Pesticide exposure and genetic variation in xenobiotic-metabolizing enzymes interact to induce biochemical liver damage

Antonio F. Hernández a,∗, Fernando Gil a, Marina Lacasaña b, c, Miguel Rodríguez-Barranco b, Aristidis M. Tsatsakis d, Mar Requena e, Tesifón Parrón e, f, Raquel Alarcón f

Article info

Keywords:
Pesticide exposure
Liver function test
Genetic polymorphisms
Gene-environment interactions
Occupational health

ARTICLE IN PRESS

Food and Chemical Toxicology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Metabolic activation of pesticides in the liver may result in highly reactive intermediates capable of impairing various cellular functions. Nevertheless, the knowledge about the effect of pesticide exposure on liver function is still limited. This study assessed whether exposure to pesticides elicits early biochemical changes in biomarkers of liver function and looked for potential gene-environmental interactions between pesticide exposure and polymorphisms of pesticide-metabolizing genes. A longitudinal study was conducted in farmers from Andalusia (South Spain), during two periods of the same crop season with different degree of pesticide exposure. Blood samples were taken for the measurement of serum and erythrocyte cholinesterase activities as well as for determining clinical chemistry parameters as biomarkers of liver function. Serum lipid levels were also measured as they may help to monitor the progress of toxic liver damage. A reduction in serum cholinesterase was associated with decreased levels of all clinical chemistry parameters studied except HDL-cholesterol. Conversely, a decreased erythrocyte cholinesterase (indicating long-term pesticide exposure) was associated with increased levels of aspartate aminotransferase and alkaline phosphatase and increased levels of triglycerides, total cholesterol and LDL-cholesterol, but reduced levels of HDL-cholesterol. Changes in liver biomarkers were particularly associated with the PON155M/192R haplotype. The obtained results therefore support the hypothesis that pesticide exposure results in subtle biochemical liver toxicity and highlight the role of genetic polymorphisms in pesticide-metabolizing enzymes as biomarkers of susceptibility for developing adverse health effects.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Pesticides are widely used to enhance food production in agricultural practice and, to a lesser extent, to control unwanted pests and disease vectors in public health. N-methylcarbamates (NMC), pyrethroids (PYR), dithiocarbamates, neonicotinoids and organophosphates (OPs) are among the pesticides most commonly used in plastic-covered greenhouses widely spread in Andalusia (South Spain) for intensive vegetable production (Hernández et al., 2003). Furthermore, NMC, OPs, PYR and neonicotinoids are, in this order, the compounds more often involved in acute pesticide poisoning in the same area and remain an important source of occupational intoxications (Hernández et al., 2010). Most of these pesticides undergo phase I reactions, rendering highly reactive molecules that may further interact with relevant molecular targets such as enzymes, nucleic acids and membrane phospholipids leading to cytotoxic changes, genotoxicity and cell necrosis, respectively (Milatovic et al., 2006; Androutsopoulos et al., 2013). Given that these metabolic reactions occur primarily in the liver microsomes, early hepatotoxicity at a biochemical level may be expected as one of the earliest toxic effects of pesticides.

The liver plays a key role in the maintenance of the homeostasis of the organism. Conventional liver test provide information about the integrity of hepatocytes, such as serum transaminases (alanine–ALT– and aspartate–AST– aminotransferases), with ALT being considered as the gold standard clinical chemistry marker of liver injury. The integrity of the biliary system is commonly assessed by measuring gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) (Ozer et al., 2008). Chronic overproduction of
reactive species induces a redox imbalance leading to increased GGT, which has also been considered as an early marker of oxidative stress in humans (Sedda et al., 2008). Given that de novo synthesis of triglycerides (TG) and their incorporation into lipoprotein particles takes place in the liver, TG might be useful for monitoring the progress of toxic damage (Provost et al., 2003). However, so far no thorough study has assessed the usefulness of TG as a marker of liver dysfunction in humans.

Significant increases in serum biochemical indices, including ALT, AST, ALP, total cholesterol, TG, low density lipoprotein cholesterol (LDL-c) and lipid peroxidation by-products have been observed experimentally after subchronic or chronic exposure to OPs (Binukumar et al., 2010; El-Gharieb et al., 2010; Kalender et al., 2005, 2010; Rezg et al., 2008). A dose-dependent increase in total serum lipids concentration has also been observed in rats treated with atrazine (Santa Maria et al., 1987).

The enzymes primarily involved in pesticide metabolism include cytochrome P450s (CYP450s), paraoxonase-1 (PON1) and glutathione S-transferases (GSTs), although the profile of the participating isoforms is different for each agent (Hernández et al., 2008). Serum cholinesterase (BChE) and carboxylesterases can also inactivate OPs and NMC acting as stoichiometric scavengers. The marked inter-individual variation in expression of these enzymes can largely influence pesticide toxicity by increasing or decreasing the sensitivity to certain compounds. Hence, particular genotypes (or phenotypes) can be used as biomarkers of susceptibility for the detection of subgroups of individuals at an increased risk of adverse health effects.

The aim of this study was to assess whether exposure to pesticides in an intensive agriculture setting may lead to changes in liver function parameters. Secondly, to ascertain whether pesticide exposure interacts with genetic polymorphisms of pesticide-metabolizing enzymes resulting in alterations of the clinical chemistry parameters studied, by using new and non-conventional statistical analysis integrating data from exposure at two different time-periods.

2. Materials and methods

2.1. Study population

A longitudinal study was conducted on greenhouse workers from Almeria (a Southeastern province of Spain) during two periods of the same crop season. In the high exposure period (October–November) large quantities of pesticides were sprayed and in the low exposure period (April–May) pesticides were less heavily sprayed. A comparative group of non-exposed subjects was selected and assessed at the same periods. Both groups of individuals were recruited from the same villages in order to eliminate differential biases related to socio-economic status and background exposure to pesticide residues. The non-exposed group was specifically interviewed to ascertaining the lack of potential exposure to either pesticides or any other industrial chemical that could modify any of the enzyme activities assessed.

A total of 190 individuals agreed to participate in the study. 135 of them being greenhouse workers (14–59 years-old) and 55 acted as non-exposed controls (aged 23–55). All individuals were identified through occupational physicians involved in their health surveillance. However, only 118 subjects provided two blood samples during the course of the crop season; of the subjects, 81 were pesticide applicators and 37 age-matched healthy controls. The primary reason of dropping from the follow-up was change of work. Individuals presenting any type of chronic disease (e.g., liver dysfunction, diabetes, renal failure, cancer) were excluded from the study to avoid any interference with the biochemical parameters measured. Subjects taking any medication on a long-term basis were also excluded. Exposed subjects performed different activities within greenhouses, including the application of pesticides. Pesticide categories more often used were NMC, PVR, diithiocarbamates, neonicotinoids and OPs.

2.2. Collection of information

The study population completed a structured questionnaire containing questions on sociodemographic characteristics (age, gender), anthropometric measures (weight and height), lifestyle (alcohol and smoking habits) and occupational features (lifetime exposure to pesticides, use of personal protective equipment and type of pesticide used). The application of questionnaires and collection of blood samples were done by nursing personnel specially trained and standardized for this purpose. They did not know the objectives of the study. All the individuals gave informed and written consent after explaining the procedures and main objectives of the study and they were also informed of their right to withdraw at any time throughout the study after which each subject participated voluntarily. The study was approved by the Ethics Committee of the University of Granada.

2.3. Samples collection

Blood samples were taken in fasting conditions by the nursing personnel using heparinized Vacutainer® tubes and Vacutainer® serum tubes. Samples were centrifuged at 2500 rpm for 20 min to separate serum and erythrocyte package. Erythrocytes were washed twice in NaCl 0.9% and diluted in an equal volume of saline. Serum and erythrocyte aliquots were stored frozen at −20 °C until analysis, not longer than 1 month thereafter.

2.4. DNA extraction and genotyping

Genomic DNA was extracted from the buffy coat layer of blood samples using a standard phenol–chloroform extraction protocol and purified DNAs were stored at −20 °C. Unit analysis, PON1 and GST polymorphisms were determined by polymerase chain reaction amplification followed by polymorphism specific restriction digestion (PON1 genotypes) or allele specific oligonucleotide probe ligation (GST polymorphisms). The resulting fragments were separated by gel electrophoresis analysis and identified by visualization of the band pattern following the procedure described elsewhere (Hernández et al., 2003 and Hernández et al., 2005).

2.5. Enzyme activities and lipid profile in serum

BChE and AChE activities were determined by the method of Ellman et al. (1961) as previously described (Hernández et al., 2005). Exposure to pesticides was characterized by distinguishing short-term and long-term exposures based on depression of BChE and AChE, respectively. BChE variants were determined by measuring the inhibition of benzoylcholine hydrolysis with dibucaine and fluoride at 240 nm (Whittaker, 1984). Serum enzymes reporters of liver function (AST, ALT, GGT and ALP), often referred to as liver function test, were determined by colorimetric assays using a Hitachi 747 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Lipid parameters, such as triglycerides (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) were measured using the same instrument following routine and automatized procedures. All analyses were performed in certified clinical laboratories.

2.6. Statistical analysis

Because the distribution of some liver function test and serum lipid parameters was skewed to the right and did not fulfill normality criteria (Kolmogorov–Smirnov test) they were log-transformed for further statistical analysis. The significance of the differences in the mean values of raw clinical chemistry parameters was tested by the Student’s t-test or the non-parametric Mann–Whitney test in the case of a non-normal distribution. Differences in lifestyle (tobacco and alcohol consumption) between the control and exposed groups were studied by the χ2 test. Geometric means (GM) of paired data in the two crop season periods studied were compared by the paired Student’s t-test and the Wilcoxon signed-rank test, depending on whether their distribution fulfilled normality criteria. Generalized estimating equation (GEE) models were developed to evaluate the association between indicators of pesticide exposure (serum and erythrocyte cholinesterases, crude exposure as a binary variable and accumulated lifetime exposure in years) and genetic polymorphisms of pesticide-metabolizing enzymes with serum biomarkers of hepatic function and serum lipid parameters. These models extend generalized linear models for the situation of correlated data (as is the case of our study, where the population was assessed at two different time-points).

Interaction effects of BChE and AChE activities with genetic polymorphisms on liver function test and serum lipid parameters were estimated by means of GEE. Potential confounding variables, including age, body mass index (BMI), tobacco and alcohol consumption (with both being considered as dichotomous covariates) were included in the adjusted models based on their biological plausibility and on current scientific literature. Significant associations were considered when the p value was <0.10. All the statistical analyses were conducted with Stata version 8.2 (StataCorp LP, Texas) and SPSS version 15.0 (SPSS Inc. Chicago, IL).

3. Results

General characteristics of the study population are described in Table 1. A comparison of levels of cholinesterases, liver function test and serum lipid profile between the exposed population and...
control for the two periods of follow-up is shown in Table 2. In general, the exposed population had higher levels of liver function test in both periods with less differences being noted for serum lipids. The percentage of subjects with serum levels of clinical chemistry parameters above the upper normal limit at the high exposure period was 28% for ALT and AST, 36% for GGT, 26% for ALP, 52% for total cholesterol, 49% for TG, 35% for HDL-c and 46% for LDL-c. When the group of subjects was compared with the group of individuals lost during follow-up, statistically significant differences were observed in AChE activity, which may potentially indicate a selection bias. Workers lost during follow-up had higher AChE levels, which could be associated to lower exposure to pesticides and may lead to overestimation of results. However, since no significant differences were observed in any other clinical chemistry parameters between both groups (those that remained in the study and those lost during follow-up) the results are not expected to be biased due to missing data.

The association between indicators of pesticide exposure and biomarkers of hepatic damage is shown in Table 3. BChE was found to be directly correlated with all liver function test and serum lipid parameters with the exception of HDL-c, where an inverse relationship was observed. In turn, AChE was inversely associated with AST and ALP and with all lipid parameters but HDL-c, with which a positive correlation was found. Among all the biochemical parameters studied, only ALT and ALP were directly associated with lifetime exposure to pesticides (expressed as years). The remaining biochemical markers showed no significant statistical association.

The exposed population had lower levels of TG as compared to non-exposed subjects, which approached statistical significance. Table 4 depicts GEE models for the effect of genetic polymorphisms of pesticide metabolizing enzymes on clinical biochemistry parameters. As key enzymes involved in metabolic detoxification of pesticides, PON1 and GST polymorphisms and BCHE variants were used as potential biomarkers of susceptibility to pesticides in the studied individuals. ALT was the only clinical chemistry parameter that showed an association with PON1 polymorphisms. GST polymorphisms failed to be associated with liver function test or serum lipids. As regards to BCHE polymorphism, carriers of unusual phenotypes had significantly higher levels of serum transaminases (ALT and AST) and GGT.

Interaction analysis between the genetic polymorphisms studied (PON1<sup>192</sup>, PON1<sup>55</sup>, PON1<sup>1068</sup>, PON1<sup>590</sup>, GSTM1, GSTT1, BCHE) and cholinesterase activities (AChE and BCHE), used as biomarkers of pesticide exposure, on clinical chemistry parameters is shown in Supplementary Tables 5–8. The significant associations demonstrated are also shown in Figs. 1–3.

Table 1 shows the significant interaction effects between BCHE activity and genetic polymorphisms of pesticide-metabolizing enzymes on liver function test. The figure shows the relative or absolute effect size on the liver enzymes (depending on whether or not they were log-transformed, respectively) for each 100 U/l decrease in BCHE activity for each particular genotype studied. Statistically significant interactions were found between BCHE and PON1<sup>192RR</sup> genotype on ALT, and AChE and ALP, indicating that for each 100 U/l decrease in BCHE activity the carriers of that genotype showed significantly higher levels of ALT and AST and lower levels of ALP. BCHE also interacted with PON1<sup>1068CC</sup> genotype on GGT, as carriers of this genotype had almost 20% lower GGT levels after short-term pesticide exposure. Regarding GST polymorphisms, we found a significant interaction of BCHE with GSTT1 + 0 genotype on both transaminases, as carriers of the null allele had significantly lower ALT and AST activities. BCHE also interacted with GSTM1 + 0 on GGT, as carriers of the null genotype had lower GGT levels, and with unusual BCHE variants on AST, indicating that for each 100 U/l decrease in BCHE activity individuals carrying unusual BCHE had about 6% lower levels of AST than subjects with the usual variant.

Table 2 shows the significant interaction effects between BCHE activity and genetic polymorphisms of pesticide-metabolizing enzymes on serum lipid parameters. Significant interactions were observed between BCHE and PON1<sup>55MM</sup> genotype on total cholesterol and LDL-c, indicating that the decrease in BCHE is associated with increased levels of these two lipids but only in subjects homozygous for the 55 M allele. BCHE also interacted significantly with PON1<sup>1068RR</sup> genotype on HDL-c, indicating that short-term exposure to anticholinesterase pesticides is associated with lower levels of this lipoprotein but only in subjects homozygous for the RR genotype. Regarding GST genotypes, the only interaction found was between BCHE and GST-M1 + 0 on HDL-c, indicating that for each 100 U/l decrease in BCHE activity, individuals null for GSTM1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>General characteristics of the study population.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Exposed</td>
</tr>
<tr>
<td>Age</td>
<td>37.4 ± 9.4</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (50.0)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (50.0)</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>27.4 ± 4.2</td>
</tr>
<tr>
<td>Tobacco consumption</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13 (33.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>26 (66.7)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22 (55.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>18 (45.0)</td>
</tr>
<tr>
<td>Total</td>
<td>55 (28.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>High exposure period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCHE</td>
</tr>
<tr>
<td>Control</td>
<td>Mean (S.D.)</td>
</tr>
<tr>
<td>Exposed</td>
<td>Mean (S.D.)</td>
</tr>
<tr>
<td>p-Value</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low exposure period</th>
<th>BCHE</th>
<th>AChE</th>
<th>ALT</th>
<th>AST</th>
<th>GGT</th>
<th>ALP</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>LDL-c</th>
<th>HDL-c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>894.9 ± 175.5</td>
<td>13566.8 ± 2009.0</td>
<td>15.2 ± 9.3</td>
<td>18.2 ± 4.8</td>
<td>46.4 ± 12.2</td>
<td>207.2 ± 36.6</td>
<td>126.9 ± 31.6</td>
<td>54.2 ± 11.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>833.0 ± 161.8</td>
<td>13177.8 ± 1922.6</td>
<td>17.4 ± 9.2</td>
<td>23.2 ± 6.3</td>
<td>54.5 ± 13.6</td>
<td>198.8 ± 46.7</td>
<td>119.9 ± 37.5</td>
<td>50.9 ± 11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value</td>
<td>0.050</td>
<td>&lt;0.001</td>
<td>0.036</td>
<td>0.001</td>
<td>0.002</td>
<td>0.312</td>
<td>0.314</td>
<td>0.114</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Range of normality and Units: BChE (pseudocholinesterase, 650–1450 U/l), AChE (Acetylcholinesterase, 9500–17500 U/l), ALT (alanine aminotransferase, <40 U/l), AST (aspartate aminotransferase, <37 U/l), GGT (gamma-glutamyl transferase, <40 U/l), ALP (alkaline phosphatase, 41–117 U/l), TG (triglycerides, 75–150 mg/dl), total cholesterol (110–220 mg/dl), HDL-c (High Density Lipoprotein-cholesterol, 35–65 mg/dl), LDL-c (Low Density Lipoprotein-cholesterol, 70–150 mg/dl).
showed an increase of about 5% in HDL-c. Other significant interactions observed were between BChE and PON1*5 allele on TG and between BChE and PON1*9 allele on total cholesterol.

Fig. 3 shows the relative or absolute effect size on the clinical chemistry parameters studied (depending on whether or not they were log-transformed, respectively) for each 10,000 U/hematocrit decrease in AChE activity for each particular genotype studied. A significant interaction was observed with PON1*108T polymorphism on AST and GGT levels. AChE activity also showed an interaction with PON1*108T and PON1*909C polymorphisms on AST. Regarding GST polymorphism, the only interaction observed was between AChE and GST-M1 = 0 on ALP, indicating that for each 10,000 U/hematocrit decrease in AChE, individuals with the null genotype showed an increase of about 13 U/l in ALP, while individuals with functional GST-M1 showed a non-significant increase of about 1 U/l. Regarding serum lipids, the only significant association found was between AChE and PON1*108T and PON1*909C polymorphisms on serum TG, indicating that for each 10,000 U/hematocrit decrease in AChE, heterozygous individuals for both genotypes exhibited significantly lower serum levels of TG.

4. Discussion

Potential risk or liver injury may occur after exposure to pesticides as most of these compounds are metabolized (either detoxified or bioactivated) in the liver. Although chronic exposure to mixtures of OP pesticides has been associated with hepatic dysfunction (Al-Sarar et al., 2009; Bhalia et al., 2006; Gokcimen et al., 2007; Gomes et al., 1999; Patil et al., 2009; Rojas-Garcia et al., 2011; Singh et al., 2011), the role of genetic polymorphisms of pesticide-metabolizing enzymes has not been addressed so far. In this study we have assessed whether exposure to pesticides in an intensive agricultural setting might induce changes in selected liver function test and serum lipids. Potential gene-environment interactions have also been assessed.

Serum BChE activity is a slightly more sensitive indicator of mixed pesticide exposure as compared to erythrocyte AChE activity (Richter et al., 1992) and is generally more rapidly inactivated by OP exposure. However, AChE is generally preferred over BChE because it is a more reliable marker of the neural target enzyme inhibition (US-EPA, 2000). Since AChE has been recognized as a biomarker for chronic toxicity in humans, it may be useful for assessing long-term pesticide exposure (Tinoco-Ojanguren and Halperin, 1998).

In this study, AChE activity was significantly decreased in the high exposure season as has been observed in similar studies (Sirivarasai et al., 2007). In addition to be inhibited by OPs and NMC insecticides, the membrane-bound enzyme AChE can be indirectly affected by lipid peroxidation induced by certain pesticides (including neonicotinoid and PYR) leading to a decreased enzyme activity (López et al., 2007; Rezg et al., 2008). Conversely, BChE, currently used as a biomarker of exposure to anticholinesterase pesticides, showed a significant increase in the period of high exposure. This paradoxical effect can be due to an enhanced BChE synthesis, resulting in serum levels 10–20% above normal baseline activity as has been previously observed after exposure to pesticides (Chen, 1972; Dorandeau et al., 2008; Harrison et al., 2009; Midtling et al., 1985; Ngai et al., 2002). This higher activity may act as a defense mechanism against regular occupational exposure to anticholinesterase pesticides and can be considered as a natural protective mechanism against sub-toxic exposures.

A reduction in AChE activity was associated with higher levels of AST and ALP. In turn, ALT and ALP activities increased with lifetime exposure to pesticides. Taken together, these findings suggest that long-term pesticide exposure may affect the liver function. By contrast, a reduction in BChE activity was associated with decreased levels of all liver enzymes, indicating that immediately after exposure anticholinesterase pesticides may interact with the enzyme molecules and alter their catalytic activities. This is consistent with previous studies where certain OP compounds (leptophos, chlorpyrifos and diazinon) inhibited ALT, AST and GGT (Enan et al., 1982). The herbicide paraquat was also found to cause a dose-and time-dependent reduction of transaminase activity (Podprasant et al., 2007).

been described as specific for toxic hepatitis (Kaloyanova and Vergieva, 1987; Provost et al., 2003) and thus, both serum lipids could be useful markers for liver dysfunction. Experimental studies have shown higher lipid levels after exposure to certain OP pesticides (Gokalp et al., 2003; Kalender et al., 2010; Slotkin et al., 2005; Sutcu et al., 2006). Besides, oxidative stress caused by pesticides may injure organs like liver, brain and pancreas, leading to impairments in metabolism of lipids, carbohydrates and proteins (Karami-Mohajeri and Abdollahi, 2010). Mechanistically, increased levels of lipid peroxidation products and reactive oxygen species can impair mitochondrial bioenergetics, resulting in liver dysfunction (Binukumar et al., 2010).

Seasonal variations have been reported for cholesterol and TG, with their levels being higher in winter than in summer (Cooney et al., 1995). In our study, no seasonal variation in serum lipids was observed for the control population, with the exception of a slight but significant reduction of HLD-c levels in the high exposure period. In contrast, the exposed population had significantly increased levels of cholesterol, TG and LDL-c in the period of high exposure to pesticides related to the low exposure period (data not shown). As occurred with controls, the exposed population also showed slight but significantly decreased levels of HLD-c in the high exposure period. In our study, a reduction in erythrocyte AChE was associated with increased levels of TG, total cholesterol and LDL-c and with reduced HLD-c. The latter lipoprotein is postulated to prevent the development of atherosclerosis by inhibiting the oxidation of LDL particles and by promoting reverse cholesterol transport. As a result, long-term pesticide exposure may be associated with a proatherogenic lipid profile and therefore it may be considered as a novel risk factor for insulin resistance and cardiovascular disease (Acker and Nogueira, 2012; Lasram et al., 2009). This finding calls for further experimental and epidemiological research.

We also studied the interaction between biomarkers of pesticide exposure (decrease in BChE and AChE) and gene polymorphisms of pesticide-metabolizing enzymes on clinical chemistry parameters. To our knowledge, this is one of the few epidemiological studies conducted so far assessing these interactions. Significant interaction effects were observed between decreased BChE activity and PON1 polymorphisms on liver function parameters (Fig. 1). PON1192RR genotype seems to play an important role in modulating short-term toxicity, because carriers of this genotype had increased levels of liver transaminases (AST and ALT) and lower levels of ALP and LDL-c. Significant interactions were also observed between decreased BChE and PON1155 polymorphism on serum lipid levels, indicating that homozygous for the PON1155 allele seem to be at an increased genetic risk upon short-term exposure to pesticides due to their higher levels of total cholesterol and LDL-c. On the other hand, carriers of the null genotype for GST (either M1 or T1) showed higher levels of ALT and GGT (Fig. 1), indicating a higher risk for cholestatic liver dysfunction after short-term pesticide exposure.

As regards to interactions between decrease in AChE activity and the genetic polymorphisms studied, PON1192RR was found to be the risk allele for hepatotoxicity because it contributed to a significant increase in levels of AST and GGT (Fig. 3). Although the PON1192RR genotype encodes a high-activity enzyme (with an apparently higher potential to protect individuals against OP exposure), the protection afforded by PON1 is substrate-dependent and related to the catalytic efficiency of the enzyme rather than to its catalytic activity (Costa and Furlong, 2010). On the other hand, when AChE decreased, higher levels of AST were observed for the PON1192RR and PON1192RR alleles whereas carriers of the GSTM1 null genotype showed increased ALP levels. More investigation is needed before drawing a definite conclusion as to whether or not these findings represent a true genetic risk upon pesticide exposure.

PON1 polymorphisms have been previously associated with hepatitis in a population exposed to pesticides (Tsatsakis et al., 2009 and Tsatsakis et al., 2011) indicating that liver function may be affected after long term pesticide exposure in individuals bearing the L55M and Q192R variants. Also, an increased risk of genotoxic effects has been reported for individuals with particular genotype combinations, such as PON1192RR and functional GSTM1 and GSTT1 (da Silva et al., 2008). Since pesticides are mainly metabolized in the liver, these effects may lead to cytoxicogen damage and further hepatocellular death.

Table 4

<table>
<thead>
<tr>
<th>Table 4</th>
<th>GEE models for the effect of genetic polymorphisms of pesticide-metabolizing enzymes on clinical chemistry parameters in all the study population.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PON1</strong></td>
<td><strong>PON1</strong></td>
</tr>
<tr>
<td><strong>QR</strong></td>
<td><strong>RR</strong></td>
</tr>
<tr>
<td>ALT*</td>
<td>0.15</td>
</tr>
<tr>
<td>AST*</td>
<td>0.01</td>
</tr>
<tr>
<td>GGT*</td>
<td>0.14</td>
</tr>
<tr>
<td>ALP</td>
<td>0.14</td>
</tr>
<tr>
<td>TG*</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL-c</td>
<td>0.05</td>
</tr>
<tr>
<td>LDL-c</td>
<td>2.45</td>
</tr>
<tr>
<td>HDL-c&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.02</td>
</tr>
<tr>
<td>LDL-c&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.46</td>
</tr>
</tbody>
</table>

Data represent β coefficients and 95% CI adjusted for gender, age, body mass index, tobacco and alcohol consumption.

<sup>a</sup> Log-transformed values. PON1192QQ, PON155LL, PON1192RR, PON1192RR and functional GSTM1, functional GSTT1 and Usual BCHE phenotypes were considered as the reference genotype in the models.

p < 0.05.

p < 0.01.
Fig. 1. Interaction effects between BChE activity and genetic polymorphisms of pesticide-metabolizing enzymes on liver function test. Data represent β coefficients, and 95% confidence intervals, adjusted for gender, age, body mass index, tobacco and alcohol consumption, calculated from multivariate GEE models. These coefficients indicate the change in biomarkers of liver function for each 100 U/l decrease in BChE levels in individuals for each particular genotype studied (for ALT, AST and GGT the change is expressed as percentage because these enzyme activities have been log-transformed). Only significant interaction terms (p < 0.10) are shown in the figure. See Supplementary material for a detailed interpretation of results.

Fig. 2. Interaction effects between BChE activity and genetic polymorphisms of pesticide-metabolizing enzymes on serum lipid profile. Data represent β coefficients, and 95% confidence intervals, adjusted for gender, age, body mass index, tobacco and alcohol consumption, calculated from multivariate GEE models. These coefficients indicate the change in serum concentration of lipids for each 100 U/l decrease in BChE levels in individuals for each particular genotype studied (except for TG, where the change is expressed as percentage because of its serum concentrations have been log-transformed). Only significant interaction terms (p < 0.10) are shown in the figure. See Supplementary material for a detailed interpretation of results.
genotype appear to be at an increased risk for liver dysfunction. Besides, carriers of the PON1192RR genotype are expected to show higher risk for an adverse lipid profile (i.e., increased levels of total-cholesterol and LDL-c) upon short-term exposure. Given that significantly decreased levels of ALT, AST, GGT and TG the change is expressed as percentage because their levels have been log-transformed). Only significant interaction terms (p < 0.10) are shown in the figure. See Supplementary material for a detailed interpretation of results.

5. Conclusion

The results of this study extend the information gathered from previous studies (Hernández et al., 2006 and Hernández et al., 2008) and taken together provide support for a subtle pesticide-induced liver dysfunction in the absence of clinically significant hepatotoxicity. The extremely large compensatory possibilities of the liver may account for the difficulty of proving a toxic effect in its early stage (Kaloyanova and Vergieva, 1987). Overall, our results suggest that regardless of the type of exposure, either short- or long-term, carriers of the PON1192RR genotype appear to be at an increased risk for liver dysfunction. Besides, carriers of the PON155MM genotype are expected to show higher risk for an adverse lipid profile (i.e., increased levels of total-cholesterol and LDL-c) upon short-term exposure. Given that significantly decreased levels of HDL-c were observed with the PON1192RR genotype after short-term pesticide exposure, a combined genetic risk for adverse cardiovascular events can be anticipated for carriers of the haplotype PON1192RR/55MM upon pesticide exposure. If these results are confirmed by further studies, the above-mentioned genotypes should be considered for the health surveillance of individuals occupationally exposed to pesticides.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was partially supported by a grant from the Council of Innovation of the Andalusian Government (Consejería de Innovación de la Junta de Andalucía). Reference Number PO9-CVI-5062.


