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Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats

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Abstract

Malathion is an insecticide of the group of organophosphate pesticides (OPs), which shows strong insecticidal effects. However, it possesses mutagenic and carcinogenic properties and shows organ-specific toxicity in relation to the heart, kidney and other vertebrate organs. The exact mechanism of the genotoxic effects of malathion is not yet known. Free radical damage is an important direct or indirect factor in several pathological and toxicological processes, including malathion poisoning. The aim of the present study was the evaluation of oxidative damage in different tissues of Wistar rats, administered intra peritoneally at doses of 25, 50, 100 and 150 mg malathion/kg, after acute and sub-chronic malathion exposure. Oxidative stress evaluation was based on lipid peroxidation by levels of thiobarbituric acid reactive substances (TBARS), protein oxidation by levels of carbonyl groups, and also on the activities of superoxide dismutase and catalase, two antioxidant enzymes that detoxity superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide, respectively. The results showed that the most sensitive targets of oxidative damage were kidney, lung and diaphragm after acute treatment, and liver, quadriceps and serum after sub-chronic treatment. Also, in general, increased lipid peroxidation measured as TBARS levels seems to be a better biomarker of oxidative stress compared to the contents of protein carbonyls after acute and sub-chronic malathion treatments. The present findings reinforce the concept that oxidative stress and particularly lipoperoxidation, are involved in OPs toxicity.

Keywords: Malathion; Intoxication; Antioxidants; Oxidative stress; Lipoperoxidation

1. Introduction

Pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors. Residual amounts of organophosphate pesticides (OPs) have been detected in the soil, water bodies, vegetables, grains and other food products (John et al., 2001; Galloway and Handy, 2003). Malathion [*O*,*O*-dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate] is one of the most widely used organophosphate pesticides for agriculture and public health programs (Ahmed et al., 2000; Galloway and Handy, 2003). As a result of the low persistence of OPs pesticides, they were introduced as replacements for the highly persistent organochlorine pesticides in the 1970s (Galloway and Handy, 2003). Malathion is known to induce excitotoxicity through its bioactivated analog, malaoxon (Hazarika et al., 2003).

Data on subjects acutely poisoned with organophosphorous compounds suggest that an impairment in neurobehavioral performance and emotional *status* may be observed as a long-term sequela (Colosio et al., 2003). Long-term exposure to low levels of OPs may produce neuropsychiatric symptoms (Salvi et al., 2003). Some studies indicate that other biochemical targets may be affected by OP insecticides (Samimi and Last, 2001; Akhgari et al., 2003). Moreover, the lipophilic nature of OPs facilitates their interaction with the cell membrane and leads to perturbations of the phospholipid bilayer structure (Videira et al., 2001).

OPs are known to cause inhibition of acetylcholinesterase (AChE) in target tissues, which consequently accumulate acetylcholine and subsequent activation of cholinergic muscarinic and

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nicotinic receptors. Hovewer, AChE inhibition does not explain all the symptoms of OPs intoxication. For example, the renal dysfunction secondary to OPs exposure in humans was found not to be correlated with the degree of cholinesterase suppression (Poovala et al., 1998). More recently, it has been postulated that OPs produce oxidative stress in different tissues through the formation of reactive oxygen species (ROS) (Banerjee et al., 1999; Ahmed et al., 2000; Akhgari et al., 2003; Abdollahi et al., 2004).

ROS such as hydrogen peroxide, superoxide anion and hydroxyl radical are produced in a number of cellular reactions and by enzymes such as lipoxygenases, peroxidases and dehydrogenases (Halliwell and Gutteridge, 1999). ROS are part of normal oxidative metabolism, but when produced in excess, they cause tissue injury including lipid peroxidation, DNA damage, and enzyme inactivation (Halliwell and Gutteridge, 1999; Dal-Pizzol et al., 2001). In addition, oxidative stress is also a process related to xenobiotic exposure and different levels of environmental contamination (Halliwell and Gutteridge, 1999). In such cases, peroxidation of membrane lipids seems to be an unavoidable process in tissue injury, and may impair antioxidant defenses, leading to oxidative damage by changing the balance between oxidants and antioxidants (Banerjee et al., 1988; Halliwell and Gutteridge, 1999; Torres et al., 2004). ROS have been implicated in important pathologies such as cardiovascular, pulmonary and autoimmune diseases, inherited metabolic disorders, cancer, and aging (Halliwell and Gutteridge, 1999). The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the main enzymatic defenses and act in concert with a panoply of non-enzymatic antioxidants (Halliwell and Gutteridge, 1999). In the present study, we have investigated the oxidative damage in different tissues after acute and sub-chronic malathion exposure of Wistar rats based on lipid peroxidation and protein oxidation on antioxidant defense enzymes.

2. Materials and methods

2.1. Animals and treatments

Adult male Wistar rats (age 2–3 months; weight 250–300 g) were obtained from our own breeding colony. They were caged in groups of 5 (each group in a cage for each specific protocol) with free access to food and water and were maintained on a 12 h light/dark cycle (lights on at 7:00 a.m.), and at a temperature of 23±1 °C. These conditions were maintained constant throughout the experiments.

The rats were divided in two protocols: acute and sub-chronic treatment. In the acute protocol malathion ($n = 20$ each protocol, being $n = 5$ each group) was administered once by intraperitoneal injection (i.p.) at doses of 25, 50, 100, and 150 mg/kg (1/9 LD_{50}) body weight of malathion. Malathion was dissolved in saline (0.9% NaCl), mixed manually and administered intraperitoneally (i.p.) in a constant volume of 1 ml/kg of body weight. In the sub-chronic protocol malathion was administered once a day for 28 days at the same doses as the acute protocol. For both acute and sub-chronic protocols a control group ($n = 10$ each protocol, being $n = 5$ each group) received an injection of saline. These doses were far below the LD_{50} for malathion and caused no overt signs of cholinergic toxicity, and there was no mortality at all the doses of malathion used. A dose of 250 mg/kg malathion administered intraperitoneally in rats does not cause signs of cholinergic toxicity (Brocardo et al., 2005). However, after oral administration of doses of 700 mg/kg, important signs of cholinergic hyperactivity, gross behavioral abnormalities and mortality in rats were observed (Hazarika et al., 2003).

The rats did not show significant weight alterations. In both protocols, 24 h after the last malathion injection the animals were killed by decapitation, blood was obtained by cardiac puncture, and liver, kidney, lung, diaphragm, and quadriceps were removed and stored at −80 °C. It was recently showed that in rat brain after an acute exposure, in which animals were killed 1, 6 and 12 h after i.p. administration of malathion dissolved in saline, only small variations in antioxidant responses (Fortunato et al., 2006a,b). The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of the Universidade do Extremo Sul Catarinense (protocol number 123/04).

2.2. Thiobarbituric acid reactive substances (TBARS)

As an indirect index of ROS production, we used the formation of TBARS during an acid-heating reaction, which is widely adopted as a sensitive method for measurement of lipid peroxidation, as described by Draper and Hadley (1990). Briefly, the samples were mixed with 1 ml of trichloroacetic acid 10% (TCA) and 1 ml of thiobarbituric acid 0.67% (TBA), then heated in a boiling water bath for 30 min. TBARS levels were determined spectrophotometrically at 535 nm. Results are expressed as TBARS (nmol/mg protein) using $\varepsilon = 153\ M^{-1}\ cm^{-1}$.

2.3. Measurement of protein carbonyls

The oxidative damage of proteins was assessed spectrophotometrically by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH), as described by Levine et al. (1990). In aliquots of 200 μl, proteins were precipitated by the addition of 100 μl 20% trichloroacetic acid (TCA) for 5 min on ice, and centrifuged at 4000 × *g* for 5 min. The pellet was redissolved in 100 μl 0.2 M NaOH, and 100 μl of 2 M HCl or 10 mM DNPH in 2 M HCl added to duplicate aliquots for blanks or the derivatizing of carbonyl groups, respectively. Samples were left for 30 min at room temperature. Proteins were precipitated with TCA, and washed three times with 500 μl 1:1 ethanol:ethyl acetate with 15 min standing periods to remove excess DNPH. Samples were redissolved in 200 μl 20 mM KH_2PO_4, pH 2.3, and the absorbance was read at 370 nm. The carbonyl content in nmol mg $protein^{-1}$ was calculated using a molar extinction coefficient of 22,000 $M^{-1}\ cm^{-1}$ at 370 nm after subtraction of the blank absorbance.

2.4. Measurement of catalase (CAT) and superoxide dismutase (SOD) activity

To determine CAT activity tissue portions were sonicated in 50 nM phosphate buffer (pH 7.0) and the resulting suspension was centrifuged at 3000 × *g* for 10 min, the supernatant was then used for enzyme assay. CAT activity was measured spectrophotometrically and expressed as UCAT/mg protein by the rate of decrease of hydrogen peroxide at 240 nm (Aebi, 1984). SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation in a reaction buffer containing 1 mM adrenaline/50 mM glycine–NaOH (pH 10.2) as described by Bannister and Calabrese (1987), and was expressed as USOD mg $protein^{-1}$.

2.5. Protein quantification

All the results were normalized by the protein content measured according to Lowry et al. (1951).

2.6. Reagents

Thiobarbituric acid, catalase, superoxide dismutase, dinitrophenylhydrazine, adrenaline, and hydrogen peroxide were purchased from Sigma (St. Louis, MO, USA). K_2HPO_4, KCl, NaCl, Na_2HPO_4, NaOH were obtained from Synt, São Paulo, Brazil and commercial grade malathion (95% purity, CAS 121-75-5) was obtained from Dipil Chemical Ind., SC, Brazil.