Hiroshima Journal of Medical Sciences
Vol. 32, No. 1, March, 1983
HIJM 32-16

A Case of Fatal Paraquat Poisoning and a Rapid Method for Analysis of Paraquat in Biological Material*)

Junko YAMAUCHI[1], Mikio YASHIKI[1], Itsuko UNE[1], Tohru KOJIMA[1], Hisao ITO[2], Shoji KAWACHI[3] and Minako OHTANI[4]

1) *Department of Legal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan*
2) *The 1st Department of Pathology, Hiroshima University School of Medicine, Hiroshima 734, Japan*
3) *Department of Anesthesiology, Hiroshima University School of Medicine, Hiroshima 734, Japan*
4) *Department of Acute Medical Service, Hiroshima University Hospital, Hiroshima 734, Japan*

(Received December 25, 1982)

Key words: *Paraquat, Poisoning, Distribution, Analysis*

ABSTRACT

In a case of fatal paraquat poisoning, the distribution of paraquat was determined by a) the column chromatographic method developed by Tompsett, b) the ion-pairing method, c) the deproteinization method both developed by Jarvie et al, and d) the precipitation method developed by the authors. The precipitation method relies on the use of a precipitate of paraquat with Reinecke's salt to extract paraquat from biological material, and the dithionite color reaction to detect paraquat. The paraquat concentrations obtained by the precipitation method approximated to those obtained by the column chromatographic method. The results indicate that the use of the precipitation method followed by the dithionite color reaction is suitable not only for forensic practice but also emergency analysis.

Since paraquat was not detected in the gastric contents collected at autopsy, and paraquat concentrations in the organs were much higher than the concentration in the heart blood, it seems that the hemodialysis and hemoperfusion were begun too late.

INTRODUCTION

Paraquat, the best known bipyridyl herbicide widely used in agriculture, has earned an undesirable reputation as a potent human poison. Many fatal cases have been reported, and several methods for the analysis of paraquat in biological material have been developed. However, these methods are complex, or inapplicable to the analysis of paraquat in whole blood and organs. A simple, rapid and reliable method was therefore developed for the quantitative analysis of paraquat in whole blood and organs, using Reinecke's salt precipitation reaction to extract paraquat and dithionite colorimetry to detect paraquat.

In a case of fatal paraquat poisoning, paraquat intracorporeal distribution was determined by a) the column chromatographic method established by Tompsett[1,8], b) the ion-pairing method[4,7], c) the deproteinization method[3,5] both developed by Jarvie et al, and d) the new method using Reinecke's salt precipitation reaction. The paraquat concentrations obtained by the new method approximated to those obtained by the column chromatographic method. The results indicate that the new method using Reinecke's salt is suitable not only for forensic practice but also emergency analysis.

CASE REPORT

A 38-year-old man ingested about 500 ml of Gramoxone®, containing 24% paraquat, at 7 : 30 a. m. on November 5, 1981 with the intention of committing suicide. At 10 a. m. the patient was taken to a general practitioner, at

*) 山内淳子, 屋敷幹雄, 宇根伊津子, 小嶋 亨, 井藤久雄, 河内正治, 大谷美奈子：パラコート中毒死の1例と生体試料中パラコートの迅速分析法

which time he was fully conscious. Gastric lavage was carried out in order to prevent further absorption of paraquat, although he had vomited the greater part of the ingested Gramoxone® immediately after ingestion. Soon after the gastric lavage, hypotension and cyanosis appeared. Anti-shock therapy was therefore begun. Urine volume, however, decreased gradually in spite of the forced diuresis. At 6 p. m. he was sent to Hiroshima University Hospital for treatment of acute renal failure. He did not respond to any kind of diuretic, and metabolic acidosis progressed. Since respiration became irregular and shallow tachypnea appeared, artificial ventilation was started at 9 p.m. Systolic blood pressure was less than 50 mmHg, and the patient showed no response to catecholamine. To protect the lungs and kidneys, hemodialysis and hemoperfusion were started at 10 p. m. to remove paraquat, urea and potassium. The patient died at 5 : 10 a. m. on November 6, however, having shown no improvement in his general condition.

PATHOLOGICAL FINDINGS

A pathological autopsy took place 8 hours after death. The left and right lungs showed acute congestion and edema. Tracheal and broncheal edema with hemorrhagic inflammation, and acute hemorrhagic and necrotizing inflammation of the esophagus and stomach were observed. The heart showed hypertrophy of the left ventricular muscle, and petechiae were observed in the pericardium.

Microscopically, centrilobular necrosis of hepatic cells and hyalinous necrosis of renal proximal tubular epithelial cells were found. In the adrenal cortex, diffuse necrotic change was observed.

ANALYSIS

1. Material

Sample: 1) The patient's blood entering and leaving hemoperfusion and dialysis was collected during therapy in a glass tube containing heparin as an anticoagulant, and the plasma was obtained after separation. 2) Intracardiac blood, gastric contents, liver, lung and kidney were collected at autopsy.

Control: Paraquat-free serum was obtained from a healthy living man, and blood and organs were collected from a cadaver at autopsy.

2. Chemicals

Paraquat dichloride: 99% (ICI Japan LTD).

Ion exchange resin column: 3 g of Dowex AG 50W (50–100 mesh, hydrogen type, Bio-Rad Laboratories) was immersed in 8N HCl, and washed with water until the eluate became neutral. The resin was packed into a 30×0.7 cm (i. d.) glass tube.

Water: Ion exchanged water.

Other chemicals: Reagent grade.

3. Analytical method

Spectrophotometry: A Shimadzu UV-200S with a matched pair of 1. 0-cm standard glass cuvettes was used.

Gas chromatography: The instrument used was a Shimadzu GC-3BF with a hydrogen flame ionization detector and a Shimadzu C-RIA for data processing. The column was a $1.7\ m \times 0.3$ cm (i. d.) glass tube packed with 2% w/w Polyethylene Glycol 20M and 5% w/w KOH on Chromosorb G(60–80 mesh). The temperature of the column was set at 180°C, and nitrogen with a flow rate of 73 ml/min was used as a carrier gas.

Gas chromatography-mass spectrometry: The instrument used was a Shimadzu GCMS 6020 with a $1\ m \times 0.26$ cm (i. d.) glass tube packed with 3% w/w OV-17 on Chromosorb G(60–80 mesh). The temperature of the injection port, column, separator and ion source was set at 200, 170, 250 and 250°C respectively. Helium with a flow rate of 20 ml/min was used as a carrier gas. The accelerating voltage was 3. 5 kV, and the ionization voltage of electron impact ionization was set at 70 eV.

4. Extraction and purification method

a) Column chromatographic method developed by Tompsett[8]

Two g of the specimen was homogenized with 4 ml of 20% trichloroacetic acid solution, and the homogenate was centrifuged at 10, 000 rpm for 20 min. The supernatant and 10 ml of 10 N HCl were poured into a 100-ml volumetric flask, and diluted with water to 100 ml. The diluted sample solution was passed through the ion exchange resin column, and paraquat absorbed by the column was eluted with 50 ml of 2. 5 N HCl and 100 ml of 5 N HCl, after washing with 100 ml of 1 N HCl and 50 ml of 2. 5 N HCl solution. The eluate collected in a glass dish was evaporated to dryness in a water bath. The residue was dissolved in 3 ml

of water, poured into a test tube, and 1.5 ml of 1 N NaOH solution containing 1% sodium dithionite was added. The absorbance at 600 nm was determined by spectrophotometry, and the paraquat concentration was obtained by plotting the absorbance against the calibration curve, which had been obtained by analyzing standard paraquat solution.

b) Ion-pairing method developed by Jarvie et al.[4]

The supernatant of the solid sample was prepared by the same procedure described in the column chromatographic method above. Two ml of the supernatant or serum was poured into a 50-ml glass-stoppered tube, 10 ml of methyl isobutyl ketone and isobutanol mixture (50 : 50, v/v) containing 0.5% sodium dodecyl sulfate was added, and then centrifuged at 3,000 rpm for 5 min, after 5 minutes' shaking. Eight ml of organic layer was poured into a 15-ml glass-stoppered tube, 0.7 ml of 2.5 N NaCl solution was added, and then centrifuged at 3,000 rpm for 5 min, after 5 minutes' shaking. The aqueous layer was transferred into a test tube, and then 2.8 ml of 2.5 N NaCl solution and 0.5 ml of 0.3 N NaOH solution containing 3% sodium dithionite were added. The absorbance at 603 nm and the difference between the absorbance at 397 nm and at 460 nm were determined by spectrophotometry. The paraquat concentration was obtained by plotting the absorbance against each calibration curve, which had been obtained by analyzing standard paraquat solution.

c) Deproteinization method developed by Jarvie et al.[5]

Two g of the solid sample was homogenized with 4 ml of water, and the homogenate was centrifuged at 10,000 rpm for 20 minutes. 1.5 ml of the supernatant or serum was poured into a 15-ml glass-stoppered tube, 1.5 ml of 10% sulfosalicylic acid solution was added, and then centrifuged at 3,000 rpm for 10 min, after mixing with a Vortex Mixer®. The supernatant (2.4 ml) was poured into a test tube, 0.6 ml of 5 N NaOH containing 2% sodium dithionite was added, and then mixed thoroughly. Absorbance at 396 nm was determined by spectrophotometry, and paraquat concentration was obtained by plotting the absorbance against the calibration curve, which had been obtained by analyzing standard paraquat solution.

d) Precipitation method developed by the authors

The supernatant of the sample was obtained in the same manner described in the column chromatographic method above. 2 ml of the supernatant was poured into a 15-ml glass-stoppered tube, 2 ml of 0.7% Reinecke's salt solution was added and then centrifuged at 3,000 rpm for 5 min, after one minute's shaking. After the supernatant was removed, 3 ml of 10 N HCl was added, and after mixing thoroughly, allowed to stand at room temperature for one hour. After centrifuging at 3,000 rpm for 5 min, the supernatant was evaporated to dryness on a hot plate. The residue dissolved in 3 ml of water was poured into a test tube, and 1.5 ml of 1 N NaOH containing 1% sodium dithionite was added. The absorbance at 600 nm was determined by spectrophotometry, and the paraquat concentration was obtained by the same procedure described in the column chromatographic method.

5. Preparation for gas chromatographic method[2,6,9]

Two ml of the sample solution containing purified paraquat was poured into a 25-ml glass-stoppered tube, 2 ml of 0.02 N nickel chloride solution and 0.3 ml of 2.6 N sodium borohydride solution were added, and after mixing thoroughly warmed at 50°C for 30 min. After cooling, reduced paraquat was extracted twice using 6 ml of diethyl ether. The ether layer was dried with sodium sulfate and then evaporated to dryness. The residue was dissolved in 100 μl of ethyl acetate containing 0.02% p-anicidine as an internal standard. One μl of the solution was analyzed by gas chromatography and gas chromatography-mass spectrometry.

6. Standard sample

200 μg of paraquat was added to each 1 g of water, control serum, blood or liver, and was analyzed by the column chromatographic method, the ion-pairing method, the deproteinization method and the precipitation method.

RESULTS

1. Recoveries and deviations

Analysis of the standard sample by each of the four methods, i.e. the column chromatographic method, the ion-pairing method, the

Table 1. Comparison of recoveries and deviations obtained by the four methods

Method	Recovery (X, %)			Deviation (S/X, %)		
	Water	Blood	Liver	Water	Blood	Liver
Column chromatography	81.2	93.1	76.9	6.09	2.84	3.61
Ion-pairing method a)	96.1	96.6	104.5	4.94	3.22	1.52
Ion-pairing method b)	96.6	93.2	100.0	2.60	7.24	2.29
Deproteinization method		83.5*			2.51*	
Precipitation method	99.4	102.8	110.5	5.64	3.10	2.78

X : Average. S : Standard deviation. n=5.
a) : Absorbance at 600 nm was used.
b) : Difference in absorbances at 397 nm and 460 nm was used.
*Serum.

deproteinization method and the precipitation method, yielded recoveries and deviations as shown in Table 1. The ion-pairing method and the precipitation method yielded good recoveries and precision.

2. Analysis of patient's sample

1) Qualitative analysis

Paraquat in the sample was extracted by the column chromatographic method, and analyzed by gas chromatography and gas chromatography-mass spectrometry. The gas chromatogram obtained by analysis of the blood sample is shown in Fig. 1. There are two big peaks corresponding to the reduced paraquat and the internal standard (p-anicidine) respectively. The mass spectrum of the peak corresponding to reduced paraquat is shown in Fig. 2. The mass spectrum is quite similar to that obtained by analyzing standard paraquat solution.

2) Quantitative analysis

Paraquat in the patient's sample was analyzed by the four methods described above. The results obtained are shown in Table 2. Paraquat concentrations obtained by the precipitation method approximated to those obtained by the column chromatographic method. The deproteinization method was inapplicable to whole blood, and the paraquat blood concentration obtained by the ion-pairing method was much higher than that obtained by the column chromatographic method. In organs, the deproteinization method and the ion-pairing method yielded paraquat concentrations considerably lower than those obtained by the column chromatographic method.

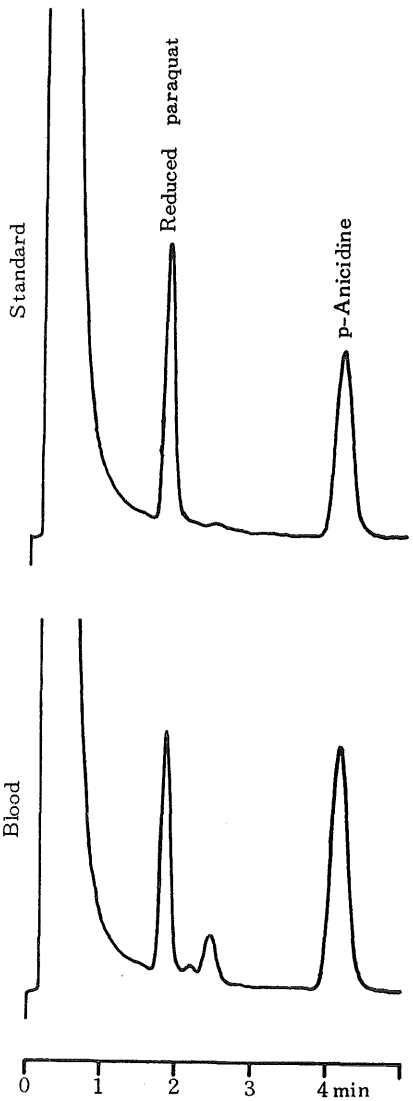

Fig. 1. Gas chromatograms.
p-Anicidine: Internal standard.

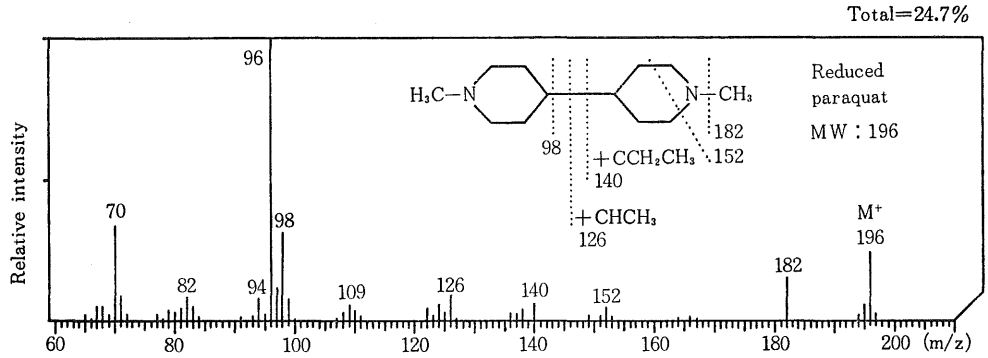

Fig. 2. Mass spectrum of the peak corresponding to reduced paraquat.

Table 2. Comparison of paraquat concentrations in the patient's sample obtained by the four methods

(unit: μg/g)

Sample	Column chromatography	Ion-pairing method a)	Ion-pairing method b)	Deproteinization method	Precipitation method
Serum 1				139.3	
Serum 2				(−)	
Blood	107.0	182.7	191.2		112.8
Lung	159.0	135.0	138.4	124.7	166.6
Kidney	318.2	206.8	203.3	255.0	335.2
Liver	380.0	337.7	320.9	322.2	401.7
Gastric contents	(−)	(−)	(−)	(−)	(−)

a) : Absorbance at 600 nm was used.
b) : Difference in absorbances at 397 nm and 460 nm was used.
Serum 1 : Serum of blood entering hemoperfusion and dialysis.
Serum 2 : Serum of blood leaving hemoperfusion and dialysis.
(−) : Not detected.

DISCUSSION

All pathological findings in this case, except for the left myocardial hypertrophy, seem to be a result of the direct effects of paraquat. Pulmonary fibrosis, which is peculiar to paraquat poisoning, was not observed, since the patient died 22 hours after ingestion of paraquat.

When the tree methods of colorimetry, ultraviolet spectrophotometry and gas chromatography for the quantitative determination of paraquat in biological material were compared, colorimetry yielded the best results. Ultraviolet spectrophotometry was useful for the determination of paraquat in a water sample. It was inapplicable, however, for the analysis of paraquat in a biological sample, since the absorption spectrum was disturbed by endogenous contaminations. Gas chromatography was inadequate for quantitative analysis, since the reduction of paraquat was incomplete, and the peak of reduced paraquat was not reproducible. Therefore, colorimetry proved to be the most accurate method for the detection of paraquat, after extraction from biological material by Reinecke's salt precipitation method.

Gas chromatography and gas chromatography-mass spectrometry were used for the qualitative determination of paraquat extracted from the patient's sample.

The results of the analysis of paraquat concentrations in the patient's sample obtained by the four methods indicate that the deproteinization method and the ion-pairing method are not suitable for analysis of paraquat in whole blood and organs. The column chromatographic method is not applicable for emergency analysis, since it is time-consuming and complicated, and requires the evaporation of a large amount of conc. HCl. The precipitation method using

Reinecke's salt seems to be suitable for emergency analysis and forensic practice, because of its simplicity, rapidity and reliability in the analysis of paraquat in whole blood and organs. The absence of paraquat in the gastric contents indicates that the patient vomited the greater part of the ingested Gramoxone® and that gastric lavage was complete. Since the concentration of paraquat in blood leaving hemoperfusion was less than the detection limit (0.5 μg/g), XAD-2 resin used in hemoperfusion absorbed not less than 99.5% of the patient's plasma paraquat. At death, however, the concentrations in organs were much higher than the concentration in blood. It seems therefore that hemoperfusion was too late to enhance the elimination of paraquat before it had caused harm.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Shoji KAWASE for his valuable advice about Reinecke's salt precipitation reaction of paraquat.

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