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EFFECTS OF CHLORPYRIFOS ON BRAIN OXIDANT/ANTIOXIDANT PARAMETERS IN PREGNANT/LACTATING RATS AND THEIR OFFSPRING

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Abstract

Description of the subject: Several studies have reported that low doses of Chlorpyrifos (CPF) target the developing brain during the period which cell division is occurring. It is well established that oxidative stress and excitatory synaptic transmission are closely related. Their disturbances are involved in most acute and chronic diseases of the central nervous system.

Objective : In the present study, we evaluated the effects of gestational CPF exposure on the oxidative stress in the offspring.

Methods : Oral administration of this pesticide at doses of 5.4mg/kg b.wt ($1/25LD_{50}$) and 13.5 mg/kg b.wt ($1/10LD_{50}$) was given 1day/2 to female rats during the entire gestation and lactation period and their pups. Several markers of oxidative stress were assessed by measuring the concentrations of brain malondialdehyde (MDA), carbonyl proteins and the activities of SOD and Catalase in pups at birth (day 0), weaning (day21) and 3 months of age (day90).

Results : These offspring had significantly higher brain MDA and carbonyl protein at days 21 and 90 compared with control offspring. At day 90, higher brain CAT and SOD activities was observed in experimental offspring compared with control offspring

Conclusion : CPF is not considered to be teratogenic at dose $1/10LD_{50}$ and $1/25\ LD_{50}$. However, the oxidative stress occurred during intra-uterine life, persisted through adulthood in offspring of rats exposed by gavage of CPF

Keywords : Chlorpyrifos ; brain ; oxidative parameters ; antioxidant enzymes

L'EFFET DU CHLORPYRIFOS SUR LES PARAMETRES OXYDANT / ANTIOXYDANT DU CERVEAU CHEZ LES RATES GESTANTES ET ALLAITANTES ET LEUR PROGENITURE

Résumé

Description du sujet : Plusieurs études ont rapporté que de faibles doses de chlorpyrifos CPF agissent au cours du développement du cerveau. Il est bien établi que le stress oxydatif et la transmission synaptique excitatrice sont étroitement liés, et sont impliquées dans des maladies aiguës et chroniques du système nerveux central.

Objectifs : Dans cette étude, les effets de l'exposition au CPF sur le stress oxydatif sont évalués chez le rat pendant la gestation, la lactation et chez la progéniture.

Méthodes : Le CPF a été administré par gavage aux doses de 5,4mg/kg de poids corporel (1/25DL50) et de 13,5 mg/kg de poids corporel (1/10DL50) aux rates gestantes, allaitantes et leur progéniture. Les marqueurs de stress oxydatif déterminés au niveau du cerveau sont le malondialdéhyde (MDA), les protéines carbonylées, et les activités du superoxyde dismutase (SOD) et la catalase chez les ratons à la naissance (jour0), au sevrage (jour21) et à 3 mois (jour90).

Résultats : La progéniture, de rates traitées au CPF, présentait une diminution des activités de la catalase et de la SOD, avec augmentation des taux de MDA et des protéines carbonylées au niveau du cerveau, à la naissance, à j21 et j90 comparée aux témoins.

Conclusion : Le CPF n'est pas considéré comme tératogène aux doses DL50/25, DL50/10, car il n'y a pas de toxicité maternelle, mais le stress oxydatif de la vie intra-utérine, a persisté à l'âge adulte chez la progéniture de rats exposés par gavage à ce pesticide

Mots clés : Chlorpyrifos ; cerveau ; paramètres oxydants ; enzymes antioxydantes

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INTRODUCTION

Pesticides are toxic chemicals that are widely used, throughout the world, in agriculture and other settings, resulting on continuing human and animal exposure. Several pesticides, especially organophosphorus insecticides (OPI) are neurotoxic, tend to cause severe "cholinergic syndrome" and affect mental health [1-2]. Otherwise, OPI exert toxicity, which has been reported to have adverse effects on the hematological and biochemical systems of human body [3]. Oxidative stress, related to an imbalance between the production of oxygen free radicals and the antioxidant defense system. It has been implicated as one of the mechanisms for the adverse health effects of OPI exposure [4]. Toxicity of OPI may induce oxidative stress leading to generation of free radicals and alteration in antioxidant system [5]. Although different studies shown that they can induce increase in oxidative damage in cells from various organs [6-7]. CPF is a broad- spectrum OPI that has win reputation in domestic, industrial and agricultural pest control [8- 9, 10]. CPF elicits a number of additional effects, including hepatic dysfunction, haematological and immunological abnormalities, embryotoxicity, genotoxicity and neurobehavioral changes [11, 12].The induction of oxidative stress is a common biochemical mechanism implicated in CPF [13, 14, 15]. It induces oxidative stress through increased levels of reactive oxygen species (ROS) and the accumulation of lipid peroxidation products in different organs [12, 4]. Several studies have reported that low doses of CPF target the developing brain during the period which cell division is occurring. It is well established that oxidative stress and excitatory synaptic transmission are closely related. Their disturbances are involved in most acute and chronic diseases of the central nervous system [16, 17]. The present study aimed to evaluate the effects of gestational CPF exposure on the oxidative stress in the offspring. Therefore, several markers of oxidative stress were assessed by measuring the concentrations of brain MDA, carbonyl proteins and the antioxidant enzyme activities of CAT and SOD in pups from chlorpyrifos-diet-fed at weaning throughout adulthood. Our present study aimed to understand how maternal gavage of CPF affects brain oxidant/antioxidant status and influences the development of diseases in the offspring.

MATÉRIALS AND METHODS

1. Chemicals

Chlorpyrifos ethyl (CE) [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate], which is being widely used as an insecticide in Algeria, was used in this study and obtained from INRAA, Algeria.

2. Animals and experimental protocol

Adult albino Wistar rats were obtained from the Pasteur Institute, Algiers, Algeria, and were used in this study. The rats were allowed to acclimatize to the laboratory environment for one week. After mating, the first day of gestation was estimated by the presence of spermatozoids in vaginal smears. Pregnant rats weighing 180 to 200 g were housed individually in plastic cages under standard conditions at 22 °C to 25°Cwith a 12 h light-dark cycle and were fed a normal diet and tape water was provided ad libitum. Pregnant rats were randomly divided into 3 groups each containing 10 animals. Group I (C/oil): Rats in the control were received orally corn oil (2mL/kg). CPF was prepared by corn oil. Group II was given CPF at a dose of 5.4 mg/kg body weight (1/25 LD_{50}) [18]. Group III was given CPF at a dose of 13.5 mg/kg b.wt. (1/10 LD_{50}) according to the protocol [19]. Animals were weighed weekly and the dose was adjusted accordingly. The route of administration selected for the study was oral gavage (1day/2) for the entire gestation and lactation days, and male offspring was followed to adulthood. This study protocol has been approved by the ethical committee of the experimental animal care at Tlemcen University. At days 0 and 21 for dams and days 21 and 90 for pups, six rats from each group were anaesthetized with intraperitoneal injection of sodium pentobarbital (60mg/kg of body weight).

Brain was dissected out, washed immediately with ice-cold physiological saline (0.9% NaCl) and weighed; one parts of the tissue immediately stored at 80°C until analysis

3. Biochemical assessment

- *Preparation of tissue sample:* Brain was homogenized in 0.9% NaCl using an Ultra Turrax tissue homogenizerto make up the 10% homogenate (w/v) and then centrifuged at 10000g at 4 °C for 20 min to obtain cytosolic fraction. Tissue homogenates (10%) were used to determine levels of malondialdehyde (MDA) and carbonyl proteins content. Cytosolic fractions of tissue homogenate (10%) were used to determine activities of antioxidant enzymes.

- *Lipid peroxidation assay:* Brain homogenate MDA (marker of lipid peroxidation) was estimated by the method of Draper and Hadley [20] using thiobarbituric acid (TBA).Absorbance was measured at 532 nm. The results were expressed as nanomoles of MDA, using the molar extinction coefficient of chromophore ($1.56\times10^5 M^{-1}cm^{-1}$).

- *Carbonyl proteins assay:* Brain homogenate carbonyl proteins (marker of protein oxidation) by the derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of stable dinitrophenyl (DNP) hydrazone adducts, which can be detected spectrophotometrically at 375 nm according to Levine et *al.* [21]. Oxidised BSA standard was used for the standard curve.

- *Antioxidant enzymes:* Superoxide dismutase (SOD, EC 1.15.1.1) activity in the cytosolic fraction of brain homogenate, was based on the ability to inhibit pyrogallol autoxidation, with one unit of SOD activity the amount that causes 50% inhibition of the oxidation of pyrogallol [22]. SOD activity was measured every 5min over 1 h at 405 nm.

The activity of catalase (CAT, EC 1.11.1.6) was assayed in the cytosolic fractions of brain homogenate by the decomposition of hydrogen peroxide according to the method of Aebi [23].

The reaction was initiated by addition of brain homogenate to the reaction mixture containing phosphate buffer (0.05 M, pH 7.2) and H_2O_2. Change in absorbance was recorded spectrophotometrically at 240. The results were expressed as unit of CAT activity corresponding to mmol of H_2O_2 decomposed per minute using the H_2O_2 standard curve.

4. Statistical analysis

The results are presented as means and standard deviations (SD). Significant differences among the groups were analyzed by Student's test, between experimental and control rats at each age. These calculations were performed using STATISTICA version 4.1 (STATSOFT). Differences were considered statistically significant at $*p < 0.05$, $**p < 0.01$.

RÉSULTATS

None of the rats treated with CPF at the dose of 5.4 mg/kg b.wt. (1/25 LD_{50}) and 13.5mg/kg b.wt. (1/10 LD_{50}) showed sign of morbidity or mortality during the studies.

1. Body weight and relative weight of brain in control and experimental rats

1.1. Mothers

The original body weight of the dams, prior the pregnancy, of the dams was similar among the three groups. Body and relative brain weights did not differ between control and mothers gavaged by CPF at 13.5 mg/kg b.wt. and 5.4mg/kg b.wt. at any age (Table 1).

Table 1. Body weight and brain relative weight in control and experimental rats at parturition (day 0) and at the end of lactation (day 21)

	Control rats	Experimental rats	
Parameter		1/10 LD_{50}	1/25 LD_{50}
Body weight (g)			
Day 0	278±11.16	285±20.21	261±9.28
Day 21	250±10.97	260±15.3	240±8.90
Brain (RW)			
Day 0	0.44±0.03	0.46±0.01	0.42±0.04
Day 21	0.68±0.02	0.72±0.03	0.70±0.01

Values are presented as means ± SD.

1.2. Offspring

At birth (day 0) and at weaning (day 21), no difference in body and relative brain weights was observed between pups. However, at 3 months of age (day 90), pups from CPF had consistently lower body and relative brain weight (Table 2).

Table 2. Post-natal changes in body weight and brain relative weight in control and experimental offspring

	Control offspring	Experimental offspring	
Parameter		1/10 LD_{50}	1/25 LD_{50}
Body weight (g)			
Day 0	4.89±0.30	5.70±0.52	4.97±0.31
Day 21	54.64±2.02	60±4.54	58.7±5.02
Day 90	300±12.23	254±22.3*	261±20.17*
Brain (RW)			
Day 21	2.01±0,02	2.03±0,02	2.04±0.03
Day 90	0.51±0,03	0.41±0,04*	0.40±0.02*

Values are presented as means ± SD. Significant differences between control and experimental offspring rats at day 0, day 21 and day 90 in each group are indicated as *$p < 0.05$.

2. Oxidative stress parameters

2.1. Mothers

Brain malondialdehyde (MDA) and carbonyl protein levels were higher, in mothers treated with the dose 13.5mg / kg and 5.4mg/kg of CPF compared with control mothers at days 0 and 21 (Table 3). Brain CAT and SOD activities were higher in CPF treatment mothers compared with control mothers throughout the experiment (Table 3).

Table 3. Oxidant/antioxidant status in control and experimental rats at parturition (day 0) and at the end of lactation (day 21)

	Control rats	Experimental rats	
Parameter		1/10 LD_{50}	1/25 LD_{50}
MDA (nmol/g tissue)			
Day 0	15.64±1.07	28.33±2.84**	23.10±1.38**
Day 21	18.44±1.52	30.34±1.67**	27.48±1.45**
Carbonyl proteins (nmol/g tissue)			
Day 0	0.54±0.01	0.70±0.02*	0.64±0.04*
Day 21	0.68±0.1	1.90±0.06**	1.6±0.1**
Catalase (U/g tissue)			
Day 0	44.33±7.20	71±3.04**	72±3.28**
Day 21	69.16±5.04	98±4.15**	86±3.40**
SOD (U/g tissue)			
Day 0	125±16.43	165±18.34*	146±16.11*
Day 21	134±15.52	206±12.86**	224±15.33**

Values are presented as means ± SD. Significant differences between control and experimental rats at day 0 and day 21 are indicated as *$p < 0.05$, **$p < 0.01$.

2.2. Offspring

Brain MDA and carbonyl protein levels increased in experimental offspring compared with controls at each age (Fig. 1). At day 90, higher MDA and carbonyl protein levels were observed in dams treated by CPF at 13.5 mg/kg b.wt. At weaning, brain CAT and SOD activities were similar in experimental offspring and controls. At day 90, however, experimental offspring treated by CPF, have significantly higher CAT and SOD activities compared with their controls (Fig. 2).

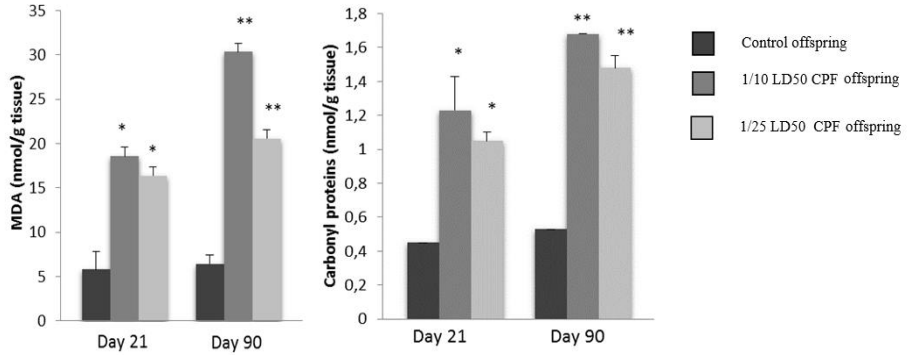

Figure 1: Brain oxidant status in control and experimental offspring

Values are means ± SD. Significant differences between experimental and control rats, at each age, are indicated by $*p < 0.05$ and $**p < 0.01$.

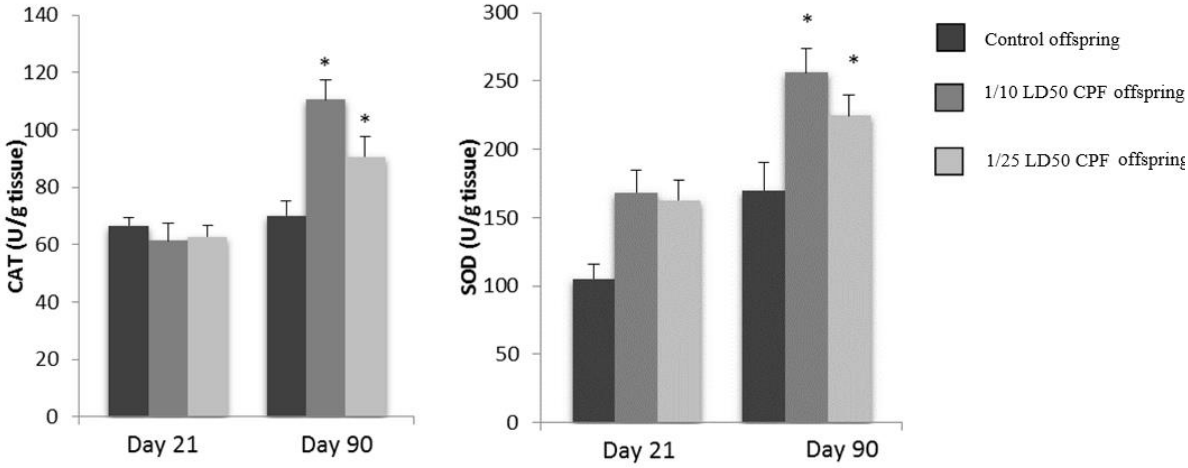

Figure 2: Brain antioxidant enzyme activities in control and experimental offspring

Values are means ± SD. Significant differences between experimental and control rats, at each age, are indicated by $*p < 0.05$.

DISCUSSION

The present study has evaluated the effect of CPF during pregnancy and lactation, in the induction of an oxidant/antioxidant imbalance on brain in the offspring of rats. The dams that received CPF orally by gavage had not a difference from body and relative brain weights after gestation and lactation periods. In this study, at day 90, the decreased body weight of offspring treated by CPF at a dose level equivalent to $1/10LD_{50}$ and $1/2/LD_{50}$was observed. These offspring also had a decrease in relative brain weight.

Our findings are in line with Goel et *al.* [24] work, that the net body weight gain of the animals intoxicated with CPF was markedly less as compared to the normal controls. In toxicological studies, body, organ and relative organ weights are important criteria for evaluation of organ toxicity [25, 26]. The reduction in body weight may be due to the combined action of cholinergic and oxidative stress [27-28].

Data reports that the central mechanism of pesticide action is changes in the cellular oxidative status. OPI can induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzyme activity [29, 30]. Free radicals have been implicated in the development of many acute and chronic diseases and in conditions involving brain or neurological tissue. Bellissimo [31] demonstrated that CPF-induced toxicity may be mediated in part by the generation of oxidative stress, and the brain is more vulnerable to oxidative stress than other tissues. Some indicators were measured to assess the oxidative stress status after CPF treatment.

Levels of MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, have been considered as an important indicator of lipid peroxidation [32]. In this study, we have shown that CPF treatment increase MDA levels in brain of the female rats at parturition and the end of lactation. Our present findings show that, in our experimental offspring the MDA levels in brain significantly increased. This observation is in accordance with other studies reported that CPF increased MDA levels on brain leading to an increase of lipid peroxidation [24].

Protein carbonyl groups are introduced via oxidation of proteins and can be used as markers for oxidatively modified proteins, and have been suggested to be a sign of tissue damage caused by oxidative stress, carbohydrate overload or both [33]. Protein carbonyl contents reflect the amount of oxidative stress that the animal has been exposed to during a long time period. In offspring of CPF treated dams, increased protein carbonyl levels indicated that free-radical-mediated oxidative damage occurred at an early stage of development.

In the present experiments, the production and activity of antioxidant enzymes also increase in relation to high oxidative stress.

CAT and SOD are the most important antioxidant enzymes, which metabolize toxic oxidative intermediates. Some studies have indicated that superoxide radicals can inhibit CAT activity and the increased H_2O_2 resulting from CAT inhibition could finally inhibit SOD activity [34]. The increased activity of SOD is known to serve as protective responses to eliminate reactive free radicals [36].

Previous studies have reported that CAT and SOD activities increased in rat tissues by OPI exposure [35, 24, 36-37]. The increase in CAT activity may have an adaptive response to oxidative stress driven by pesticides, and may be explained by their influence on hydrogen peroxide as substrate, which is formed in the process of dismutation of superoxide anion radicals [38]. The destruction of superoxide anion (O_2^-) and hydrogen peroxide by SOD and CAT improves induced toxicity of CPF in the same way as substances capable of scavenging hydroxyl radical. This is according with our results which have shown that exposure to CPF induced increased level of the brain antioxidant enzymes of experimental mothers and their offspring. The increased CAT and SOD activities might be associated with toxicity of CPF on rat brain tissue.

CONCLUSION

In view of the data of our result, it be concluded that CPF neurotoxicity could be attributed of the generation of free radicals. This increased brain oxidative stress that occurred during intrauterine life, persisted into adulthood in offspring.

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