Chemico-Biological Interactions 259 (2016) 160–167

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Activity and determinants of cholinesterases and paraoxonase-1 in blood of workers exposed to non-cholinesterase inhibiting pesticides

David Lozano-Paniagua [a], Antonio Gómez-Martín [b], Fernando Gil [a], Tesifón Parrón [c], Raquel Alarcón [c], Mar Requena [c], Marina Lacasaña [d, e, f], Antonio F. Hernández [a, *]

[a] Dept. Legal Medicine and Toxicology, University of Granada School of Medicine, Spain
[b] Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Spain
[c] University of Almería School of Health Sciences, Spain
[d] Escuela Andaluza de Salud Pública, Spain
[e] CIBERESP, Spain
[f] ibs.GRANADA, Spain

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form
27 March 2016
Accepted 4 April 2016
Available online 7 April 2016

Keywords:
Esterases
Paraoxonase-1
Cholinesterases
Pesticides
SNPs

ABSTRACT

Pesticide exposure has been associated with different adverse health effects which may be modulated to some extent by paraoxonase-1 (PON1) activity and genetic polymorphisms. This study assessed seasonal variations in PON1 activity (using paraoxon –POase–, phenylacetate –AREase–, diazoxon –DZOase– and dihydrocoumarin –DHCase– as substrates), erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (using butyrylthiocholine –BuChE– and benzoylcholine –BeChE– as substrates. The study population consisted of intensive agriculture workers regularly exposed to pesticides other than organophosphates and non-exposed controls from Almería (Southeastern Spain). The effect of common genetic polymorphisms of *PON1* and *BCHE* on paraoxonase-1 and cholinesterase activities toward different substrates was also assessed. Linear mixed models were used to compare esterase activities in agricultural workers and control subjects over the two study periods (high and low exposure to pesticides). The significant decrease in AChE and increase in BuChE and BeChE activities observed in workers with respect to control subjects was attributed to pesticide exposure. Workers also had higher levels of AREase, DZOase and, to a lesser extent, of POase, but showed decreased DHCase activity. While *PON1 Q192R* and *PON1 -108C/T* gene polymorphisms were significantly associated with all PON1 activities, *PON1 L55M* showed a significant association with AREase, DZOase and DHCase. *BCHE-K* (Karlow variant) was significantly associated with lower BeChE activity (but not with BuChE) and *BCHE-A* (atypical variant) showed no significant association with any cholinesterase activity. These findings suggest that increased PON1, BuChE and BeChE activities in exposed workers might result from an adaptive response against pesticide exposure to compensate for adverse effects at the biochemical level. This response appears to be modulated by *PON1* and *BCHE* gene polymorphisms.

1. Introduction

Pesticides are largely used in agriculture to enhance food production and, to a lesser extent, to control unwanted pests and disease vectors in public health. However, these compounds are often associated with toxicity in non-target species including humans. Occupational pesticide handlers, as a result of mixing or applying these compounds, are at an elevated risk for adverse health effects due to the potential for high exposure events or chronic low-level exposure through skin contact, inhalation or accidental ingestion [42].

Organophosphorus (OPs) and methylcarbamate insecticides are known to be specific inhibitors of acetylcholinesterase (AChE) enzyme activity. They became the most widely used pesticides since the removal of organochlorine pesticides from use; however, the use of OPs is decreasing in many countries as a result of their restriction or banning because of a better understanding of their

* Corresponding author. Department of Legal Medicine and Toxicology, University of Granada School of Medicine, Health Sciences Technological Park, Avda. de la Investigación, 11, 18016 Granada, Spain.
E-mail address: ajerez@ugr.es (A.F. Hernández).

http://dx.doi.org/10.1016/j.cbi.2016.04.008
0009-2797/

long-term health hazards.

The use of biomarkers is gaining growing interest as an integrated measure of exposure and/or adverse health effects because of difficulties in identification of exposure sources and the need of more integrated data for risk assessment. Hence, biomonitoring data are valuable to make associations with the risk of adverse outcomes. Occupational monitoring programs for cholinesterase depression generally rely on measuring the activity of either of two common blood cholinesterases: erythrocyte acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BuChE), which serve as proxy measurements for nervous system AChE. Furthermore, decreases in enzyme activity between two different time-points of a crop season (pre- and post-exposure or high versus low pesticide use) can be used as a quantitative estimation of exposure. This procedure allows to overcome the substantial variability in baseline cholinesterase levels in the general population, with BuChE activity having greater reproducibility and lower variability than AChE activity [42]. However, AChE can be successfully applied in human biomonitoring as it is easy to measure and sensitive, shows a dose-dependent response to pesticide exposure and is linked to health adverse effects [27].

Over the last years, a number of chemicals other than OPs and carbamate insecticides have been increasingly reported to decrease AChE activity, including pesticides from different chemical families (e.g., pyrethroids, triazines and paraquat), heavy metals, polycyclic aromatic hydrocarbons, detergents and different classes of nanoparticles (reviewed in Ref. [27]. Thus, mixed exposures to different compounds may produce a significant reduction in AChE activity, eventually leading to additive anticholinesterase effects. This indicates the need to reconsider the usefulness of AChE in biomonitoring and risk assessment as this biomarker could provide an integrative measurement of the overall risk posed by the whole burden of chemicals present in the environment [27].

BuChE provides protection from adverse effects of OPs and methylcarbamates insecticides, as well as other chemicals, binding them stoichiometrically (molecule-for-molecule), thereby sparing circulating levels of AChE. In turn, paraoxonase-1 (PON1) affords protection against OPs by catalytically inactivating the bioactive oxon metabolites of OPs. The marked inter-individual variation in expression of these enzymes can largely influence pesticide toxicity by increasing or decreasing the sensitivity to these compounds [25]. The *BCHE* gene shows multiple nucleotide variations with the wild type “U” (usual) allele being the most common one, followed by the K, A, and F mutations [41]. PON1 is a calcium-dependent enzyme associated with serum HDL particles exhibiting esterase, lactonase and peroxidase activity. These activities might explain the antioxidant and anti-inflammatory potential of the enzyme, which plays a relevant role in the maintenance of a low oxidative state in the blood [10,34].

Reduced serum arylesterase and paraoxonase activities (two substrate-specific assays for measuring PON1 function) have been linked to increased systemic oxidative stress and related clinical conditions [32]. The wide interindividual variation in PON1 serum levels is influenced by genetic factors (such as polymorphisms in the *PON1* gene, which account for more than 60% of the interindividual variation in enzyme concentration and activity), nutritional factors (intake of antioxidants), environmental agents (like exposure to oxidant agents) and lifestyle [9]. PON1 is also modulated by some pathophysiological events such as inflammation and oxidative stress [34]. Hernández et al. [18] reported that chronic pesticide exposure might result in long-lasting oxidative stress and that polymorphic genes encoding PON1 and BChE are relevant genetic determinants of pesticide toxicity that significantly interact with exposure to modify antioxidant enzyme activities.

This study was aimed to assess a number of esterase activities, namely erythrocyte AChE, plasma BuChE and BeChE, and serum PON1 towards a number of substrates, as well as the major genetic polymorphisms of *BCHE* and *PON1*in a cohort of greenhouse workers exposed to low toxicity pesticides under an integrated production system for achieving a sustainable use of pesticides.

2. Material and methods

2.1. Study population

A longitudinal study was conducted on a cohort of 259 individuals ranging in age 18–66 years from Almeria coastline (Southeastern Spain), where about 18,000 ha are devoted to intensive agriculture under plastic greenhouses. After being contacted during their scheduled annual occupational health survey, they all volunteered to participate in the study. The exposed group consisted of 175 greenhouse workers involved in pruning, weeding, thinning and pesticide application inside the greenhouses (averaged area of 1 ha) under an integrated production system. Insecticides and fungicides were the functional class of pesticides most often used for the crops grown over the study period (tomato, cucumbers and zucchini). The major chemical classes of insecticides used were macrocyclic lactones (abamectin, spinosad), neonicotinoids (imidacloprid, acetamiprid), pyrethroids (cypermethrin, deltamethrin) and others (indoxacarb, azadirachtin, spiromesifen, Bacillus thuringiensis). On the other hand, the fungicides more frequently used for the aforementioned crops included: triazoles (tebuconazol, triadimenol, miclobutanil), anilino-pyrimidines (cyprodinil, mepanipyrim, pyrimethanil), copper salts (copper oxychlroride) and others (phenyl pyrrole, thiophanate methyl, fluopicolide, chlorthalonil, propamocarb, dimethomorph, azoxystrobin).

The control group consisted of 84 healthy individuals having no previous or current occupational exposure to pesticides, recruited from the Center for Prevention of Occupational Hazards from Almería province. To minimize any difference in background exposure to pesticides, control subjects were recruited from the same area as the greenhouse workers. Two agricultural periods of a crop season were surveyed: low exposure [May–June 2011], when pesticides were used occasionally, one or two applications per month, and high exposure [October–November 2011], when pesticides were regularly used on a weekly basis.

2.2. Sample collection

Blood samples were collected by venopuncture after a fasting period of 10 h at the time of the clinical examination for each study period. Samples were stored in a portable fridge and brought to the laboratory within 4 h. An aliquot was taken separately for determination of AChE, another aliquot for serum separator tubes (with clot activator) and the remainder was immediately centrifuged at 400 × g for 20 min to separate plasma and the erythrocyte package, which were further stored frozen at −40 °C for determination of esterase activities and genetic polymorphisms.

2.3. Enzyme activities in blood

Erythrocyte AChE was determined following the method of Ellman modified by Worek et al. [47]. Briefly, acetylthiocholine (0.48 μM) was used as a substrate in a reaction mixture containing phosphate buffer pH 8 (90 mM final concentration), dithiobisnitrobenzoic acid (DTNB, 0.32 mM) and ethopropazine (0.02 mM) to inhibit plasma cholinesterase (final concentration is given for all reagents). The reaction mixture was incubated at 25 °C for 5 min and then 0.17 ml of thawed whole blood diluted 1:100 with