Neurotoxic Effects of Chlorpyrifos and the Possible Protective Role of Antioxidant Supplements: an Experimental Study

Ghada Ghanem El-Hossary; Sahar Mahmoud Mansour; Anisa Saleh Mohamed

Pharmacology and Histology Departments, Research Institute of Ophthalmology, Giza, Egypt

Abstract: Chlorpyrifos is used increasing as a pesticide with increasing concern about being a neurotoxicant. The aim of the present study is to evaluate the morphological changes produced by chlorpyrifos in brain and optic nerve tissues and the possible protective effects of vitamins C and E. Forty male Wister rats were divided into four groups including two control groups. The other two groups were intoxicated with chlorpyrifos as a single oral dose of 63 mg/kg. One group was left untreated and the other group was treated once daily with 250 mg/kg vitamin C by intramuscular (IM) injection and 150 mg/kg vitamin E by intraperitoneal (IP) injection for 7 days. The results indicated that brain tissue of animals intoxicated by chlorpyrifos showed pyknotic nerve cell nuclei with partially occluded blood vessels surrounded by perivascular edema. The optic nerve showed degenerative changes in the form of increased vacuolations, thick pial septa and abnormal myelin sheath. Treatment with vitamins C and E produced marked improvement in brain tissue while the optic nerve specimens still displayed persistent degenerative changes. In conclusion, using chlorpyrifos can produce morphological changes in brain and optic nerve tissues. Combined vitamins C and E supplementation could protect brain tissue but were not so useful in protecting against chlorpyrifos-induced optic nerve damage.

Key words: chlorpyrifos, vitamin C, vitamin E, Neurotoxicity, Neuroprotection.

INTRODUCTION

The widespread exposure of humans to organophosphorus pesticides, such as chlorpyrifos, has raised increasing concern about their neurotoxicity particularly in the developing brain[1]. It was shown that chlorpyrifos inhibits DNA synthesis, mitosis, neurite outgrowth and neural cell replication and differentiation[6]. It also interferes with signaling cascades including serotonergic, cholinergic and catecholimergic pathways[3]. In addition, chlorpyrifos inhibits glial cell replication, gliogenesis and glioma cell differentiation and disrupts the normal pattern of glial cell development[4]. Moreover, chlorpyrifos produces inflammatory activation of astrocytes which play critical roles in the proper function of the brain[1]. Organophosphates act by inhibition of cholinesterase enzyme leading to cholinergic hyperstimulation with its associated symptoms. However, many neurotoxic effects involve mechanisms unrelated to this mechanism and are elicited even at exposures below the threshold for cholinesterase inhibition[6]. Oxidative stress has been implicated as a contributing mechanism in development of different neurotoxic effects of chlorpyrifos such as seizures[7], developmental neurotoxicity[6], retinal toxicity[9] and nephrotoxicity[10].

In addition, some antioxidants were reported to ameliorate different chlorpyrifos-induced toxic effects[7,8,10,11,12]. Oxidative stress is one of the factors that can lead to DNA damage and apoptosis[13]. In fact, some reports attributed the neurotoxic effects of chlorpyrifos to its apoptotic effects[14,15].

The diversity of mechanisms underlying the toxicity of chlorpyrifos is one of the reasons that urge investigators for identification of target organs that may contribute to the understanding of the mechanism of action. Since there is limited research investigating the microscopic effects of chlorpyrifos on the brain tissue and the optic nerve, so the objective of this study is to evaluate the toxicity of the administered dose of chlorpyrifos in adult male rats based on the results of the histological investigation. The protective effects of combined vitamins C and E supplementation will also be assessed histologically.

MATERIALS AND METHODS

Induction of Chlorpyrifos Acute Toxicity: Chlorpyrifos (Chlorofet from Vapco, Jordan) was reconstituted in corn oil to get a 1% concentration to be administered in a dose of 63 mg/kg as a single oral dose by a stomach tube[16].

Corresponding Author: Ghada Ghanem El-Hossary, Pharmacology Department, Research Institute of Ophthalmology, Giza, Egypt
E-mail: gghossary@yahoo.com
Animals: Forty male Wistar rats weighing 140-150 grams, aged 3 months, were used. They were individually housed in separate cages under standardized temperature (25-28 °C), humidity (50%-60%) and light conditions (12 hours light–dark cycles). They were fed the standard diet and water for seven days. All care and handling were in accordance with institutional guidelines for use of animals in ophthalmic and vision research with approval of the institutional authority for laboratory animal care.

Animals were divided into four groups; each was consisting of ten rats. Group I- Animals received an equivalent volume of saline by IM and IP injections once daily for 7 days (negative control). Group II- Animals were treated once daily with 250 mg/kg ascorbic acid (vitamin C from Memphis, Egypt) by IM injection and 150 mg/kg alpha tocopherol (vitamin E from Sigma, Germany) by IP injection for 7 days (positive control). Group III- Chlorpyrifos was administered once orally by a stomach tube in a dose of 63 mg/kg on the 1st day of experiment (untreated chlorpyrifos toxicity). Group IV- Chlorpyrifos was administered as mentioned above and the animals were treated concomitantly once daily with vitamin C and vitamin E via IM and IP injections respectively for 7 days (the same doses as in group II).

Histological Examination: The animals were anesthetized by ether and sacrificed by cervical dislocation. Specimens of brain tissue were collected and processed for paraffin sections and stained by haematoxyline and eosin (Hx & E). Optic nerve specimens were processed for semithin sections and stained by toluidine blue (TB). Slides were examined under Olympus light microscope and photographed by Olympus camera.

RESULTS AND DISCUSSION

Histopathological examination of specimens taken from groups I and II (negative and positive controls respectively) showed normal appearance. The cerebral cortex showed variable sizes of pyramidal cells. The intercellular area is occupied by nerve fibers and neuroglial cells (Fig. 1). The optic nerve of control group showed densely packed nerve fibers with regular myelin sheath. Thin pial septa were seen between the compact nerve axons (Fig. 2). In group III (untreated chlorpyrifos toxicity), histopathological examination of cerebral cortex cells revealed clumping of chromatin of their nuclei giving rise to the formation of some pyknotic (dense) nuclei of nerve cells. Meanwhile, the majority of neurocytes had vesicular nuclei. Fine vacuoles were also seen in some neurocytes. Partially occluded blood capillaries surrounded by perivascular edema could be noticed (Fig. 3). In optic nerve specimens was a striking observation of thick pial septa that were compressing the nerve axons. Moreover, the increased vacuolations and abnormally disrupted myelin sheath were clearly obvious (Fig. 4).

In the current study, the histopathological changes of cerebral cortex cells (pyknotic nuclei and vacuolations) are in accordance with previous reports. Some authors reported focal pyknosis of nuclei in neurocytes of cortex cerebri and cerebellum as a result of dermal application of chlorpyrifos and cypermethrin in rats[14]. In addition, chlorpyrifos was reported to produce histopathologic manifestations of cytotoxicity in neuroepithelium of cultured rat embryos. As examination of sections from forebrain and hindbrain revealed cytoplasmic vacuolation, enlargement of intercellular spaces and the presence of a significant number of apoptotic cells. The observed pyknotic nuclei are indicative of DNA damage and they occur most probably when the cells are exposed to toxic or biological insults. These insults lead to activation of apoptosis and the cells die in a programmed fashion. Chlorpyrifos was reported to produce apoptosis in the retina[9], in cultured rat embryos[14] and in immune cells[15] which is in agreement with the results of the present study. Chlorpyrifos was also reported to produce severe oxidative stress[17] which can be an additive factor producing DNA damage and finally apoptosis[18].

In the present investigation, the presence of edema around some blood capillaries can be an indicative of disruption of the blood brain barrier. In fact, disruption of the blood brain barrier with alteration of its integrity and structure were previously reported effects of chlorpyrifos[19] which may further support our results. The striking documentation was the compression of nerve axons by thick pial septa and deformity of myelin sheath in the optic nerve. To the best of our knowledge, such dramatic structural alterations due to chlorpyrifos administration have not been reported previously. These dramatic effects can be due to the severe oxidative stress induced by chlorpyrifos which can lead to lipid peroxidation of both myelin sheath and cell membrane leading to their destruction.

In animals of group IV (chlorpyrifos toxicity treated with a combination of vitamins C and E), cerebral cortex specimens exhibited marked improvement with almost normal morphological appearance of nerve cells with only residual fine vacuolations of pyramidal cells (Fig. 5). On the other hand, the optic nerve specimens displayed persistent degenerative changes in the form of severe vacuolations and myelin sheath deformity (Fig. 6). In consonant with the present study many reporters tried to treat or ameliorate the toxic effects of chlorpyrifos.
on different tissues. El-Shazly and associates\[20\] have successfully used combined vitamins C and E in vivo to protect against acute chlorpyrifos toxicity and noticed improved erythrocytes reduced glutathione, serum malondialdehyde which are indicative of oxidative stress and soluble FAS ligand which is indicative of apoptosis. They also noticed improved histological picture of retina and kidney as compared to untreated toxicity model\[20\]. In addition, there was improved retinal cellular apoptosis, DNA damage and retinal lipid peroxidation when Yu and colleagues\[9\] used combined vitamins C and E in vivo to protect the rat retina from acute chlorpyrifos toxicity which is in agreement with the results of the present study. Others reported marked improvement of levels of erythrocyte antioxidants when a combination of vitamins C and E was used along with melatonin in vivo to protect rat erythrocytes against chlorpyrifos toxicity\[11\]. Moreover, a combination of vitamins C and E protected against chlorpyrifos-induced nephrotoxicity. Oncu and associates\[10\] noticed preserved kidney tissue architecture and improved levels of antioxidants in renal tissue of rats intoxicated with chlorpyrifos.

**Conclusion:** The results of the present study clearly demonstrate that oral administration of chlorpyrifos leads to histopathological changes in brain tissue as well as optic nerve. Although the brain tissue was well protected against these changes by a combination of vitamins C and E, these antioxidant vitamins were not so useful in protecting against chlorpyrifos-induced optic nerve damage. Further studies are suggested to test for other potential neuronprotective agents and their value in prophylaxis of chlorpyrifos toxicity in other organs.

**Fig. 1:** Light micrograph of control cerebral cortex showing pyramidal cells of variable sizes. The intercellular area is occupied by nerve fibers and neuroglial cells (Hx & E X500).

**Fig. 2:** Light micrograph of control optic nerve showing regularly myelinated axons (TB X500).

**Fig. 3:** Light micrograph of cerebral cortex of group III (untreated chlorpyrifos toxicity) showing marginal clumping of chromatin of nuclei of most neurons (M), while few cells have pyknotic nuclei (P). Partially occluded blood capillaries surrounded by perivascular edema are obvious (arrow) (Hx & E X500).

**Fig. 4:** Light micrograph of semi-thin sections of optic nerve in group III (untreated chlorpyrifos toxicity) showing compression of nerve axons by thick pial septa (S), increased vacuolations (V) and abnormally formed myelin sheath (arrow) (TB X500).
Fig. 5: Light micrograph of cerebral cortex of group IV (chlorpyrifos toxicity treated with a combination of vitamins C and E) showing nearly normal morphological appearance of nerve cells with residual fine vacuolations of pyramidal cells (Hx & E X500).

Fig. 6: Light micrograph of semi-thin sections of optic nerve in group IV (chlorpyrifos toxicity treated with a combination of vitamins C and E) showing persistent degenerative changes in the form of severe vacuolations and myelin sheath deformity (d) (TB X500).

REFERENCES


