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Potential for Error when Assessing Blood Cyanide Concentrations in Fire Victims*

REFERENCE: Moriya F, Hashimoto Y. Potential for error when assessing blood cyanide concentrations in fire victims. J Forensic Sci 2001;46(6):1421–1425.

ABSTRACT: The present study explores toxicologic significance of blood cyanide concentrations in fire victims. Headspace gas chromatography was used for cyanide detection. Analysis of blood samples from ten fire victims (postmortem interval = 8 h to 3 to 5 d) detected zero to 11.9 mg/L of cyanide and a large difference in cyanide concentrations among victims. Carboxyhemoglobin (COHb) saturation was in the range of 24.9 to 84.2%. To examine the effects of methemoglobinemia and postmortem interval on blood cyanide concentrations in fire victims, an experiment was carried out using rabbits as the animal model. The rabbits were sacrificed by intramuscular injection of 1 mL/kg 2% potassium cyanide 5 min after intravenous injection of 0.33 mL/kg of 3% sodium nitrite (Group A, $n = 3$) or physiological saline (Group B, $n = 6$). Average methemoglobin contents immediately before potassium cyanide administration were 6.9 and 0.8% in Groups A and B, respectively. Average cyanide concentrations in cardiac blood at the time of death were 47.4 and 3.56 mg/L, respectively. When blood-containing hearts of the rabbits ($n = 3$ for Group B) were left at 46°C for the first 1 h, at 20 to 25°C for the next 23 h and then at 4°C for 48 h, approximately 85 and 46% of the original amounts of blood cyanide disappeared within 24 h in Groups A and B, respectively. After the 72-h storage period, 37 and 10%, respectively, of the original amounts of cyanide remained in the blood. When the other three hearts in Group B were left at 20 to 25°C for the last 48 h without refrigeration, cyanide had disappeared almost completely by the end of the experiment. The present results and those published in the literature demonstrate that the toxic effects of cyanide on fire victims should not be evaluated based solely on the concentration in blood.

KEYWORDS: forensic science, forensic toxicology, cyanide, blood cyanide concentrations, fire victims, methemoglobin, carboxyhemoglobin, gas chromatography, rabbits, animal model

A significant quantity of hydrogen cyanide in addition to carbon monoxide is known to be produced during house fires, with pyrolysis or combustion of nitrogen-containing materials such as newly developed building materials, polyurethane, and polyacryronitrile, responsible for the hydrogen cyanide production (1–3). The concentrations of cyanide in the blood of fire victims occasionally exceed those of persons who die after ingestion of cyanide compounds or inhalation of hydrogen cyanide (4–6). In fire victims, however, hemoglobin is converted into methemoglobin (MetHb) to various degrees (5.7 to 31.6% of total hemoglobin) by oxides of nitrogen produced during combustion (7). Little is known about the effects of such levels of methemoglobin on blood cyanide concentrations in fire victims.

It has been well documented that cyanide remains very stable in blood stored in a vial at 4°C and that it becomes unstable when the temperature is increased (8–10). However, there are no reports quantitatively showing the stability of cyanide in the blood of fire victims.

Thus, the present study explores the effects of slight methemoglobinemia, typically caused by fire gases, and of the postmortem interval on blood cyanide concentrations in fire victims.

Materials and Methods

Apparatus

A Shimadzu gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan) equipped with a GS-Q column [30 m × 0.537 mm i.d. (J&W Scientific, Folsom, CA)] and a flame thermionic detector was employed for quantification of cyanide. The temperature of both injection port and detector was 160°C. The column temperature was programed as follows: the initial temperature of 100°C was maintained for 1 min, then increased to 140°C at a rate of 10°C/min, and the final temperature was maintained for 1 min. The carrier gas was helium at a flow pressure of 70 kPa.

A double beam spectrophotometer (UVIDEC-430, Japan Spectroscopic Co., Ltd., Tokyo, Japan) was used to determine the contents of carboxyhemoglobin (COHb) and MetHb.

Human Autopsy Cases

We examined blood cyanide concentrations and COHb saturation in ten fire victims (Cases 1–10). Analysis of cyanide levels was performed for various blood samples within 1 h after autopsy or within 48 h of storage of the blood samples at 4°C after autopsy. COHb saturation was determined in cardiac blood samples within 1 h of autopsy. Cases 1 to 8 were victims of house fires. The decedents in Cases 9 and 10 were found in a burnt car. Postmortem intervals were estimated to be 8 to 12 h in Cases 1 to 4, 15 to 18 h in Cases 5 to 8, 2 d in Case 9, and 3 to 5 d in Case 10. The corpses were placed at room temperature (around 20°C) until autopsy after being recovered from the scene. The cause of death was diagnosed as burns in Cases 1 to 9 and carbon monoxide poisoning in Case 10.

Animal Experimentation

Male rabbits (2.6 to 3.3 kg) were slightly anesthetized by intramuscular injection of 25 mg/kg sodium pentobarbital and were in-

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* This study was supported by the President Fund of Kochi Medical School Hospital.

Received 14 Sept. 2000; and in revised form 30 Nov. 2000 and 22 Jan. 2001; accepted 23 Jan. 2001.

travenously given 0.33 mL/kg of 3% sodium nitrite (Group A, $n = 3$) or of physiological saline (Group B, $n = 6$). After 5 min of these intravenous injections, blood samples (0.2 mL) were drawn from the auricular veins to determine MetHb contents. Then, the rabbits were sacrificed by intramuscular injection of 1 mL/kg 2% potassium cyanide. Immediately after death of the animals, a thoracotomy was performed, and large vessels around the hearts were ligated and the hearts removed. A 0.1-mL aliquot of blood was drawn from the right cardiac chambers for the determination of cyanide concentrations by needle puncture. In Group A, the hearts were left at 46°C for the first 1 h, at 20–25°C for the next 23 h and then at 4°C for the last 48 h. In Group B, the hearts of three rabbits (Group B1) were left under the same conditions as Group A; the hearts of the other three rabbits (Group B2) were left at 46°C for the first 1 h and then at 20–25°C for the next 71 h. Blood samples (0.1 mL each) were taken from the right cardiac chambers by needle puncture at 1, 6, 24, 48, and 72 h of storage for the determination of cyanide concentrations. Our treatment and disposal of the experimental animals followed The Principles of Laboratory Animal Care in Kochi Medical School.

Quantitation of Cyanide

Headspace gas chromatography was performed (10). To a 15-mL glass vial, 0.1 to 0.5 mL of blood and 0.5 mL of 1 mg/L acetonitrile (internal standard) in distilled water were added. The vial was capped with Teflon-coated silicone rubber and then sealed by crimping an aluminum cap. A 0.2-mL aliquot of 50% phosphoric acid was introduced through the silicone rubber using a 1-mL disposable syringe. The vial was then incubated in a water bath at 55°C for 15 min and 0.5 mL of air phase was injected into the gas chromatograph.

Determination of MetHb and COHb Contents

Spectrophotometric methods described by Sato (11) and Ishizu et al. (12) were used for the determination of MetHb and COHb contents, respectively.

Results

Human Autopsy Cases

Table 1 shows blood cyanide concentrations and COHb saturation in Cases 1 to 10. The blood cyanide concentration was very site dependent. In Cases 1 to 4 in which the postmortem interval was 8 to 12 h, the mean values for blood cyanide concentration and COHb saturation were within the ranges of 0.26 to 1.30 mg/L and 30.5 to 48.3%, respectively. In Cases 5 to 8 in which the postmortem interval was 15 to 18 h, the mean values for blood cyanide concentration and COHb saturation were within the ranges of 0.56 to 5.38 mg/L and 29.2 to 69.0%, respectively. In Case 9 in which the postmortem interval was approximately 2 d, the average blood cyanide concentration was 0.54 mg/L and COHb saturation in blood from the left cardiac chambers was 84.2%. In Case 10 in which the postmortem interval was 3 to 5 d, no cyanide was detected in any of the blood specimens other than pulmonary arterial blood. The average COHb saturation was 72.4%.

Animal Experimentation

Average MetHb contents in blood immediately before potassium cyanide administration were 6.9 ± 1.7 and 0.8 ± 0.9% in Groups A and B, respectively (Fig. 1). Rabbits in Group A survived longer (16.0 ± 7.9 min) than those in Group B (5.8 ± 0.7 min) after cyanide administration (Fig. 2). Blood cyanide concentrations at

TABLE 1—*Blood cyanide concentrations and carboxyhemoglobin (COHb) saturation in ten fire victims.*

Site of Blood Sampling	Cyanide Concentration (mg/L)									
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Pulmonary arteries	1.99	NE	NE	1.26	1.21	4.26	6.09	1.78	0.54	0.26
Pulmonary veins	2.42	0.73	NE	2.05	1.26	7.89	NE	2.24	0.98	ND
Left cardiac chambers	1.01	NE	0.17	1.08	0.47	4.02	5.45	1.68	0.22	NE
Right cardiac chambers	0.79	0.21	0.44	0.59	0.07	1.42	7.08	1.09	NE	NE
Aorta	2.03	NE	NE	1.69	0.78	4.00	6.53	1.32	0.95	ND
Inferior vena cava	0.51	NE	NE	0.05	ND	1.16	1.78	1.42	NE	ND
Iliac arteries	NE	NE	NE	NE	NE	NE	NE	NE	0.12	NE
Iliac veins	1.23	NE	NE	NE	NE	NE	2.08	0.28	NE	NE
Femoral arteries	0.62	NE	NE	1.82	NE	1.25	11.9	NE	0.41	ND
Femoral veins	1.12	0.16	0.18	1.88	0.15	0.85	2.10	0.40	NE	ND
Mean value	1.30	0.37	0.26	1.30	0.56	3.11	5.38	1.28	0.54	0.04
Average COHb (%) in cardiac blood (range)	42.4 (41.6–43.1)	30.5 (28.5–32.4)	48.3 (41.7–54.8)	38.0 (33.9–42.0)	48.3 (46.0–50.5)	69.0 (65.9–72.0)	68.3 (67.4–69.1)	29.2 (24.9–33.4)	84.2* —	72.4 (72.3–72.4)
Postmortem interval	8 h	12 h	12 h	12 h	15 h	15 h	15 h	18 h	2 days	3–5 days

NE = not examined.
ND = not detectable.
*Blood in the left cardiac chambers was only analyzed.

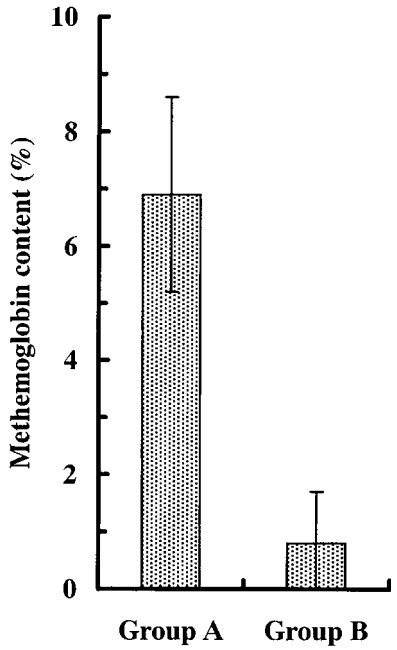

FIG. 1—*Methemoglobin contents in the peripheral venous blood of rabbits 5 min after intravenous injection of 0.33 mL/kg 3% sodium nitrite (Group A,* n = *3) or of physiological saline (Group B,* n = *6). Each column represents the mean ± SD.*

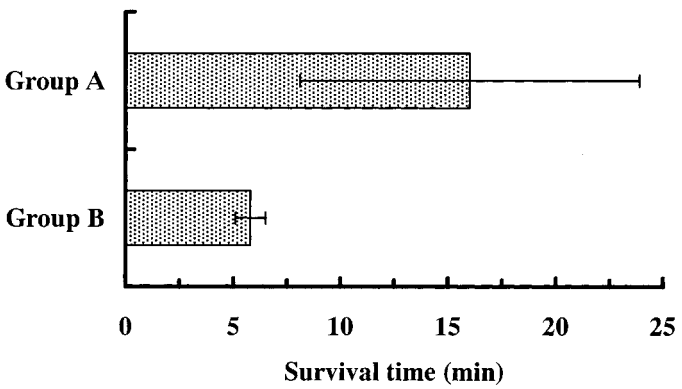

FIG. 2—*Survival time of rabbits given 1 mL/kg of 2% potassium cyanide, intramuscularly, 5 min after intravenous injection of 0.33 mL/kg 3% sodium nitrite (Group A,* n = *3) or of physiological saline (Group B,* n = *6). Each column represents the mean ± SD.*

the time of death were much higher in Group A (47.4 ± 14.3 mg/L) than in Group B (3.56 ± 0.39 mg/L) (Fig. 3).

Figures 4 and 5 show the stability of cyanide in blood in the rabbit hearts. Approximately 85, 47, and 45% of the original amount of blood cyanide disappeared within 24 h in Groups A, B1, and B2, respectively. After the 72-h storage period, approximately 10 and 37% of the original amount of cyanide remained in the blood in Groups A and B1, respectively; blood cyanide disappeared almost completely in Group B2.

Discussion

Fire victims are known to show a variety of blood cyanide concentrations depending on the circumstances of the fire and on the age and physical conditions of the victims. In fact, in Cases 1 to 8 in which the postmortem interval was short (within 18 h), a wide range of blood cyanide concentrations were observed. In Cases 6 and 7 where average blood cyanide concentrations were higher than 2.7 mg/L, which is thought to be a lethal level in individuals ingesting cyanide compounds or in those inhaling hydrogen cyanide gas, COHb saturation (near 70%) was at a lethal level. A similar tendency was observed by Shiono et al. (6) in fire victims. This indicates that a large volume of hydrogen cyanide gas may be produced under the circumstance of a fire in which a large quantity

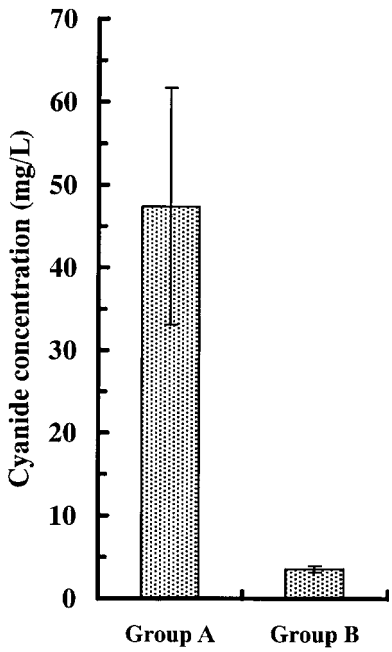

FIG. 3—*Cyanide concentrations in cardiac blood taken immediately after death of rabbits given 1 mL/kg of 2% potassium cyanide, intravenously, 5 min after intravenous injection of 0.33 mL/kg 3% sodium nitrite (Group A,* n = *3) or of physiological saline (Group B,* n = *6). Each column represents the mean ± SD.*

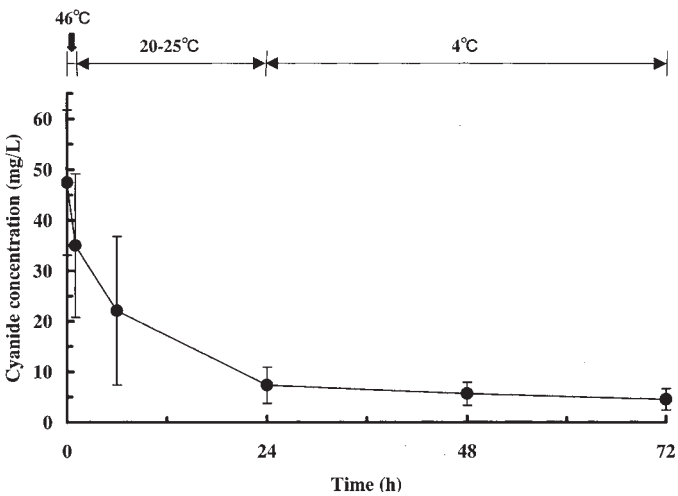

FIG. 4—*Changes in blood cyanide concentrations in the hearts of rabbits (Group A,* n = *3) sacrificed by intramuscular injection of 1 mL/kg of 2% potassium cyanide 5 min after intravenous injection of 0.33 mL/kg of 3% sodium nitrite. Each point represents the mean ± SD.*

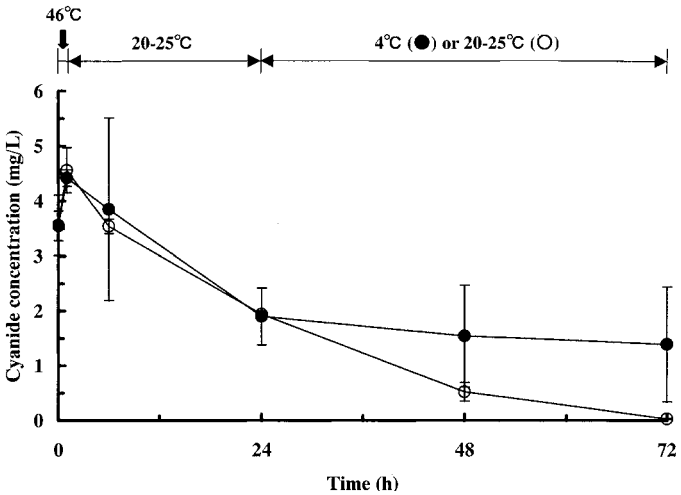

FIG. 5—*Changes in blood cyanide concentrations in the hearts of rabbits (Group B1, ●, n = 3 and Group B2, ○, n = 3) sacrificed by intramuscular injection of 1 mL/kg of 2% potassium cyanide 5 min after intravenous injection of 0.33 mL/kg of physiological saline. Each point represents the mean ± SD.*

of carbon monoxide is formed. Thus, it is strongly suggested that cyanide played only a small role in the death in the aforementioned two cases. Seto et al. (13) reported artefactual production of hydrogen cyanide and carbon monoxide gases during heat denaturation of reduced hemoglobin. In our fire victims, whose vascular blood was completely liquid, however, this postmortem effect might have been minimal.

In fire gases, oxides of nitrogen, which are also contained to various degrees, cause methemoglobinemia (7). Although we did not measure the contents of MetHb in our cases, Katsumata et al. (7) detected 5.7 to 31.6% of MetHb from all 12 fire victims they examined. MetHb can be formed postmortem by heat denaturation of hemoglobin (13). However, in liquid blood samples negligibly affected by heat, it is reasonable to assume that detected MetHb was produced by inhalation of oxides of nitrogen during the fire. Cyanide is rapidly combined with MetHb and this bound form of cyanide no longer exerts toxic action. We compared peak height ratios of cyanide (range of cyanide concentrations: 0.4 to 1.2 mg/L) to the internal standard in MetHb-free human blood and those in the blood spiked with sodium nitrite at a concentration of 0.2 g/L (MetHb content: 65%). No difference in peak height ratio was observed between the two blood samples at each concentration of cyanide. These results demonstrate that total cyanide (free form and bound form of cyanide) is detected by our headspace gas chromatography. Thus, blood cyanide concentrations in fire victims should not be evaluated merely by comparison with those obtained from individuals who were poisoned purely by cyanide compounds or hydrogen cyanide gas.

In the present study, sodium nitrite-treated rabbits showed extremely high concentrations of blood cyanide. Hemoglobin contents of rabbits are in a range of 11 to 14 g/dL (14). In rabbits' blood containing about 7% MetHb, 12 to 16 mg/L cyanide can be trapped by MetHb to form cyanmethemoglogin. Tadic (15) reported when sodium nitrite was injected intravenously to rats 30 min after subcutaneous administration of 20 mg/kg of sodium cyanide, marked reactivation of brain cytochrome oxidase activity was observed. Prevention of MetHb formation by toluidine blue did not affect the reactivating ability of sodium nitrite. Thus, sodium nitrite may exert antidotal effects on cyanide by more complex mechanisms than simple MetHb formation. Sodium nitrite itself may protect brain cytochrome oxidase from cyanide binding or enhance a reaction of cyanide with serum proteins (16). It is suggested that sodium nitrite effectively works as an antidote of cyanide even at concentrations as low as several percent.

The difference in cyanide concentrations among blood samples in each fire victim (Cases 1 to 9) was too large to be explained by circulatory conditions during the fire alone and by inhalation of fire gases in which the cyanide concentration is fluctuating moment by moment. It was strongly suggested that an irregular postmortem decrease in blood cyanide concentration is largely responsible for the site-to-site differences in blood cyanide concentrations. In Case 10 in which the postmortem interval was estimated to be 3 to 5 d, hydrogen cyanide in addition to carbon monoxide might have been produced at a large quantity during the fire because the victim was found in a car, in which the interior was burnt, but the glass windows were not broken. Although COHb saturation was approximately 70%, a negligible amount of cyanide was detected only in pulmonary arterial blood. Thus in this case, cyanide present at the time of death might have disappeared almost completely before autopsy.

To obtain basic data on the postmortem stability of blood cyanide in corpses, we conducted an experiment using blood-containing hearts of rabbits sacrificed by potassium cyanide. The reason why the hearts were left at 46°C for the first 1 h of the stability test was to reproduce circumstances similar to the hearts of fire victims exposed to high temperatures without causing heat coagulation of blood. The rate of disappearance of cyanide was greater in cardiac blood containing high concentrations of cyanide (Group A) than in that containing low concentrations of cyanide (Groups B1 and B2). In the hearts of Groups B1 and B2, a slight elevation in the blood cyanide concentration was temporally observed postmortem. This phenomenon is likely due to postmortem diffusion of cyanide from the myocardium into the blood, although the cyanide concentration in the myocardium was not determined. There may be little possibility of postmortem formation of cyanide due to degeneration of reduced hemoglobin, because the temperature of 46°C, at which the hearts were left for the first 1 h after death was well below the temperature that causes degeneration of hemoglobin (13). A plausible reason why the aforementioned phenomenon was not observed in the hearts of Group A is that disappearance of blood cyanide was much greater than the diffusion of myocardial cyanide. The mechanism underlying the disappearance of cyanide from cardiac blood is unclear. However, we believe that our animal models are useful for estimating how much cyanide disappears from fire victims' blood between death and blood sampling. From the results of the stability test, it was strongly suggested that more than 40% and almost 100% of original blood cyanide disappeared during 24 h and 3 d, respectively, in the deceased fire victims left at around 20°C and that blood cyanide is fairly stable when the bodies are stored at 4°C as well as when blood obtained from fire victims is preserved in glass vials at 4°C (8–10).

In conclusion, we determined that the contribution of cyanide to the death of a fire victim should be determined while taking into account burns, COHb saturation, MetHb content, the temperature at which the body has been left, postmortem interval, and the age and physical condition of the victim.

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