Chemosphere 92 (2013) 1117–1125

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The role of plant metabolism in the mutagenic and cytotoxic effects of four organophosphorus insecticides in *Salmonella typhimurium* and in human cell lines

Josefina Cortés-Eslava [a], Sandra Gómez-Arroyo [a,*], Francisco Arenas-Huertero [b], Saúl Flores-Maya [c], Martha E. Díaz-Hernández [a], María Elena Calderón-Segura [a], Rafael Valencia-Quintana [d], Jesús Javier Espinosa-Aguirre [e], Rafael Villalobos-Pietrini [a]

[a] Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510 México, D.F., Mexico
[b] Laboratorio de Biología Molecular, Departamento de Patología, Hospital Infantil de México Federico Gómez, México, D.F., Mexico
[c] Laboratorio de Recursos Naturales Citogenética-Vegetal-UBIPRO, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Av. De los Barrios No. 1, Los Reyes Iztacala, Tlalnepantla 54090, Edo. de México, Mexico
[d] Facultad de Agrobiología, Universidad Autónoma de Tlaxcala, Av. Universidad No. 1, Col. La Loma X. Tlaxcala, Mexico
[e] Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510 México, D.F., Mexico

HIGHLIGHTS

- Insecticides metabolized by plants were evaluated in Ames test and human cells.
- Plant metabolites of organophosphorus insecticides were mutagenic in TA98 strain.
- A decrease in cytotoxicity in human cells was obtained with plant metabolites.
- A "protecting effect" of plant metabolism was observed in human cells.

ARTICLE INFO

Article history:
Received 16 March 2012
Received in revised form 9 January 2013
Accepted 14 January 2013
Available online 22 February 2013

Keywords:
Promutagens
Coriandrum sativum
Organophosphorus insecticides
HaCaT cells
NL-20 cells

ABSTRACT

This study used a cell/microbe co-incubation assay to evaluate the effect of four organophosphorus insecticides (parathion-methyl, azinphos-methyl, omethoate, and methamidophos) metabolized by coriander (*Coriandrum sativum*). The reverse mutation of *Salmonella typhimurium* strains TA98 and TA100 was used as an indicator of genetic damage. Treatments with these insecticides inhibited peroxidase activity in plant cells by between 17% (omethoate) and 98% (azinphos-methyl) and decreased plant protein content by between 36% (omethoate) and 99.6% (azinphos-methyl). Azinphos-methyl was the most toxic when applied directly. In the Ames test, treatments applied directly to strain TA100 killed the bacteria; however, the presence of plant metabolism detoxified the system and permitted the growth of bacteria. In strain TA98, plant metabolites of insecticides were mutagenic. This result suggests that the tested pesticides produce mutations through frameshifting. The same pesticides were applied to human skin (HaCaT) and lung (NL-20) cell lines to evaluate their effects on cell viability. Pesticides applied directly were more cytotoxic than the combination of pesticide plus coriander metabolic fraction. Omethoate and methamidophos did not affect the viability of HaCaT cells, but azinphos-methyl and parathion-methyl at 100 and 1000 μg mL^{-1} significantly decreased viability ($p < 0.05$). The NL-20 cell line was remarkably sensitive to the direct application of insecticides. All of the treatment conditions caused decreases in NL-20 cell viability (e.g., viability decreased to 12.0% after parathion-methyl treatment, to 14.7% after azinphos-methyl treatment, and to 6.9% after omethoate treatment). Similar to the Ames test, all of the insecticides showed decreased toxicity in human cells when they were cultured in the presence of plant metabolism. In conclusion, when the studied organophosphorus insecticides were plant-metabolized, they induced mutations in the bacterial strain TA98. In human cell lines, plant metabolism reduced the cytotoxic properties of the insecticides, and human keratinocytes were more resistant to mortality than bronchial cells.

* Corresponding author. Address: Laboratorio de Genotoxicología Ambiental, Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510 México, D.F., Mexico. Tel.: +52 55 5622 4051; fax: +52 55 5616 0789.
E-mail address: slga@atmosfera.unam.mx (S. Gómez-Arroyo).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.01.058

1. Introduction

Organophosphorus pesticides are common compounds involved in poisoning (Bello-Ramírez et al., 2000). They act by inhibiting the hydrolysis reaction performed by acetylcholinesterase, an enzyme that is essential for central nervous system function in insects and humans. This inhibition leads to accumulation of the neurotransmitter acetylcholine, causing interruption of nervous impulses in the synapses (Eyer, 2003). Moreover, organophosphate compounds have alkylating properties due to the presence of electrophilic sites that are able to react with nucleophilic sites on DNA in the cells (Wild, 1975).

It is known that plants are capable of bio-concentrating environmental agents and activating pro-mutagens into toxic metabolites (Plewa et al., 1993). These capabilities increase concerns that plants could also activate agrochemicals and environmental agents, thereby introducing new mutagens into the human food chain (Plewa, 1978). The cell/microbe co-incubation assay described earlier by Plewa et al. (1983) is a very sensitive method for investigating plant metabolism through the use of whole plant cells in suspension culture as an activating system and microbial cells (e.g., the Ames test *Salmonella typhimurium* strains) as the genetic indicator organism. Results obtained by Cortés-Eslava et al. (2001) showed the efficiency of cultured *Coriandrum sativum* cells in metabolizing organophosphorus insecticides that induced mutagenicity. Promutagen activation by plants has also been studied *in vitro* using an extract of *Vicia faba* root (the S10 microsomal fraction) (Calderón-Segura et al., 1999).

Many human epidemiologic and animal studies have shown that exposure to several insecticides is positively correlated with endocrine disruption (Lemaire et al., 2004), reproductive and immune dysfunction (Ayub et al., 2003), and cancer (Kalantzi et al., 2004). The routes of exposure to insecticides are ingestion, inhalation, and absorption through skin. Therefore, it is important to test the effect of these compounds in *in vitro* models. Skin exposure can be evaluated using the spontaneously immortalized human keratinocyte cell line HaCaT (Boukamp et al., 1988). This well-characterized cell line is still able to proliferate and differentiate, and it has been shown to be a good model in toxicology (Delescluse et al., 1988). These cells show good predictive results and constitute an appropriate model for studying several insecticide effects (Ledirac et al., 2005). However, as mentioned above, inhalation is one of the most important routes of exposure to insecticides and particularly to the organophosphorus insecticides that are widely used in Mexico (Gómez-Arroyo et al., 2000; González et al., 2002; Martinez-Valenzuela et al., 2009; Palacios-Nava et al., 1999). Some of these insecticides, such as parathion-methyl, are classified by the WHO (2004) as Ia (“extremely hazardous”), while others, such as azinphos-methyl, omethoate, and methamidophos, are classified as Ib (“highly hazardous”). These compounds can be metabolized by plants. Metabolites or their residues can then remain stored in edible plants and be ingested by humans, where the metabolic machinery can transform these compounds into more toxic products (Plewa et al., 1988, 1993; Cortés-Eslava et al., 2001;Gómez-Arroyo et al., 2007). Because of these concerns, the aim of this study was to evaluate the effect of the organophosphorus insecticides parathion-methyl, azinphos-methyl, omethoate and methamidophos, applied directly and metabolized by coriander (*C. sativum*) cells, on the induction of reverse mutations in *Salmonella thyphimurium* and in the viability of human skin and lung cell lines.

2. Materials and methods

2.1. Chemicals

4-Nitro-*o*-phenylenediamine (NOP) and diethyldithiocarbamic acid sodium salt (DEDTC) (CAS numbers 99-56-9 and 20624-25-3, respectively) were purchased from Sigma Chemical (St. Louis, MO). Parathion-methyl, azinphos-methyl, omethoate, and methamidophos (CAS numbers 298-00-0, 86-5-0, 122852-42-0, and 10265-92-6, respectively) were bought from Bayer of México. Guaiacol (CAS number 90-05-1) was purchased from REASOL of México. Hydrogen peroxide (CAS number 7722-84-1) was purchased from J.T. Baker of México. All other reagents used were of analytical grade. Coriander seeds used to generate coriander cell cultures were acquired from the supply central of México City.

2.2. Coriander (C. sativum) cell preparation

Coriander seeds were germinated and sowed in a greenhouse. When the plants reached 10–15 cm, shoots were cut and placed under sterile conditions in Murashige and Skoog (1962) modified medium (MX) containing ascorbic acid (0.01 mg mL^{-1}) as an antioxidant, 2-methyl-4-chlorophenoxy propionic acid (0.03 mg mL^{-1}), and benzyl amine purine (0.3 μg mL^{-1}). Cuttings were maintained in this solid medium for 4 weeks. The callus were placed aseptically in liquid medium and then transferred to fresh medium every week. The cultures were grown in suspension in a dark chamber at 27 °C with constant shaking (120 rpm). Later, 3 g of cells was incubated in 100 mL of fresh liquid medium with constant agitation for 7 days. Finally, the fresh mass of the cell suspension was adjusted to 100 mg mL^{-1} in MX^- medium (without 2,4-dichloridephenoxyacetic acid (2,4-D)) for the assay and placed on ice until used (⩽30 min). All changes were made under sterile conditions. Coriander cell culture was the metabolic factor used for the transformation of pesticides.

2.3. Preparation of bacterial suspensions

S. typhimurium tester strains TA98 and TA100 were maintained frozen at −80 °C as recommended by Maron and Ames (1983). The cells were incubated in LB medium for proliferation, and master plates were prepared. Strain genetic markers were determined in all mutagenic experiments according to the method described by Zeiger et al. (1981). In our laboratory, the number of spontaneous revertant colonies per plate ranged from 25 to 40 for the TA98 strain and from 103 to 172 for the TA100 strain.

The mutagenic activities of parathion-methyl, azinphos-methyl, omethoate, and methamidophos were determined using the coriander plant cell/microbe co-incubation assay with NOP aromatic amine, whose mutagenicity is increased by plant metabolism, as a positive control. Bacteria were grown overnight in Oxoid Nutrient Broth No. 2 at 37 °C with shaking at 120 rpm and washed in 100 mM potassium phosphate buffer (pH 7), and the density of the bacterial suspension was determined spectrophotometrically and adjusted to 1×10^{10} colony-forming units mL^{-1} in phosphate buffer. The experiments were performed under yellow light to avoid photo-oxidation of NOP and the insecticides (Nishi and Nishioka, 1982).

2.4. Plant cell/microbe co-incubation assay

The conditions used for co-incubation were as described by Plewa et al. (1983, 1988) and Wagner et al. (2003). In brief, plant cells were grown, harvested and prepared as previously described using a coriander cell suspension of 100 mg mL^{-1}. For the co-incubation assay without metabolism, 100 μg (co-incubation flask)$^{-1}$ of NOP and increasing concentrations of insecticides (10, 50, 100, 250, 500 and 1000 μg (co-incubation flask)$^{-1}$) were added in each case to 2.25 mL of MX^- modified medium. Based on preliminary assays, these concentrations gave the most significant activation without affecting bacterial viability. Bacterial viability was determined by observing the background lawn of bacterial growth (De