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Point-of-care identification of organophosphates in gastric juice by ambient mass spectrometry in emergency settings

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ABSTRACT

Background: For emergency management, it is important to promptly identify the organophosphate ingested by self-poisoning patients since different organophosphates cause intoxication through different mechanisms and require different therapeutic strategies. This study aimed at the development of a point-of-care ambient mass spectrometric approach for rapid identification of organophosphate(s) in gastric juice for emergency management.

Methods: Six organophosphate insecticides that are commonly ingested by self-poisoning patients in Taiwan were examined. The sample solutions were prepared and diluted with human gastric juice. A direct metallic probe was dipped and removed immediately from the sample solution. The probe was then inserted into the thermal desorption-electrospray ionization/mass spectrometry (TD-ESI/MS) to detect the analyte on the probe.

Results: Since no pretreatment of the specimen was required, the sampling processes followed by thermal desorption-electrospray ionization and mass spectrometric analysis of the organophosphate in the gastric juice were completed within 30 s. The detection limit of the organophosphates is at the 10–100 parts per billion level. Good linearity was observed between the corresponding changes in mass spectrometric signal intensities and the changes of organophosphate concentrations within the range of 5–1000 parts per billion. The high efficiency of this ambient mass spectrometric platform was further confirmed when a real sample of the drained gastric lavage fluid of a patient who suffered from ingestion of chlorpyrifos was collected in the emergency room and tested with this cutting-edge technique.

Conclusions: The results suggested that TD-ESI/MS is promising in promptly providing toxicological information to assist succeeding medical management in an emergency room.

1. Introduction

1.1. Not all organophosphates are the same in poisoning

Since the banning of organochloride pesticides in 1950's, organophosphates have become one of the most commonly used pesticides worldwide. World Health Organization (WHO) has categorized the intoxicating organophosphates into three classes: extremely hazardous (Class I), moderately hazardous (Class II), and slightly hazardous (Class III) [1,2]. > 3 million acute poisoning cases per year

Abbreviations: WHO, (World Health Organization); AChE, (acetylcholinesterase enzyme).; GC/MS, (gas chromatography/mass spectrometry).; LC/MS, (liquid chromatography/mass spectrometry).; TD-ESI/MS, (thermal desorption-electrospray ionization/mass spectrometry).; TPP, (triphenyl phosphate).; MRM, (multiple reaction monitoring).; TFDA, (Taiwan Food and Drug Administration).; LOD, (limit-of-detection).; RSD, (relative standard deviation).; DESI, (desorption electrospray ionization).; DART, (direct analysis in real time).; ELDI, (electrospray-assisted laser desorption ionization).

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occurred worldwide by following ingestion of Class I and II organophosphates. Most of these intoxicated cases happened in Asia and led to approximately 10% mortality. Previous studies indicated a positive relationship between the WHO hazard class and mortality in organophosphate poisoning cases [3]. However, the high fatality could be due to delay in diagnosis or improper management in the emergency room.

Organophosphates poison insects and mammals primarily by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. For human beings, organophosphate intoxication leading to a broad spectrum of clinical symptoms and severe poisoning is potentially lethal. In the early intoxicated stage, organophosphates bind to AChE and some phosphorylated acetylcholinesterase can be de-phosphorylated by an antidote pralidoxime. As time progresses, the enzyme phosphoryl bond will be strengthened by losing one alkyl group from the phosphoryl adduct, a process called aging, and pralidoxime reactivation is therefore no longer possible [4]. This phenomenon mandates early identification of the ingested organophosphates in the emergency room, and this could be achieved by the development of a sensitive and specific point-of-care test conducted at the bedside of the patient [5].

Although organophosphates are generally considered within a single group entity, it has been reported that different organophosphate pesticide has unique chemical and biological characteristics and leads to different outcomes after being ingested [3–6]. For examples, the organophosphates like methamidophos, fenthion, dimethoate and monocrotophos are known to be exceptionally prone to storage in fat tissue, prolonging the need for antidote as stored pesticides tend to be released back into the circulation. Intermediate syndrome occurs after the intoxication to these organophosphates [7–9]. Dichlorvos and chlorophos have been implicated as causes of delayed neuropathy and follow up of patients should be taken at least four weeks after acute intoxication [10,11]. In vivo degradation of trialkylphosphorothioates can cause restrictive lung disease [12]. Methyl-parathion, parathion, mevinphos, dimecron, crufomate and carbophos have been reported to have significant cardiac toxicity and require specific cardiac care [13–18]. In vitro and animal studies have demonstrated potentiation or additive effects when two or more organophosphates are absorbed simultaneously. Enzymes critical to the degradation of one are sometimes inhibited by its co-ingestant [19,20]. Patients with dimethyl organophosphate poisoning have a different course and outcome compared with patients with diethyl organophosphate poisoning [6]. Therefore, even though organophosphates share common mechanisms of cholinesterase inhibition and cause similar symptoms, there is a wide range of toxicity in these agents, making their specific identification and management important.

1.2. Identification of ingested poison before absorption

A number of clinical strategies have been applied to diagnose the exposure to organophosphates including the estimation of acetylcholinesterase activity in blood, the fluoride reactivation of the inhibited enzyme, and the digestion of the inhibited enzyme followed by the detection of phosphoryl protein adduct [5]. However, all these methods are effective only after certain amount of the ingested pesticides have entered the bloodstream through gastrointestinal absorption.

Traditionally, identification of the pesticides in emergency department is performed by analyzing blood samples with gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) [21,22]. Although both techniques are reliable, laborious and time-consuming sample pretreatment processes such as solvent extraction, filtration, concentration, fractionation, and derivatization are needed to avoid the interferences from predominant biological compounds in blood [21–23]. Pesticides are usually identified within hours and laboratory reports are therefore not prompt enough to be used for immediate resuscitation in the emergency department.

Recently, the development of ambient mass spectrometry dramatically reduces the analytical time from hours to seconds, as ambient mass spectrometry requires minimum or no sample pretreatment. The techniques have already evolved as an effective analytical tool in bioanalysis where complex biological samples are analyzed in a rapid and direct fashion with minimal sample preparation at ambient conditions [24–29]. Thermal desorption-electrospray ionization/mass spectrometry (TD-ESI/MS) is an ambient mass spectrometric technique which is capable of characterizing residue pesticides on fruits and vegetables without extraction and separation [30,31]. In TD-ESI/MS, analytes were collected by sweeping a metallic probe across the sample surface. Analytes on the probe were thermally desorbed in a preheated oven. The desorbed analytes were subsequently carried by a nitrogen stream to join an electrospray plume and ionized via reactions with charged solvent species to form analyte ions.

Since the detection of ingested organosphosphates in blood can be achieved only after the pesticides have entered the bloodstream through gastrointestinal absorption, gastric juice instead of blood specimen should be targeted. Gastric lavage is routinely given to all organophosphate-poisoned patients in many Asian countries, and a number of randomized controlled studies have been published showing the improvement in patient outcome, in particular reducing the risk of death [32–36]. In patients with change of consciousness, gastric lavage could be done after airway protection with endotracheal intubation. In this study, six organophosphates that are commonly ingested by self-poisoning patients in Taiwan were tested by using TD-ESI/MS. The aim of this study is to validate its role in rapid identification of different types of organophosphate in human gastric juice.

2. Materials and methods

2.1. Reagents and standard

Over-the-counter organophosphate insecticides were purchased from Sinon Corporation (Taichung, Taiwan) with different purities (in parentheses). According to epidemiological statistics, these were the most commonly ingested pesticides consumed by self-poisoning patients in Taiwan, including methamidophos, acephate, dimethoate, iprobenfos, diazinon and chlorpyrifos (details in Supplemental material). Triphenyl phosphate (TPP), purchased from the Sigma-Aldrich Corporation (St. Louis, USA) was used as internal standard. The chemical structure of the pesticides was shown in Fig. 1.

The solvents including methanol, acetone, and hexane (HPLC-grade) were purchased from Merck (Darmstadt, Germany). Acetic acid was purchased from Sigma-Aldrich (St. Louis, USA). Distilled deionized water (purified with a PURELAB Classic UV from ELGA, Marlow, UK) was used to prepare the electrospray solution.

2.2. Sample preparation

Gastric juice specimens (15 mL each) were collected from 10 volunteers who received routine gastroendoscopic examination after informed consents were obtained under the approval of the institutional review board of the Kaohsiung Medical University Hospital (KMUH-IRB-20130004). The gastric juice specimens were stored under −80 °C before the studies. Stock solutions of six organophosphates were prepared by diluting each of them with distilled deionized water. It is common practice to treat this kind of intoxicated patients by gastric lavage with normal saline. In order to mimic this clinical practice, the gastric juice specimen was diluted with normal saline prior to further experiments and the stock solutions of organophosphate(s) were spiked into diluted gastric juice to make the sample solutions containing different concentration of pesticides. The collection of this clinical sample of gastric lavage content was approved by the Institutional Review Board of the Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20160063).