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HUMAN AND EXPERIMENTAL TOXICOLOGY

In consideration of the publication in the above journal Human and Experimental Toxicology the contribution entitled: "Severe Fenthion Intoxications Due to Ingestion and Inhalation With Survival Outcome"

by (all authors’ names): ...........
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Case report
Severe fenthion intoxications due to ingestion and inhalation with survival outcome

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Two cases of severe fenthion intoxication are presented. The first is a case of a psychiatric patient who attempted suicide with ingestion of the compound, and the second case was of a child exposed to the chemical agent by air spraying. Both patients were treated in the intensive care unit with atropine and pralidoxime and finally survived. Fenthion blood levels on admission were 2.7 and 0.95 μg/mL, respectively. Different concentrations of pralidoxime were added to the first patient’s poisoned serum in order to assess in vitro the effect of pralidoxime on cholinesterase reactivation. The clinical and toxicological data of the poisonings are discussed, as well as the potential therapeutic use of pralidoxime in organophosphate intoxication. Human & Experimental Toxicology (2002) 21.

Key words: fenthion; ingestion; inhalation; mass spectrometry; poisoning; pralidoxime

Introduction

Organic insecticide poisoning remains one of the major health issues in both developing and developed communities.1 A great proportion of acute poisoning cases are caused by exposure to pesticides, especially organophosphates and carbamates.2,3 In Crete, many different pesticides are currently used in agriculture in order to raise crop quality. Due to the extensive use of these agents, there are many reported cases of intoxication caused either by accident or on purpose.4–8

The mechanism of action of organophosphates is based on inhibition of the acetyl-cholinesterase enzyme. Three main types of associated neurotoxicity have been demonstrated: acute cholinergic crisis, delayed neurotoxicity and intermediate syndrome.5

This study presents the clinical and laboratory data of two cases of acute poisoning caused by the organophosphate agent, fenthion [0,0-dimethyl-0(4-methyl mercapto-3 methyl phenyl)phosphorothioate]. The first case is a suicide attempt performed by a psychiatric patient, and the second is the case of a child who was exposed to the chemical agent by air spraying.

These cases were selected because of their being interesting, since both patients had long treatment in the Intensive Care Unit with atropine and pralidoxime and finally survived. The therapeutic use of pralidoxime is discussed based also on the results of the in vitro tests that were carried out during the hospitalization of the first patient.

Case history 1

A 67-year-old male was presented to the Emergency Room with vomiting, perspiration and salivation, 1 h after ingestion of an unknown quantity of fenthion. The patient suffered from chronic psychosis and had a history of numerous admissions in psychiatric wards. While under treatment with biperiden, chlorpromazine, thioridazine and amitriptyline, he attempted to commit suicide through ingestion of the organophosphate agent. Body temperature was 36.2°C, heart rate was 78/min and blood pressure was 122/64 mm Hg. Physical examination revealed pinpointed pupils, reduced muscle tone and tendon reflexes.

The patient was admitted to the Intensive Care Unit with respiratory failure and was placed on mechanical ventilation (Dräger IPPV-PC). The initial treatment...
consisted of gastric lavage with H2O and activated charcoal (for the first 24 h after admission), enemas with lactulose and skin washing. Moreover, 2 mg of atropine was administered within 15 min and subsequently continued as infusion of 1 mg/h. Pralidoxime mesylate (Contrathion®) was also administered in noncontinuous infusion of 4 g/24 h. The rest of the therapy included diuretics, fluids and electrolytes. Upon admission, the laboratory data were as follows: FIO2 0.45, pO2 152 mm Hg, pCO2 34 mm Hg (IPPV ventilation), [HCO3−] 21 mEq/L, hematocrit (Hct) 40.7%, hemoglobin (Hb) 13.8 g/dL, white blood cell (WBC) count 17500 mm−3, prothrombin time (PT) 12.9 s, activated partial thromboplastin time (aPTT) 25.4 s, serum potassium ([K+] 4.3 mEq/L, serum sodium ([Na+] 136 mEq/L, serum creatinine ([Cr]) 0.9 mg/dL, serum chloride ([Cl−]) 102 mEq/L, serum calcium ([Ca++]) 9.2 mg/dL, serum glucose ([gluc]) 115 mg/dL, alanine aminotransferase (ALT) 17 U/L, aspartate aminotransferase (AST) 10 U/L, serum potassium ([K+] 4.3 mEq/L, serum sodium ([Na+] 136 mEq/L, serum creatinine ([Cr]) 0.9 mg/dL, serum chloride ([Cl−]) 102 mEq/L, serum calcium ([Ca++]) 9.2 mg/dL, serum glucose ([gluc]) 115 mg/dL, alanine aminotransferase (ALT) 17 U/L, aspartate aminotransferase (AST) 10 U/L, γ-glutaminate transference (γ-GT) 21 U/L, creatinine kinase (CK) 134 U/L, serum lactate dehydrogenase (LDH) 212 U/L and serum alkaline phosphatase (ALP) 79 U/L. Samples of blood and urine were sent for toxicological analysis. Fenticon blood and urine levels on admission were 2.7 and 0.5 μg/mL respectively, with a serum acetyl-cholinesterase activity on admission was 124 U/L. Fenthion was detected in blood (0.95 μg/dL on admission) and urine, but not in stomach content. On the fifth day, the patient regained self-respiration and a Venturi mask 35% O2 was placed. The child was discharged from the hospital 5 days later.

### Case history 2

A 13-year-old child, male, was engaged with a group of adults spraying their fields with fenthion for 7 days. On the last day, he complained of fatigue and had a fainting episode half an hour after taking a bath. Upon arrival, the child presented with symptoms of nausea, abdominal pain and vomiting. There were no signs of peritonitis or meningitis. Blood pressure was 130/80 mm Hg, heart rate was 102/min and body temperature was 36.2°C. Shortly afterwards, the child experienced a seizure episode, developed respiratory distress and was admitted to the Intensive Care Unit.

Mechanical ventilation was initiated (Dräger IPPV-PC), and based on the clinical signs and the relatives’ statement, great suspicion of organophosphate poisoning was established. A gastric lavage was performed with activated charcoal and the skin was washed. The patient was also administered atropine (1-mg bolus and then as infusion of 0.5 mg/h) and pralidoxime (infusion of 1.5 g/24 h).

The laboratory results on admission were: FIO2 0.37, pO2 102 mm Hg, pCO2 36 mm Hg, [HCO3−] 22 mEq/L (IPPV ventilation), Hct 36.5%, Hb 13.2 g/dL, WBC 7390 mm−3, PT 11.5 s, aPTT 16.9 s, [Na+] 138 mEq/L, [K+] 3.6 mEq/L, [Alb] 4.4 g/dL, BUN 17 mg/dL, Glucose 130 mg/dL, Glutamic Oxaloacetic Transaminase (AST) 17 U/L, Aspartate Amino Transferase (ALT) 12 U/L, AST 15 U/L, γ-GT 11 U/L, CK 371 U/L, LDH 706 U/L and ALP 122 U/L.

The child was evaluated daily for the severity of his condition (APACHE II and GCS score) and samples of blood, urine and stomach contents were sent for toxicological analysis. The serum cholinesterase activity on admission was 124 U/L. Fenthion was detected in blood (0.95 μg/dL on admission) and urine, but not in stomach content. On the fifth day, the patient regained self-respiration and a Venturi mask 35% O2 was placed. The child was discharged from the Intensive Care Unit 4 days later.

### Methods

#### Chemicals

Fenthion, diazinon and dimethoate were obtained from Riedel-de Haen and Promochem (Postfach, Wesel, Germany). Chloroform, n-hexane, acetone and dichloromethane were analytical grade obtained from Lab-Scan (Stillorgen Industrial Park, Dublin, Ireland), as well as methanol (HPLC grade). Merck (Darmstadt, Germany) provided acetonitrile.

#### Sampling and general toxicological screening

The biosamples from the poisoned patients were gastric fluids (Levine), blood and urine. General toxicological screening included head space GC for volatiles, immunoassays techniques (Abbott TDx and ADx) and automated liquid chromatography REMEDI HS Drug Profiling System (Bio-Rad Laboratories, USA) for basic, amphoteric and acidic drugs and color tests for chemicals. Acetyl-cholinesterase levels in blood and serum were also monitored.
diazinon (MS) and dichlorvos (NPD) were used. For recovery studies and calibration curves 0, 0.25, 0.5, 1, 2, 4, 8 and 16 ng/mL were prepared. For recovery studies and calibration curves diazinon (MS) and dichlorvos (NPD) were used.

Preparation of standard curves

The injection volume was 1 μL. The elution solvent was chloroform:methanol and 2 mL of distilled water. The sample was separated and evaporated to remove acetone. The remaining water solution was either 1) filtered and then extracted twice by 6 mL of solvent system hexane:dichloromethane (2:1), or 2) reextracted using Techelut SPE column, C18 (200 mg) cartridges. The conditioning of cartridges was done with 2 mL of methanol and 2 mL of distilled water. The sample was eluted from the column with a flow rate of 1 mL/min. The elution solvent was chloroform:methanol (9:1). The extract (1 or 2) was dried using sodium sulfate, filtered and then evaporated to dryness. The residue was reconstituted in 0.1 mL of methanol containing internal standard and used for GC-NPD quantification and EI MS confirmation. The injected volume was 1 μL.

Preparation of blood samples for chromatography

Five milliliters of blood sample and 20 mL of acetone were homogenized with the help of an Ultra-Turrux homogenizer (10,000 rpm). The homogenate was centrifuged (3000 rpm, 10 min) and then the supernatant was separated and evaporated to remove acetone. The remaining water solution was either 1) filtered and then extracted twice by 6 mL of solvent system hexane:dichloromethane (2:1), or 2) reextracted using Techelut SPE column, C18 (200 mg) cartridges. The conditioning of cartridges was done with 2 mL of methanol and 2 mL of distilled water. The sample was eluted from the column with a flow rate of 1 mL/min. The elution solvent was chloroform:methanol (9:1). The extract (1 or 2) was dried using sodium sulfate, filtered and then evaporated to dryness. The residue was reconstituted in 0.1 mL of methanol containing internal standard and used for GC-NPD quantification and EI MS confirmation. The injected volume was 1 μL.

Serum acetyl-cholinesterase measurements

A kit from Boehringer Mannheim (Mannheim, Germany) was used. Blood samples were centrifuged immediately in order to obtain serum. The contents of bottle 1 (butyrylthiocholine iodide, phosphate buffer, pH 7.7, and dithiobis; nitrobenzoate in granular form) were dissolved in 3 mL of redistilled water and brought to 37°C; 20 μL of serum was added and the contents were mixed and poured into a cuvette within 30 s. Using a stopwatch, the time (Dt) in seconds required for an absorbance increase of DA 0.100 at 405 nm was measured. Each measurement was repeated three times and a mean value for Dt was obtained. This was used to calculate the activity of acetyl-cholinesterase in units per liter. Reference values range between 3.500 and 8.500 U/L. Absorbance measurements were performed against distilled water.

Preparation of blood samples for chromatography

For the first test, 10 μg/mL Contrathion was added to the first patient’s serum on admission and the activity of cholinesterase was measured after 30, 60, 90, 120 and 150 min. The second test measured the activity of serum cholinesterase after the introduction of pralidoxime.

In vitro evaluation of the cholinesterase activity

For the first test, 10 μg/mL Contrathion® was added to the first patient’s serum on admission and the activity of cholinesterase was measured after 30, 60, 90, 120 and 150 min. The second test measured the activity of serum cholinesterase after the introduction of pralidoxime.

Instrumental method

The quantitative determinations were performed on a gas chromatograph model Carlo Erba, Vega Series 6000, with nitrogen–phosphorus detector (NPD) connected to Varian Data system 4400. The column that was used was a DB-5, 30 m x 0.32 mm, 0.25-μm film thickness capillary column. Helium (99.9999%) was used as the carrier gas with flow rate of 2.4 mL/min. The injector and detector temperatures were 270 and 300°C, respectively. The column initial temperature was 180°C for 3 min, then increased to 230°C at 10°C/min and was held at 230°C for 35 min, increased to temperature of 270°C with step 5°C/min and maintained at 270°C for 5 min. Under these conditions, the retention time of dichlorvos was 7.35 min and that of fenthion was 33.56 min. The recovery for fenthion was 78% (y=34.052x−3949, r²=0.9946) and detection limits were 1 ng/mL.

Positive ion chemical ionization mass spectrometric confirmatory analysis was performed on a Finnigan Matt GCQ system equipped with a DB-5 (30 m x 0.25 mm x 0.25 μm) capillary column. Pure helium (99.9999%) with a velocity of 20 cm/s was used as a carrier gas. One microliter of the solution was injected into the system in the splitless mode and was analyzed under the following conditions: The column temperature was initially held at 180°C for 3 min, raised to 230°C at 10°C/min, held for 2 min, and was finally raised to 300°C, at 20°C/min, where it remained stable for 2 min. The injector temperature was 270°C. The transfer line temperature was set at 275°C. The mass spectrometer acquisition parameters were: ion source 200°C, electron impact ionization 70 eV and electron multiplier voltage 1700 V. The mass spectrometer was operated at the selected ion monitoring mode and programmed for the detection of m/z 179 and 304 for diazinon (internal standard), and 278 and 125 for fenthion. Under these conditions, diazinon eluted at time t=6.30 min, and fenthion at time t=8.88 min.

<table>
<thead>
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<th>Pralidoxime (μg/mL)</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>16</th>
<th>32</th>
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<tr>
<td>Cholinesterase activity (U/L)</td>
<td>250±2</td>
<td>287±2</td>
<td>296±3</td>
<td>316±2</td>
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Table 1  Serum cholinesterase activity (±SD) of the patient who ingested fenthion (blood sample drawn on admission) versus time after the addition of 10 μg/mL pralidoxime

Table 2  Cholinesterase activity (±SD) measured in the serum of the patient who ingested fenthion (blood sample drawn on admission) after 30 min of incubation with different concentrations of pralidoxime
of pralidoxime in different concentrations. More specifically, 0, 4, 8, 16 and 32 µg/mL pralidoxime were introduced to the patient's serum and the enzyme activity was measured after incubation of 30 min at temperature of 30°C, according to the guidelines of the Boehringer Ingelheim Kit.

**Results**

What is concluded from the first *in vitro* test is that cholinesterase activity was stabilized nearly 90 min after the introduction of 10 µg/mL pralidoxime. At that time, the activity of serum cholinesterase was 417 U/L. The highest rate of increase in enzyme activity was seen in the first 30 min of incubation (Table 1). The second test showed that cholinesterase reactivation was greatest after incubation of 10 µg/mL pralidoxime. Higher concentrations of the oxime offered no notable advantage (Table 2).

The toxicological data of the poisoning cases are given in Figures 1 and 2. Serum cholinesterase activity reached 95% of the lower reference value within 24 days for the first patient and 5 days for the second. Fenthion blood levels over time are also presented.

**Discussion**

This study reported on two cases of fenthion intoxication with survival outcome. The first one was a typical suicide attempt with ingestion of the compound, whereas the second refers to a child who was poisoned due to inhalation and transdermal absorption of fenthion. The child’s intoxication cannot be attributed to ingestion of the toxic agent based on the gastric fluids analysis. Although in most of the published cases of organophosphate poisoning the toxic compound was ingested,4,5,9-12 there are also a few cases where the intoxication was due to inhalation13 or dermal exposure.14-16 Other uncommon ways of poisoning include subcutaneous17 and intravenous injection18 of the compound in the context of a suicide attempt.

The adult patient presented with typical muscarinic and nicotinic signs and symptoms of organophosphate poisoning, in contrast to the second case where abdominal pain and vomiting were the most outstanding symptoms, next to the fainting episode the child had. This is in accordance with the reported difference in clinical presentation of cholinesterase inhibitor intoxication between adults and young children.13 In children, the most common signs are those of CNS depression and hypotonia, and so the absence of classic muscarinic effects should not exclude the possibility of cholinesterase inhibitor poisoning.

The first patient presented symptoms of intoxication shortly after the ingestion of fenthion. The child’s poisoning, on the contrary, appeared only after a week of daily air exposure to fenthion. Although organophosphates are quickly absorbed through the skin and mucous membranes,15 signs and symptoms of acute toxicity after dermal exposure appear much later compared to those after ingestion.14 Moreover, fenthion is known to have a highly cumulative effect, probably due to its high solubility in fat tissue.19 One may, therefore, assume that the child was being exposed to subtoxic levels of the organophosphate agent throughout the week, resulting in clinical poisoning on the seventh day of spraying. The presenting blood levels of fenthion in our cases were lower than those previously reported, which range between 2.9 and 4.8 µg/mL.4,5,20 The concentrations of fenthion in proximal segments of total hair samples taken from the patients 15 and 20 days after the admission to the intensive care unit were 0.1–0.3 and 0.6–0.9 ppm for the first and the second patient, respectively.16,21

In both cases, initial treatment consisted of gastric lavage, activated charcoal and administration of
atropine and pralidoxime. Hemoperfusion was not initiated since previous studies have concluded that only minimal amounts of the lipophilic insecticides are removed. A recent report suggests that in cases of severe organophosphate poisoning complicated with serious pulmonary or cardiovascular dysfunction, percutaneous cardiopulmonary support may prove to be beneficial.

Oximes are considered to be cholinesterase reacti-

vators and thus specific therapeutic agents in cases of organophosphate poisoning. There are some reports, however, questioning their effect and necessity in treatment of organophosphate intoxication. According to the literature, oximes may be beneficial when administered in low doses early in the course of the poisoning. Despite cases of little effect on plasma cholinesterase, oximes have also been shown to reduce diaphragmatic muscle necrosis in experimental organophosphate intoxication and should, therefore, be administered in all cases of organophosphate poisoning. In cases of diazinon toxicosis in fowl, atropine alone was partially effective, whereas 2-pralidoxime alone or in combination with atropine was extremely efficacious. Factors affecting the effectiveness of oximes include the specific type of organophosphate causing the intoxication, the time of initiation of the treatment and serum cholinesterase levels the following days.

Based on pharmacokinetic data, the assumed therapeutic concentration of pralidoxime is 4 µg/mL. In our in vitro tests, pralidoxime did reverse the effect of fenitrothion on patient’s serum cholinesterase and the cholinesterase reactivation was found to be greatest within the first 30 min of incubation. We also noticed a substantial increase in cholinesterase activity after the addition of 4, 8 or 10 µg/mL pralidoxime, whereas concentrations of 16 or 32 µg/mL offered no additional effect. In view of the previous findings, one might argue that the dosage of pralidoxime should be adjusted in order to achieve serum pralidoxime levels that range from 4 to 10 µg/mL.

In the course of hospitalization, none of the patients developed the intermediate syndrome of organophosphate poisoning, which is regarded to be common in fenitrothion intoxication cases. This is characterized by acute respiratory paresis, weakness in the territory of multiple motor cranial nerves, weakness of neck flexor and proximal limb muscles and depressed tendon reflexes. Neither patient has also developed delayed neurotoxicity so far. Despite the severity of the poisonings, both patients survived.

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