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Field Study

Paraoxonase 1 Correlates with Butyrylcholinesterase and Gamma Glutamyl Transferase in Workers Chronically Exposed to Pesticides

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Abstract: Paraoxonase 1 Correlates with Butyrylcholinesterase and Gamma Glutamyl Transferase in Workers Chronically Exposed to Pesticides: Manel ARAOUD, *et al*. Laboratory of Biochemistry 05/UR/09-09, Faculty of Medicine of Monastir, Tunisia—Objectives: Agricultural workers chronically exposed to complex mixtures of pesticides are at increased risk of acute and chronic toxicity of these compounds. Enzyme activities are among the biomarkers that may be used to detect the effects of pesticides before adverse clinical health effects occur. The aims of this study were to ascertain the relationships between paraoxonase 1 (PON1) and other serum enzymes and to investigate whether long-term exposure to pesticides affects these relationships in Tunisian agricultural workers. **Methods**: The activities of butyrylcholinesterase (BChE), aminotransferases, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK) were measured in plasma from workers chronically exposed to pesticides using an Integra 400 plus™ system. PON1 activity was measured using konelab 30™ system. **Results**: Significant increases in aminotransferases, CK, LDH and ALP activities were found in workers exposed to pesticides. However, BChE and PON1 activities were decreased significantly in these subjects. In addition, PON1 activity was positively correlated with both BChE and GGT activities in these workers. **Conclusion**: This study suggests that pesticides lead to alteration of serum enzymes and that chronic exposure to pesticides might contribute to explain the positive correlation between PON1 and GGT, perhaps in order to protect BChE and simultaneously induce detoxification of pesticides.
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Key words: Agricultural workers, Butyrylcholinesterase, Enzymes, Gamma glutamyl transferase, Paraoxonase1, Pesticides

Pesticides are used extensively throughout the world, and all people are inevitably exposed to these toxics through environmental contamination or occupational use. Therefore, agricultural workers chronically exposed to complex mixtures of pesticides are at increased risk of acute and chronic toxicity of these compounds.

Enzyme activities are among the biomarkers that may be used to detect the effects of pesticides before adverse clinical health effects occur[1)]. Cholinesterases are the most used enzymes as indicators of organophosphate (OP) exposure, in either acute or chronic toxicity[2)]. Altuntas *et al*. (2002) reported the increase in the activities of aminotransferases in workers engaged in an agriculture and health program due to effect of methidathion[3)].

Exposure to pesticides for a prolonged period affects the normal functioning of different organ systems, such as the liver and lung, and possibly produces characteristic clinical effects such as hepatitis and dyspnea[4)]. In addition, an association of pesticide exposure with changes in serum enzymes reflecting cytotoxicity, such as aminotransferases, lactate dehydrogenase (LDH) and creatine kinase (CK), has been reported in farmers engaged in intensive agriculture[1)].

Humans are diverse in their responses to exogenous exposure because of variability in the rate of metabolism, depending on the enzymes involved in the metabolic pathways. Serum gamma glutamyl transferase (GGT) plays an important role in the metabolism of pesticides[5)]. GGT as well as acetylcholinesterase (AChE) activities in lymphocytes of human cases of poisoning were found to be altered following acute intoxications by malathion,

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lindane and propoxur [5]. GGT was also considered to be among the oxygen free radical (OFR) enzymatic scavengers that protect against oxidative stress induced by acute or chronic pesticide exposure[6].

Paraoxonase 1 (PON1), another antioxidant enzyme, plays a major role in hydrolyzing OP esters, carbamate pesticides and nerve agents as well as oxidized lipids and pharmaceutical drugs. Animal studies have shown that raising rat plasma PON1 levels by intravenous administration of partially purified rabbit PON1 protected the rats against cholinesterase inhibition by paraoxon and chlorpyrifos-oxon[7]. This esterase was considered to be an important biomarker of susceptibility toward toxic agents especially OP pesticides[8]. PON1 levels were indeed found to be important to consider when trying to predict sensitivity to specific exposure[9].

The activities of PON1 and cholinesterase were decreased in applicators of pesticides[10]. It was therefore necessary to study the relationship between the enzymes concerned by pesticide in order to find, at least, a plausible explanation of the mechanisms, which are still largely unknown, by which these compounds can contribute to chronic events. Hence, the aims of this study were to ascertain the relationships between PON1 and other serum enzymes and to investigate whether long-term exposure to pesticides affects these relationships in Tunisian agricultural workers.

Subjects and Methods

Study subjects and samples collection

The study group consisted of 58 agricultural workers from 18 to 72 yr of age (40 ± 13 yr) who were chronically and occupationally exposed to pesticides in the Sahel region of Tunisia. The control population consisted of 89 healthy subjects without previous occupational exposure to pesticides or any other industrial chemicals and who were from 25 to 65 yr of age (39 ± 10 yr). There were no significant differences between the workers and control groups in regard to age and gender. All individuals were offered health examinations consisting of a medical history and a physical examination. Individuals presenting any chronic diseases were excluded from the population studied in order to avoid any interference with the parameters measured.

Blood samples were collected in heparinized tubes by venipuncture at the time of clinical examination. The samples were stored in a portable refrigerator and taken to the laboratory. Plasma was separated by centrifugation and then stored at –20°C until analysis.

All participants who agreed to participate in the study gave their informed consent and were allowed to drop out whenever they wanted. The study was approved by the Ethics Committee of the department of medicine.

Biochemical parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) and butyrylcholinesterase (BChE) were analyzed on an Integra 400 plus™ system (Roche Diagnostics).

Assay of PON1 activity

Serum PON1 activity was measured according to the modified method of Santanam and Parthasarathy[11] using a Konelab 30™ system. Briefly, serum (5 μl) was added to freshly prepared tris-NaOH buffer (0.26 M, pH 8.5) containing 0.5 M NaCl, 1.2 mM paraoxon and 25 mM calcium chloride. After thirty seconds of incubation at 37°C, the liberation of p-nitrophenol (ε=18.053 M^{-1} cm^{-1}) was followed at 405 nm for six minutes (at 54 second intervals).

Statistic analysis

All statistical analyses were performed with a statistical analysis program (SPSS 17.0). Log-transformed data were used for the statistical analysis after ascertaining that the parameters were approximately log-normally distributed. The Student's *t*-test was used to compare group means of parameters. The categorical variables were compared using the χ^2 test. The Pearson correlation coefficient was used to evaluate the degree of association between variables. Statistical significance was accepted for p values < 0.05.

Results

General information about the control group and agricultural workers are summarized in Table 1. There were no significant differences between the exposed workers and the control group in regard to age, gender, smoking habits and alcohol consumption. The mean duration of exposure to pesticides in the workers group was 19 ± 11 yr. These workers were versatile; they were involved in the preparation, storage and spraying of pesticides in the crop. Their daily duration of exposure to pesticides exceeded seven hours for two or three days per week. Of these workers, 90% reported irregularities moment and during pulverization of pesticides, and sometimes as needed, they pulverized several times per day. They were mostly exposed to a mixture of pesticides from different families, and 97% of these individuals used more than one pesticide at a time. Most of the pesticides used were organophosphorus and carbamates compounds (Table 2).

Table 3 shows the mean values of enzyme activities in the worker and control groups. Significant decreases in the BChE and PON1 enzyme activities were found in workers compared with the control group. However, the AST, ALT, CK and LDH activities were significantly

Table 1. General characteristics of the controls and exposed workers

Parameters	Controls	Exposed workers	*p* value
Numbers	89	58	–
Age (yr) (mean ± S.D)	39 ± 10	40 ± 13	0.761
Gender (n)			0.357
Female	10	11	
Male	79	47	
Smoking status (n smokers)	51	40	0.108
Alcohol consumption (n of consumers)	64	42	0.921

Table 2. Chemical class, common name, activity and World Health Organization (WHO) classification of pesticides frequently used by the studied population

Chemical class	Common name	Activity	Class (WHO)*	User (%)
Organophosphorus	Chlorpyriphos-ethyl	I	II	73
	Dimethoate	I, Ac	II	4
	Methamidophos	I, Ac	Ib	22
	Methidathion	I	Ib	19
	Cadusafos	Ne	Ia	19
Carbamates	Methomyl	I	Ib	71
	Propamocarb	F	NC	5
	Carbofuran	I	Ib	23

I: Insecticide, Ac: Acaricide, F: Fungicide, Ne: Nematicide. *: Ia: extremely hazardous, Ib: highly hazardous, II: moderately hazardous, III: slightly hazardous, NC: Not classified (according to the WHO classification of pesticides).

Table 3. Serum enzyme activities (log transformed) of the control group and exposed workers

	Controls	Exposed workers	*p*
PON1	5.31 ± 0.78	4.76 ± 1.23	0.004
BChE	9.02 ± 0.34	8.88 ± 0.31	0.003
ALT	2.18 ± 0.73	2.53 ± 0.48	<0.001
AST	2.81 ± 0.32	3.11 ± 0.27	<0.001
GGT	3.23 ± 0.28	3.22 ± 0.36	NS
CK	4.71 ± 0.48	4.91 ± 0.58	0.010
LDH	5.42 ± 0.43	5.73 ± 0.43	<0.001
ALP	4.29 ± 0.48	4.44 ± 0.36	0.021

Paraoxonase1 (PON1), Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) and butyrylcholinesterase (BChE). NS: not significant.

increased in the workers compared with the control subjects.

Serum PON1 activity was correlated with BChE activity (r=0.332, p=0.012) in the agricultural workers (Fig. 1), but not in the control group (r=0.084, p=0.438). In addition, Fig. 2 shows a positive correlation between the PON1 and GGT activities (r=0.398, p=0.002) in the workers, but no such correlation was found in the control group (r=0.160, p=0.137). Positive correlations were also found between the GGT and BChE activities for the

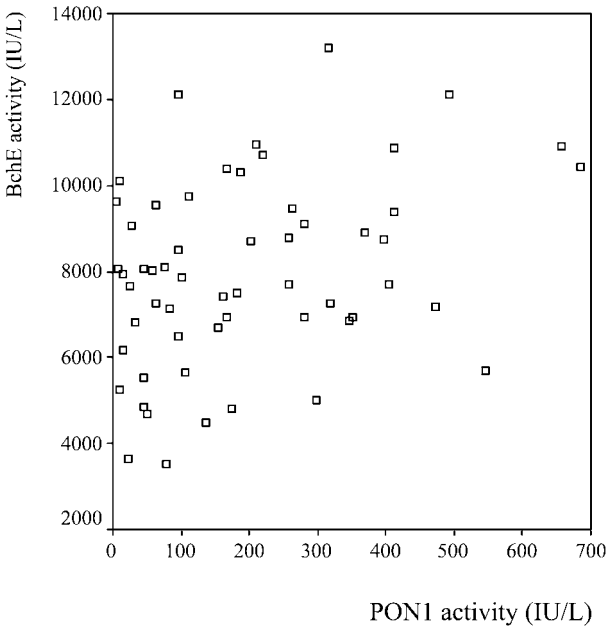

Fig. 1. Correlation between the PON1 and BChE activities in workers exposed to pesticides (r=0.332, p=0.012).

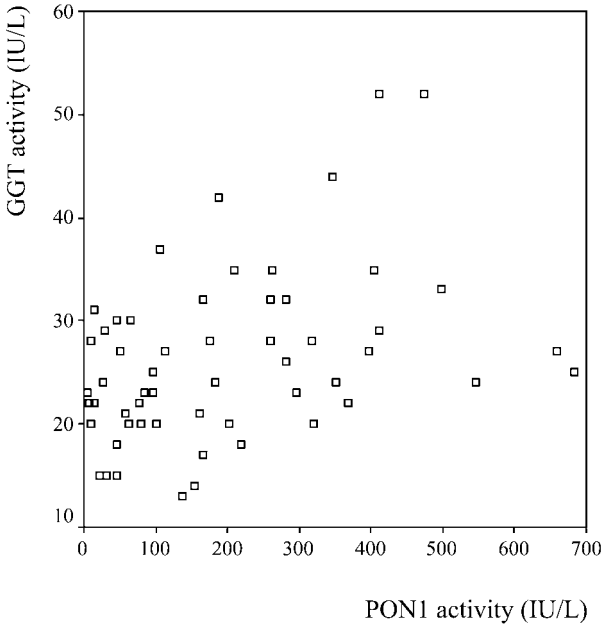

Fig. 2. Correlation between the PON1 and GGT activities in workers exposed to pesticides (r=0.398, p=0.002).

control group (r=0.396, p<0.01) and the workers (r=0.283, p=0.03).

Discussion

The present study revealed that certain serum enzymes in agricultural workers are influenced by long-term exposure to pesticides. Serum PON1 activity was significantly decreased in the serum of workers compared with the control group. These findings are in agreement with those of Hernandez *et al*.[10]. PON1 has been reported to hydrolyze OP, carbamates and aromatic esters[8–10]. The metabolic activation of pesticides to highly reactive intermediates might account for the decreased PON1 activity, as this enzyme can be inactivated by these compounds after oxidative stress challenge[10]. However, Zhou *et al*. (2007) reported that long-term exposure to OP pesticides can inhibit butyrylcholinesterase (BChE) and carboxylesterase but exerts no inhibitory effect on PON1 activity[12].

Likewise, the BChE activity was significantly decreased in the workers compared with the control subjects. Cholinesterase activity measurements are the most common way of determining organophosphorus and carbamate exposure in humans. It has been reported that AChE is better than BChE for the assessment of chronic exposure to OP. However, serum BChE activity has been reported to be a slightly more sensitive and suitable indicator of mixed exposure than red blood cell acetylcholinesterase activity[1, 10]. Additionally, although the effect of pesticides on BChE lasts at most a few weeks, BChE is precluded from full recovery when workers are continuously exposed to pesticides on a weekly basis[1].

In our study, there were no significant differences between the two studied populations in regard to GGT activity. This finding is in agreement with the results of Ranjbar *et al*. (2002), who reported no significant changes in GGT activity of manufacturing workers chronically exposed to pesticides and a control group[13]. Whereas, in a study of acute insecticide (malathion, lindane, propoxur) poisoning cases, Seth *et al*. (2001) indicated that the activities of GGT and acetylcholinesterase in human lymphocytes were altered following acute exposure to pesticides[5]. The effect on these enzymes was more pronounced in the malathion-exposed cases[5, 6]. These observations may suggest that alteration of the levels of GGT perhaps depends on the type of intoxication as well as on the type of pesticide used.

Significant increases were found for AST, LDH and CK, reflecting the risk of cytotoxicity. This finding suggests also that chronic exposure to pesticides may cause muscle damage or myopathy. Our results are consistent with those of Hernandez *et al*.[1]. Friendman *et al*. (2003) reported an elevation in CK in patients more than 10 yr after acute exposure to anticholinesterase compounds[14]. These authors hypothesize that this elevation was a sentinel event for impending muscle damage and necrosis. An increased risk of liver dysfunction was reported after spraying of herbicides in Vietnam, and the effect was suggested by the increase in AST, ALT and LDH activities[15].

Several studies have shown that PON1 has a major role in the detoxification of OP compounds[8, 9, 16, 17]. In the present study, the decrease in PON1 activity in the workers group was correlated with BChE activity. Our findings are in agreement with those of other studies[18, 19]. These studies revealed a correlation between PON1 and BChE in OP poisoning cases but indicated that this correlation was lower in subjects acutely exposed to OP than in subjects chronically exposed to OP insecticides. In addition, in high level acute exposure to pesticides, no additional time is available for enzyme detoxification[19]. Thus, PON1 is more effective against low levels of toxic agents in chronic exposure to pesticides. On the other hand, Hernandez *et al*. (2004) did not find any correlation between AChE and PON1 activities in greenhouse workers exposed to pesticides[10].

In our study, PON1 was significantly correlated with GGT but not with the other liver enzymes in the worker group. This correlation was not found in the control subjects. This finding suggests that chronic exposure to pesticides might contribute to explain the relationship between these two antioxidant enzymes. Recently, Lee *et al*. (2006, 2008) reported that organochlorine pesticides are positively related to serum GGT, within its reference interval, in the general population without any occupational exposure, and hence, this enzyme may be a biomarker of exposure to various environmental xenobiotics that are conjugated with GSH, including pesticides[21, 22]. This enzyme belongs to antioxidant defence system and has been implicated in the metabolism of pesticides[5].

Thus, PON1 and GGT enzymes may interact together in order to simultaneously induce the detoxification of pesticides and perhaps be part of the total antioxidant defence mechanism to protect the body against oxidative stress induced by toxic pesticide compounds.

Conclusion

In conclusion, significant increases in aminotransferases, CK, LDH and ALP activities were found in workers exposed to pesticides. However, the BChE and PON1 activities were decreased significantly in these subjects. These findings suggest that pesticides lead to alteration of serum enzymes. In addition, PON1 activity was correlated with both the BChE and GGT activities in these workers. Thus, we suggest that chronic exposure to pesticides might contribute to explain the positive correlation between PON1 and GGT, perhaps in order to protect BChE and simultaneously induce detoxification of pesticides.

Further investigations are necessary to confirm these initial findings in order to fully ascertain the mechanisms by which PON1 and GGT may interact to act against the adverse effects of pesticide compounds.

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