INTOXICATION WITH DIAZINON

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Abstract. Acute poisoning with organophosphoric compounds (OP) still remains an issue for the doctors, due to its’ high prevalence and sternness, many cases evolving to death. This occurs because the organophosphoric compounds are frequently used as insecticides in agriculture and in the household. They are extremely toxic and one of the most frequent types of contamination is through the lung, by inhalation of substances. More often the organophosphoric compound is not so poisonous for humans than a metabolite of the compound. The diagnosis of poisoning is established based on two components: the clinical and analytical diagnostic. Clinical diagnostic refers to the clinical symptoms; it is called the cholinergic toxidrome. The analytical diagnostic concerns, on one hand, the level of pseudo cholinesterase and, on the anther hand, the identification of the organophosphoric compound. In the last years, great progress has been achieved in order to identify these compounds and their metabolites. The biological monitoring of pesticides is performed using Gas Chromatography coupled with Mass Spectrometry (GC-MS).

Key words: OP compounds, GC-MS, cholinesterase

Introduction

Organophosphorus compounds (OP) are a group of chemical substances widely used in industrial and domestic surroundings. They exert their main toxicological effects through irreversible inactivation of esterases in the central nervous system. As a result, Ach accumulates in the synaptic cleft, causing overstimulation of the cholinergic receptors and consequently impending neurotransmission (Bouchard M., 2006). Human exposure to OP occurs through multiple routes: dermal, oral or by inhalation.

To obtain an indication of the exposure, we can measure the compounds or their metabolites in blood or urine (Gil F. & Pla A., 2001). The first synthesized products had increased potency, being extremely toxic to both humans and animals. That is why researchers sought to synthesize less toxic compounds. Hence new compounds were integrated, but their efficiency decreased in time due to the fact that insects developed resistance to them (Tudosie M.S., et all, 2014). However, these insecticides have remained extremely toxic to humans. Sometimes, not the mere compound but its metabolites prove to be more toxic (Voicu V., et al. 1998). For example, tunic pesticides do not have an important effect on cholinesterase, but, through metabolism, under the action of P450 cytochrome, they become phosphate compounds, which are strong cholinesterase inhibitors. In the case of accidental exposure to pesticides, it is useful to identify the compound and its metabolites as well as to correctly assess the degree of toxicity.

A lot of research has been done to determine whether there is a link between exposure to pesticides and various diseases. One of the methods to determine an exposure to pesticides is to measure the plasmacholinesterase and the erythrocyte acetylcholinesterase, reflecting the capacity of absorbed organophosphoric compound to inhibit these blood enzymes. However, it is well recognized that this is a relatively insensitive indicator of an absorbed dose of OP compound, (Rei S.J. & Watts R.R., 1981; Nutley B.P. & Cocker J., 1993; Hardt J. & Angerer J. 2000, Cocker J., et all, 2002 ). Blood cholinesterase activity needs at least 15% depression from an individual’s normal level of plasma or erythrocyte enzyme activity to be considered indicative of pesticide over-exposure. In addition, the collection of blood samples is sometimes considered as invasive (Cocker J., et al 2002).

The measurement of urinary biomarkers of organophosphoric exposure is of special interest, being non-invasive and highly sensitive. Experimental studies...
in volunteers, indicate that within a few hours following exposure to organophosphoric compound, whatever the route-of-entry, organophosphoric metabolites became easily measurable in urine, even at absorbed doses well below those necessary to induce any sign of toxic effects (Morgan D.P., et all, 1977; Richter E.D., et all, 1992; Carrier G. & Burnet R.C., 1999, Bouchard M., et all, 2003, 2006). The laboratory methods used to assess OP compounds are based on gas chromatography (GC), coupled with mass spectrometry (MS), (GC-MS). Biological monitoring through these methods is a useful tool in establishing the presence and magnitude of exposures.

At present, there are is a significant number of OP compounds, for which exposures need to be assessed. Moreover, the contamination of small children, in whose case biological samples are more difficult to collect, is becoming increasingly problematic nowadays. Hence the need to develop new methods that may enable the collection of maximum amount of information from a limited amount of urine, this being the main biological product used for the biological monitoring of chemical substances such as pesticides (Barr D.B., et al., 2006).

Many currently used methods, described by scientific literature focus on the determination of certain pesticides and their metabolites. Over the past few years, GC methods which identify a higher number of markers belonging to more classes of compounds and requiring smaller amounts of urine, have been developed. These methods are of interest to epidemiological studies because they could help identify an increasing number of pesticides people are exposed to, and provide a statistical interpretation of the exposure effects on the population's health.

New methods for pesticide detection, using HPLC-MS or GC-MS, with quantification using isotope dilution like the multiclass method, the dialkilphosphat method and the phenols method are currently being developed (Barr D.B., et al., 2006).

Recent studies concluded that the development of multi residue methodologies, which may screen simultaneously an increased number of different classes of pesticides is of great value for monitoring the population's exposure to pesticides. Moreover, a trend in the development of such methodologies during the last couple of years can be observed.

**GC-MS method**

Operational parameters for GC

- **Injector temperature - 300°C**
- **Interface GC-MS temperature - 260°C**
- **Carrier gas - He**
- **Column flow - 1.2 ml/min**
- **GC Column - DB5 M5 (30m X 0.25 mm X 0.254 μm)**

The GC oven temperature program is shown in Table I.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rate (°C/min)</th>
<th>Waiting time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>0.00</td>
<td>1</td>
</tr>
<tr>
<td>250</td>
<td>5.00</td>
<td>17.00</td>
</tr>
</tbody>
</table>

**Table I. The GC oven temperature program**

- **Mass range 50 – 450 amu**
- **Manifold temperature - 80°C**
- **Ion trap temperature - 170°C**
- **Ionisation current – 10 μA**
- **Acceleration voltage – 70 eV**
- **Mass range 50 – 450 amu**

**Analytical determinations**

Confirmation of the compounds' identity of the is based on comparing the mass spectrum and the abundance of reference ions of each identified analyte in the sample, with those of standards, using a library of spectra. For spectroscopic identification the mass spectra library Pfleger Maurer was used Weber (PMW), a library specialized for the toxicological compounds and their metabolites. Also, other libraries were used: NIST2000 and Wiley6, both for confirmation of obtained mass spectra using PMW library, and for identification of some compounds that had no spectrum in the PMW library.

The organophosphorus compounds are extracted from urine using liquid-liquid method, with a synergist mixture of dichloromethane: dichlorethane: chlorophorm 1:1:1 v/v and a phosphate buffer.

The quality control of this method is performed by adding an internal standard, midazolam.

The extract purification is carried out by centrifugation 10 min. to 2500rot/min. and is followed by sample concentration to 1000C. The residue is taken up in the mixture of three solvents 500ul.

In the GC-MS system, it is inject 1μ from the analyte.

**Case report**

Male patient, 51 years, presenting: nausea, vomiting, salivation, headache, abdominal cramps, after accidental ingestion of a small amount of an unknown substance, two hours earlier. The patient is admitted in ATI II Toxicology Department.

His breathing and cardiac functions were normal, SpO2 = 97%, without O2 supplement, PA = 125/75 mmHg, HR = 66 - 76/min. He had mitotic pupils. Upon investigating about the ingestion, he told the medical team, that three days earlier his wife had made a pest control of the house and she asked the company responsible for this to hand her the small bottle of insecticide. We had the suspicion that insecticide could probably be an organophosphoric compound. We determined the pseudo cholinesterase which turned out with a light decreased value, at 3000 u/l. The reference level for male at this laboratory is between 4000 and 12600 u/l. We collected 50 ml urine sample for GC-MS method.

Figure 2. presents the ion chromatography, the mass spectra of diazinon and its metabolite, hidroxidiazinone, obtained from a urine sample taken from the patient N.G.

Diazinon is a thiophosphoric type of insecticide. It was synthesized to replace another compound – DDT. Diazinon metabolism is accomplished by oxidation dioxzon, much more aggressive than initial insecticides. Because it is a fat-soluble compound, its toxicity manifests over 24 hours from exposure (depending on the amount of poison), by mobilization of fat tissue.

In the first 24 hours, the therapy required gastric...
lavage, in order to remove from the stomach xenobiotics, volume and electrolytic rebalancing, associated with symptomatic treatment: antispastic, analgesic, antiemetics, gastric protection, and diuretic. Atropine was administered, 1 mg iv, after 1 mg subcutaneous every 6 hours. Plasma was not administered, the level of pseudo cholinesterase being close to normal.

After 24 hours, we repeated the determination of pseudo cholinesterase. The patient still presented headache, nausea and, in addition, myalgia appeared, especially in the neck. The Psche was 3800u/l. The symptomatic treatment was continued and obidoxime was administered- 4mg/kg in the first 20 min. and after 0,5mg/kg/h.

The evolution was favourable, with remission of symptoms after 48 hours.

After 5 days, the patient was discharged with recommendations for periodic neurological control.

Diagnosis is based on analytical and identification organophosphoric compounds and their metabolites by GC-MS screening of urine produced.

The extraction technique used is convenient for GC-MS, to yield very low interference. The mass detector has high selectivity and offers significant spectral information, which provides unambiguous identification of organophosphoric compounds and their metabolites in urine. Evaluated results of operational parameters indicate that the method provides high accuracy and precision.

It is necessary to monitor organophosphoric compounds and their metabolites in urine. Since these compounds are involved in acute intoxication that poses a threat to life- given their presence in the human body their impact is similar to that in gas warfare (organophosphoric compounds more aggressive).

The clinical case presented in this paper, is important for the determination of the chemical compound, which was produced by intoxication. Also, knowing the chemical compound the medical team could apply the rproper treatment. The difference between insecticides that are organophosphoric, organocarbamat or otherwise is that each ingestion requires a certain therapeutic conduct which obviously varies from case to case. A simple determination of pseudo cholinesterase levels may lead to inappropriate or ineffective treatment, with negative results on the evolution of the patient. It is known that low levels of pseudo cholinesterase may be associated with other conditions, such as liver impairment, without any exposure to insecticides or carbamate type insecticide exposure, in whom obidoxime is wrong.

The establishment of intoxication level produced by a certain compound is useful for follow-up, especially in terms of associated diseases. Neurological damage, poisoning with accompanying organophosphoric compounds have an evolutionary characteristic, occurring later than the time of exposure.

In recent years, researchers’ interest of on exposure to this type of compounds has grown and expanded mainly through biological monitoring among children, given the potential increased occurrence of diseases which are believed to be related to pesticide exposure, Parkinson’s disease, brain tumours, types of leukaemia, lymphomas, liver and respiratory damage.

For the future, our medical team tries to develop, in our own laboratory methods to determinate OP compounds. Our focus is on ways to extend the kind of samples that can be used by these methods.

The ability to detect pesticides and other chemicals in new matrices such as meconium, amniotic fluid and umbilical cord results in the demonstration that such biologic media are useful specimens for biomonitoring of environmental pollutant.

Discussions

The establishment of analytical toxicology laboratories with a broad database and faster laboratory and poison records, is an important step in the development of emergency medicine. Frequently used on a wide range, organophosphoric compounds penetrate the circuit biogeochemical ecosystems their harmful effects impacting the food chain and humans.

Advanced methodologies capable of detecting a larger number of organophosphate through a single determination should be implemented.

Fig. 2. Total ion chromatography and MS spectrum for hydroxydiazinone

References


