Case report

An accidental death due to Freon 22 (monochlorodifluoromethane) inhalation in a fishing vessel

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Abstract

A case of accidental Freon 22 (monochlorodifluoromethane) poisoning in a fishing vessel is reported. Forensic autopsy revealed severe pulmonary edema and congestion (left lung; 576 g, right lung; 740 g). GC–MS analysis clearly showed that the deceased inhaled Freon 22 gas prior to his death. Freon 22 concentration was 169 ± 7.0 μg/ml in the heart blood. The distribution pattern of Freon 22 in tissue samples was similar to that in previously reported cases. The brain had the highest concentration of Freon 22 followed by the spleen, liver, kidney and lung, respectively.

Histopathologically, Oil red O staining of the liver showed many small, positive red areas in the cytosol, which have been reported in other cases of Freon 22 poisoning. However, Schmorl staining revealed that most areas of Oil red O positivity were lipofuscin granules. Lipofuscin in the liver, which closely relates to aging and other cell stresses, could have a relevance to Freon 22 exposure, but further experimental studies are needed to confirm it.

Keywords: Monochlorodifluoromethane; Freon 22; Postmortem distribution; Liver; Fat droplets; Lipofuscin

1. Introduction

Monochlorodifluoromethane (Freon 22) is a chlorofluorocarbon, which has been widely used as a refrigerant for freezers, refrigerators and air conditioners [1]. It is a colorless, non-flammable gas with a slightly ethereal odor at room temperature and has a high vapor density (3.03; air = 1) [2]. According to the classification of solvent and gas toxicity by Underwriters Laboratories Inc., Freon 22 belongs to group 5a, and the toxicity is in the same class with Freon 11 (1,2-dibromotetrafluoroethane) and carbon dioxide [3]. Although Freon 22 is believed to have a low toxicity compared to other chlorofluorocarbons, fatalities caused by accidental inhalation have been reported, especially in fishing vessels [4–8].

We had a forensic autopsy case of a man who died accidentally while maintaining a freezing system in a fishing vessel. We present the details of the case and a toxicological analysis of Freon 22 in the body. The cause and manner of death are discussed based on the toxicological and histopathological findings.

2. Case history

The chief engineer and a deck-hand of a deep-sea trawler were found unconscious in their vessel at about 08:30 am. They had been working in the vessel from 07:30 that morning to prepare for the next voyage. Their colleagues rescued them. The deck hand, who was lying on the middle deck of the vessel, survived (Fig. 1B). The chief engineer who was on the bottom deck died (Fig. 1 A) at 10:22 am after hospitalization. The colleagues did not notice any strange odor in the cabins, but they nearly fainted within a minute of entering the cabin.

Three months before the fatal incident, the freezing system of the vessel had a gas-leaking accident. Although it was repaired, a further Freon gas-leak was expected because the system was too old. The police only investigated the scene the next day after ventilating the cabin space for their own safety, therefore, the oxygen and Freon 22 concentrations in the cabin at the time of the accident are not known.

3. Forensic autopsy

A judicial forensic autopsy of the chief engineer was performed about 24 h after his death. The deceased was a
38-year-old man weighing 64 kg and 174 cm tall. There was severe pulmonary edema and congestion (left lung: 576 g, right lung: 740 g). Mucosal hemorrhages were noted in most parts of the small intestine. There were no specific findings in other organs except for severe congestion. Tissues were formalin fixed and examined by HE and Schmorl stainings after an ammonia-ethanol treatment to remove formaldehyde-derived artifacts [9]. Oil red O staining of the liver tissue was also performed using cryostat sections [10]. For toxicological analysis, blood and tissue samples were collected in thick plastic bags (Unipack G-4, Seinichi Co. Ltd., Japan), vacuum-sealed and immediately stored at −30 °C until analysis.

4. Toxicological analysis

4.1. Chemicals

Standard Freon 22 gas (99.9%) was purchased from Fukuhoteisan Co., Fukuoka, Japan and used for calibration. All solvents and chemicals were of analytical grade and purchased through local suppliers. Standard Freon 22 gas was transferred into a glass vial by the water displacement method. Briefly, a glass vial was submerged in tap water and the water in the vial was displaced by the standard Freon 22 gas. The vial was then sealed with a Teflon rubber under water. The exact volume of Freon 22 was taken from the vial by using gas-tight syringes and used for calibration. The volume of Freon 22 was converted into weight according to the Avogadro's law.

4.2. Sample preparations

One milliliter of defrosted blood and 1 g each of sliced frozen tissue was sealed in a glass vial with a silicon septum. For quantitative calibrations, exact volumes of pure Freon 22 gas were transferred into sealed vials containing 1 ml of blace blood using gas-tight syringes. Each vial was heated at 50 °C in an electric aluminum block heater (Eira Co. Ltd., Tokyo, Japan) for 15 min and its 100 µL of the headspace were introduced onto the GC–MS by using a gas-tight syringe. Each sample and calibration vial was prepared in triplicate.

4.3. GC–MS conditions

GC–MS analysis was performed by Shimadzu QP-5000 (Shimadzu Co., Kyoto, Japan). The GC conditions were as follows: splitless injection mode; column, DB-WAX capillary column, 30 m × 0.25 mm i.d., 250 nm film thickness; injection port temperature, 180 °C; carrier gas, helium; flow rate, 1.2 ml/min; column temperature, 30 °C. The MS conditions were as follows: full scan mode for qualification (m/z 30–150), selected ion monitoring (SIM) mode for quantification (m/z 51); ionization, EI; interface temperature, 230 °C.

5. Results

5.1. Histological findings

HE staining of the lung showed severe congestion and edema. There were no marked findings in other organs except for congestion. Oil red O staining of the liver showed many small positive red areas in the cytosol (Fig. 2A). Schmorl staining revealed that most portions of the Oil red O staining positive areas were lypofuscin granules (Fig. 2B).

5.2. Toxicological analysis

The presence of Freon 22 in the blood and tissues was confirmed by the GC–MS analysis. Freon 22 was eluted at a retention time of 1.4 min and the obtained mass chromatogram was the same as that obtained from authentic Freon 22 (Fig. 3). No other volatile substances, including fluorocarbons, were detected in any of the samples. Linearity of the calibration curve was observed from 0 to 380 µg/ml of pure Freon 22 with a correlation coefficient of 0.995 (Fig. 4). Results of Freon 22 analysis are shown in Table 1. The concentration of Freon 22 in blood was 169 ± 7.0 µg/ml. In tissue samples, the highest Freon 22 level was observed in
6. Discussion

It is well recognized that halogenated hydrocarbons including Freon 22 depress the central nervous system and induce both cardiac arrhythmias and excessive secretion in the trachea [11–13]. These direct toxic effects of the chemical could be a cause of death. On the other hand, due to the heavy vapor density of Freon 22, it displaces air and results in insufficient oxygen concentration, which could also be a cause of death.

In the present case, toxicological analysis clearly showed that the deceased inhaled Freon 22 gas prior to his death. The autopsy findings excluded suffocation due to excessive secretions in the trachea from the causes of death. However, toxicological and histological findings could not fully elucidate the manner or the direct cause of death. First of all, as in most A. Koreeda et al. / Forensic Science International 168 (2007) 208–211

Table 1
Freon 22 concentrations in blood and tissue (µg/ml or g)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Freon 22 concentration (µg/ml or g)</th>
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<tbody>
<tr>
<td>Heart blood</td>
<td>169 ± 7.0</td>
</tr>
<tr>
<td>Brain</td>
<td>199 ± 51.2</td>
</tr>
<tr>
<td>Lung</td>
<td>60 ± 3.8</td>
</tr>
<tr>
<td>Liver</td>
<td>131 ± 4.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>143 ± 10.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>72 ± 6.2</td>
</tr>
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Fig. 4. (A) SIM (m/z 51) chromatograms of (1) heart blood, (2) standard Freon 22, (3) blanc blood. (B) A typical standard curve of Freon 22 in blood.

Fig. 2. Oil red O (A) and Schmorl (B) staining of liver tissue. Oil red O staining (A) showed various sizes of red particles in the hepatocytes. Schmorl staining (B) revealed that most of them were lipofuscin (dark blue pigments). CV; central vein.

Fig. 3. Total ion chromatogram of a heart blood sample and its mass chromatogram of a peak of RT 1.40 min (Freon 22).
previously reported cases [4–8], both Freon 22 and oxygen concentrations at the scene were unknown, and asphyxia due to low oxygen concentrations can not be confirmed as a cause of death. Secondly, autopsy findings specific to Freon 22 poisoning have not yet been reported. Granular fat droplets positive to Sudan III staining in the hepatocytes were reported in similar Freon 22 poisoning cases, but the mechanisms of the findings are controversial [4–7]. Morita et al. reported two Freon 22 poisoning cases and suspected that the fat droplets in the liver were produced by the effect of Freon 22. Other reports suggested that they were not specific to the toxicity of Freon 22 [5–7]. Kazama et al. showed in animal experiments that fine fat droplets appeared temporarily in the course of inhaling Freon 22, but it disappeared immediately after stopping the inhalation and they were different from hepatic toxicity reported in chronic exposure to chloroform and other chlorinated hydrocarbons [5].

In the present case, similar pathological findings were observed in the liver. However, most of them were positive to Schmorl staining, which is specific to lipofuscin pigment. This has not been mentioned in similar previous case reports [4–8]. Lipofuscin is located in lysosomes and contains products of the peroxidation of unsaturated fatty acids, which closely relate to aging and other cell stresses [14]. It is reported that the existence of lipofuscin in tissues could be a marker of chronic exposures to low concentration of chemical substances [15]. High Freon 22 concentration in the liver may produce a cell stress. However, experimental studies will be needed to clarify whether Freon 22 has relevance to lipofuscin appearance. The cause of mucosal hemorrhages in the small intestine was not clear. Stress reactions due to a direct effect of Freon 22 or hypoxic conditions of the body may have contributed to the lesions. Further histopathological investigations are needed to clarify it.

Freon 22 concentration in HB was 169 ± 7.0 μg/ml. It was within the ranges of concentrations reported in other fatal cases, which were from 78 to 577 μg/ml in blood samples [4–8]. Also, a distribution pattern of Freon 22 in tissues was similar to those fatal cases from the literature [4–7]. Freon 22 levels were high in blood, brain and liver; and low in lung and kidney, which does not contradict the lipophilic property of Freon 22. The high concentration of Freon 22 in blood and tissues suggested that the death was not instant. Resuscitation attempts may be partly a reason for the low concentration in the lung.

Because of their potential effects on ozone concentrations in the stratosphere, production of many chlorofluorocarbons has been stopped or scheduled to discontinue. However, they are industrially important and widely used as refrigerants, propellants, washing solvents for electronic devices and so on [1]. In order to prevent accidental deaths due to inhalation of Freon gases, alarm systems for gas leakages and oxygen concentration in work places are essential, especially for closed spaces like the fishing vessel presented here.

Acknowledgment

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References