Cyanide concentrations in blood and tissues of fire victims

Thu Thu Htike[a,b], Fumio Moriya[a,c], Satoru Miyaishi[a,*]

[a] Department of Legal Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Shikata-cho 2-5-1, Kita-ku, Okayama 700-8558, Japan

[b] Department of Forensic Medicine, University of Medicine, Mandalay, 30th Street, Between 73rd & 74th Streets, Chanayetharzan Township, Mandalay, Myanmar

[c] Department of Nursing, Faculty of Nursing, Kawasaki University of Medical Welfare, Matsushima 288, Kurashiki, Okayama 701-0193, Japan

* Corresponding author.

E-mail address: miyaishi@md.okayama-u.ac.jp (miyaishi)

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Abstract

Cyanide poisoning has been regarded to contribute the fatal outcome in fire victims. The toxicity of inhaled hydrogen cyanide (HCN) at the cellular level was evaluated considering the impact of methemoglobin (MetHb) produced by fire gases. Cyanide (CN) concentrations and total hemoglobin contents were measured in right heart blood (RHB) and seven organs/tissues (basal ganglia, brain stem, heart, lung, liver, kidney and psoas muscle) collected from 20 fire fatalities. MetHb and carboxyhemoglobin saturations were also measured in RHB. The amount of CN probably bound to the cytochrome c oxidase (CCO-CN) of the tissue cells was extrapolated from CN and hemoglobin contents in RHB and organs/tissues, MetHb saturation in RHB and binding capacity of MetHb for CN. CN concentrations in RHB showed a wide range with the highest concentration of 8.927 μg/mL. The lung contained the largest CN content among organs/tissues with the mean concentration of 2.219 μg/g, then the heart (0.259 μg/g) and it was lower than 0.100 μg/g in others. Exceedingly large amount of CN in the lung could be explained by high hemoglobin content, being the port of entry of HCN and postmortem diffusion of fire gases. CCO-CN was theoretically present in about 20% of organ/tissue samples, most commonly in the basal ganglia (10 samples, with the mean of 0.059

μg/g) followed by heart (eight samples, with the mean of 0.109 μg/g). No CCO-CN was found in liver and kidney. HCN might have the effect on brain and heart.

Key words: cyanide; methemoglobin; cytochrome c oxidase; fire fatality

1. Introduction

During fires, hydrogen cyanide (HCN) can be produced by combustion of nitrogen-containing materials such as plastics and nylon [1–4]. With the increasing use of those materials in modern buildings, probable cyanide (CN) poisoning among victims of house fires has been considered as an important issue and many researches claimed that CN concentrations in fire victims were high enough to produce fatal results [5–9]. On the other hand, oxides of nitrogen in fire gases also produce methemoglobin (MetHb) which has great affinity for CN; about 1% of MetHb can bind 2 mg/L of CN thereby detoxifying it [8,10,11]. This means that CN in the blood might exist as cyanmethemoglobin and has no toxic action in case of fire fatalities. Although we have pointed out this possibility and Giebułtowicz et al. also reported the same in 2017 [12], there has been no discussion on the role of MetHb in CN toxicity among fire victims in heretofore reported literature other than those mentioned above.

Outside the blood stream, CN diffuses into body water and acts as a chemical asphyxiant at the cellular level by binding to cytochrome c oxidase (CCO) [12–14]. Tissue CN levels, therefore, can directly reflect the degree of CN toxicity caused by inhalation of HCN in fire victims. In the present study, we investigated CN concentration in the blood and various

organs/tissues of fire victims in combination with MetHb contents taking its effect into consideration, and the toxicity of CN was discussed.

2. Materials and Methods

2.1. Sample collection

Blood and seven organ/tissue samples were collected from 20 fire victims who died at the scene; those were the right heart blood (RHB), basal ganglia, brain stem, heart, lung, liver, kidney and psoas muscle. Among victims, ten were male and ten were female, the age ranged from 20s to 90s years old and the postmortem intervals varied from one to four days. Causes of death were death due to fire in 13 cases and death due to burn in seven cases. Samples were also collected from 20 non-fire fatalities which were not related to cyanide poisoning as control. The deceased were 10s to 80s years old, thirteen of them were male and seven were female. Causes of death were asphyxia (7/20), death by injury (4/20), death by internal cause (4/20) and others (5/20). Postmortem interval varied from 1.5 to five days. Samples were provided to toxicological analysis such as CN, total hemoglobin (total Hb), carboxyhemoglobin (CO-Hb) and MetHb immediately after collection.

2.2. Determination of total Hb contents, and CO-Hb and MetHb saturations in the collected materials

Total Hb content and CO-Hb saturation in RHB were measured with an automatic hematology analyzer (Celltac E, Nihon Kohden Corp., Tokyo, Japan) and a CO-oximeter (Avoximeter 4000, ITC, MA, USA), respectively. MetHb not binding to CN (free MetHb) was measured with RHB by a spectrophotometric method developed by Sato et al. [15] using UV spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan). Saturation of bound MetHb in the form of cyanmethemoglobin was calculated as follows:

$$Bound\ MetHb\ in\ RHB\ (\%) = \frac{Total\ CN\ in\ RHB(\mu g/mL) * MW\ of\ Hb * 100}{Total\ Hb\ in\ RHB(\mu g/mL) * MW\ of\ CN * 4}$$

MW: molecular weight

4: the number of subunits of Hb

Combination of bound and free MetHb was regarded as total MetHb in RHB.

Total Hb contents in organ/tissue samples were determined from the supernatant of their homogenates using Hemocue Plasma/Low Hb System (Hemocue Co., Ängelholm, Sweden); free MetHb concentration was regarded as same level as that in RHB.

2.3. Pretreatment of samples for measuring CN concentration

Organ/tissue samples were homogenized with distilled water at 0°C using a disperser (LK-21, Yamato Scientific CO., Ltd., Tokyo, Japan). The ratio of tissue to distilled water was 1 : 1

(other than psoas muscle) to 1 : 2 (psoas muscle). Blood (0.5 mL) or tissue homogenate (0.5 g) was put into a 10 mL glass vial with 0.5 mL of acetonitrile (1 μg/mL) as internal standard and 25 μL of distilled water or KCN solution (20 μg/mL). The vial was sealed with Teflon-coated silicone rubber septum and plastic screw cap. After injecting 0.2 mL of 50% phosphoric acid through the septum using 28 G needle, the vial was incubated at 58°C for 30 minutes.

2.4. Determination of CN concentration

Total CN concentrations in autopsy materials were measured by the previously reported headspace gas chromatography [10]. About 0.5 mL of headspace gas in the vial described in the previous section was injected into the gas chromatograph (GC-2014, Shimadzu Corp., Kyoto, Japan) equipped with GS-Q column (0.530 mm × 30 m, Agilent Technologies Inc., CA, USA) and a flame thermionic detector. The lower limit of quantitation (LLOQ) of the method was 0.028 μg/mL.

The amount of CN which did not bind to MetHb but to CCO (CCO-CN) of tissue cells was extrapolated from CN, cyanmethemoglobin and free MetHb concentrations using the following formula.

$$CCO - CN(\mu g/g) = Total\ CN\ in\ tissue(\mu g/g) - \frac{Total\ MetHb\ in\ RHB(\mu g/g) * Total\ Hb\ in\ tissue(\mu g/g)}{MW\ of\ Hb * 4 * MW\ of\ CN}$$

MW: molecular weight

4: the number of subunits of Hb

2.5. Statistical analyses

Statistical analyses were performed using Microsoft Excel 2016 (Microsoft Corporation; USA) and SPSS software (IBM SPSS statistics, version 20.0, USA). The relationship between variables was evaluated by linear regression analysis and 2-group comparison was made by Mann-Whitney test.

2.6. Ethical approval

This study was approved by the institutional ethics committee at the authors' University (approval number 1807-114).

3. Results

CN concentration, total Hb content, CO-Hb and MetHb saturations in RHB of 20 fire victims are shown in Table 1. CN concentrations in RHB of 20 fire victims varied from below LLOQ of the analytical method used (0.028 μg/mL) to 8.927 μg/mL. CO-Hb and total MetHb saturations were 41.8 % and 2.0%, respectively, in average. Figure 1 and 2 showed the relationship between CN concentration and CO-Hb or MetHb in RHB respectively. Table 2 shows total CN concentrations and total Hb contents in the organ/tissue samples in average. The lung contained the highest concentration of CN (2.219 ± 2.248 μg/g), and it was followed by the heart, kidney, basal ganglia, liver, brain stem and psoas muscle in the decreasing order of CN concentrations. Total Hb content was also the highest in the lung (94.80 ± 37.01 mg/g) and that of basal ganglia, brain stem, heart and psoas muscle was lower than 10.00 mg/g. When 20 victims were divided into two groups, with and without exposed thoracic cavity, the significant difference of total CN contents in the lung was observed between these groups with Mann-Whitney U test ($p < 0.01$, Fig. 3). CCO-CN in organs/tissues was found in about 20% of the samples. The number of samples containing CCO-CN was the largest in basal ganglia (10 samples) followed by the heart (eight samples) as shown in Table 3. Possible concentrations of CCO-CN as the mean in the basal ganglia and heart were 0.059 ± 0.041 μg/g and 0.109 ±

0.195 μg/g, respectively. CCO-CN concentration was the highest in the lung (0.503 ± 1.118 μg/g). All samples of the liver and kidney contained no CCO-CN.

4. Discussion

It is well known that CN poisoning is a type of chemical asphyxia caused by the disturbance of cellular respiration and the essential mechanism is the inhibition of CCO activity due to its binding to this enzyme [12–14]. It is also known that MetHb can detoxify CN by binding this poisonous substance immediately in blood (formation of cyanmethemoglobin) and the nitrites are widely used therapeutic agent for CN poisoning as MetHb formers [13,16,17]. Fire gases contain a considerable amount of nitrogen oxides and CN in blood was reported to be bound to produced MetHb in fire victims [8,10,11]. Therefore hitherto reported discussion on CN toxicity among fire victims only using its concentration in the blood [6,9,18] seems inappropriate. It should be discussed using the content of CN in parenchymal cells in organs/tissues, which does not bind to MetHb but to CCO. However, CCO-CN cannot be separately determined from CN existing as cyanmethemoglobin by the instrumental analysis. In this study, we determined total CN, total Hb and free MetHb in blood as well as total CN and total Hb in organs/tissues simultaneously. Then CCO-CN contents in organs/tissues were theoretically calculated and the role of CN in fire victims was discussed.

In the present study, CN content in RHB varied widely among the victims (1.627 ± 2.126 μg/mL) with the highest concentration of 8.927 μg/mL. This range was similar to that in the

previous reports on the blood CN concentrations in fire fatalities [8,19,20]. CN concentrations in the blood samples of 20 non-fire cases as control ranged from that lower than LLOQ to 0.053 μg/mL being within previously reported physiological level which is below 0.25 μg/mL [6]. There were five fire cases in which blood CN concentrations were within physiological range. Such finding was reported previously and the plausible explanation is the different characteristics of fire incidents [8,11,21]. Concentration of CN in RHB did not express the significant correlation with CO-Hb and MetHb concentrations as being reported previously [11].

Total CN in seven types of organs/tissues was also measured in 20 fire victims simultaneously with that in RHB. The largest content of total CN was detected in the lung (2.219 ± 2.248 μg/g) and it was followed by the heart (0.259 ± 0.298 μg/g). Mean total CN in all other organs/tissues were lower than 0.100 μg/g and the lowest was observed in psoas muscle (Table 2). An important reason why total CN in the lung was remarkably higher than that in other samples was an excessively large content of blood in this sample as shown in Table 2; CN largely exists as cyanmethemoglobin [12,22]. Additionally, the lung is the port of entry for fire gases and it is directly exposed to HCN during antemortem period. Postmortem diffusion of HCN from the airway or through the burnt out thoracic wall may also occur [8,23].

In the present study, total CN concentrations in the lung was significantly higher in the victims with exposed thoracic cavity than those with intact thoracic wall ($p < 0.01$).

CCO-CN in the tissue cells was calculated using the values of total CN concentrations and Hb contents in RHB and respective organs/tissues and measured MetHb concentration in RHB. In the present study, it was assumed that CN in organs/tissues, which was not in the form of cyanmethemoglobin, would bind to CCO to estimate the possible maximum toxicity of CN at the cellular level. However further investigation is required because there was a possibility of CN in organs/tissues existing as free CN, which could not be affirmed by the present study. The number of CCO-CN containing samples was different among organs/tissues of 20 fire cases: most commonly in the basal ganglia (10/20) followed by the heart (8/20), lung and psoas muscle (4/20 each) and brain stem (3/20). Total CN in the basal ganglia was markedly lower in concentration than the lung and heart, but it had the highest frequency of containing CCO-CN among investigated seven organs/tissues. Although the frequency of containing CCO-CN was not so high in the brain stem, it was present in some samples while total CN content in the brain stem was relatively low. Therefore, the brain showed the presence of CCO-CN in total 13 of 40 samples which was the second largest frequency among organs/tissues. These facts suggested that CN might have toxic effect in the brain, despite its low total content. Inhaled

HCN might also have effect on the heart because eight samples were positive for CCO-CN with the highest concentration of 0.766 μg/g.

On the contrary, no CCO-CN was detected in the liver and kidney samples, though there was no considerable difference in average total CN between them and the brain and muscle samples. One of the possible explanations for this finding was the high activity of rhodanese, an important cyanide metabolizing enzyme, in the liver and kidney as shown in Table 2. Our experience of measuring rhodanese activity was in concordance with the report of Aminlari et al. [24].

The concentration of CCO-CN observed was highest in the lung (0.503 ± 1.118 μg/g) and it was notably higher than the others (0.028 ± 0.050 μg/g in the psoas muscle to 0.109 ± 0.195 μg/g in the heart) as same as in total CN. A plausible reason of high CCO-CN content in the lung might also be the postmortem exposure to fire gases. However, it should be confirmed by further experiment. On the other hand, there were only four lung samples contained CCO-CN suggesting that CN present in this sample largely existed in blood in the pulmonary vessels rather than in the parenchymal cells.

According to our knowledge, there has been one case report investigating CN content in organ. However, the organ investigated was only the lung and effects of MetHb and rhodanese

activity were not taken into consideration [25]. Consequently, this study is the first systematic study on CN concentrations in organs/tissues with subsequent consideration of the toxic effects exerted by this chemical among fire-related deaths.

HCN in the fire gases might not be as poisonous as that was described in the previous literatures which based on CN concentration in blood only, because CCO-CN could not be found in some organs in many cases. In some cases, the victims inhaled a large amount of HCN at the fire scene resulting in high CN concentration in RHB, however, it was probably detoxified by MetHb and unable to reach mitochondrial level to exert toxic action.

5. Conclusion

CN concentration in seven organs/tissues of 20 fire fatalities was determined together with CN concentration and MetHb and Hb contents in RHB and Hb content in organs/tissues. Then, CN possibly bound to CCO of tissues was calculated theoretically. CCO-CN was found in some samples especially in the brain and heart so inhaled CN might have toxic effect at cellular level in fire victims. Therefore, CCO-CN in fire fatalities should be taken into consideration to evaluate the role of HCN on cause of death in fire fatalities, although it is necessary to perform further study on the form of CN (free or binding to CCO) in the organs/tissues.

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Legends to Figures

Fig. 1. Correlation between cyanide concentration and carboxyhemoglobin saturation in right heart blood (RHB) of fire victims

Fig. 2. Correlation between cyanide concentration and total methemoglobin content in right heart blood (RHB) of fire victims

Fig. 3. Box-plot comparing total cyanide (total CN) concentration in lung samples of fire victims with and without exposed body cavity

Lower and upper box boundaries: 25th and 75th percentiles, respectively; line inside box: median

There was a significant difference in total CN concentration between two groups by Mann-Whitney U test.

Table 1. Cyanide (CN) concentrations, hemoglobin (Hb) contents, saturations of carboxyhemoglobin (CO-Hb), free and bound methemoglobin (MetHb) in right heart blood of 20 fire victims

Case No.	Age	Sex	Cause of death	PMI[a] (hour)	CN (μg/mL)	Hb (g/dL)	CO-Hb (%)	Free MetHb[b] (%)	Bound MetHb[c] (%)
1	80s	M	Death due to fire	96	0.649	2.9	74.8	0.3	1.4
2	40s	F	Death due to fire	60	8.927	16.9	69.7	0.5	3.3
3	80s	F	Death due to burn	36	0.376	12.4	1.4	6.2	0.2
4	50s	F	Death due to fire	36	1.036	11.3	71.5	0.7	0.6
5	90s	F	Death due to fire	60	1.050	8.5	75.0	0.4	0.8
6	20s	M	Death due to fire	72	0.060	12.6	41.4	1.0	0.0
7	60s	M	Death due to burn	48	0.097	10.6	14.1	0.6	0.1
8	80s	M	Death due to burn	48	0.473	4.8	9.5	3.6	0.6
9	70s	F	Death due to fire	48	2.662	8.8	58.6	0.5	1.9
10	60s	F	Death due to fire	72	2.839	14.5	45.4	1.1	1.2
11	60s	M	Death due to fire	36	2.163	13.1	74.0	0.6	1.0
12	90s	F	Death due to burn	48	0.067	13.4	2.6	0.5	0.0
13	90s	M	Death due to fire	36	2.874	21.2	50.3	0.7	0.8
14	60s	M	Death due to fire	60	4.630	15.2	57.4	0.6	1.9
15	70s	M	Death due to fire	36	2.148	9.2	38.8	0.3	1.4
16	70s	F	Death due to burn	36	<LLOQ[d]	8.3	6.8	0.8	0.0
17	70s	M	Death due to fire	84	0.499	5.6	66.7	1.7	0.6
18	90s	F	Death due to burn	84	0.803	8.6	2.5	1.4	0.6
19	90s	M	Death due to burn	96	0.123	10.2	0.5	1.0	0.1
20	60s	F	Death due to fire	84	1.064	15.1	74.4	0.4	0.4

[a] Postmortem interval.

[b] MetHb saturation measured by spectrophotometry.

[c] MetHb saturation extrapolated from CN concentration and Hb content of right heart blood.

[d] Lower limit of quantitation (0.028 μg/mL).

All deceased were victims of building fire except Case 7 who incinerated himself in the open field.

Table 2. Total cyanide (total CN) concentrations and total hemoglobin (total Hb) contents in the organ/tissue samples of 20 fire victims in the present study and rhodanese activity in different organs/tissues of humans reported [24]

Tissue	Total CN (μg/g) Mean ± SD[a]	Total Hb (mg/g) Mean ± SD	Rhodanese activity (U/g tissue)
Basal ganglia	0.091 ± 0.084	4.03 ± 3.15	0.28 ± 0.12[b]
Brain Stem	0.063 ± 0.088	4.68 ± 3.97	0.28 ± 0.12[b]
Heart	0.259 ± 0.298	9.76 ± 4.33	ND[c]
Lung	2.219 ± 2.248	94.80 ± 37.01	0.48 ± 0.16
Liver	0.086 ± 0.193	32.91 ± 19.74	3.44 ± 0.86
Kidney	0.094 ± 0.120	27.81 ± 13.65	7.64 ± 0.95
Psoas muscle	0.051 ± 0.096	6.55 ± 1.81	0.40 ± 0.10[d]

[a] Total CN below the lower limit of quantitation of analytical method used (0.028 μg/g) was treated as 0.
[b] Activity in the brain.
[c] Not determined.
[d] Activity in the muscle.

Table 3. Theoretically calculated concentration (μg/g) of cyanide (CN) that possibly bound to cytochrome c oxidase (CCO) in the tissue cells

Case No.	Basal ganglia	Brain stem	Heart	Lung	Liver	Kidney	Psoas muscle
1	0.071	0	0.167	0	0	0	0.013
2	0.062	0.150	0.766	1.899	0	0	–
3	–	–	0	0	0	0	0
4	0.096	0.138	0.109	0	0	0	0
5	0.049	–	0.088	0	–	0	0.130
6	–	–	0	0	–	–	–
7	–	–	–	0	–	–	0.014
8	–	–	0	0	–	–	–
9	0.128	0	0.221	0.971	–	0	–
10	0.045	0	0	0	–	–	–
11	0.041	0	0.086	3.804	0	0	–
12	–	–	–	–	–	–	–
13	0.091	–	0.114	2.876	–	–	–
14	0.020	0	0.084	0	–	0	–
15	0	0	0	0	–	0	–
16	–	–	–	0	–	0	–
17	0.107	0.108	0	0	–	–	0.010
18	–	–	–	0	–	–	–
19	–	–	–	0	–	0	–
20	0	–	0	0	–	–	–
Mean ± SD[a]	0.059±0.041	0.044±0.067	0.109±0.195	0.503±1.118	0±0	0±0	0.028±0.050

–: It was impossible to calculate because total CN concentration in the sample was below lower limit of quantitation (LLOQ) of the analytical method used (0.028 μg/g).
[a] The samples with total CN concentration below LLOQ were excluded.
Concentration of CN that probably bound to CCO in the tissue cells was extrapolated from CN concentration and methemoglobin content in right heart blood and total CN concentrations and hemoglobin contents in the organs/tissues.

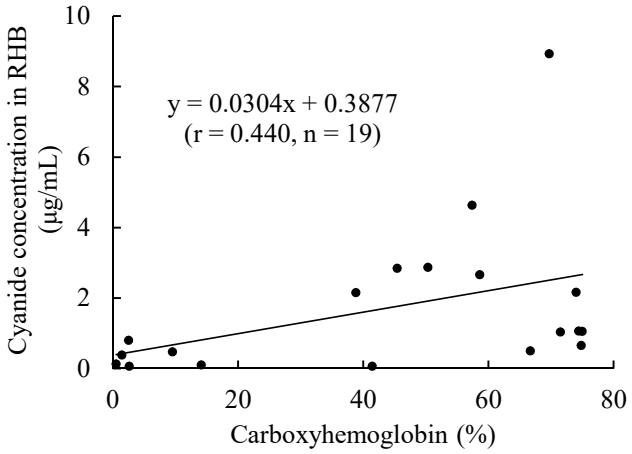
y = 0.0304x + 0.3877
(r = 0.440, n = 19)
Cyanide concentration in RHB (μg/mL)
Carboxyhemoglobin (%)
0
2
4
6
8
10
0
20
40
60
80

Fig. 1

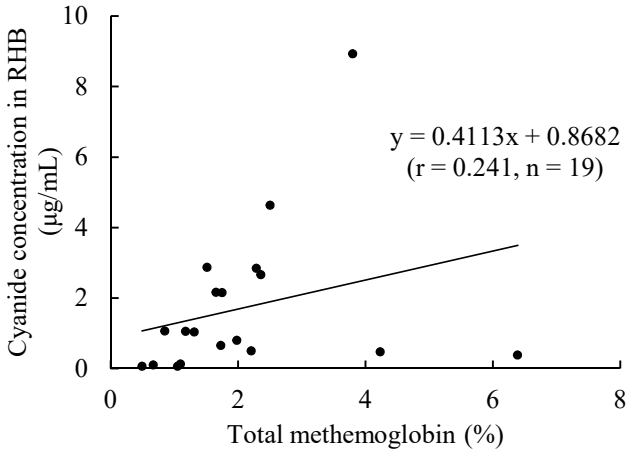
Cyanide concentration in RHB (μg/mL)
Total methemoglobin (%)
y = 0.4113x + 0.8682
(r = 0.241, n = 19)
0
2
4
6
8
10

Fig. 2

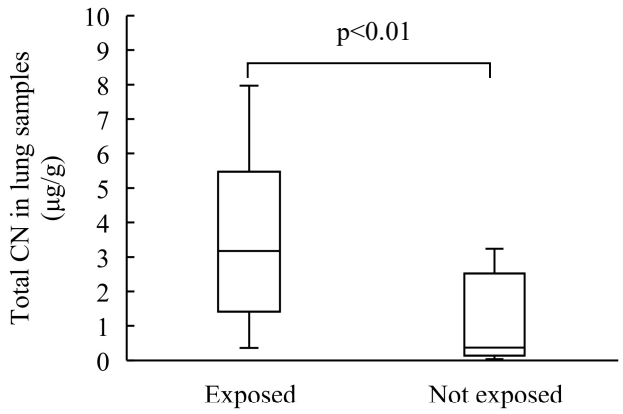
p<0.01
Total CN in lung samples (µg/g)
10
9
8
7
6
5
4
3
2
1
0
Exposed
Not exposed

Fig. 3