British Journal of Medicine & Medical Research
4(4): 949-956, 2014

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Effect of Dimethoate and Chlorpyrifos in Hepatic and Renal Function of People Belonging to Risk Groups in Iraklia Serres (N. Greece)

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Authors' contribution

This work was carried out in collaboration between all authors. Author GA did all literature survey, selection, collection of blood samples and laboratory analysis. Authors TA, SM and DL are designed the study and supervised the work. Authors EA, NA, FE part of experimental work and first draft of manuscript. Authors DP, NA and GA performed the statistical analysis and the final manuscript. All authors read and approved the final manuscript for publication.

Original Research Article

Received 3rd July 2013
Accepted 15th August 2013
Published 28th October 2013

ABSTRACT

Aims: There is little evidence concerning the effects of organophosphates in the liver of healthy individuals, and the existing researches come to contradictive results. In this study, we evaluated the influence of organophosphates (Dimethoate, Chlorpyrifos) in liver and renal function of healthy exposed workers, not experiencing symptoms of serious intoxication.

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Study Design: Measure serum activity of the liver function monitoring enzymes SGPT, SGOT, γ-GT and ALP and serum concentration of the renal function indicative biomarkers urea and creatinine.
Place and Duration of Study: Sample were collected in Health Care Greece of Iraklia Serres and analyzed in Department of Medical Laboratory Studies Alexander Technological Educational Institute of Thessaloniki.
Methodology: Blood samples were collected from 112 individuals, randomly selected from villagers of N. Greece. 42 of them were organophosphates (OP) applicators aged less than 50 years old (mean age 37 years old) and 42 were OP applicators older than 50 years old (mean age 58 years old); while 28 individuals (13 of them were less than 50 years old and 15 older than 50 years) were not OP applicators and used as control groups.
Results: A remarkable and statistically significant increase ($P < 0.05$) in the main liver-function monitoring enzymes (SGOT, SGPT, γ-GT) was observed in exposed people compared to the control group. Increase in ALP values compared to not exposed individuals was not observed. Concerning the kidneys, data analysis shows that there is not any significant effect on their operation by the use of OP.
Conclusion: The age of OP applicators and the time past between the application and the measure of blood serum seems to play an important role in the values of hepatic enzymes. While the renal indicators seemed not so much affected, as organophosphates are rapidly metabolized in human organism.

Keywords: Organophosphates; SGOT; SGPT; γ-GT; ALP; urea; creatinine; farm workers.

1. INTRODUCTION

Organophosphate (OP) compounds are a diverse group of chemicals which are used as pesticides. Most of them contain an inorganic thiophosphate group which is transformed to its oxo analogue following replacement of the S with O during the first phase of oxidative metabolism in the liver. Cytochrome P450 enzymes are involved in both oxidative desulfuration of the phosphorothionate and oxidation of the thioether group to sulfoxide [1], while other enzymes also seam to take part in metabolism [2]. The oxo-metabolite of OP pesticides is usually more toxic than the initial compound [3]. Since, most organophosphate are lipophilic, in a second phase they are converted to sulfate- or glucuronate conjugates in order to undergo urine excretion. Glutathione-mediated deakylation may also be involved [4]. Although, some organophosphates have a short half life and may be cleared from the body within hours, some others may have a half life of several days or weeks. It is believed that the more lipophylic OPs may be deposited in fat tissue and gradually released [5,6,7].

Inhibition of cholinesterase by organophosphoric pesticides or their metabolites plays a key role in toxicity. However, inhibition of other enzymes, such as neuropathy target esterase or other beta esterases and the direct effects of organophosphates on tissues are also important [8]. The symptoms are divided into three phases, acute cholinergic phase involving bronchorrhoea, salivation, and sweating, bronchoconstriction, bradycardia, vomiting and increase in gastrointestinal motility, cramps and diarrhea, muscle misfunction, headache, insomnia, giddiness, confusion, and, in severe exposures, convulsions, coma and respiratory depression [9], the intermediate syndrome that occurs 1-4 days after acute phase, involving respiratory function [10] and the organophosphate-induced delayed neuromyopathy which occurs 7-21 days after exposure and concerns long nerve function, affecting peripheral muscles [11]. Though, the effects observed during acute phase are

mainly due to the accumulation of the neurotransmitter acetylcholine within central nervous system or in peripheral junctions, mainly affecting muscarinic sites and nicotinic sites, the two other phases seam to have other etiology, involving alterations in nicotinic receptor function and in neuropathy target esterase, respectively. Other mechanisms, among which oxidative stress induction, have been found to be implicated in organophosphate toxicity [12]. Liver toxicity has been mentioned by some scientists [13]. Acute pancreatitis, rabdomyolysis and renal dysfunction were observed in certain cases [8,14].

Liver and renal damage are not among the main consequences of organophosphate poisoning. However, liver accepts and metabolizes organophosphates through oxidation and sulfate or glucuronate conjugation and may undergo oxidative damage.

There is little evidence concerning the effects of organophosphates in the liver of healthy individuals, and the existing researches come to contradictive results [14,15]. In this study, we evaluated the influence of organophosphates (Dimethoate, Chlorpyrifos) in liver and renal function of healthy exposed workers, not experiencing symptoms of serious intoxication, by measuring serum activity of the liver function monitoring enzymes SGPT (glutamate pyruvate transaminase, ALT), SGOT (glutamate oxaloxalate transaminase, AST), γ-GT (γ-glutamyl transferase) and ALP (alkaline phosphatase) and serum concentration of the renal function indicative biomarkers urea and creatinine.

2. MATERIAL AND METHODS

Blood samples were collected from 112 individuals, randomly selected from villagers of N. Greece. Forty-two of them were organophosphates (OP) applicators aged less than 50 years old (mean age 37 years old) and forty-two were OP applicators older than 50 years old (mean age 58 years old); while twenty-eight individuals (13 of them were less than 50 years old and 15 older than 50 years) were not OP applicators and used as control groups. No one of them is alcoholic or has any hepatic diseases. A few smokers are included in all four groups. For each individual two samples of serum was taken, the first in September, just after OP application (5 – 10 days) and the second one, three months later (during January).

SGOT, SGTP, ALP, γGT, Creatinin and Urea were estimated using colorimetric photometric Kits purchased by DiaSys (product No 2601, 2701, 0401, 2801, 1711, and 3101 respectively).

For each one of the above mentioned serum parameters, one - way ANOVA was applied in the control groups, to estimate possible influences of time and age in the non applicators population. Then t-test applied between each group of applicators and the respective control for possible differences. In order to examine if the age of the applicators has any influence on the above mentioned serum parameters we made t-test between different age groups (a vs b) for September and January, respectively. In addition, paired t-test applied in each age group of applicators (a, b) between the first and the second measurement (September vs January), in order to test possible influence of the time.

3. RESULTS AND DISCUSSION

One way ANOVA between control groups, for each one of the above mentioned serum parameters, shows that the null hypothesis (all mean are equal) can't be rejected ($P>0.05$),

so it could assumed that time and age has no influence in any one of the measured serum parameters in non applicators groups.

In table 1, the estimated means and SD of all the measured serum parameters (SGTP, cSGTP, SGOT, cSGOT, γGT, cγGT, ALP, cALP, Creatinin, cCreatinin, Urea and cUrea) are given separately for each age group and time of sample collection. Further more, significant statistical differences are signed in the same table.

SGPT: According to table 1 it could be assumed that the applicators of both ages were affected by the uses of organophosphates compared to their control, with an exception of SGTPb2. Applicators aged less than 50 years old have significant higher values (P=0.002 for September measurement and P=0.014 for January measurement) compared to the respective ones of b groups (age >50). Although there are no statistical significant differences for each group of applicators between first and the second measurement, a tendency of reducing SGTP concentration could be observed (fig. 1). SGTP values of OP applicators older than 50 years (b group) are lower than the respective ones of a group and further more, the most of them are lower than the maximum international acceptable value, 41 U/L, (table 1, fig. 2).

SGOT: According to table1 it could be assumed that the applicators of both ages were affected by the uses of organophosphates compared to their control, with an exception of SGOTb2. There are not significant statistical differences in time between the first and the second measurement for each group of OP applicators and between ages as well. The profile of each individual separately shows similar behavior (table 1, fig. 1).

Table 1. The estimated means, SD and statistical significant differences of all the measured serum parameters.

	Age (a<50)		Age (b>50)	
	September (1)	**January (2)**	**September (1)**	**January (2)**
SGPT[1]	36.48 ± 23.64 $^{*,\#}$	34.21 ± 19.58 $^{*,\#}$	23.38 ± 11.39 $^{*,\#}$	24.92 ± 13.71 $^{\#}$
cSGPT[1]	18.77 ± 7.88	19.81 ± 9.35	17.97 ± 7.65	19.03 ± 9.81
SGOT[1]	20.38 ± 7.89*	19.48 ± 7.35*	18.47 ± 7.19*	19.73 ± 8.66
cSGOT[1]	16.79 ± 5.26	16.83 ± 5.62	17.38 ± 4.09	18.96 ± 5.61
γGT[1]	39.14 ± 20.44*	40.26 ± 21.79*	32.31 ± 29.17*	33.55 ± 41.48
cγGT[1]	20.23 ± 8.92	21.62 ± 8.33	19.33 ± 5.42	21.67 ± 7.06
ALP[1]	178.55 ± 38.90$^{\#}$	187.4 ± 38.36$^{\#}$	161.64 ± 46.47$^{\#}$	166.83 ± 44.57$^{\#}$
cALP[1]	166.62 ± 38.30	165.62 ± 39.27	157.2 ± 33.0	152.47 ± 39.5
Creatinin[2]	1.05 ± 0.12 §	1.1 ± 0.11*,§	1.05 ± 0.14	1.09 ± 0.16
cCreatinin[2]	0.96 ± 0.23	0.96 ± 0.21	0.99 ± 0.22	0.99 ± 0.16
Urea[2]	31.26 ± 8.78 $^{\#}$	32.33 ± 7.44 $^{\#}$	39.67 ± 12.90 $^{\#}$	36.91 ± 8.16 $^{\#}$
cUrea[2]	34.85 ± 8.42	34.62 ± 8.38	39.87 ± 6.35	39.73 ± 7.83

1 units are expressed in U/L
2 units are expressed in mg/dL
** significant difference vs control (t-test, P<0.05)*
 # significant difference between age groups of applicators (a vs b) for September and January, respectively (t-test, P<0.05)
§ significant difference in the same age group of applicators between the first and the second measurement (1 vs 2) paired t-test (P<0.05)

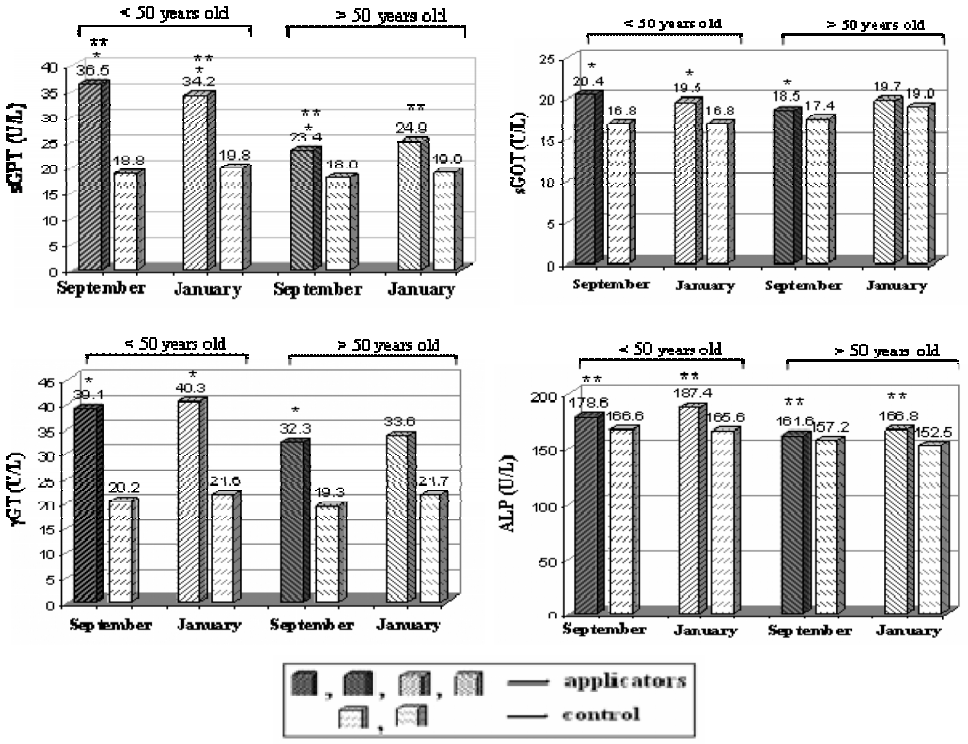

Fig. 1. Effect of OP in the liver enzymes (SGPT, SGOT, γGT, ALP) of different aged applicators in two time periods a) just after of the OP application (September) and b) three months later (January)

** significant difference vs control (t-test, P<0.05)*

*** significant difference between age groups of applicators for September and January, respectively (t-test, P<0.05)*

γGT: According to table1 it could be assumed that the applicators of both ages were affected by the uses of organophosphates compared to their control, with an exception of γGTb2. Although there are not significant statistical differences in time between the first and the second measurement for each group of OP applicators and between ages as well, there is a tendency of reducing the concentration of γGT in the serum of individuals aged more than 50 years (table 1, fig. 1).

ALP: Applicators aged less than 50 years old have significant higher values (P=0.045 for September measurement and P=0.014 for January measurement) compared to the respective ones of b groups (age >50). Further more, the mean value of ALP concentration in serum of applicators aged less than 50 years is out of the range of the maximum international acceptable value of 171 U/L (Table 1, fig. 1). A statistical significant increase in ALP values compared to not exposed individuals was not observed. However, mean ALP value exited normal high level in blood samples of the first collection in the younger group.

Creatinin: According to table1 it could be assumed that statistical significant differences appeared only between the first and the second measurement of applicators aged less than 50 years old as well as between the creatinin a2 vs cCreatinin a2 (table 1, fig 2).

Urea: According to table1 it could be assumed that there are not exist statistical significant differences between applicators and non applicators in both ages. The only statistical significant differences are these between the age groups (table 1, fig. 2).

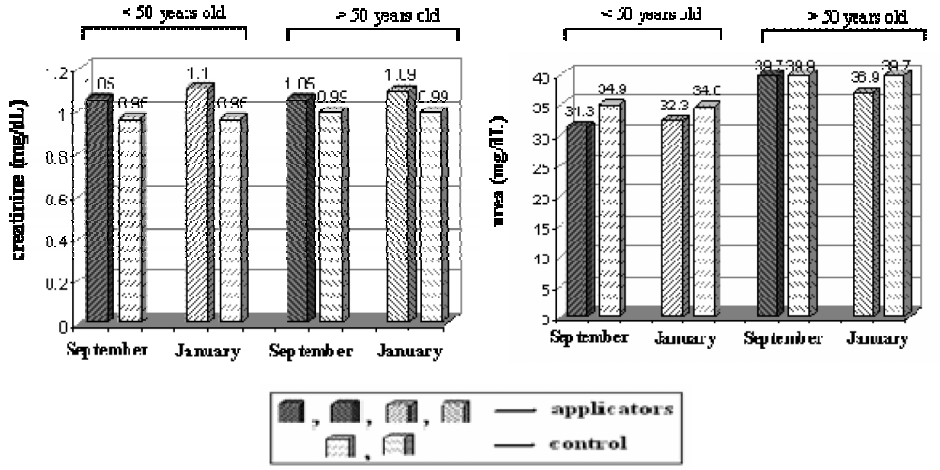

Fig. 2. Effect of OP in the kidneys enzymes (Creatinine and Urea) of different aged applicators in two time periods a) just after of the OP application (September) and b) three months later (January)

4. CONCLUSION

According to the results, a remarkable and statistically significant increase ($P < 0.05$) in the main liver-function monitoring enzymes (SGOT, SGPT, γ-GT) was observed in exposed people compared to the control group. Since, all values were within the normal range, the results do not indicate a considerable liver dysfunction or damage. However, increase of transaminases within the reference range, have been proposed as indicative for unhealthy liver function in some cases (15). A higher increase was observed in younger individuals, reaching 94.2% in SGPT and 93.6% increase in γ-GT values, during the first days after exposure (first blood collection). A much lower increase of 21.4% in SGOT values was observed, supporting the liver origin of transaminases. Serum transaminase elevation due to alterations in liver function may be the result of hepatic cell damage and enzyme leaking to the circulation or transaminase overexpression as a response to endoplasmic reticulum (ER) stress [16]. A slight decrease in serum transaminases and γ-GT values was observed in blood samples collected three months after exposure indicating gradual, although slow, recovery and return of liver function to the control rates.

Older individuals, responded differently in exposure to OP. A lower, though statistically significant increase in enzyme values was observed in blood samples collected a few days after exposure. More precisely, 30% increased SGPT, 6.3% increased SGOT and 67.4% increased γ-GT mean values were observed compared to the not-exposed control group. However, no significant change in enzyme profile was observed three months later. The overall profile points to a less extended initial effect of organophosphates in the liver of older individuals and a lower recovery rate of the affected tissue. A limited recovery possibility may be expected as a result of aging. However, initial limited effect, is an interesting observation which may need further investigation. Since, serum transaminases may increase as a result

of overexpression induced by ER stress and not only due to hepatic cell death, lower levels may indicate limited overexpression possibility [16].

Concerning the kidneys, data analysis shows that there is not any significant effect on their operation by the use of OP.

The increase of hepatic transaminases observed in this study is in correlation with increase in SGPT values observed in young exposed tobacco Pakistan workers mentioned in the literature (14). However, higher increase in SGOT values and statistically significant increase in urea and creatinine were also observed in that case. Variations in specific organophosphates used may result in differences in the measured biomarkers reflecting a different effect on liver and kidney. On the other hand, low and not statistically significant increase in SGPT and SGOT values was observed in a case of thiaziphos and acephate exposed young industrial workers [17], also outlining the importance of specific organophosphate and extent of exposure in the specific tissue effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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