Effects of Routes of Administration on the Cyanide Concentration Distribution in the Various Organs of Cyanide-Intoxicated Rats

KEICHI YAMAMOTO, YOSHIKO YAMAMOTO, HIROYUKI HATTORI and TOMOYUKI SAMORI

Department of Legal Medicine, Faculty of Medicine, Kyoto University, Kyoto 606


Cyanide concentrations in various organs (the blood, liver, lung, spleen and brain) of the rats intoxicated by ingestion or inhalation were determined with a slightly modified microdiffusion method. In the inhalation experiment, a rat in an exposure chamber inhaled HCN produced by addition of NaCN solution to H$_2$SO$_4$. For each route two different doses were used. The time to death was shorter when doses were larger. Cyanide concentrations were higher in per os group than in inhalation group in all the organs tested. The concentration in the liver was most sensitive to the route of administration; it was the highest among the organs tested after per os administration, but low after inhalation. In the lung it was significantly higher than that of the blood when cyanide was inhaled. It was concluded that, when it is necessary to determine whether cyanide was ingested or inhaled, at least the lung and liver must be analyzed for cyanide contents in addition to the blood and stomach content.

Small but significant amounts of cyanide are present in the blood of the majority of victims of fires (Wetherell 1966; Fukui 1969). The cyanide is considered to be released from nitrogen-containing polymers exposed to heat (Wetherell 1966; Yamamoto 1975). On fire victims with high cyanide concentrations approaching to lethal concentration, under certain circumstances, it will be necessary to establish whether the cyanide was inhaled or ingested. Cyanide concentrations in the stomach content and/or in the blood can be served for the above differential diagnosis. Since cyanide distribution pattern in various body tissues is considered to depend on whether the toxicant was ingested or inhaled, the distribution study also undoubtedly contributes to resolving the above question.

The microdiffusion method with a Conway dish (Feldstein and Klendshoj 1954) has been widely used for determination of cyanide concentration in the biological samples. When tissue homogenates are analyzed for cyanide contents with conventional Conway dishes, however, there arises sometimes troubles, that is, because tissue homogenate is usually less viscous than the blood, contact of the

Received for publication, July 10, 1981.
homogenate with H$_2$SO$_4$ may occur in the outer chamber before the dish is sealed. A further possible trouble with a conventional Conway dish is that NaOH solution in the central chamber is easily contaminated with fluid crossing over the wall from the outer chamber when the dish is rotated at a speed beyond a certain limit. In the present study, modified Conway dishes were made and a simple device was used in order to prevent the contents of the outer chamber from being mixed before sealing the dish.

**MATERIALS AND METHODS**

Male Wistar rats weighing each about 200 g were used. Animals for per os experiments were deprived of food 15 hr before experiments.

**Per os study.** Rats received cyanide radical of 7 mg or 21 mg per kg body weight as NaCN-saline solution. The solution was injected into the stomach at a volume of 10 ml per kg body weight for each dose group. After administration, the rat was placed in a cage.

**Inhalation study.** Since the experimental apparatus has been detailed in the previous report (Yamamoto and Kuwahara 1981), only brief account is given in the present report. The apparatus for exposure consists of two, transparent plastic boxes of the same shape and capacity (measuring 15 cm × 15 cm × 11 cm) connected with each other by a piece of flexible plastic tube. One box is animal chamber, in which an animal is exposed, and the other is gas chamber. HCN was produced in the gas chamber by addition of NaCN to H$_2$SO$_4$. From a buret inserted into the gas chamber through its upper hole, about 10 ml of NaCN solution (8.5 mg/ml or 25 mg/ml) was added into a dish containing 1 ml of H$_2$SO$_4$. It took on average about 30 sec for an addition. During the first few min, the atmosphere in the chamber was stirred by pushing in and out the plunger of a syringe connected to the sampling hole of the animal chamber. Gas sample (exactly 15 ml) was withdrawn by a glass syringe 3, 5, and 8 min after start of exposure. The cyanide concentration in the gas sample was determined by the following method (Yamamoto and Kuwahara 1981)

**Cyanide concentration determination in the gas sample.** As a sampling vessel, a 30 ml capacity of Erlenmeyer flask with two straight side tubes, each of which can be closed by turning a glass stop-cock, was used. The orifices of these side tubes are on the opposite sides of the flask chamber. Prior to starting a sampling procedure, 2 ml of 0.1 N NaOH solution was put into the flask and the vessel was closed with a silicon rubber stopper. After the inside pressure of the vessel had been reduced to a subatmospheric level by aspirating with a large-capacity glass syringe connected to a side tube, a gas sampler containing accurately 15 ml of sample was attached to a side tube and the sample was completely transferred into the flask by opening a stop-cock. Immediately after completion of a sample transfer, the stop-cock was opened for very short time for equating the inside pressure with the ambient pressure, and the flask was manually shaken for short time. After 2 hr of standing at room temperature, the solution was analyzed for its cyanide concentration.

**Sampling of specimen.** In per os group as well as inhalation group, after respiration of the animal had stopped ultimately, the chest was opened and the blood was taken from the right heart by a heparinized syringe. The brain, lung, liver, and spleen were removed immediately after blood sampling. After being dipped into physiologic saline and blotted with filter paper, each organ was weighed by an electronic reading balance (Shimadzu Libror ED-200MO). To respective organs except liver, 5 ml of physiologic saline was added and the mixture was homogenized in an ice-bath. To the entire liver, 20 ml of physiologic saline was added and the mixture was similarly treated. Homogenates were subjected to cyanide determination procedure.

**Conway dish.** The Conway dish used in the present study measures 6 cm in internal diameter and 2 cm in depth, it being deeper than conventional one by 1 cm. After 2 ml of 0.1 N NaOH solution was put into the central chamber, the outer chamber was divided
Different Administration Routes and Organ Cyanide Levels

into two zones by smearing a very small amount of vaseline at two positions approximately opposite to each other across the central chamber. To one zone a 2 ml aliquot of sample (homogenate or blood) was placed and to the other zone 1 ml of H$_2$SO$_4$ was added. Vaseline prevented the contents from being mixed before sealing the dish. After sealing the dish, the entire unit was rotated gently on a slide test rotator for about 1 min to mix the contents of the outer chamber. After 2 hr of standing at room temperature, NaOH solution in the central chamber was analyzed for cyanide concentration by apyridine-pyrazolone method.

RESULTS

Experimental data are summarized in Tables 1 and 2.

**Per os study.** The time to death was 10.3 min for 7 mg group and 3.3 min for 21 mg group. The survival time of the larger dose group was shortened to a third of that of the smaller dose group. When the both dose groups were combined (hereafter, termed as combined data), the liver showed the highest cyanide concentration and the difference from the blood was significant. The concentrations in the spleen as well as of the brain were significantly lower than the concentration in the blood. The lung appeared to show a higher value than the blood, but the difference was not significant. Between the two dose groups there was no significant difference in concentration in the organs except the liver.

**TABLE 1. Cyanide concentrations in various organs (per os study)**

<table>
<thead>
<tr>
<th>Organs</th>
<th>7 mg/ml (n=10)</th>
<th>21 mg/ml (n=9)</th>
<th>“Combined”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (µg/ml)</td>
<td>5.01±1.61</td>
<td>4.79±2.04</td>
<td>4.91±1.78</td>
</tr>
<tr>
<td>Liver (µg/g wet wt.)</td>
<td>7.53±1.45 (161±50)</td>
<td>10.40±2.17 (230±47)</td>
<td>8.89±2.30 (194±59)</td>
</tr>
<tr>
<td>Lung (µg/g wet wt.)</td>
<td>5.44±2.27 (114±46)</td>
<td>6.30±2.19 (146±66)</td>
<td>5.85±2.22 (129±57)</td>
</tr>
<tr>
<td>Spleen (µg/g wet wt.)</td>
<td>2.48±0.98 (53±22)</td>
<td>1.76±0.50 (36±12)</td>
<td>2.09±0.88 (45±19)</td>
</tr>
<tr>
<td>Brain (µg/g wet wt.)</td>
<td>1.31±0.47 (27±8)</td>
<td>1.76±0.70 (40±8)</td>
<td>1.52±0.47 (33±10)</td>
</tr>
<tr>
<td>Time to death (min)</td>
<td>10.3±4.3</td>
<td>3.3±2.1</td>
<td></td>
</tr>
</tbody>
</table>

Rats received orally 7 mg/kg or 21 mg/kg of CN radical as NaCN-saline solution. “Combined” indicates the combined data of the two groups. Mean±s.d. are given. Figures in parentheses indicate the concentration in each organ as percentages of that of the blood.

**TABLE 2. Cyanide concentrations in various organs (inhalation study)**

<table>
<thead>
<tr>
<th>Organs</th>
<th>8 mg/ml</th>
<th>25 mg/ml</th>
<th>“Combined”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (µg/ml)</td>
<td>2.91±0.41</td>
<td>3.11±0.51</td>
<td>3.01±0.46</td>
</tr>
<tr>
<td>Liver (µg/g wet wt.)</td>
<td>2.19±0.77 (76±29)</td>
<td>2.11±1.10 (68±32)</td>
<td>2.15±0.93 (72±30)</td>
</tr>
<tr>
<td>Lung (µg/g wet wt.)</td>
<td>4.78±1.61 (170±69)</td>
<td>4.08±2.24 (141±104)</td>
<td>4.42±2.02 (156±88)</td>
</tr>
<tr>
<td>Spleen (µg/g wet wt.)</td>
<td>0.58±0.38 (20±9)</td>
<td>0.79±0.81 (28±24)</td>
<td>0.68±0.60 (24±18)</td>
</tr>
<tr>
<td>Brain (µg/g wet wt.)</td>
<td>1.34±0.54 (45±14)</td>
<td>1.56±0.38 (50±10)</td>
<td>1.45±0.47 (48±12)</td>
</tr>
<tr>
<td>Time to death (min)</td>
<td>9.6±2.3</td>
<td>5.4±0.9</td>
<td></td>
</tr>
</tbody>
</table>

Rats inhaled HCN produced by addition of NaCN solution (8 mg/ml or 25 mg/ml) to H$_2$SO$_4$. The numbers of animals used were 12 for either groups. For details, refer to the note to Table 1.
Inhalation study. The time to death was 9.6 min for 8.5 mg/ml group and 5.4 min for 25 mg/ml group. The mean HCN concentrations in the exposure room at 3, 5, and 8 min after exposure were 330, 375, and 364 ppm's, respectively, for the lower dose group, while those at 3 and 5 min were 1,160 and 1,200 ppm's, respectively, for the higher dose group. For either group, cyanide concentrations in the exposure chamber approximated to their maximal values 3 min after addition of NaCN. In terms of the combined data (8 mg/ml and 25 mg/ml) the lung showed the highest concentration among the tissues tested and the difference from the blood was significant. The cyanide concentrations of all the other organs were significantly lower than the blood level. Between the two concentrations used, there was no significant difference in any organs tested.

Comparisons between oral route and inhalation route. In terms of the combined data, the concentration was significantly higher in the blood, lung and liver in the per os group. The difference between the routes of administration was most marked in the liver, per os group showing three times higher value than inhalation group. As to the other organs, per os group was higher, but the difference was not significant. In order to reveal any difference, if present, of the distribution pattern between the different routes of administration, a relative value (%) was defined as a ratio of the concentration of an organ concerned to that of the blood and in terms of this parameter two administration routes were compared. Per os group was significantly higher in the liver and spleen. On the other hand, the reverse held true with the brain. With the lung there was no significant difference between the two routes.

In all the organs tested there was no significant correlation between the survival time and the relative value.

**Discussion**

In a previous paper (Yamamoto and Yamamoto 1977) we have already discussed a cause of the difference in the blood cyanide concentrations between different administration routes (per os and inhalation routes). In inhalation the absorption of cyanide into the body entirely depends upon ventilation. On the other hand, in per os route, cyanide is absorbed according to the concentration gradient, independent of ventilation which is in usual cases significantly reduced at the later stage of intoxication. Furthermore, in per os case, postmortem diffusion of cyanide from the stomach must be taken into account.

As to the concentration difference between the two routes, the liver and lung seem to be worthy of mention. The liver was the organ in which the cyanide concentration was at the greatest degree dependent on the routes of administration among the organs tested. Compared with the blood, a significantly higher value was obtained in per os route. On the other hand, when cyanide was inhaled, a lower value was recorded. The above feature of the liver can be explained as follows: In the first place, its location near the stomach is
favorable for its concentration increase by diffusion. The significant concentration difference between the two dose groups is considered to support the above view. Secondly, in inhalation its location far apart from the lung accounts for its low cyanide concentration.

The cyanide concentrations of the lung, irrespective of the administration routes, were higher than that of the blood, although in per os study the difference was not significant. Since this organ is a sole entrance to the body for cyanide when it is inhaled, it is no wonder that it showed a high cyanide concentration. Rich supply of the blood is considered to contribute to the high concentration in this organ. Contribution of the blood cyanide to the cyanide concentration of an entire organ except the central nervous system is apparent from the experiment by Ballantyne et al. (1972) on organs perfused with physiologic saline. Unfortunately, data on the lung are not included in their report.

The cyanide concentrations of the brain as well as the spleen were significantly lower than that of the blood, irrespective of the administration routes. However, comparisons based on the relative value revealed the characteristics of these two organs, which can be explained by the relationship between the location of the organ concerned and the entrance of cyanide. Cyanide concentration of the spleen seems to depend on the species examined (Ballantyne et al. 1974); in contrast to the rat and the rabbit, high concentrations of this organ have been reported in human (Sunshine and Finkle 1964).

In practical cases, in which we usually deal with bodies with much longer postmortem intervals than that in the present animal experiment, it must be taken into consideration that considerable changes in cyanide concentration and distribution must have occurred before autopsy is performed. Therefore, direct application of the present result to practical cases may be inappropriate. From the present study, however, it can be well concluded that, in cases which require determination as to whether cyanide was inhaled or ingested, at least the lung and liver must be analyzed in addition to the blood and stomach content for cyanide contents.

References