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Effect of Chronic Exposure of Parathion/Paraoxon Pesticides on Testicular Functions in Spraying Workers

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ABSTRACT

Objectives: Manufacturing and spraying workers of organophosphate pesticides (OPs) could suffer from testicular spermogenesis and steroidogesis dysfunctions particularly when their degradation is subnormal.
Aim: Exploration of spermatogenic and endocrinologic testicular functions and paraoxonase/arylesterase (PON-1/ARY) degrading activities of the organophosphate parathion and its oxon in their middle age sprayers.
Patients and methods: Forty five parathion (PAR)/ paraoxon (PARO) sprayers (exposed group), provided the laboratory by their semen and blood samples during their working in PAR/PARO spraying. Of them 20 men had intestinal bilharziasis. Also, 20 healthy volunteers who were never exposed to these OPs (nonexposed reference group) provided similar samples. Semen quality and serum pituitary-testicular axis hormones and PON-1/ARY enzymatic activities were determined in the nonexposed control group as well as before and after 1 and 3 months of PAR/PARO exposure as sprayers.
Results: Parathion/paraoxon spraying workers had subnormal sperm concentration, motility%, viability% and morphology % 3 months after exposure. Sperm anomalies were not significantly different in presence of bilharziasis in comparison to non-bilharzial sprayers. In addition, PAR/PARO sprayers showed significantly decreased serum total and free testosterone concentrations with a negative feed-back increase of serum FSH and LH levels 3 months after exposure. Also subnormal serum PON-1 and ARY levels were found after prolonged PAR/PARO exposure due to exhaustion of the system by genetic damage.
Conclusion: Chronic PAR/PARO exposure induced subnormal semen quality, pituitary-testicular endocrinal function and circulating PON-1/ARY activities with unfavorable reproductive outcomes. Prevention of this occupational hazard is recommended.
KEYWORDS: Organophosphate, Parathion, Paraoxon, Paraoxonase, Arylesterase, Cholinesterase.

INTRODUCTION

Organophosphate (OP) compounds are widely used insecticides and pesticides in agriculture, health campaigns and urban pest control [(1)]. Almost all organophosphates require metabolic activation by their oxidative desulfuration to form the corresponding active oxon. Workers in OP manufacturing or spraying frequently showed subnormal semen quality causing decreased fertility rate and increased embryo loss [(2,3)].

Paraoxonase (PON-1) and arylesterase (ARY) are considered two separate enzymes in spite of their marked biological similarities. Plasma PON-1 catalyzes the hydrolysis of the bioactive organophosphate metabolite paraoxon to nontoxic products as p-nitrophenol and diethyl-phosphoric acid. At the same time, ARY is another aromatic esterase that inactivates the same OP but unlike PON-1 is not inhibited by cholinesterase inhibitors as phesostigmine (eserine) [(4)]. These enzymes are synthesized in the liver and circulate in the blood carried by the plasma high density lipoprotein (HDL). Both enzymes showed a wide variation in their activities among the different populations due to genetic polymorphism [(5)].

Prolonged OP exposure may induce decreased serum PON-1/ARY concentrations. However, different individuals and species have different OP hydrolyzing abilities[(1)] depending on the circulating esterase activity state on OP exposure[(6)]. Such a prolonged exposure can induce different harmful reactive oxygen species (ROS)[(7,8)], many chronic diseases such as atherosclerosis or diabetes mellitus[(8)], DNA fragmentation[(1,9,10)] and paraoxinase gene polymorphism or damage[(1,5,11)].

In this respect, the relation between OP exposure duration with PON-1/ARY bioactivity and male reproduction was poorly studied among Egyptian sprayers who had schistosomiasis, although OP intoxication and portal schistosomiasis represent an occupational health problem in them.

So assessment of testicular spermatogenic and endocrinal functions with circulating PON-1/ARY OP-deactivation capacity in PAR/PARO-Egyptian sprayers were the aim of the present study.

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SUBJECTS AND METHODS

Forty five agriculture PAR/PARO spraying male workers from northern Nile Delta Governorates were recruited in the present study. Of them 20 sprayers suffered from schistosoma mansoni infection (as shown by direct stool, rectal snip smear or IHA serum antibody tests). All donors had normal testes, epididymis and ductus deferens anatomy. Moreover, none of them had genital infection, significant varicocele or hydrocele. However, they did not follow perfectly the recommended measures for OP spraying. Informed concents were obtained from all investigated subjects. The laboratory was provided by their semen and blood samples after chronic PAR/PARO exposure as follows.

I- Semen samples:

After 3 days of sexual abstinence, semen samples were obtained from all sprayers after 30 days of PAR/PARO exposure (presenting an in vivo exposure of their spermatids and mature spermatozoa to OP). Of them, 39 workers gave second semen samples after 3 months of PAR/PARO exposure (reflecting an in vivo exposure during a complete spermatogenic cycle). Semen samples in between these two samples were not required for the laboratory.

Computer Assisted Semen Analyzer [Weili Color Sperm Analysis System-ALJY-9000, China] was used to determine sperm quality of the fresh semen samples (within one hour of ejaculation). Also, sperm vitality% was determined by eosin-y staining while seminal smears were stained by Papanicolaou method to assess sperm morphology [12].

II- Blood samples:

Seven ml fasting venous blood were withdrawn from every spraying subject to be used as follow:
1.6 ml blood was added into a tube containing 0.4 ml of 3.8% sodium citrate anticoagulant solution. After mixing and centrifugation, the blood cell deposit was separated and washed with saline three times. Then the red blood cells (RBCs) homogenate was used for erythrocyte acetylcholinesterase (AChE) estimation [13].

The remaining blood was left to clot and serum was separated after centrifugation and kept in a deep freeze at -70ºC till used for:

- Male sex hormones assay: IMMULITE 1000 (solid phase 2-site chemiluminescent immunometric assay) was used for determination of serum follicular stimulating (FSH) and luteinizing (LH) hormones [14] as well as total and free testosterone [15].
- PON-1[4] and ARY [16] activities assay:

Serum paraoxonase assay was performed after addition of 1.0 mol NaCl solution to the samples to stimulate serum PON-1 activity during its estimation. One unit of paraoxonase is its amount which produces 1.0 μmol of p-nitrophenol from hydrolysis of paraoxon substrate solution at 25°C per min.

Serum arylesterase assay: The rate of formation of phenol from phenyl acetate substrate using pH 8-TNE buffer was determined by spectrophotometer. One unit of arylesterase equals 1.0 micromol of phenol formed per min.

At the same time semen and blood samples were obtained from 20 healthy men who were never exposed to OPs (nonexposed control). They were matched in age and BMI with the OP spraying workers (Table 1). These samples were managed as those of the OP sprayers.

Statistical analyses:

SPSS for windows program version 11 (SPSS Inc., Chicago, USA) was used for calculation of:

1) Differences of seminal and serum mean values between sprayers (patients) and nonsprayers (controls) using the ANOVA test.
2) Correlations between conventional semen quality parameters (ejaculate volume and sperm concentration, motility, viability, and morphology) after OP exposure of donors for one and 3 months.
3) Significance at $p < 0.05$.

RESULTS

- The main demographic criteria of the spraying workers and healthy reference groups are shown in Table (1).
- Table (2) shows that the erythrocyte acetylcholinesterase activity was significantly lower ($p< 0.01$) in OP sprayers with relatively poor protective measures after three months exposure to PAR/PARO on comparison to the corresponding value of the unexposed control. At the same time, serum FSH and LH concentrations were significantly increased while serum total and free testosterone concentrations were significantly decreased in the sprayers after three months of exposure to PAR/PARO on comparison to the corresponding control values.
- Table (3) shows that spraying workers had subnormal sperm concentrations, motility % and viability % with high abnormal morphology % one hour after seminal collection in comparison to those of the normal volunteers. Seminal pictures showed no significant difference between bilharzial and non bilharzial spraying donors after one or months of OP exposure.

- Table (4) shows that serum PON-1 and ARY activities were significantly decreased after PAR or PARO exposure for three months.

Table (1): Demographic characteristics in agriculture PAR/PARO spraying workers and normal control groups.

	Data	
Characteristics	**Sprayers**	**Controls**
Age in years (X±SD):	34.5 ± 8.0	33.6±5.4
Body mass index in kg/m2 (X±SD):	24.2 ± 2.0	24.5±3.0

- NO significant difference between sprayers and unexposed control data (p>0.05)

Table (2): Erythrocyte AChE and serum pituitary-testicular hormones (FSH, LH and testosterone) concentrations in male spraying farmers after one and three months of PAR/PARO exposure Vs unexposed control group.

Variables	Unexposed control	One month exposure	Three months exposure
RBC Acetyl cholinesterase(U/h)	1.6±0.4	1.2±0.4	**0.7±0.2***
• **Pituitary – Testicular Axis function tests:**			
FSH (mIU/ml)	**4.8±1.6**	**5.7±1.8**	**7.9±2.1***
LH (mIU/ml)	**3.3±1.5**	**4.0±1.5**	**6.5±1.6***
Total Testosterone(ng/dl)	**587.6±91.7**	**490.4±63.7**	**343.5±60.8***
Free Testosterone(ng/dl)	**23.7±6.3**	**19.9±4.1**	**15.8±3.8***

* Significant from the control value (P<0.05)

Table (3): Semen quality in the agriculture PAR/PARO spraying workers versus reference data of OP-nonexposed volunteers.

Parameter	Control (20 subjects)	One month OP exposure (45 subjects)	3 months OP exposure (39 subjects)
Volume (ml)	2.8±1.0	2.9±0.8	**2.9±1.0**
Concentration$X10^6$/ml	74.5±19.1	66.1±11.4	**51.1±12.4***
Abnormal Morphology %	16.2±7.1	20.2±9.6*	**30.4±10.7***
Motility %	66.9±13.8	58.3±12.1	**41.7±9.5***
Vitality %	41.9±10.1	33.7±6.5	**22.5±4.6***
Linearity of motility:			
• **Straight line (u/s)**	61.2±8.0	54.2±9.2	**40.4±6.7***
• **Curl line (u/s)**	**38.8±6.1**	**45.8±8.4**	**59.6±9.0***

* Significant difference in comparison to normal group (p<0.000) and one month PAR/PARO exposure (p<0.001).

Table (4): Sodium chloride stimulated serum PON-1 and ARY activities (mean±SD) in agriculture PAR/PARO spraying workers and normal controls.

Data	Paraoxonase activity (U/L)	Arylesterase activity (U/L)
Normal control	571.9±66.8	158.8±40.3
Spraying workers:		
after one month exposure	610.0 ± 45.2	162.8 ± 25.9
after three months exposure	312.5 ± 38.1**	80.1 ± 19.7**

**: Significant from normal control and after one month exposure (P<0.000).

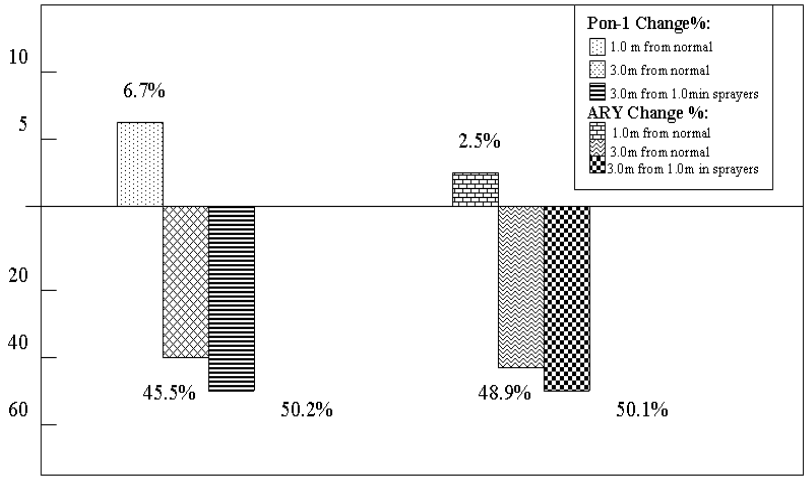

Figure (1): Percent of decrease of serum paraoxinase (PON-1) & arylesterase (ARY) activities from normal after PAR/PARO exposure for one and three months

DISCUSSION

Paraoxon (diethyl-o.p-nitrophenylphosphate) is the active neurotoxic metabolite of the inactive organophosphate parathion. It has a potent anti-cholinergic action and can be absorbed by the gastro-intestinal, cutaneous and pulmonary pathways[17]. However, the testicular activities of parathion and its oxon requires.

In the present study (Table 2), significant subnormal erythrocyte AChE activity was found among the sprayers after PAR/PARO prolonged exposure. However AChE levels were still higher than required to cause clinical neurotoxic manifestations. Decrease of RBC (true) acetyl cholinesterase activity was a concentration dependant of PARO and a biochemical indicator of organo-phosphate content in the body [7.17,18].

Also, the results of the present study showed lower circulating total and free testosterone concentrations and higher serum gonadotropins (FSH and LH) levels in the PAR-PARO sprayers than the corresponding reference values (Table 2). This negative feed back hyper gonadotropinemia could support the residual spermatogenesis [2,19] in these cases. However, Kamijima et al.,[19] found significantly higher than normal of serum testosterone concentrations after OP exposure during the off-season though FSH and LH concentrations were normal during both busy and off seasons.

Sperm cells at their late stages of maturation are sensitive to OP toxicity. In consequence, subnormal semen quality has been reported in OP manufacturing workers[2] environmentally OP exposed men[20] and agricultural insecticide sprayers of OP [19,21].

In this study (Table 3), after one month of PAR/PARO exposure, sperm count, motility%, straight line velocity and morphology% were subnormal. Meanwhile continuous OP exposure for three months exaggerated these unfavorable sperm reactions. Simple portal schistosomiasis did not deteriorate seminal picture in these sprayers. It has been reported that PAR/PARO toxic responses varied according to the exposed spermatozoal stage of development. Because spermatogenesis in men lasts about 2.5 months [2,19], one month of PAR/PARO exposure could induce changes during epididymal spermatids and spermatozoal maturation. On the other hand, 3 months of PAR/PARO exposure induced sperm cell developmental changes in their whole spermatogenesis cycle (early in meiosis and late in epididymal spermatid maturation). In such condition, spermatid and sperm maturations are at risk of genetic damage due to reduced defense mechanisms as DNA-repair [22] and antioxidant bioactivities [23,24]. In turn, after prolonged exposure to OP, spraying farmers were susceptible to unfavorable reproductive outcomes due to OP-adverse effects on semen quality [2].

In the present study, middle age Egyptian sprayers of PAR/PARO (Table 4 & Figure 1) induced an increase but not significant of PON-1 and ARY circulating levels during the first month of exposure. However, this was followed by a significant subnormal and dose dependent serum PON-1/ARY levels after the third month of exposure. This may be due to exhaustion of these hepatic OP-esterase production and subsequent enhancement of OP chronic toxicity. PAR/PARO may be accumulated in the circulation inducing many adverse effects among which testicular dysfunctions. In agriculture male workers, OP prolonged exposure decreased serum PON-1/ARY concentrations. However, different individuals and species confer different OP hydrolyzing abilities [1]. Meanwhile, Mackness et al. [6] found decreased capacity to detoxify OPs due to induced subnormal circulating esterase in gulf war syndrome. In turn, analysis of both these esterases in blood can assess the individual susceptibility or resistance to PAR /PARO intoxication.

In management of these cases, strict personal protective measures and withdrawal of chronic intoxicated subjects from the spraying team together with intake of antioxidant supplements, antibilharzial therapy and proper stimulation of spermatogenesis were advised.

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تأثير التعرض المزمن للباراثيون - الباراأوكسون على وظائف الخصية فى عمال رش الإبادة

المقدمة: تستخدم مركبات الفوسفات العضوية بصورة واسعة النطاق فى مقاومة حشرات وفطريات الزراعة والمساكن رغم تأثيرها المرضى على المستخدمين لها.

هدف البحث: دراسة علاقة معدلات المنى وهرمونات المحور النخامى - التناسلى الذكرى مع الباراأوكسينيز والاريل إستريز فى العاملين برش مبيدى الحشرات الباراثيون والباراأوكسون الشائعين الاستخدام.

أشخاص وفحوص الدراسة: 45 رجلا ممن يعملون فى رش مبيدى الباراثيون والباراأوكسون. اجريت تحاليل المنى و هرمونات المحور النخامى التناسلى الذكرى وأنزيمى الباراأوكسينيز – الاريل استريز لكل افراد المجموعة بعد شهر ثم ثلاثة شهور من التعرض المستمر لهذين المبيدين وكذا أجريت نفس التحاليل لعشرين شخصا من الذين لم يتعرضوا لمثل هذه المبيدات كمجموعة ضابطة وكان 44,4% من عمال الرش مصابين بالبلهارسيا المعوية المزمنة.

النتائج: أظهر عمال الرش انخفاضا ملحوظا فى حيوية الحيوانات المنوية مع زيادة فى عدد الحيوانات المشوهة بالمقارنة بمثيلاتها فى المجموعة الضابطة. كما وجد بالدم انخفاض ذو دلالة احصائية فى هرمون التيستيرون مع زيادة فى هرمونى الغدة النخامية المنشطين لهذا الهرمون وكذلك اوضحت النتائج نقص فى نشاط الباراأوكسينيز والاريل استريز المحللين لمبيدى الحشرات.

الاستنتاج: ان التعرض المزمن للباراثيون أو الباراأوكسون قد يؤثر على خصوبة الرجال وتزداد حدة هذا التأثير مع طول فترة التعرض كما ان التعرض يقلل من نشاط الانزيمين المسؤلين عن تكسير مركبى الفوسفات المذكورين فى الدم مما يجعل العاملين بالرش عرضة للعقم.