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Oxidative stress and cholinesterase inhibition in plasma, erythrocyte and brain of rats' pups following lactational exposure to malathion

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ABSTRACT

The organophosphorus (OP) pesticide malathion is a highly neurotoxic compound. Some studies have reported neurotoxicity signs after *in utero* exposure to OP pesticides. However there is no evidence of the exclusive contribution of the lactational exposure to malathion as a possible cause of neurotoxicity in rats' pups. In this respect, we investigated the exclusive contribution of malathion (200 mg/kg, *b.w.*) exposure through maternal milk in rat pups during lactation. We evaluated the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), as well as on biochemical parameters related to the oxidative stress such lipoperoxidation and antioxidant enzyme activities as superoxide dismutase (SOD) and catalase (CAT) in the brain, plasma and erythrocytes of rats' pups at 21st postnatal day (Pnd). These parameters were also evaluated in the same tissues but at 51 Pnd. Our results showed that the malathion exposure during lactation induced a high inhibitory effect of the brain, plasma and erythrocyte AChE and BChE activities in rat pups. Many changes were observed in the biochemical parameters related to the oxidative stress for pups brain, plasma and erythrocyte. The present study shows, for the first time, that the exposure of postnatal pups to malathion *via* lactation inhibits the activity of brain, plasma and erythrocytes cholinesterase in the pups. These findings suggest that malathion exposure during lactation induced a cerebral alterations and oxidative stress in rat pups.

1. Introduction

Since the industrial revolution, extensive use of pesticides has led to serious environmental contamination and potential health hazards including acute and chronic cases of human poisoning (Kamath and Rajini, 2007). Organophosphate (OP) insecticides, chemical compounds which inhibit acetylcholinesterase (AChE), constitute one of the large extensively used classes of pesticides employed for agricultural and landscape pest control. Due to the wide accessibility of OPs, toxic effects in human have been detected (Tsatsakis et al., 1998; De-Bleecker et al., 1993). In fact, OPs were primarily recognized for their aptitude to provoke toxicity in mammals through inhibition of AChE and subsequent activation of cholinergic receptors (Costa et al., 2006). OPs could also exert toxic effects on many other tissues and organs including brain (Kalender et al., 2007), liver and soft tissues (Gomes et al.,

Abbreviations: MDA, malondialdehyde; SH, groupements sulfhydryl of protein; AChE, acetyl cholinesterase; BChE, Butyrylcholinesterase; SOD, superoxide dismutase; CAT, catalase; POD, total peroxidase; CNS, central nervous system; TBARS, thiobarbituric acid reactive substances; RBCs, red blood cells; OP, organophosphorus.

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1999). Moreover, the lipophilic nature of OPs facilitated their interaction with cell membrane and led of the phospholipids bilayer structure perturbations of most visceral organs (Videira et al., 2001). Brain was one of the target organs of experimental animals attacked by acute, sub-chronic and chronic exposure to OPs (Chanda et al., 1995). Besides, it has been postulated that OPs produced oxidative stress in different tissues, such as brain of rats and mice, through the formation of reactive oxygen species (ROS) (Sivapiriya et al., 2006; Sulak et al., 2005). Neurotoxicity signs have been reported after the *in utero* exposure to some OPs pesticides (Chanda et al., 1995). However, there is no evidence of the exclusive contribution of the lactational exposure to malathion as a possible cause of neurotoxicity in the rats' pups. Importantly, the presence of OPs pesticides, including malathion has been reported, in bovine, mouse and human milk (Sanghi et al., 2003) showing the relevance in terms of human health concerns. Recently, the residue of malathion and its analog (malaoxon) were found in many foods including cow's milk and mice (Battu et al., 2004; Salas et al., 2003). Although data on acute, sub-acute and chronic toxicity of malathion in our laboratory were well documented. However, it was recently shown that malathion is capable of altering cerebral and metabolic functions in adult rats leading to oxidative damages (Rezg et al., 2007; Lasram et al., 2008).

Hence, the present study aimed to investigate the putative effect of malathion on cerebral function and oxidative stress of pups from exposure during lactation.

2. Materials and methods

2.1. Chemicals

Malathion (fyfanon 50 EC 500 g/l), commercial grade, was was obtained from the Agricultural Struggle Center, Tunis, Tunisia. 5,5-dithio bis (2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCh), *S*-butyrylthiocholine iodide (BTCh) trichloroacetic acid (TCA), KOH, ethanol, ether, Coomassie G250, bovine serum albumin, orthophosphoric acid 85%, hemoglobin and NaCl were obtained from Sigma–Aldrich Co. (Germany).

2.2. Animals and treatment

Wistar rats from SIPHAT (Tunis, Tunisia), weighing 230–250 g, were used for this study in accordance with the local ethics committee of Tunis University for use and care of animals in conformity with the NIH recommendations. The animals were housed in standard cages (40 cm × 28 cm × 16 cm) under controlled conditions: 12:12 h light–dark, 20–22 °C, food and water available *ad libitum*. Primiparous females were placed three per cage with one male breeder and vaginal smears were examined daily in the morning (between 09:00 and 10:00 h). The day 0 of pregnancy was confirmed by the presence of a vaginal smear of both vaginal cells typical of the oestrous stage and spermatozoa. Malathion was dissolved in corn oil and administrated per orally (p.o.) during lactation (for 21 days). Injection volume was 1 mL/kg *b.w.*

Pregnant females were placed one per cage and divided into four groups as follows:

Group I and III: received malathion (200 mg/kg, *b.w.*, p.o.) during lactation (21 days).
Group II and IV: received corn oil during lactation (21 days).

Animals of group I and II were sacrificed at 21 Pnd) For group III and IV sacrifice was performed at 51 Pnd. Brain, liver, muscle were rapidly excised and homogenized in phosphate buffered saline, pH 7.4, for biochemical determination. Blood was also collected and plasma was separated from erythrocytes and both processed for biochemical determinations.

2.3. Biochemical determinations

2.3.1. Hematologic parameters

A hematocrit measurement was carried out in capillary tubes centrifuged with HEMATOCRIT 20 Hettich for 15 min at 1000 × *g*. Hemoglobin concentrations in whole blood were spectrophotometrically analyzed at 540 nm for the cyanomethemoglobin (Zijlstra and Kampen, 1960). Blood samples were mixed with 5 mL Drabkin's solution (0.1% sodium bicarbonate, 0.005% potassium cyanide and 0.02% potassium ferricyanide) for hemoglobin determination.

2.3.2. Glycemia and glycogen assays

Glucose was measured by the glucose oxidase and peroxidase (Lott and Turner, 1975a,b) using quinoneimine as a chromogen. The amount of plasma glucose is related to amount of quinoneimine, which is measured spectrophotometrically at 505 nm (Lott and Turner, 1975a,b).

For determination of glycogen, 0.5 g of liver was extracted with 3 mL of 30% KOH, incubated for 30 min at 100 °C, and then brought to acid pH by addition of 20% trichloroacetic acid. Precipitated protein was removed by centrifugation for 10 min at 3000 × *g*. Glycogen was precipitated by ethanol and weighed. The results were expressed in g of glycogen/100 g of liver (Good et al., 1933).

2.3.3. Cholinesterase activities assays

Acetylcholinesterase (E.C.3.1.1.7) and butyrylcholinesterase (E.C.3.1.1.8) activities were determined at 25 °C in 0.1 M phosphate buffer (pH 7.4) with 0.3 mM DTNB and 1.0 mM ATCh or BTCh according to Ellman method (Ellman, 1961).

2.3.4. Antioxidant activity assays

The activity of superoxide dismutase (SOD) in plasma, erythrocyte and brain tissue of control and treated rats was assayed by the spectrophotometric method of Misra and Fridovich, 1972. Catalase (CAT) activity was measured by the modified method of Aebi (1984).

2.3.5. Measurement of oxidative stress markers

2.3.5.1. *Lipid peroxidation*. Lipid peroxidation is detected by the determination of malondialdhyde (MDA) production determined by the method of Begue and Aust (1978). Homogenates of liver or lung were centrifuged at 1000 × *g* for 10 min at 4 °C to sediment cell debris and mitochondrial samples, were suspended in PBS, pH 7.4, mixed with BHT–TCA (trichloroacetic