Urinary Elimination Kinetics of Acephate and its Metabolite, Methamidophos, in Urine after Acute Ingestion

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ABSTRACT

Introduction: Acephate (AP) is a widely available organophosphorus (OP) insecticide considered to have low mammalian toxicity. In plants and insects, AP is metabolized extensively to methamidophos (MP), a more potent OP insecticide. The limited mammalian metabolism of AP to MP has been studied in laboratory rat models and suggests that initial formation of MP from AP may inhibit further formation. No case reports of human ingestion with urine AP and MP levels have been previously published.

Case Report: A 4-year-old male being evaluated for altered mental status and head trauma was noted to have muscarinic and nicotinic cholinergic signs. Further history suggested possible ingestion of a commercial AP product at an unknown time. Ingestion of AP was confirmed by the presence of urinary AP and MP and severely depressed red blood cell (RBC) cholinesterase and pseudocholinesterase activity levels. The patient initially received atropine in two 0.02 mg/kg IV boluses, then was started on 0.05 mg/kg IV per hour and titrated accordingly to clinical signs of cholinergic toxicity. Pralidoxime was also given at 20 mg/kg IV bolus, followed by an infusion of 10 mg/kg per hour. The patient required mechanical ventilation for 18 days and atropine infusion for 20 days. After a complicated intensive care unit course, he recovered and was discharged after a total of 32 days of hospitalization.

Methods: Four urine samples collected at different times were analyzed for AP and MP by using high-performance liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry. Kinetic calculations were performed by using standard equations.

Results: Suspected ingestion was confirmed by the presence of AP and MP in urine. The amount of MP found in urine suggests some limited human metabolism to this more toxic compound.

Conclusions: Urinary elimination kinetics of AP demonstrates low metabolic conversion of AP to MP in humans.

INTRODUCTION

Acephate (AP) is one of the few commercially available, over-the-counter organophosphorus (OP) insecticides in the US. It is considered one of the safest OP insecticides because of its low mammalian toxicity, with a lethal dose (LD50) of 360 mg/kg in mice and 900 mg/kg in rats [1]. The selective toxicity of AP is due reportedly to differential bioactivation and metabolism in insects compared to mammals; however, the exact mechanism is still unknown. In insects, AP is rapidly and extensively metabolized to a more potent OP insecticide—methamidophos (MP)—which has a lower LD50 of 14–30 mg/kg in mice and rats, thus

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a higher toxicity than AP [2,3]. The currently favored hypothesis is that AP metabolizes to MP in insects by insect carboxyamidase enzymes differently than it is metabolized in other species. For example, mammalian carboxyamidase metabolism of AP is slower, resulting in decreased formation of MP. In addition, some animal experiments show that MP itself acts as a carboxyamidase inhibitor, which prevents further formation of MP, thus acting as its own feedback inhibitor [4].

Like the toxicity of other OP insecticides, the toxicity of AP and MP results from inhibition of acetylcholinesterase (AChE) activity, resulting in excessive buildup of the neurotransmitter acetylcholine at the level of the neuronal synapse and resultant "cholinergic crisis." Further animal experiments have shown that AP may bind to a different allosteric site on mammalian AChE and not insect AChE, which may protect the active site from MP binding and causing its toxic effects [2,5].

Reports of acute human exposures to AP are extremely limited. Earlier reports issued by the Food and Agriculture Organization of the United Nations have indicated that urinary levels of MP and other metabolites are measureable in human subjects exposed to AP in formulation plants [6]. Other reports indicate that chronic exposure in workers in industrial AP manufacturing has resulted in measurable urinary levels of AP, but not MP, and no depression of AChE activity [7]. More recently, postmortem levels of AP and MP were reported in two cases of suicidal ingestion of AP [2,3].

Although AP and MP have been detected in urine after acute and chronic exposures, human toxicokinetic data of AP and MP have not been studied in detail [2,3]. A case report of acute AP ingestion by a 4-year-old child and the subsequent clinical course is provided. Toxicokinetic data interpretation from this case report are consistent with the hypothesis of feedback inhibition of carboxyamidase by MP, which has not been previously reported in humans.

CASE REPORT

A mother found her 4-year-old son to be unresponsive after suffering a forehead hematoma from a fall one day prior. Emergency medical services (EMS) providers noted that the patient had altered mental status and lethargy. Upon arrival at the local hospital, he was noted to have profound, generalized weakness and depressed gag reflex. He was placed on mechanical ventilation for airway protection. Intravenous (IV) fluids and vasopressors were started for hypotension. His past medical history was significant for diabetes insipidus treated with chlorothiazide and amiloride. Initial blood chemistries were remarkable, with elevated sodium of 162 mEq/L, chloride of 115 mEq/L, bicarbonate of 15 mEq/L, blood urea nitrogen (BUN) of 11.4 mmol/L (32 mg/dL), and creatinine of 194.5 µmol/L (2.2 mg/dL). The initial radiological interpretation of the computed tomography (CT) scan of his head showed a frontal contusion and a possible small frontal subdural hematoma.

The patient was transferred to a pediatric trauma center for neurosurgical management. MRI of the brain and spine at the trauma center showed no abnormalities, and additional review of the initial CT showed no subdural hematoma. However, the patient was noted to have episodes of sinus bradycardia down to 50 beats per minute, miosis, increased mucus secretions, and diarrhea. He was treated with 2 doses of atropine of 0.02 mg/kg IV for his bradycardia. A toxicological cause of his apparent cholinergic crisis was then suspected. During examination of the patient, the toxicology team also observed nicotinic signs including muscle fasciculations and paralysis. Pralidoxime at 20 mg/kg IV bolus was then started, followed by a continuous infusion of 10 mg/kg per hour. Continuous infusion of atropine was also required at 0.05 mg/kg IV per hour and was titrated to signs of cholinergic toxicity. After several hours, mild improvement of respiratory and gastrointestinal secretions was observed. A detailed exposure history found that the mother had mixed an insecticide in a sports drink bottle and left it unattended on top of the refrigerator. Further communication with the store of purchase revealed that the product was Acephate 75. The presence of urinary AP and MP confirmed ingestion of this OP insecticide (*Table 1*). The patient's initial red blood cell (RBC) cholinesterase level was 7 U/g Hgb (normal range: 25–52 U/g Hgb), and his pseudocholinesterase level was 82 U/L (normal range: 2900–7100 U/L). We attributed the rest of his metabolic and electrolyte abnormalities to the patient's history of diabetes insipidus coupled with severe insensible fluid loss, which eventually improved with crystalloid IV fluid infusions and supportive care. Also, time of ingestion of AP was unknown but could have been up to 24 hours prior to presentation.

The patient was extubated on the fourth day of treatment but required reintubation for respiratory distress, although pralidoxime and atropine were continued. The diagnosis of intermediate syndrome was considered; however, the findings of fever, a new pulmonary infiltrate, and increased leukocytosis with left shift made pneumonia more likely. Although pralidoxime was stopped on hospital day 5, atropine infusion was continued due to excessive respiratory secretions and episodes of bradycardia. The patient required mechanical ventilation for an additional 18 days and atropine for a total of 20 days. His cholinesterase levels increased steadily to normal ranges by his 21st day in the hospital (RBC cholinesterase of 35 U/g Hgb and pseudocholinesterase of 4000 U/L). He was discharged 32 days after hospital admission.

Table 1: Urinary Concentrations of Acephate (AP) and Methamidophos (MP) in a 4-Year-Old Patient Who Ingested a Commercial AP Preparation

Time from presentation (hrs)	AP (µ/mL)	MP (µg/mL)	Ratio (MP/AP)
0	607.4	13.17	0.02
14.5	206.2	4.08	0.02
28.7	9.37	0.19	0.02
57.9	13.92	0.26	0.02

MATERIALS AND METHODS

Sample Preparation and Analysis

Urine: Four urine samples were collected at the time of the patient's presentation at the outside hospital (time 0) and at 14, 28, and 57 hours at the trauma center. The method used to prepare and analyze the urine samples has been described previously [8]. Briefly, urine samples (2 mL) were spiked with 25 µL of the labeled internal standards, mixed, then frozen. After the samples were frozen, they were placed in a lyophilizer overnight to remove the water content of the samples (Labconco, Kansas City, MO). The dried residue was reconstituted in dichloromethane, filtered, dried again with nitrogen, then reconstituted in methanol. The analysis was performed using high-performance liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry (HPLC-APCI-MS/MS) with isotope dilution calibration.

Pesticide formulations: To evaluate the possible presence of MP in the AP solution prior to ingestion, commercially available AP formulations were purchased. Orthene PCO (Valent USA, Walnut Creek, CA) and Acephate 75 (Control Solutions, Pasadena, TX) were bought from local hardware stores in the same vicinity of the patient's home and analyzed. Individual solutions of the commercial acephate were prepared by weighing out 1 mg and dissolving it in 100 ml of acetonitrile. Commercial solutions were analyzed within 24 hours of preparation. For the analysis, 25 µL of the sample solution was combined with 25 µL of labeled internal standard solution. The mixture was dried in a TurboVap LV, reconstituted with 50 µL of methanol, and transferred to auto-injection vials. The same (HPLC-APCI-MS/MS) procedure was used for analysis of the diluted commercial materials.

Data Analysis

The urinary elimination constant and half-life were calculated by using standard pharmacokinetic equations: $C_t = C_o e^{-kt}$. Results were also graphed and analyzed with Sigma Plot 10 (Systat Software, Chicago, IL).

RESULTS

The urinary concentrations of AP and MP in the patient samples are shown in Table 1. Although detected in the highest concentrations in the earliest samples collected, AP and MP were detectable in all available samples. In addition, the ratio of AP to MP remained relatively constant over the time period tested, suggesting that the elimination kinetics of the two chemicals were similar.

The half-life and urinary kinetic elimination (K_{elim}) constant of AP and MP that were calculated from the urinary data are shown in Table 2. Both AP and MP exhibited similar half-lives and urinary elimination kinetics. The biological half-lives were about 10 hours, and the K_{elim} was roughly 0.06. Urinary concentrations of methamidaphos were about 2% of the concentrations of acephate, regardless of the sampling time.

To account for the possibility that the urine MP measured was from ingestion of an AP solution contaminated with MP, we analyzed two commercially available products. MP was present in solution at less than 0.5% (*Table 3*).

Table 2: Calculated Urinary Elimination Kinetic Data for Acephate and Methamidophos

	Half-Life (hours)	K_{elim} (1/hours)
Acephate	10.62	0.065
Methamidophos	10.22	0.068

K_{elim} = elimination constant

Table 3: Methamidophos (MP) Content Measured in Commercial Acephate Products

Product	Measured MP	Ratio MP/AP
Orthene PCO (>94% AP, 5% inert)	0.37%	0.004
Acephate 75 (75% AP, 25% inert)	0.10%	0.001

DISCUSSION

Commercial Acephate Preparations: Orthene PCO and Acephate 75

MP content in both readily available commercial preparations of AP was analyzed to consider the possibility of MP contamination during manufacturing or during spontaneous conversion of AP to MP during storage. Several earlier reports in the literature cited elevated AP levels and its metabolites during its industrial manufacturing. The studies were contradictory in that some reported detectable MP in commercial products, while others reported only AP and no MP. One commercial preparation (Acephate 75) that the patient purportedly ingested had a ratio of MP/AP of 0.4%. This is about 4-fold less than the MP/AP of 2% found in the patient's urine, suggesting that some acephate was metabolized to MP in the patient, but the metabolism was not extensive.

Urinary Elimination Kinetics of AP

Consistent with findings reported previously in animal experiments, AP and MP seem to be eliminated according to first-order kinetics (*Figures 1 and 2*, $R^2 = 0.9881$ and $R^2 = 0.9916$, respectively). The urinary elimination half-life and elimination constant of AP and MP are calculated to be around 10 hours and 0.06 L/hours, respectively. This finding has not been previously reported in humans. Most OP insecticides are moderately lipophilic and after acute exposure, a small percentage can accumulate in fat stores [9]. Cases have been reported of other OP insecticides, such as fenthion, that have caused delayed cholinergic crisis after unmetabolized compounds from adipose tissue have been mobilized back into the vascular compartment [10–12].

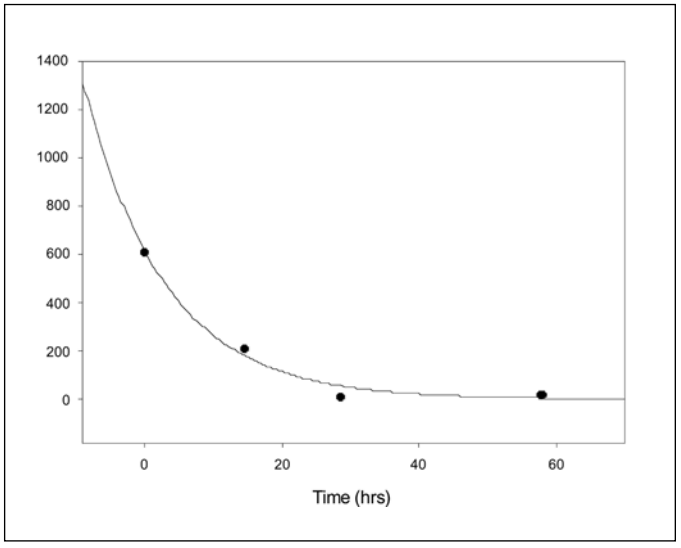

Figure 1: Plot showing first-order kinetics of AP concentration fitted to equation $y = ae^{-bx}$. $R^2 = 0.9881$.

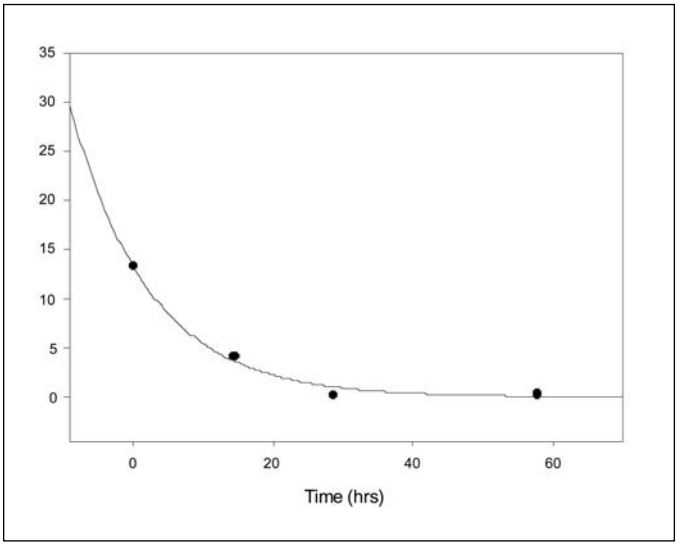

Figure 2: Plot showing first-order kinetics of MP concentration fitted to equation $y = ae^{-bx}$. $R^2 = 0.9916$.

The nearly identical half-life and urinary elimination constants suggest that a common elimination pathway affects both AP and MP. This assumption is reasonable because MP is almost identical in structure to AP, except for an electron-rich acetyl group. Although this moiety most likely does not affect the rate of elimination, it is believed to play a significant role in acetylcholinesterase inhibition. As postulated previously, this additional moiety may bind to an allosteric electron-deficient site on the mammalian AChE, altering its structure and, therefore, protecting it against phosphorylation by MP [5].

As previously reported and postulated by others [3,4], the toxicokinetics from this case are consistent with the hypothesis that:

(1) AP is metabolized to MP after ingestion in humans. The ratio of MP to AP detected in our patient's urine represents a 5-fold to 20-fold increase over the ratio of MP to AP contained in commercially available acephate products, Orthene PCO and Acephate 75, respectively.
(2) Metabolism of AP to MP occurs initially but may be subsequently turned off by its own feedback inhibition. Assuming that AP and MP have similar elimination processes, the constant ratio of MP to AP during their elimination does not support AP being a simple substrate for the ongoing formation of MP by carboxyamidases. If this was the case, AP concentrations subsequently would fall as MP either increases or remains the same, resulting in an *increasing* MP to AP ratio over time. Based on our results, human feedback inhibition of AP by MP is purely speculative since further analysis of blood, serum, tissue, and urine levels of MP and AP would be required to elucidate this process further.

A limitation of this study is that the exact time the patient ingested AP is unknown but thought to be up to 24 hours prior to presentation; the exact amount of AP ingested is also unknown. Although the AP and MP levels obtained at the four time points are indeed sufficient in determining the urinary elimination kinetics, levels at additional time points following ingestion would have allowed more certainty in the elimination kinetics calculations. In addition, spot urine samples may not reflect total bodily elimination adequately. Creatinine correction was also not available, which potentially could have altered our conclusions.

CONCLUSION

Case reports of human AP oral ingestions are extremely rare in the literature. We report a nonfatal acute ingestion of AP by a pediatric patient who experienced significant morbidity. The patient required a prolonged hospital stay with mechanical ventilation despite treatment with atropine and pralidoxime.

The authors have no potential financial conflicts of interest to report.

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