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Forensic Toxicology

A sudden death related to 1,1-difluoroethane inhalation—A case report and brief review of the literature

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ABSTRACT

1,1-difluoroethane (DFE; HFC-152a, Freon® 152a) is used as a propellant in gas dusters, and it is sometimes misused as a recreational "drug" to induce an altered mental state. Herein we describe the forensic autopsy case of a man who may have died due to DFE inhalation. No specific external injuries were detected during the autopsy, and internal examination revealed pulmonary edema and intrapleural effusion. Gas chromatography-mass spectrometry analysis detected DFE in the decedent's heart blood. The DFE concentrations (μg/mL or g) in blood and tissue samples were 74.8 in heart blood, 137.4 in femoral blood, 58.8 in lung, 108.7 in the liver, and 89.5 in muscle. In brain and adipose the DFE levels exceeded the upper limit of quantification (150 μg/g). The DFE concentrations in the present case did not exceed those in previous cases of poisoning with DFE alone, and they were similar to those of previous cases involving combined factors. Considering the autopsy findings and the DFE concentrations in the blood and tissue samples, we concluded the cause of death was drowning due to loss of consciousness resulting from DFE inhalation. The difference between DFE concentrations in heart blood and femoral blood suggests that he may have died after he stopped inhaling DFE rather than during inhalation. While the number of poisoning deaths due to DFE in Japan cannot be reliably ascertained, the regulation of certain drugs such as nitrite esters (RUSH®) may have inadvertently resulted in some people misusing DFE, which is more accessible than illicit drugs.

1. Introduction

1,1-difluoroethane (DFE; also known as HFC-152a and Freon® 152a) is a chlorofluorocarbon substitute that is used as a propellant in aerosol sprays and gas dusters. It is a colorless, odorless, and extremely flammable gas. DFE can also cause frostbite on contact [1]. Spray propellants such as DFE, propane, and butane are sometimes misused, especially among young people in many countries, who report that inhaling them can induce euphoria and elation [2,3]. Halogenated hydrocarbons such as DFE are known to cause symptoms resembling drunkenness and depress the central nervous system [4], as well as inducing arrhythmias due to electrolyte imbalance [5]. By way of toxicity affecting the cardiovascular system, their inhalation can cause cardiac disorder and result in lethal accidents.

According to the National Poison Data System maintained by the American Association of Poison Control Center, there were 997 cases of poisoning and 18 deaths due to intentional inhalation of Freon® and other propellants in 2016 in the United States [6]. The total number of cases of DFE poisoning that have occurred in Japan is unknown, but 1–7 lethal cases have been reported per year since 2008, and most have been men aged in their 20 s to their 40 s [7]. Only a relatively small number of DFE poisoning reports have included DFE concentrations in blood and tissue samples, because quantifying DFE in such samples is problematic due to its high volatility and rapid elimination. Herein we report a forensic autopsy case of a man who may have died due to DFE inhalation. DFE concentrations in blood and tissues were determined via headspace gas chromatography-mass spectrometry (GC–MS) performed using a validated method. Possible causes of death are discussed below in comparison with previous reports. Relationships between the regulation of recreational drugs and DFE abuse in Japan are also discussed.

2. Case report

2.1. Case history

A 28-year-old man with no medical history was found dead in a short-stay hotel. He was lying in a bathtub in the right lateral decubitus position and his face was under the water, which was mixed with sexual lubricant containing vomit and red-brown mucus. Beside the bathtub there were six propellant gas cans for an airsoft gun containing DFE, a bottle of lubricant, and a dildo. The deceased had been arrested 2 years prior for using an illicit drug named RUSH®, a recreational drug consisting of alkyl nitrites. Moreover, his family had previously seen him bite the nozzle of a spray can and inhale the gas emitted from it. A judicial autopsy was performed to clarify the cause of death.

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2.2. Autopsy findings

A forensic autopsy was performed 1 day after the corpse was found. The deceased was 171 cm tall and weighed 86.8 kg. The body had decomposed and was distended. The only specific external injury was abrasion of the epidermis of the forehead. Internal examination revealed pulmonary edema (right lung 890 g, left lung 522 g) with pleural effusion (right 170 mL, left 30 mL). The lungs were overinflated, and mucus that was similar to the fluid in the bathtub dripped from the cut surface. The postmortem interval was estimated to be 2–3 days. No ethanol or other drugs were detected in the blood via GC–MS or liquid chromatography-mass spectrometry. GC–MS detected DFE in the heart blood. Blood and tissue samples were collected for subsequent toxicological analysis, and were immediately frozen then maintained at -30 °C prior to that analysis.

3. Materials and methods

3.1. Reagents

Standard DFE (0.2 mg/mL in methanol) and internal standard 1,1,1-trichloroethane (1 mg/mL in methanol) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All other materials were analytical grade and were purchased through local suppliers.

3.2. Toxicological analysis

Toxicological analysis was performed using the method described in Sasaki et al. [8], with slight modifications. Briefly, blood samples were thawed and frozen tissue samples were sliced at a thickness of approximately 0.1 cm prior to analysis. Sample vials containing 1.4 mL distilled water and either 0.1 mL blood or 0.1 g tissue were prepared then tightly sealed (n = 4). For quantitative calibrations standard vials of swine blood containing various concentrations of DFE standard methanol solution (0, 20, 50, 70, 100, and 150 μg/mL blood) were prepared. Ten microliters of 1,1,1-trichloroethane methanol solution (1 mg/mL) was added to each vial via a gas-tight syringe as an internal standard.

Headspace GC–MS analysis was performed using Agilent GC7890A, 5977MSD, and an Agilent G1888 network headspace sampler utilizing a DB-WAX column (30 m, 0.25 mm I.D., 0.25 μm film thickness) with helium as the carrier gas. The oven temperature was increased from 35 °C to 180 °C at a rate of 30 °C/min. Injection was performed using split mode (9:1). The mass spectrometer (MS) was used in electron ionization mode with an electron energy of 70 eV. In the MS procedure, scan mode was used for qualification (m/z 40–200) and selected ion monitoring mode was used for quantification (DFE m/z 65; internal standard m/z 61).

3.3. Method validation

A 5-point calibration curve was constructed by plotting the peak area ratio of DFE to the internal standard against DFE concentration, and fitting was performed via least-squares linear regression without a weighting factor. Precision (%) and accuracy (%) were determined by analyzing three control samples containing known amounts of DFE. Precision was expressed as the relative standard deviation of the concentration values. Accuracy was expressed as the percentage of differences between the observed concentrations and the expected concentrations. The limit of quantification was evaluated based on the precision data recorded below 15 %.

4. Results and discussion

Total ion current chromatography of heart blood is shown in Fig. 1a. DFE with mass spectrometry spectra shown in Fig. 1b were detected at 1.1 min. DFE quantification was successfully performed within a limit of quantification ranging from 20 to 150 μg/mL, with accuracy and precision both acceptable at < 15 % (Table 1).

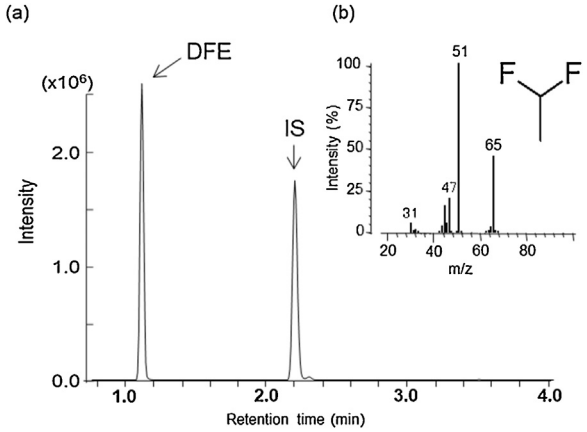

Fig. 1. Gas chromatography-mass spectrometry total ion current chromatogram of the heart blood sample (a) and mass chromatogram of the peak at 1.1 min, which identified 1,1difluoroethane (b). The peak of 1,1,1-trichloroethane as an internal standard was detected at 2.2 min.
DFE, 1,1-difluoroethane; IS, internal standard.

DFE concentrations in blood and tissue samples in the present case and in previously reported DFE poisoning cases [8–14] are shown in Table 2. In the current deceased the DFE concentration in heart blood (74.8 μg/mL) was comparatively lower than it was in previously reported poisoning cases. In tissue sample analyses DFE was detected in every tissue tested, and the results suggested that the decedent inhaled a substantial amount of DFE prior to death. The DFE concentrations in adipose tissue and brain were higher than the upper limit of quantification (150 μg/g), and concentrations in lung and muscle were lower than the concentrations in other tissues. The DFE distribution pattern was consistent with the characteristics of lipophilic chemicals, and it was similar to those of some previously reported fatal cases (cases 4 and 5 in Table 2) [12,13].

The causes of death in cases 1 [9] and 2 [10] in Table 2 were DFE poisoning combined with other factors such as trauma or intoxication with other drugs. In case 1 involving a motor vehicle, the surviving passenger reported that the decedent had been inhaling DFE while operating that motor vehicle and had had a car accident because she lost control of the car. Conversely, in cases 3–7 [8,11–14] in Table 2 there were no other factors related to death apart from DFE poisoning. In the present case the DFE concentration in heart blood was similar to those in cases 1 and 2 in Table 2, but it was substantially lower than the concentrations reported in cases 3, 6, and 7. Moreover, DFE concentrations in tissues suggested that the decedent inhaled a substantial amount of DFE prior to death. Considering the autopsy findings and the DFE concentrations in blood and tissue samples, we concluded the cause of death was drowning due to loss of consciousness resulting from DFE inhalation.

In the current case the DFE concentration in heart blood (74.8 μg/mL) was much lower than that in femoral blood (137.4 μg/mL). There are two possible explanations for this large difference in blood DFE concentrations; antemortem pharmacokinetics and/or postmortem diffusion rate. Based on the antemortem pharmacokinetics of DFE, the concentration in heart blood is considered to be higher than it is in femoral blood during the inhalation of

Table 1
Validation data for the quantitative determination of 1,1-difluoroethane (n = 3).

	Concentration (μg/mL)	Accuracy (%)	Precision (%)
DFE	20	5.7	2.5
	50	−12.5	4.1
	70	−9.1	6.8
	100	−6.9	1.5
	150	6.4	2.6

DFE, 1,1-difluoroethane.

Table 2
1,1-difluoroethane concentrations (μg/mL or g) in blood and tissue samples in the present case and previously reported lethal poisoning cases.

	Present case	Case 1 [9]	Case 2 [10]	Case 3 [11]	Case 4 [12]	Case 5 [13]	Case 6 [8]	Case 7 [14]
Heart blood	74.8	85.6*	91	122.7*			546	591
Femoral blood	137.4	29.8	145	83.5	136.3	324.9		481
Brain	> 150.0	11.7		43.8	117.5	285.5	372	
Lung	58.8			91.1	60.3		212	
Liver	108.7	27.9		92.7	87.6	161.6	634	
Adipose	> 150.0			29.8	235.7	407.2		
Muscle	89.5			80.5				
Urine						74.7	70.2	
Cause of death	Drowning	Accidental trauma	Acute DFE and diazepam intoxication	DFE intoxication	DFE intoxication	Acute DFE poisoning	Acute DFE poisoning	DFE intoxication

DFE, 1,1-difluoroethane.
* Aortic blood in those cases.

DFE. The concentration in heart blood is considered to be lower than it is in femoral blood in excretion phase because DFE absorption/excretion occurs via the lungs. In case 1 in Table 1 the DFE concentration in heart blood (85.6 μg/mL) was higher than that in femoral blood (29.8 μg/mL), and that patient is known to have died during DFE inhalation [9]. Conversely the patient in case 2 in Table 1 was resuscitated, and the DFE concentration in heart blood (91 μg/mL) was lower than that in femoral blood (145 μg/mL) [10]. Therefore, it is possible that the deceased in the present case died after he stopped inhaling DFE rather than during DFE inhalation. The difference in postmortem diffusion rate in heart blood and femoral blood may also explain the difference between the two blood DFE concentrations. The DFE concentration in femoral blood may decrease slowly due to a high level of DFE in adipose tissue surrounding the vessel, whereas the concentration in heart blood may decrease rapidly because DFE diffuses easily to air space in the heart and chest cavity. In the present case the postmortem interval was estimated to be 2–3 days, and postmortem diffusion may have influenced the DFE concentration in heart blood. Even though postmortem changes would influence DFE concentrations in blood samples, the determination of DFE in heart and femoral blood provide helpful information with regard to determining whether deceased individuals died during DFE inhalation or after they stopped inhaling DFE.

According to the National Research Institute of Police Science only 3 deaths due to DFE poisoning were reported in Japan from 2003 to 2007, whereas from 2008 to 2016 the total number of deaths was 26 [7]. Notably, new legal restrictions on nitrite esters (RUSH®) were instigated in 2008 in Japan. After the regulation of RUSH® there was an increase in recreational inhalation of nitrous oxide (laughing gas), and its distribution and use for non-medical purposes has been restricted since 2016 [15]. It is well known that spray propellants are a “gateway drug” in young people, but it is also possible that some people have shifted to misusing volatile substances such as DFE, propane, or butane instead of illicit drugs, because they are more accessible.

In conclusion, herein we have described a case of DFE poisoning and the DFE concentrations detected in blood and tissue samples. The DFE concentration in heart blood was similar to the concentrations in two previously reported cases in which other factors as well as DFE poisoning contributed to death. Determination of DFE concentrations in heart blood and femoral blood may provide helpful information with regard to determining whether deceased individuals died during DFE inhalation or after they stopped inhaling DFE. The prevention of misuse of volatile substances such as DFE is crucial for public health.

Declaration of Competing Interest

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