An unusual autopsy case in which the consumption of organophosphate insecticide was not the direct cause of death

Akina Nara (mailto:akina@tohoku-mpu.ac.jp)
Tohoku Medical and Pharmaceutical University: Tohoku Ika Yakka Daigaku

Chiho Yamada
Tohoku Medical and Pharmaceutical University: Tohoku Ika Yakka Daigaku

Manami Suyama
Tohoku Medical and Pharmaceutical University: Tohoku Ika Yakka Daigaku

Yu Kozakai
Tohoku Medical and Pharmaceutical University: Tohoku Ika Yakka Daigaku

Masaki Yoshida
Kyorin University: Kyorin Daigaku

Kaori Iwahara
Nihon Shika Daigaku Seimei Shigakubu: Nihon Shika Daigaku

Tetsuya Takagi
Tohoku Medical and Pharmaceutical University: Tohoku Ika Yakka Daigaku

Research Article

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Abstract

In acute poisoning cases involving the ingestion of organophosphate insecticides such as fenitrothion and malathion, serum cholinesterase (ChE) activity is remarkably decreased, thus representing a useful indicator of the direct cause of death. In the present case, a man in his early 70s tried to committed suicide via the oral ingestion of both fenitrothion and malathion. Fenitrothion and malathion concentrations in cardiac blood were 2.63–2.98 and 0.31–0.58 µg/mL, respectively. However, the serum ChE level was 200 IU/L, which was not considerably lower than the normal range in males (242–495 IU/L). Conversely, we confirmed a positive reaction for *Streptococcus pneumoniae* using a urinary antigen detection kit. Moreover, histopathological analysis of both the left and right lungs revealed extensive inflammatory cell infiltration into the alveolar space. The autopsy and histopathological findings indicated that the direct cause of death was severe bacterial pneumonia caused by the infection of *S. pneumoniae*. This is an unusual autopsy case in which the oral ingestion of both fenitrothion and malathion was not the direct cause of the death, and might have rapidly exacerbated respiratory decline.

Introduction

Organophosphates (OPs) have been used as agricultural insecticides globally [1–3]. In humans, the inhibition of acetylcholinesterase (AChE) by OP insecticides, such as fenitrothion and malathion, causes the accumulation of acetylcholine (ACh), and leads to the overstimulation of the muscarinic receptors that induces miosis [2, 4]. Although patients’ pupils were miotic (1–2 mm) in clinical emergency cases of OP poisonings [5, 6], in forensic autopsy cases, miosis was not always been observed owing to the elapsed time after death [7–9]. Conversely, the serum cholinesterase (ChE) activity markedly decreased in cases of fatal OP poisonings [7–9]; thus, the measurement of the deceased's serum ChE levels is used to determine the direct cause of death in acute poisoning cases caused by the ingestion of OPs [10].

In Japan, pneumonia is the fifth leading direct cause of death, and death attributable to pneumonia most commonly occurs in the elderly [11, 12]. In elderly Japanese patients, *Streptococcus pneumoniae* (*S. pneumoniae*) is the most frequently detected pathogenic species [13]. Extensive inflammatory cell infiltration into the alveolar space is a typical histopathological finding in the lungs of patients with bacterial pneumonia caused by *S. pneumoniae* [14, 15].

Here, we report an unusual autopsy case of a man in his 70s who died of bacterial pneumonia caused by *S. pneumoniae*, and the oral ingestion of both fenitrothion and malathion was believed to have hastened his death.

Case Report

One day, a man in his early 70s was diagnosed with dementia in a hospital. Following the diagnosis, he called his wife and expressed suicidal ideation, and went missing on the same day. Five days after his disappearance, he was found dead on a forest road approximately 17 km away from his house.
Autopsy Findings

On external examination, the man was 167 cm tall, and he weighed 57.2 kg. The diameters of his left and right pupils could not be measured because of severe corneal opacity. On internal examination, a volatile odor typical of organic solvents was detected from the tongue and bronchi. On the trachea and both the left and right bronchial mucosa, a milky white liquid was attached. A 275-mL of coagulated cardiac blood was noted to be dusky red in color. In the stomach, 250 mL of a greenish white muddy fluid with a volatile odor were found. The left and right lungs weighed 730 and 390 g, respectively, and the inferior lobes of both lungs were muddy white in color. The remaining organs exhibited no pathological findings highlighting the direct cause of death.

Hematoxylin–eosin (HE) staining of both the left and right lungs revealed the extensive infiltration of inflammatory cells into the alveolar space of the inferior lobes (Fig. 1A). Additionally, detachment of the bronchiolar mucosal epithelial cells was observed in the lungs (Fig. 1B).

The serum ChE level was 200 IU/L (normal range in males: 242–495 IU/L). For the detection of S. pneumoniae antigens in urine to diagnose S. pneumoniae infection, an IMMUNOCATCH™ S. pneumoniae rapid diagnostic test kit (Eiken Chemical Co., Ltd, Tokyo, Japan) was used, and a positive result was obtained. The alcohol concentration in the cardiac blood and urine was <0.1 mg/mL, as determined by gas chromatography. Drug screening using cardiac blood was performed with the LC/MS/MS Rapid Toxicology Screening System Ver. 3 (Shimadzu, Kyoto, Japan) and the results revealed the presence of caffeine, fenitrothion, and malathion.

Materials And Methods

Chemicals and Reagents

Fenitrothion, fenthion, and malathion were obtained from Sigma–Aldrich (Tokyo, Japan). Acetonitrile, ammonium formate, formic acid (abt. 99%), methanol, and ultrapure water were all analytical grade, and they were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). A methanolic solution of fenthion was used as the internal standard (IS) for both fenitrothion and malathion. Stock solutions of fenitrothion, fenthion, and malathion (mg/mL) were prepared in methanol.

Sample Preparation

For drug analyses at the time of autopsy, whole blood was obtained from both the left and the right cardiac chambers. Blank whole human blood, which was purchased from KAC Co., Ltd. (Kyoto, Japan), was screened for fenitrothion, fenthion, and malathion, none of which was detected in the samples.

Calibration curves were prepared by spiking whole blood (100 µL) with appropriate volumes of the previously mentioned working standard solutions to produce calibration curve points equivalent to 0.2, 0.5, 1, 5, 10, and 100 µg/mL for fenitrothion and to 0.0005, 0.005, 0.01, 0.05, 0.1, and 0.5 µg/mL for malathion. Quality control (QC) samples at 0.2 (lower limit of quantification; LLOQ), 0.4 (low), 2
medium), and 50 (high) µg/mL for fenitrothion were prepared in bulk by spiking the appropriate working standard solutions into whole blood (100 µL). Malathion QC samples were also prepared at 0.0005 (LLOQ), 0.001 (low), 0.02 (medium), and 0.2 (high) µg/mL. All samples were extracted using a Micro Volume QuEChERS Kit for LC/MS (Forensic) (Shimadzu). The extraction procedure for QC, calibration curve, and autopsy samples were prepared as described previously [16].

**LC-MS/MS conditions**

Qualitative and quantitative analyses were performed using a Nexera X2 HPLC system coupled with an LCMS-8045 triple quadrupole mass spectrometer (Shimadzu). Chromatographic separation was achieved using a Kinetex® XB-C18 column (100 × 2.1 mm i.d.; particle size, 2.6 µm; Phenomenex, Torrance, CA, USA) with a Security Guard ULTRA cartridge system (UHPLC C18 for 2.1 mm ID column; Phenomenex) maintained at 40°C. The mobile phases consisted of 10 mM ammonium formate with 0.1% formic acid in water (A) and in methanol (B). The flow rate and the elution gradient were conducted as described previously [16]. The mass spectrometer was operated on the positive mode with an electrospray ionization interface. The ionization source conditions were conducted as described previously [16]. The multiple reaction monitoring (MRM) mode was used to detect analytes. Two product ions (m/z), namely a quantifier and a qualifier, were monitored for each compound in the MRM transitions (Table 1). Both the product ions and collision energy were optimized via the post-column infusion of each compound's methanolic solution (Table 1). Labsolutions Insight Ver. 3.10 SP1 software (Shimadzu) was used for the quantitative analysis of all data.

**Method Validation**

The limit of detection (LOD) and LLOQ, which corresponded to signal/noise ratios of ≥ 3 and ≥ 10, respectively, were calculated as the concentrations of analytes in QC samples. The recoveries of fenitrothion or malathion were determined by comparing the peak area of fenitrothion or malathion extracted from the spiked blood samples with the mean peak area of the recovery standards. The matrix effects of fenitrothion or malathion were determined by comparing the peak area of the recovery standard with the mean peak area of the standard solution at the same concentration. To determine the accuracy and precision, QC samples at each concentration of the analytes were analyzed over 3 days. The percentage deviations of the means from the true values were determined as the relative error and relative standard deviation and served as measures of the method's accuracy and precision.

**Results**

As shown in Table 2, all the results obtained with this method using whole blood were linear and sensitive for each analyte. Additionally, validated data (Tables S1 and S2) met the criteria indicated in the US Food and Drug Administration guidelines [17]. Table 3 summarizes the quantitative results for fenitrothion, and malathion, which were detected in left and right cardiac blood from the autopsy.

**Discussion**
Generally, both fenitrothion and malathion are rapidly metabolized, and their elimination half-lives in blood are 0.8–4.5 h and 3–6 h, respectively [18, 19]. In addition, 10 µg/mL concentration of fenitrothion or malathion decreased by >25% or 100%, respectively, after 24 h at room temperature [20]. Therefore, in forensic autopsy cases of fenitrothion or malathion poisoning, the detected blood concentrations of these compounds may be lower than the ingested ante-mortem blood concentrations because a certain period has passed since death. Several previously reported fenitrothion or malathion fatal poisoning cases based on cadaveric blood or serum collected from the femoral vein, or the cardiac chambers [21–25] were not presented (Table 3). Therefore, it was difficult to determine the fatal cardiac blood concentrations of fenitrothion or malathion. However, the cardiac blood fenitrothion concentrations in our case was ranged of 2.63–2.98 µg/mL. In view of the time elapsed since his death, we thought that these values were within the range of fenitrothion intoxication concentrations. Conversely, the cardiac blood malathion concentrations were relatively low compared with the findings in other fatal malathion poisoning cases (Table 3). In our case, the outside temperature of the area in which he was found was 24.5°C; thus, malathion might have decomposed after his death.

In humans, butyrylcholinesterase (BuChE), also known as serum ChE, is produced is secreted into serum [10, 26]. Acute poisoning of fenitrothion or malathion in humans is often fatal and life-threatening, and the serum ChE activities were remarkably reduced to a few % of the normal values [18, 21]. Cadaveric ChE levels based on blood samples collected within 24 h after death were slightly decreased [27]. However, in fatal OP poisoning cases, serum ChE levels were decreased considerably below the normal range [7–9]. In our case, the serum ChE level was 200 IU/L, which was not reduced compared with that in previous reported cases of OPs acute poisoning. Serum ChE levels in males gradually decrease with age, ranging 200–450 IU/L in 70-year-olds [28]. Considering the age of the studied subject, this finding appeared to reflect age-related declines in serum ChE activity rather than OP-related decreases.

In Japan, the causative microorganisms of pneumonia in the elderly are more diverse than those in the young [14, 29]. As a parameter indicating the severity of pneumonia, lung weight on autopsy has been considered useful [30]. In the present case, the left lung weight on autopsy was 730 g, which was more obviously weighted than that of non-pneumonia. To identify the causative pathogens in fatal autopsy cases of pneumonia, histopathological examination using lung tissue can identify specific pathogens and corroborate the microbiological diagnosis [31]. In autopsy cases of patients who died of pneumonia, the inferior lobes of the left and right lungs were the most common sites of pneumonia [32]. As microscopic findings in the patients with pneumococcal pneumonia, intra-alveolar fibrinous exudates with neutrophils and mononuclear cells and marked capillary congestion have been observed [15]. We observed white turbid inferior lobes in both lungs during the autopsy. Also, the remarkable inflammatory cell infiltration, distended capillaries, and congestion with prominent neutrophilic infiltration and erythrocytes were found within the alveolar space of both lung lobes. Because the presence of desquamated bronchiolar mucosal epithelial cells in the lungs on histopathology was observed (Fig. 1B), it was believed to reflect the oral ingestion of fenitrothion and malathion, and these compounds were aspirated intratracheally. In OP poisoning, the impairment of the diaphragm and thoracic skeletal muscles cause respiratory paralysis, and high ACh concentrations in the central nervous system cause
respiratory depression [2]. Therefore, in our case, it may be rapidly exacerbated respiratory decline following the oral ingestion of fenitrothion and malathion.

*S. pneumoniae* is the most common causative microorganism of community-acquired pneumonia (CAP) in the elderly [13, 15, 29, 30]. The diagnosis of pneumonia caused by *S. pneumoniae* is traditionally obtained through culture-based investigations; however, *S. pneumoniae* is difficult to isolate, and bacteremic pneumococcal pneumonia comprises only one-fourth of all cases of CAP [32]. For these reasons, an *S. pneumoniae* urinary antigen detection kit is recommended for identifying the causative agent in patients with CAP [32, 33]. The IMMUNOCATCH™ *S. pneumoniae* pneumococcal urinary antigen test is a useful tool for the qualitative detection of *S. pneumoniae* capsular antigen [32]. We confirmed a positive reaction for *S. pneumoniae* with this kit using urine collected at autopsy and identified *S. pneumoniae* as the causative agent of bacterial pneumonia.

In conclusion, we determined that the direct cause of death in the present case was severe bacterial pneumonia caused by *S. pneumoniae* infection, and the oral ingestion of both fenitrothion and malathion was further and rapidly exacerbated respiratory function, hastening death. This unusual case provides that organophosphorus pesticide intoxication was not necessarily the direct cause of death.

**Key Points**

1. 1. Acute poisoning associated with the ingestion of fenitrothion or malathion is typified by remarkable reductions in serum cholinesterase (ChE) activity.

2. 2. *Streptococcus pneumoniae* is the most frequently detected pathogenic species of community-acquired pneumonia in elderly Japanese patients.

3. 3. In our case, a man in his 70s died of bacterial pneumonia caused by *Streptococcus pneumoniae*, and the oral ingestion of both fenitrothion and malathion was considered to have hastened his death.

4. 4. The serum ChE level in this case appeared to reflect age-related declines in serum ChE activity rather than organophosphate-related decreases.

**Declarations**

**Ethics declarations:** The authors declare that they have no conflict of interest.

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**Informed consent:** The article does not include participants from whom informed consent was required.

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**Tables**

Due to technical limitations, the tables are only available as a download in the supplemental files section.