions, as well as formalin-fixed tissues, are among the most frequently found specimens in forensic science and pathology laboratories. Therefore, identification of poisons from these materials is of considerable importance to forensic science. Although several methods have been developed for determining paraquat in biological specimens [*15–26*], little attention has been paid to analysis of paraquat in formalin because the interference of formaldehyde limits the availability of this material.

Since paraquat is a water-soluble chemical, it is reasonable to postulate that it might be released from tissues into formalin solution during the fixation process. This study provides evidence that a portion of tissue paraquat is, in fact, released into formalin and that, therefore, the preserved formalin solution also could be used for identifying paraquat. Moreover, the determination of paraquat in formalin solution by the proposed method is much simpler than determination in formalin-fixed tissues [*10*].

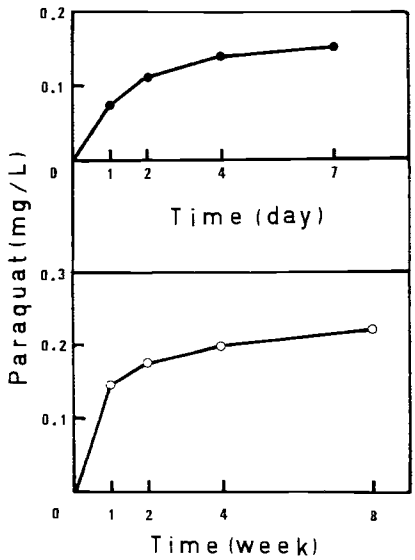

FIG. 2—*Release of tissue paraquat into formalin solution in Case 3.*

On the other hand, the forensic toxicologist is often requested to estimate the amount of a drug ingested by a decedent or to predict the elapsed time between drug administration and death on the basis of drug concentrations in postmortem blood or tissue samples, or both. However, in medicolegal practice, the samples of formalin-fixed tissues, together with the formalin, may arrive after various storage times. This investigation demonstrated that paraquat levels in the formalin solution increased with increasing storage time. This means that paraquat levels in the formalin-fixed tissues will decrease with increasing fixation periods. Therefore, the forensic science investigator must interpret paraquat levels from formalin-fixed tissues or formalin solutions cautiously. The author suggests that the combined paraquat concentrations of both the formalin-fixed tissues and formalin solutions will accurately reveal the total paraquat in postmortem tissues. The results of this investigation might provide important information for the forensic toxicologist to use in interpreting the analytical results from formalin-fixed specimens.

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