Laboratory Assessment of Poisoning with a Carbamate Insecticide

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We discuss a case of a 17-year-old white male who intentionally ingested a tick and flea insecticide and was admitted to the emergency room unconscious, with signs and symptoms of cholinergic toxicity. Capillary gas chromatography and electron-impact mass fragmentographic analysis of the patient's urine and serum demonstrated the presence of polyethylene glycol and propoxur (α-isopropoxyphenyl N-methylcarbamate), a carbamate-based cholinesterase inhibitor commonly used in insecticides. The patient fully recovered, but only after a complicated hospital course. We also discuss the laboratory assessment and clinical treatment of poisoning with carbamate and organophosphate insecticides.

The four major classes of insecticides in common use are the nictotines, organochlorines, pyrethrins, and cholinesterase (EC 3.1.1.8) inhibitors. Since the 1972 Environmental Protection Agency ban of DDT (chlorophenothane), an organochlorine, the cholinesterase inhibitors have been increasingly used as insecticides, and reports of poisonings by cholinesterase inhibitors have increased. In a 1984 survey of poison-control centers in the U.S.A., a total of 20,726 incidents of toxic exposures to cholinesterase inhibitors was recorded (1).

The two major types of cholinesterase inhibitors are the organophosphates and the carbamates. The organophosphates have found more widespread use and are more often implicated in acute poisonings by insecticides. However, the carbamates have recently been recognized as an important cause for acute poisonings (2). In 1984, 3033 toxic exposures to carbamates were recorded (1), and in 1986, the largest recorded outbreak in the United States of a food-borne insecticide-associated illness was attributed to a carbamate (3).

We report here a case of intentional ingestion of propoxur, a commonly used carbamate-based pet insecticide, and we discuss the clinical and laboratory evaluation as well as treatment of poisonings with carbamates and organophosphates.

Case Report

A previously healthy 17-year-old white male was admitted to the emergency room with a presumptive diagnosis of respiratory insufficiency and a seizure disorder. A family member discovered the patient unconscious on the floor, exhibiting labored breathing and myoclonic jerks of all extremities, only an hour after the patient last appeared in his usual state of health. The family denied that the patient was on any medications or that there was any history of ethanol or illicit drug use.

The patient was given four ampules (1.6 mg) of naloxone and one ampule (25 g) of 50% dextrose, with no response. On physical examination, the patient was unresponsive and had no outward signs of physical trauma. The vital signs were remarkable for a blood pressure of 160/80 mmHg, a pulse rate of 118/min, and a rectal temperature of 38°C. On ophthalmological examination, bilateral pinpoint pupils that were minimally reactive to light were observed. Increased oral secretions were noted during the examination of the mouth. The lungs were clear to auscultation, but the patient had very small respiratory movements. He had a regular heart rhythm, but with frequent premature ventricular contractions. The abdominal and rectal examination did not reveal any abnormalities except that the patient was noted to be incontinent, with a watery stool. The extremities appeared cyanotic and there was increased perspiration. During the neurologic examination, the patient was unresponsive to pain, had eyes deviated to the right with a negative oculocephalic reflex, showed no gag or deep-tendon reflexes, had downturned toes during the planter reflex, and exhibited myoclonic jerks of all extremities.

Analysis of the patient's blood gases revealed a severe respiratory acidosis with a pH of 7.02, a $P_{CO_2}$ of 96 mmHg (12.8 kPa), a $P_O_2$ of 35 mmHg (4.66 kPa), and a carbon dioxide content of 25 mmol/L.

Analysis of the patient's serum showed a [Na+] of 139 mmol/L, a [K+] of 4.1 mmol/L, a [Cl -] of 99 mmol/L, a [HCO_3 -] of 23 mmol/L, a urea nitrogen concentration of 110 mg/L (1.6 mmol of urea per liter), a creatinine concentration of 11 mg/L (97 μmol/L), a glucose concentration of 2.31 g/L (12.8 mmol/L), and an increased calculated anion gap of 17 mmol/L. The serum osmolality, measured about 2 h after admission, was 298 mOsm per kilogram of water, with a calculated osmolar gap (4) of 5 mOsm/kg. A dipstick test of the patient's urine showed a pH of 5.0 and was negative for ketones.

The patient was intubated soon after admission and was artificially respirated with 100% oxygen. He developed a grand-mal seizure, which was controlled with lorazepam and phenytoin. Analysis of a sample of cerebrospinal fluid and a computerized tomographic scan of the patient's head showed no evidence of any central nervous system abnormalities.

An initial toxicologic screen by thin-layer chromatographic analysis of the patient's urine was positive only for an unidentified diffuse smear starting at the origin of the thin-layer plate. Analyses of serum for volatile substances by head-space gas chromatography and for ethylene glycol by gas chromatography were negative. A brown oily residue was found after evaporation of an organic extract of the

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patient's urine. Gas chromatography–mass spectroscopy (GC-MS) analysis of the residue revealed the presence of polyethylene glycol and 2-isopropoxyphenol, a metabolite of propoxur (2-isopropoxyphenyl N-methylcarbamate).

Approximately 8 h after admission, the patient spontaneously awoke and denied any drug or toxin ingestion. The patient's family, subsequently, discovered a partially empty bottle of tick and flea insecticide for dogs in the patient's bedroom and disclosed that the patient had a history of depression and a previous suicide attempt with aspirin. GC-MS analysis of a sample of the insecticide matched the spectrum found for the substance extracted from the patient's urine. A retrospective analysis of a serum sample obtained from the patient on the day of admission showed a depressed pseudocholinesterase (EC 3.1.1.7) activity of 1260 U/L (reference interval: 2436–4872 U/L), whereas analysis of a serum sample obtained three days after admission showed a normal value, 3430 U/L.

The patient responded well to supportive therapy alone and did not receive any specific therapy for the insecticide poisoning because of a quick resolution of symptoms. The patient was fully recovered by the fourth day after admission and was discharged under psychiatric care.

Materials and Methods

The urine drug screen was done with the Toxi Lab Kit as described by the manufacturer (Marion Laboratories, Kansas City, MO 64114). The serum assay for volatile substances was done by head-space gas chromatography (6). Serum ethylene glycol was assayed by gas chromatography with use of a Porapak Q column (6). Serum pseudocholinesterase was assayed colorimetrically with use of propionylthiocholine and dithiobis(2-nitrobenzoic acid) (7).

We extracted the patient's urine and serum for GC-MS analysis, using the Toxi Tube A dichloromethane–bicarbonate solvent extraction system at pH 9.0. We mixed 2 mL of either urine or serum, to which were added 20 μL of internal standard solution (0.25 mg of cyheptamide per milliliter of methanol), with 3 mL of de-ionized water. After this mixture was shaken continuously for 2 min in a Toxi Tube A extraction tube, the organic layer was evaporated at room temperature with air, and the resulting oily residue was then reconstituted with 50 μL of methanol.

The GC-MS analysis was done on a Model 5890 GC (Hewlett Packard) with a 5970 Mass Selective Detector. The data system was an HP59970 Chem Station computer equipped with an HP9133 disc drive. For data acquisition and manipulation we used standard software supplied by the manufacturer, and for the library search we used Revision E of the National Bureau of Standards' Mass Spectral Library. The mass selective detector was operated in the electron impact mode at 70 eV with an ion source temperature of 200 °C and on an m/z range from 40 to 450 atomic mass units. Autotuning of the instrument was performed daily with perfluorotributylamine. For sample analysis, the electron multiplier voltage of the detector was set at 200 V above the autotune voltage. A direct capillary system was used without a jet separator, and the GC-MS interface temperature was kept at 285 °C. For chromatographic separations we used an SPB-5 (15 m × 0.25 mm i.d.) fused-silica column (Supelco, Bellefonte, PA 16823) with the following temperature ramp: 50 °C for 0.8 min followed by 30 °C per min until 145 °C was reached, held at that temperature for 1 min, followed by 17 °C/min up to 285 °C and held for 10 min. The injector temperature was main-
tained at 265 °C, and the volume of organic extract injected was 1 μL. Helium was the carrier gas with flow maintained by a head pressure of 41.4 kPa (6 psi).

Results and Discussion

GC-MS analysis is often the most useful test, as it was in this case, in the toxicologic investigation of insecticide poisonings because of its high sensitivity and specificity and its broad applicability (8). GC-MS analysis of a urine extract from the patient in this case resulted in a total ion chromatogram that consisted of seven major peaks (Figure 1). The last set of six major peaks, with retention times from 5.4 min to 13.5 min, had mass spectra that were consistent with the presence of polyethylene glycol, the vehicle used in the insecticide. These peaks apparently represent a series of polyethylene glycol polymers with different molecular masses. The polyethylene glycol was probably responsible for the diffuse smear noted on the thin-layer chromatogram, but probably did not contribute significantly to the toxicity of the insecticide (9). Polyethylene glycol is not believed to be metabolized to ethylene glycol or other acids that can cause an increased-anion-gap metabolic acidosis (9). The increased anion gap in this patient could be the result of accumulated lactic acid, which was not measured but would probably have been increased because of the severe respiratory acido-
sis and the seizure the patient suffered.

The mass spectrum of the first peak in the total ion chromatogram with a retention time of 3.8 min is shown in Figure 2. The mass spectrum had a molecular ion peak of 152 Da and a base peak of 110 Da. This spectrum is that of 2-isopropoxyphenol, a metabolite of propoxur (Figure 3), which can be produced by hydrolysis of the parent drug (10). GC-MS analysis of an extract of the patient's serum also showed the presence of polyethylene glycol and 2-isopropoxyphenol (data not shown). GC-MS analysis of the tick and flea insecticide found in the patient's room showed a nearly identical total ion chromatogram to that found with the patient's urine sample (Figure 4), and analyses of the major peaks showed the presence of polyethylene glycol and 2-isopropoxyphenol with the same retention times and mass spectra as found in the patient's urine sample. The mass spectrum of a small peak at 6.5 min from the total ion chromatogram of the tick and flea insecticide (Figure 4) had a molecular ion of 209 Da, a base peak of 110 Da, and matched the known spectrum for propoxur, the parent

![Figure 1. Total ion chromatogram of urine extract](image-url)
The depressed pseudocholinesterase activity in the patient's serum was also another laboratory feature of the carbamate poisoning. Assay of pseudocholinesterase is a rapid screening test for the presence of cholinesterase inhibitors, and a depression of pseudocholinesterase activity greater than 50%, which was seen in the patient in this case report, is indicative of poisoning with cholinesterase inhibitors (18). However, decreased pseudocholinesterase activity is not as reliable for the diagnosis of carbamate poisonings as it is for organophosphate poisonings. Carbamates cause only a temporary enzyme inhibition by a reversible carboxylation of the enzyme, whereas organophosphates cause an irreversible phosphorylation and permanent inhibition of pseudocholinesterase (19, 20). Another problem in the interpretation of values for serum pseudocholinesterase for both types of poisonings is that the test lacks specificity in that infections, liver disease, advanced carcinoma, pregnancy, and genetic variability can all result in low pseudocholinesterase values (18, 20). Although it is technically difficult, analysis of erythrocyte acetylcholinesterase (EC 3.1.1.7), which is the same isoenzyme that is found in nerve synapses, has been suggested to be superior to pseudocholinesterase monitoring, because it more accurately reflects the neurotoxicity of cholinesterase-inhibitor poisonings (21).

Another screening test for detection of carbamates, which was not done in this case report, is a fluorescent spot test (5, 22). The primary use of this test is in the detection of mebrobamite, but it can also detect carbamates, particularly in filtered stomach contents or washings. In addition, methods for the detection of the metabolites of cholinesterase inhibitors by filter tests have been described (14). These rapid screening tests are most applicable in situations where cholinesterase inhibitor poisoning is suspected clinically.

Carbamates and organophosphates exert their toxic effect by inhibition of acetylcholinesterase, which results in cholinergic toxicity, owing to an accumulation of acetylcholine at the nicotinic, muscarinic, and central nervous system receptors for acetylcholine. Table 1 lists the major signs and symptoms of acute cholinergic toxicity for each type of receptor. The patient presented in this case had many of the reported signs and symptoms of cholinergic toxicity and among the most prominent were miosis, increased salivation, increased sweating, fecal incontinence, respiratory depression, seizures, and unconsciousness. An unusual aspect of this case was the prominent central nervous system manifestations exhibited by the patient, which may be related to the large dose of carbamate ingested. Unlike the

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<th>Table 1. Manifestations of Cholinergic Toxicity</th>
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organophosphates, the carbamates penetrate the blood–brain barrier poorly and usually do not result in severe central nervous system symptoms unless they are present in high concentrations (12, 23, 24). Another difference between the two types of cholinesterase inhibitors is that carbamates cause only a transient period of symptoms, usually less than 6 h, because of the quick hydrolysis of the inhibited carbamylated acetylcholinesterase to the free active enzyme and carbamic acid (17, 19, 20).

The major therapeutic modalities for cholinesterase-inhibitor poisoning, after supportive care and steps to remove unab sorbed toxins from the gastrointestinal tract or the skin, are treatment with atropine and the cholinesterase reactivator pralidoxime (12, 13). Atropine is often given empirically, based on the clinical presentation alone before the toxicologic investigation is complete, but it should not be used until after cyanoxygen has been treated because, in the presence of hypoxia, it can produce ventricular fibrillation. Atropine, a cholinergic antagonist, reverses the effect of excess acetylcholine at the muscarinic receptors and is given in large doses intravenously every 10 to 30 min until resolution of symptoms or until signs of mild atropinization appear (skin flushing, dry mouth, dilated pupils). Because atropine has no effect on nicotinic receptors, pralidoxime is often added to the treatment of these patients. If used within 48 h of the exposure, pralidoxime can reverse the inhibition of the phosphorylated acetylcholinesterase by forming an oxime-phosphonate complex and releasing the free active enzyme (18, 20). In contrast to the organophosphates, treatment of carbamate poisonings with pralidoxime is not thought to be necessary because of the quick reversal of the inhibition of acetylcholinesterase that occurs with carbamates. In fact, in the case of poisoning by carbaryl, a commonly used monomethyl carbamate, pralidoxime has been reported to have a deleterious effect on the treatment of these poisonings (25, 26). However, for dual toxic exposure to carbamates and organophosphates and for toxic exposures to unidentified cholinesterase inhibitors, many toxicologists advocate the use of pralidoxime (12, 25, 27).

In summary, as carbamate-based compounds are finding more widespread use, poisonings by carbamates are becoming a more frequently encountered problem. Because carbamate poisonings are potentially lethal and because of the need to institute specific treatment quickly for maximum therapeutic effectiveness, it is important for toxicologists to recognize carbamates as potential agents in poisonings and to provide an effective and rapid means for their detection.

References

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