Ecotoxicology and Environmental Safety 162 (2018) 121–128

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Biomarkers of oxidative stress in blood of workers exposed to non-cholinesterase inhibiting pesticides

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

[a] Dept. Legal Medicine and Toxicology, University of Granada School of Medicine, Spain
[b] University of Almería School of Health Sciences, Spain
[c] Escuela Andaluza de Salud Pública, Spain
[d] CIBERESP, Spain
[e] ibs.GRANADA, Spain

ARTICLE INFO

Keywords:
Thiobarbituric acid reactive substances (TBARS)
Total thiol groups (SHT)
Ferric reducing ability of serum (FRAS)
Gamma glutamyl transpeptidase (GGT)
Pesticides
Oxidative stress
Lipid peroxidation

ABSTRACT

In occupational settings workers are often exposed to pesticides at relatively high doses compared to environmental exposures. Long-term exposure to pesticides has been associated with numerous adverse health effects in epidemiological studies, and oxidative stress is often claimed as one of the underlying mechanisms. In fact, different pesticides have been reported to induce oxidative stress due to the generation of free radicals and/or alteration in antioxidant defense enzymes. The present study examined greenhouse workers regularly exposed to diverse pesticides under integrated production system, and a group of controls of the same geographic area without any chemical exposure. Two different periods of the same crop season were assessed, one of high exposure (with greater use of pesticides) and other of low exposure (in which a less use of these compounds was made). Non-specific biomarkers of oxidative stress, e.g. thiobarbituric acid reactive substances (TBARS), ferric reducing ability of serum (FRAS), total thiol groups (SHT), gamma-glutamyl transpeptidase (GGT) and paraoxonase-1 (PON1) were measured in serum samples from all study subjects, alongside erythrocyte acetylcholinesterase (AChE). Results are suggestive of a mild increase in oxidative stress associated with pesticide exposure, which was compensated by an adaptive response to raise the antioxidant defenses and thus counter the detrimental effects of sustained oxidative stress. This response led to significantly increased levels of FRAS, SHT and PON1 in greenhouse workers relative to controls. Furthermore, AChE was decreased likely as a result of oxidative stress as workers did not use organophosphate insecticides.

1. Introduction

Pesticides, like other environmental pollutants, at relevant exposure levels have been associated with a variety of adverse health outcomes, in which oxidative stress is considered as one of the key mechanisms involved their pathogenesis. Most pesticides, including organophosphates (OPs), bipyridyl herbicides, pyrethroids (PYR), neonicotinoids and organochlorines (OCs) have been shown to produce oxidative molecules and/or to disturb the antioxidant defense systems of the body, ultimately leading to oxidative stress (Pearson and Patel, 2016; Poljšak and Fink, 2014; Wang et al., 2016, 2018). Prolonged or sustained oxidative stress is detrimental to cells and can contribute to loss of their biological function. At the organism level, these changes are thought to be major pathophysiological factors for long-term diseases such as carcinogenesis, neurodegeneration, and cardiovascular, respiratory, renal, endocrine and reproductive disorders (Jabłońska-Trypuć, 2017; Mostafalou and Abdollahi, 2013).

Since highly reactive species cannot be determined in body fluid samples because of their short half-life, indirect biomarkers of oxidative stress (or their oxidation by-products) are measured instead. The by-product most often measured is malondialdehyde (MDA), one of the main lipid hydroperoxides produced by the peroxidative degradation of polyunsaturated fatty acids from cell membranes containing two or more double bonds.

The general state of oxidative stress can be determined by measuring the total antioxidant capacity (TAC) of the organism (e.g., the ferric reducing ability of serum, FRAS). The FRAS test measures TAC from non-enzymatic antioxidants, with ascorbic acid and uric acid

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

https://doi.org/10.1016/j.ecoenv.2018.06.074
Received 21 February 2018; Received in revised form 15 June 2018; Accepted 25 June 2018

being the main contributors (Gawron-Skarbek et al., 2015). Since reduced glutathione (GSH) levels are not measured with the FRAS assay (Ranjbar et al., 2002a), total thiol (SHT) groups should be measured as a complementary biomarker. Thiols are endogenous compounds with at least one sulfhydryl group responsible for their antioxidant effects as it can be readily oxidized. GSH is the most abundant thiol compound that can be measured by the SHT assay.

Gamma glutamyl transpeptidase (GGT) is an enzyme located on the outer surface of the plasma membrane of numerous cell types that is involved in the homeostasis of GSH, thus contributing to the antioxidant defense (Lim et al., 2004). On the other hand, paraoxonase-1 (PON1) is a HDL-associated protein known to have protective effects on lipid peroxidation but also confers protection against free radicals. Hence, it can be considered as a member of antioxidant enzyme systems playing an important role in the protection of proteins and lipids against oxidative damage (Hernández et al., 2013a).

This study assessed a number of biomarkers of oxidative stress (TBARS, FRAS and SHT) and antioxidant enzymes (GGT and PON1) in greenhouse workers from Almeria (South-eastern Spain) involved in intensive agriculture. Intensive agriculture represents an optimal scenario to study adverse health effects from long-term exposure to pesticides. However, in the study area, less toxic pesticides were used as integrated production systems were in place in order to achieve a sustainable use of pesticides.

2. Material and methods

2.1. Study population

A longitudinal study was conducted on a cohort of 266 individuals ranging in age 18–66 years from Almeria coastline. Almeria is one of the most important areas of intensive agriculture in Spain, with 37,575 ha of greenhouses devoted to fruits and vegetables crops, which represents around 57% of the total greenhouse area in Spain (Glass et al., 2012). The main features of the study population have been reported elsewhere (García-García et al., 2016). Briefly, after being contacted during their scheduled annual occupational health survey, 266 individuals volunteered to participate in the study. These consisted of 175 greenhouse workers carrying out farmer activities (40 h per week) such as pruning, weeding, thinning and pesticide application in the confines of plastic greenhouses (with an average area of 1 ha each) under an integrated production system.

The main crops grown in the greenhouses of the study area were tomato, cucumbers and zucchini. Insecticides and fungicides were the functional class of pesticides most often used over the crop season studied. The major chemical classes of insecticides used were macrocyclic lactones (abamectin, spinosad), neonicotinoids (imidacloprid, acetamiprid), pyrethroids (cypermethrin, deltamethrin), *N*-methylcarbamates (methomyl) and others (indoxacarb, azadirachtin, spiromesifen, *Bacillus thuringiensis*). Likewise, the fungicides more frequently used were triazoles (tebuconazole, triadimenol, miclobutanyl), anilino-pyrimidines (cyprodinil, mepanipyrim, pyrimethanil), copper salts and others (phenyl pyrrole, thiophanate methyl, fluopicolide, chlorthalonil, propamocarb, dimethomorph, azoxystrobin). Notably, almost all these pesticides have the potential to produce oxidative stress and no anticholinesterase insecticides were used with the only exception of methomyl (García-García et al., 2016).

The control group consisted of 91 healthy individuals living in the same area than greenhouse workers but without previous or current occupational exposure to pesticides. They were recruited from the Center for Prevention of Occupational Hazards of Almeria province. Two periods of a crop season were surveyed: low exposure (which extended from May to June 2011), when pesticides were used occasionally, one to two applications per month, and high exposure (which extended from October to November 2011), when pesticides were used regularly on a weekly basis. Individuals with a clinical diagnosis of chronic diseases or tumors were excluded. The study was approved by the Research Ethics Committee of the University of Granada (reference 29/2009) and was in agreement with the Declaration of Helsinki for International Health Research. All participants signed an informed consent form after being informed about the objectives of the study and their right to drop from the study at any time.

2.2. Sample collection

Blood samples were collected by venipuncture after a fasting period of 10 h in each study period, when the clinical examination for occupational health surveillance was carried out. Samples were stored in a portable fridge, shipped to the laboratory within 4 h and centrifuged to separate serum. Then, samples were divided into single-use aliquots. One of them was taken for immediate determination of GGT activity and the other aliquots were stored frozen at − 40 °C for further determination of TBARS, FRAS, SHT, PON1 and AChE in the next three months.

2.3. Biochemistry assays

2.3.1. Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation products (MDA) produced by oxidative damage to lipids was determined by using 2-thiobarbituric acid (TBA) as a derivatization reagent. This reaction produces MDA-TBA adducts which can be determined by high-performance liquid chromatography coupled to a fluorescence detector (HPLC-FD) (Agarwal and Chase, 2002). A Perkin Elmer (Norwalk, USA) HPLC was used equipped with a Hypersil ODS (C18) column 10 cm × 0.46 cm and a guard column (Hewlett-Packard, USA) was used for this assay. The mobile phase consisted of 40:60 (v/v) methanol-K_2HPO_4/KOH (50 mM and pH 6.8) at a flow rate of 1.0 ml/min. The fluorescence detector was set at an excitation wavelength of 515 nm and emission wavelength of 553 nm. Standards samples were prepared through serial dilution of a stock solution of 1,1,3,3-tetraethoxypropane (TEP) with 40% ethanol solution. Serum and standard samples were derivatized with a 42 mM TBA solution in 40% ethanol solution. Previously 50 µl of butylated hydroxitoluene (BHT) were added to 50 µl of sample to prevent in vitro oxidation. Sample tubes were vortex mixed and 400 µl of 0.44 mM perchloric acid were added (adjusted to pH 3.5 with KOH). Samples were vortex mixed once again and heated for 20 min on a 50 °C water bath. Then, 100 µl of TBA were added and vortex mixed during one minute. Samples were then placed in a 100 °C dry bath incubator for one hour and then cooled on an ice bath for 5 min. Finally, derivatized samples were extracted with 500 µl of 1-butanol and the supernatant was injected into the chromatographic system. TBARS levels were expressed as µmol MDA/l serum.

2.3.2. Ferric reducing ability of serum (FRAS)

This test assessed the total antioxidant capacity of blood by measuring the power of serum to reduce Fe^{3+} to Fe^{2+} ion at low pH according to Benzie and Strain (1996). This method is based on the use of 2,4,6-(2-tripyridyl)-1,3,5-triazine (TPTZ) which forms a colored complex with Fe^{2+}. Briefly, FRAS reagent consisted of acetate buffer (300 mM, pH 3.6), TPTZ (10 mM) and $FeCl_3$ (20 mM, in 40 mM HCl) freshly prepared every day (ratio 10:1:1). This reagent was kept protected from light until use. The reaction was started by adding 110 µl of 1/4 serum diluted with distilled water to 830 µl of FRAS reagent. Then, the mixture was incubated at 37 °C for 10 min. The complex Fe^{2+}-TPTZ formed was spectrophotometrically measured at 593 nm and compared with a standard curve obtained with ferrous sulfate solutions tested in parallel. Units were expressed as µmoles de Fe^{2+}/l serum.

2.3.3. Total thiol groups (SHT)

SHT in serum were determined spectrophotometrically according to Ellman (1959) using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as