Zinc Attenuates Malathion-Induced Depressant-like Behavior and Confers Neuroprotection in the Rat Brain

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Malathion is an organophosphate widely used as an insecticide in agriculture and in public health programs, causing risk to human health. As was recently reported, malathion induces depressant-like behavior and oxidative damage to the brain of rodents. Given the relevance of searching for neuroprotective agents against such damage, this study was therefore undertaken to investigate the neuroprotective potential of zinc in dealing with malathion-related toxicity. Female Wistar rats were exposed to malathion (50 and 100 mg/kg, ip) and/or zinc chloride (ZnCl₂; 5 mg/kg, ip) for 3 days. Malathion produced a depressant-like effect, observed by the increased immobility time in the forced swimming test (FST), without affecting total locomotor activity and rearing in the open-field. However, malathion administered at 50 mg/kg reduced the central time in the arena and at the dose of 100 mg/kg reduced the central locomotion. These effects were completely reversed by ZnCl₂. Exposure to malathion (50 mg/kg, ip) and/or ZnCl₂ did not affect AChE activity in the hippocampus, cerebral cortex, and blood. Malathion (50 mg/kg, ip) alone caused some harmful effects, such as (1) an increase in lipid peroxidation and a reduction of glutathione peroxidase activity in the cerebral cortex, (2) reduction of glutathione reductase activity in the hippocampus, and (3) changes in the structure of chromatin in the dentate gyrus, all effects attenuated by ZnCl₂. In conclusion, these results clearly show that zinc administration is able to attenuate some neurochemical, morphological, and behavioral effects induced by malathion, notably the malathion-induced depressant-like effect in the FST.

Key Words: malathion; zinc; depression; oxidative injury; pesticides; organophosphates.

Malathion is among the organophosphate (OP) compounds most widely used as a pesticide in agriculture, in veterinary practice, and as an ectoparasiticide applied against human body lice (Maroni et al., 2000). Its widespread use has raised concerns over its potentially adverse effects on the health of humans, animals, wildlife, and fish (Flessel et al., 1993). Malathion has been pointed as one of the main contaminants in cases of OP intoxication in Santa Catarina, a state in Southern Brazil, according to unpublished data obtained from a Toxicological Information Center (Centro de Informações Toxicológicas—CIT) hosted by the Hospital Universitário, Florianópolis, SC.

Acute toxic effects induced by malathion pesticides are mainly attributable to the inhibition of acetylcholinesterase (AChE) in the nervous tissue with a consequent increase in the levels of acetylcholine, causing several cholinergic symptoms (Kwong, 2002). It is important to consider that acute OP poisoning may lead to chronic sequelae, including anxiety and depression (Stallones and Beseler, 2002), while long-term exposure to OP may produce neuropsychiatric symptoms, including depression (Salvi et al., 2003). Preclinical studies also indicate that malathion, administered acutely (100–250 mg/kg, ip) or repeatedly for 7 days (25–100 mg/kg, ip), causes depressant-like behavior in the forced swimming test (FST) (Assini et al., 2005). Another study has shown that malathion administered chronically for 28 days (25–150 mg/kg, ip) also caused a depressant-like behavior in the FST (Ramos et al., 2006). The FST is the most widely used animal test predictive of antidepressant action (Cryan et al., 2002). Besides this ability, the FST has been shown to be sensitive to several experimental models of predisposition to depression, including the exposure of rodents to chronic mild stress and to substances that are associated with increased risk to cause depression in humans, such as amphetamine and interferon-alpha (Cryan et al., 2003; Makino et al., 2000). These findings raise the possibility that the increase in the immobility time elicited by malathion in the rat FST may be of clinical relevance.

Preclinical and clinical studies have shown that depression can lead to atrophy and cell loss in limbic brain structures, including the hippocampus (Duman, 2004). Moreover, functional imaging studies reveal that hippocampal volume is decreased in patients with depression (Duman, 2004; Saylam et al., 2003), suggesting that neurogenesis is altered in depression. Antidepressant treatment increases adult neurogenesis in the hippocampus, especially in the dentate gyrus (Dranovsky and Hen, 2006; Warner-Schmidt and Duman, 2006).
Zinc is a trace element present at high concentrations in the central nervous system, particularly in the hippocampal mossy fibers (Choi and Koh, 1998). Many studies have been reported indicating an important role of zinc in the psychopathology and therapy of depression. In serum, lower zinc concentration has been found in patients with depression (Maes et al., 1999), and treatments with antidepressants were able to normalize serum zincemia (Maes et al., 1997). Moreover, zinc supplementation enhanced the efficacy of antidepressant therapy (Nowak et al., 2003). Several studies have shown that acute treatment with zinc produces an antidepressant-like effect in mice and rats in the FST (Kroczyk et al., 2001; Nowak et al., 2003; Rosa et al., 2003), in the tail suspension test in mice (Rosa et al., 2003), and in the olfactory bulbectomy model in rats (Nowak et al., 2003). Moreover, similarly to antidepressants, chronic administration of zinc hydroaspartate (65 mg/kg, for 14 days) induces brain-derived neurotrophic factor gene expression in the cerebral cortex (Nowak et al., 2004) and increases the synaptic zinc level in the hippocampus of rats (Szewczyk et al., 2006). Indeed, some studies suggest zinc as a beneficial agent against brain damage (Aschner et al., 2001; Cabre et al., 1999), although an excess of zinc may produce cytotoxic effects (Choi et al., 2000).

Although zinc has been proposed as an antidepressant agent, to our knowledge, no study was performed until now to investigate whether zinc produces an antidepressant-like effect against the depressive-like behavior and also against a morphological damage to the hippocampus induced by a depressogenic substance. Regarding this issue, malathion was used in this study to cause such effects, making possible to investigate a possible antidepressant role of zinc. Neurprotection afforded by zinc was searched by investigating the antioxidant defense system, lipid peroxidation, and the activity of AChE in the hippocampus and cerebral cortex of rats. We also investigated whether ZnCl₂ produces any protective action against malathion-induced chromatin condensation in dentate gyrus of rats, considering the involvement of this structure in the pathophysiology of depression.

MATERIALS AND METHODS

Animals. Naïve female Wistar rats with no pregnancy history, approximately 3 months old (weighing 180–220 g) were housed in plastic cages in a temperature-controlled room (22°C–27°C) with free access to food and water and maintained on a 12-h light/dark cycle (lights on at 7:00 A.M.). Considering that the main focus of our study was to investigate the ability of zinc to attenuate the depressive-like effects of malathion exposure, female rats were used, taking into account that depression affects more women than men (Weissman et al., 1996). The rats were randomly distributed into specified experimental groups. All procedures used in this study were performed after approval of the protocol by the Ethics Committee of the Institution, and every effort was made to minimize animal suffering.

Drugs and treatment. The following chemicals were used: commercial grade malathion, 500 CE (95% purity, CAS 121-75-5, Dipil Chemical Ind., Brazil); nicotinamide adenine dinucleotide phosphate (reduced form; NADPH); nicotinamide adenine dinucleotide phosphate (oxidized form, NADP⁺); glucose-6-phosphate, tert-butylhydroperoxide (tBOOH), magnesium chloride, oxidized (GSSG) and reduced (GSH) glutathione; chloro-2,4-dinitrobenzene (CDNB); glutathione reductase (GR); acetylthiocholine iodide; bovine serum albumin (BSA); 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB); Hoechst 33258; malondialdehyde (MDA); and 2-thiobarbituric acid from Sigma Chemical Co, St. Louis, MO. Trichloroacetic acid, toluidine blue, and zinc chloride (ZnCl₂) were purchased from Merck (Darmstadt, Hesse, Germany).

In order to choose a dose of malathion to be used in this study, rats were treated once a day for 3 consecutive days with an ip injection of malathion (50; 100 or 250 mg/kg). Thirty minutes later, the training session of the FST was carried out, and 24 h later, rats were submitted to the test session of the FST.

With the purpose to investigate the possible protective effect of ZnCl₂ against malathion-induced behavioral alterations, rats received three daily ip injections of saline (control), malathion (50 or 100 mg/kg) and/or ZnCl₂ (5 mg/kg) in a volume of 1.0 ml/kg body weight. All groups of rats received two contralateral ip injections. The second injection was given immediately after the first one. Thus, rats were given saline and saline (control group), ZnCl₂ and saline, malathion and saline, and malathion and ZnCl₂. The doses of malathion were selected based on the dose-response curve in the FST, and the dose of zinc was chosen based on preliminary experiments in our laboratory. Malathion doses used did not produce overt signs of toxicity in rats.

Rats were killed by decapitation on the 4th day, 24 h after the last malathion (50 mg/kg, ip) and/or ZnCl₂ (5 mg/kg, ip) injection. One hour after the FST, hippocampal and cerebral cortices from rats previously exposed to this test were removed and homogenized 1:2 (wt/vol) in 20mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.4, for biochemical analysis.

For histochemical analysis, rats treated with malathion (50 mg/kg, ip) and/or ZnCl₂ (5 mg/kg, ip) for 3 consecutive days were anesthetized with sodium pentobarbital (100 mg/kg) and perfused transcardially, first with physiological saline and then with a fixative solution containing 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brains were removed and postfixed in 4% paraformaldehyde at 4°C for 18–24 h. The tissues were then block and embedded in paraffin according to the standard histological techniques.

All the behavioral, biochemical, and morphological analyses were performed by the staff blind to the dose groups.

Forced swimming test. The studies were carried out on rats according to the method of Porsolt et al. (1978), with minor modifications. Behavioral naïve rats were individually forced to swim in open plastic cylinders (height 40 cm, diameter 30 cm) containing 25 cm of water, maintained at 25°C ± 1°C. The pretest session was carried out 30 min after the last malathion and/or ZnCl₂ injection. In the pretest session, rats were allowed to swim for 15 min and then returned to their home cages. In the test session, 24 h later, rats were again submitted to the FST, with immobility time measured during 5 min. Each rat was judged to be immobile when it remained floating motionless in the water, making only those movements necessary to keep its head above water.

Open-field test. The open-field apparatus consisted of a white-painted plywood arena measuring 32.5 cm in height and 97 cm in diameter. The floor of the arena was divided into 19 units by black lines and lighted from above by one red bulb. In the open-field, each behavioral naïve rat was placed in the center of the open-field and the following variables were recorded, for 5 min: number of line crossing with four paws (total locomotion), number of central squares (i.e., away from the walls) crossed (central locomotion), number of rearing responses, and time in the central area (central time). The open-field test was carried out 24 h after the last malathion and/or ZnCl₂ dosing. The behavior of each animal was recorded by video camera positioned above the open-field and monitored in another room via closed circuit TV camera.

Determination of AChE activity. AChE activity was measured by the method of Ellman et al. (1961), using acetylthiocholine iodide as a substrate in homogenates of hippocampus and cerebral cortex centrifuged at 2300 × g for 15 min. Each sample was taken from one animal and assayed in triplicate. The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm through...
the release of the thiol compound which when reacted with DTNB produced the color-forming compound thionitrobenzoic acid. For the determination of blood AChE activity, rats were anesthetized with sodium pentobarbital (100 mg/kg), and blood was collected from rat liver port vein in heparinized tubes and stored at −20 °C. After thawing, samples were hemolyzed in ice-cold water for 30 min and centrifuged 10,000 × g for 10 min. The supernatant was assayed for AChE activity according the method described by Ellman et al. (1961) and normalized by hemoglobin (Hb) content. The Hb concentration was measured at 540 nm as cyano-met-Hb form.

**Antioxidant enzyme assays.** Glutathione peroxidase (Gpx) activity was measured by the Wendel (1981) method, using tBOOH as a substrate. NADPH disappearance was monitored by a spectrophotometer at 340 nm. Glutathione-S-transferase (GST) activity was assayed by the procedure of Habig and Jakoby (1981), using CDNB as a substrate. The assay was conducted by monitoring the appearance of the conjugated complex of CDNB and GSH at 340 nm. GST activity in brain homogenates was determined by the method described by Carlberg and Mannervik (1985). The reduction of GSSG in the presence of NADPH was measured spectrophotometrically at 340 nm. The activity of glucose-6-phosphate dehydrogenase (G6PDH) was determined by means of the absorbance increase induced by the reduction of NADP+ to NADPH at 340 nm (Glock and McLean, 1953).

**Total glutathione assay.** Total glutathione (GSH-t) was measured by the GR-DTNB recycling assay described by Akerboom and Sies (1981). Values were obtained based on a standard curve with known GSSG concentration.

**Measurement of thiobarbituric acid reactive substances levels.** As an index of lipid peroxidation, the formation of thiobarbituric acid reactive substances (TBARS) was measured in hippocampal and cerebral cortical crude homogenates by the method of Ohkawa et al. (1979). The results were expressed in terms of the extent of MDA production.

**Protein determination.** The protein content in hippocampal and cerebral cortical homogenate samples was quantified by the method of Bradford (1976), using BSA as a standard.

**Nuclear staining with Hoechst 33258.** Fixed hippocampi of treated and control rats (n = 3) were dehydrated in graded ethanol and embedded in paraffin. Blocks were oriented and tissue sections cut in the coronal plane at 5 µm thick in series of 10 sections to analyze the apoptotic cells. The sections were rinsed with phosphate-buffered saline (PBS) and then incubated for 20 min at room temperature with Hoechst 33258 (1 µg/ml in PBS). The sections were dipped in a 0.1% gelatin-glycerin solution and visualized under a fluorescence microscope. For histological control, samples were stained with toluidine blue (1%). Normal cells were identified by intact round-shaped nuclei with diffuse fluorescence and apoptotic cells by highly refringent smaller nuclei with fluorescent staining.

**Statistical analysis.** All data are expressed as mean ± SEM. Data were analyzed statistically by one-way ANOVA for the experiments dealing with malathion effects in the FST and two-way ANOVA for malathion × ZnCl2 experiments. When the overall test of significance led to a rejection of the null hypothesis, a post hoc comparison test was carried out to determine the source of the effect. This analysis was based on the Duncan’s test. Values were considered significant when p < 0.05.

**RESULTS**

**Forced Swimming Test**

The dose-response curve for malathion administered once a day for 3 consecutive days is shown in Figure 1. Malathion at all doses tested produced an increase in the immobility time in the FST, consistent with a depressant-like effect. Based on this result, we choose the lowest doses of malathion for the further experiment, in which the possible protective effect of ZnCl2 against malathion-induced depressant-like behavior was investigated. Thus, the immobility time in FST of rats treated with malathion (50 and 100 mg/kg, ip) and/or ZnCl2 (5 mg/kg, ip) for 3 days is shown in Figure 2. The malathion-induced increase in immobility time was completely prevented by treatment with ZnCl2.

**Open-Field Test**

The effects of malathion and/or ZnCl2 on spontaneous locomotor activity in rats are shown in Figure 3A and 3D. Malathion and ZnCl2, alone or in combination, did not interfere with total locomotion and rearing responses. However,
Malathion administered at 50 mg/kg reduced the central time in the arena and at the dose of 100 mg/kg reduced the central locomotion. These effects were completely reversed by ZnCl₂ (5 mg/kg, ip).

AChE Activity

Table 1 shows the effect of repeated administration (3 days) of malathion and/or ZnCl₂ on AChE activity. Malathion and ZnCl₂, alone or in combination, did not significantly alter the activity of AChE in the cerebral cortex, hippocampus, and blood of rats.

Nuclear Staining in the Dentate Gyrus of Malathion-Treated Rats

The specific DNA stain, Hoechst 33258, was used to assess changes in chromatin structure following exposure to malathion and/or ZnCl₂. As shown in Figure 4, we qualitatively demonstrated that nuclei of dentate gyrus in animals treated with saline (Fig. 4B) observed by fluorescence microscopy were large and exhibited diffuse staining of the chromatin. The image for the group treated with ZnCl₂ showed few cells with condensation of nuclear chromatin (Fig. 4C). In contrast, nuclei of dentate gyrus of animals treated with malathion (Fig. 4D) showed a variety of abnormal morphology, including highly condensed and fragmented chromatin, which is a typical feature of chromatin condensation (i.e., apoptosis). This morphological profile was attenuated by the treatment of animals with ZnCl₂ (Fig. 4E).

Antioxidant Enzyme Activity and Measurement of GSH-t Levels

Table 2 shows the influence of repeated administration (3 days) of malathion and ZnCl₂, alone or in coadministration, on antioxidant enzymes and on the levels of GSH-t in the cerebral cortex and hippocampus of rats. The cerebral cortical activity of GPx decreased slightly, yet significantly, in the malathion-treated group, while coadministration of ZnCl₂ prevented that effect. In the hippocampus, the decrease in the...
activity of GR in the malathion-treated group was reversed by the treatment with ZnCl₂.

Malathion, in combination with ZnCl₂, significantly reduced the activity of GST in the hippocampus of rats. Other changes were not observed in the studied parameters.

**TBARS Measurements**

Table 3 shows a significant increase in the TBARS levels in the cerebral cortex of rats treated with malathion, which was reversed by the treatment with ZnCl₂. Malathion and ZnCl₂, alone or in combination, did not significantly alter the levels of TBARS in the hippocampus of rats.

**DISCUSSION**

In this study, rats exposed to malathion for 3 days showed a pronounced increase in immobility time in the FST, an indication of depressant-like behavior. This result is similar to previous findings by our group, showing that malathion administered acutely (100–250 mg/kg, ip) or repeatedly (25–100 mg/kg, ip) for 7 days caused depressant-like behavior in the FST (Assini *et al.*, 2005). This result is also similar to the one reported by Ramos *et al.* (2006), in which rats exposed chronically to malathion for 28 days (25–150 mg/kg, ip) exhibited a depressant-like behavior in the FST. The increase of immobility time caused by malathion in the FST cannot be attributed to a locomotor effect caused by this OP since no changes in total locomotion were apparent in malathion-exposed rats in the open-field test. The depressant-like effect of malathion in the FST is remarkable and may be of toxicological importance, considering that depression in

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**TABLE 2**

<table>
<thead>
<tr>
<th>Tissue/treatment</th>
<th>GR</th>
<th>GPx</th>
<th>GST</th>
<th>G6PDH</th>
<th>GSH-t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>22.44 ± 0.85</td>
<td>15.23 ± 0.78</td>
<td>76.47 ± 5.97</td>
<td>25.30 ± 0.66</td>
<td>1.77 ± 0.25</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>20.84 ± 0.43</td>
<td>16.47 ± 0.94</td>
<td>89.50 ± 11.20</td>
<td>29.28 ± 3.60</td>
<td>1.63 ± 0.14</td>
</tr>
<tr>
<td>Malathion</td>
<td>21.87 ± 0.92</td>
<td>13.51 ± 1.02</td>
<td>80.53 ± 5.01</td>
<td>26.13 ± 0.90</td>
<td>1.55 ± 0.24</td>
</tr>
<tr>
<td>Malathion + ZnCl₂</td>
<td>21.71 ± 1.18</td>
<td>16.32 ± 0.65</td>
<td>85.06 ± 2.19</td>
<td>30.28 ± 2.25</td>
<td>1.93 ± 0.06</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>26.05 ± 0.78</td>
<td>15.99 ± 1.06</td>
<td>61.05 ± 3.4</td>
<td>15.16 ± 0.62</td>
<td>0.82 ± 0.09</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>24.13 ± 0.85</td>
<td>13.86 ± 0.85</td>
<td>57.16 ± 3.7</td>
<td>15.37 ± 0.83</td>
<td>0.88 ± 0.12</td>
</tr>
<tr>
<td>Malathion</td>
<td>20.83 ± 0.56</td>
<td>13.04 ± 0.67</td>
<td>53.56 ± 3.9</td>
<td>13.96 ± 0.80</td>
<td>0.89 ± 0.11</td>
</tr>
<tr>
<td>Malathion + ZnCl₂</td>
<td>23.73 ± 1.06</td>
<td>13.79 ± 0.67</td>
<td>47.52 ± 4.8</td>
<td>14.01 ± 0.63</td>
<td>1.16 ± 0.17</td>
</tr>
</tbody>
</table>

Note. Antioxidant enzyme activities are expressed as nmol/min/mg protein and GSH-t levels are expressed as μmol/g wet tissue. Values are expressed as ± SEM, n = 5–6.

*p < 0.05 as compared with saline-treated control.

*p < 0.01 as compared with saline-treated control.

*p < 0.01 as compared with malathion-treated group.
TABLE 3
TBARS Levels Measured in Cerebral Cortex and Hippocampus of Animals Treated with Malathion (50 mg/kg, ip) and/or ZnCl₂ (5 mg/kg, ip) for 3 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cerebral cortex</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline/saline</td>
<td>197.4 ± 5.7</td>
<td>174.2 ± 8.3</td>
</tr>
<tr>
<td>ZnCl₂/saline</td>
<td>204.2 ± 8.6</td>
<td>163.2 ± 11.2</td>
</tr>
<tr>
<td>Saline/malathion</td>
<td>244.4 ± 9.8</td>
<td>144.0 ± 8.3</td>
</tr>
<tr>
<td>ZnCl₂/malathion</td>
<td>188.6 ± 7.7</td>
<td>154.6 ± 11.8</td>
</tr>
</tbody>
</table>

Note. TBARS levels are expressed as nmol/mg wet tissue. Values are expressed as mean ± SEM; n = 5.

*p < 0.01 as compared with saline-treated control.
bp < 0.01 as compared with malathion-treated group.

human beings is reported in many populations exposed to OP pesticides (Reidy et al., 1992; Stallones and Beseler, 2002) and is supposed to be a possible cause of suicides by rural workers exposed to these agents (Parron et al., 1996).

Another important finding of our study is that the depressant-like behavior induced by malathion (50 and 100 mg/kg, ip) was completely reversed by simultaneous treatment with ZnCl₂. ZnCl₂ per se did not cause any alteration of immobility time in the FST and did not cause behavioral alterations in the open-field test. It is also in line with data in literature showing that zinc produces an antidepressant-like effect in rats (Kroczyka et al., 2001) and mice (Kroczyka et al., 2001; Rosa et al., 2003). Moreover, the antidepressant potential of zinc is indicated by several studies showing that it may have a significant role in the modulation of depression in humans (Maes et al., 1997, 1999; Nowak et al., 2003; Nowak and Szewczyk, 2002).

Regarding the results obtained in the open-field test, it is noteworthy that malathion administered at 50 mg/kg reduced the central time in the arena and at the dose of 100 mg/kg reduced the central locomotion. The open-field arena is generally considered to be a stressful, fear-arousing environment. More anxious, emotional animals tend to ambulate less and stay away from the central part of the arena in such conditions (Walsh and Cummins, 1976). These results are somewhat in accordance with Assini et al. (2005) that showed the anxiogenic-like responses in the elevated plus-maze after acute (100 mg/kg, ip) and repeated (25 mg/kg, ip) exposure to malathion. In our study, malathion-exposed rats exhibited an anxiogenic-like response in the open-field, as compared to control rats, an effect completely reversed by ZnCl₂. Thus, the results obtained in the FST and open-field test clearly indicate a protective effect of zinc regarding malathion-induced behavioral impairments.

OPs are among the most commonly used and most acutely toxic pesticides. They evoke a consistent pattern of physical symptoms but also have acute psychological and neurobehavioral effects, such as anxiety, depression, and cognitive impairments (Stallones and Beseler, 2002). OP inhibits AChE activity in the central and peripheral nervous systems, resulting in the stimulation of cholinergic synapses (Carr et al., 2001). Malathion is described as a pesticide of low toxicity. This is consistent with the fact that no overt signs of cholinergic toxicity were observed in rats in our study. Moreover, no alterations in AChE activity in cerebral cortex, hippocampus, and blood was showed in animals exposed to this pesticide for 3 days, which impacts the current view that OP toxicity is based on AChE inhibition (Kwong, 2002; Ramos et al., 2006). The absence of AChE inhibition in the repeated exposure at relatively low doses of malathion employed in the present study, associated with a clear depressant-like effect, may suggest that other biochemical targets may be responsible for the observed effects (Fortunato et al., 2006) and does not support the notion that increased immobility time in the FST caused by malathion is associated with the activation of cholinergic receptors by accumulated acetylcholine following AChE activity (Assini et al., 2005; Ramos et al., 2006), whereas short-time treatment, as the present one, seems to fall within an adaptive response in the AChE. This statement is supported by results from our group (unpublished data). It appears that this putative adaptive response is overcome after more extended exposure.

Antioxidant status is one possible alternative target of OP toxicity. It is clearly demonstrated in literature that exposure to malathion and other OP increases the levels of lipid peroxidation products (TBARS) in erythrocytes, liver, and brain of rats (Akhgari et al., 2003; Brocardo et al., 2005; Fortunato et al., 2006; Goel et al., 2005), which is in agreement with the findings of this study. OP exposure also leads to a clear imbalance in the antioxidant status found in the brain (Brocardo et al., 2005; Sharma et al., 2005), liver (Goel et al., 2005), and erythrocytes (John et al., 2001). Lower GST activity was also found in the brain of OP-treated animals (Brocardo et al., 2005; Timur et al., 2003). These data are in agreement with our observation that GPx in the cerebral cortex and GR in the hippocampus showed a mild reduction in activity after malathion exposure. In our protocol, GST activity in the hippocampus was significantly diminished only when ZnCl₂ and malathion were coadministered, indicating that ZnCl₂, when in association to malathion, can produce some adverse effect, at least regarding GST activity. As expected for our protocol, 3-day exposure to malathion (50 mg/kg, ip) produced less pronounced effects than found previously with an acute exposure to a higher dose of malathion (250 mg/kg, ip) (Brocardo et al., 2005). Despite the less prominent effects in...
the antioxidant status, this study points to the role of mechanisms other than AChE inhibition, which should be further considered in regard to malathion neurotoxicity and the neuroprotective effects of zinc. However, our results do not allow us to establish a clear relationship between the reversal of the malathion-induced behavioral effects by zinc with the antioxidant status in the hippocampus. Indeed, a clearer neuroprotection afforded by zinc concerning oxidative stress was observed in the cerebral cortex.

The protective effect of ZnCl₂ is in line with the fact that zinc can protect against oxidative damage, although other mechanisms cannot be ruled out. The most remarkable examples were found in hepatic tissue. Livers of Wilson’s disease patients benefit from zinc treatment by attenuating lipid peroxidation and improving oxidative markers (Farinati et al., 2003). In animals, hepatic protection has been clearly shown in OP (Goel et al., 2005) and alcohol (Kang and Zhou, 2005) exposure, but few studies (Brocardo et al., 2005) have addressed the neuroprotective effect of zinc in vivo.

Although acute treatment with ZnCl₂ (5 mg/kg, ip) produced some adverse effects, such as decreased AChE, GST, and GR activity (Brocardo et al., 2005), when animals were treated for 3 days using identical ZnCl₂ concentration, no changes were observed in the biochemical parameters evaluated. These results suggest that repeated treatment produced lower toxicity than acute protocols. An interaction between malathion and ZnCl₂, leading to lower GST activity in the hippocampus, and a slight zinc-induced increase in the chromatin condensation, were the only ZnCl₂ adverse effects observed. On the contrary, ZnCl₂ afforded protection, reversing the biochemical alterations induced by malathion exposure.

The hippocampus, especially in the dentate gyrus region, has been identified as a major target site for numerous initiators of damage caused by various forms of insult, including ischemia, disease processes, excitotoxicity, and environmental factors (Harry and Lefebvre d’Hellencourt, 2003). Within the hippocampal formation, the dentate gyrus is one of the few brain structures where the production of new neurons occurs even in the adult mammalian brain (Eriksson et al., 1998). Moreover, the behavioral changes in depression are associated with alterations in the hippocampal function, and treatment with antidepressants has been shown to induce neurogenesis in the dentate gyrus (Duman, 2004). In the present study, degenerating neurons in the dentate gyrus region were found in rats exposed to malathion. In line with our findings, Abdel-Rahman et al. (2004) reported that malathion caused neuronal degeneration in the hippocampus of rats exposed to this pesticide chronically (30 days to 44.4 mg/kg of malathion by dermal route). The malathion-induced morphological alterations in the dentate gyrus observed in our study were attenuated in the group exposed to malathion/ZnCl₂, although ZnCl₂ per se produced a mild damage. It is possible that a higher dose of ZnCl₂ would produce a stronger damage since some studies in the literature report that excess of zinc may be cytotoxic (for review, see Choi and Koh, 1998). However, the morphological findings clearly suggest that zinc has a neuroprotective effect against malathion-induced damage.

In summary, ZnCl₂ completely reversed the malathion-induced depressant-like effect in rats without changes in the locomotor activity, which can be considered as an antidepressant effect. In addition, malathion-exposed rats exhibited an anxiogenic-like response in the open-field that was also reversed by ZnCl₂. Regarding the biochemical analysis, the effect of malathion was associated with no alteration in AChE activity in the cerebral cortex, hippocampus, and blood of rats exposed to this pesticide for 3 days. Therefore, we suggest that the cholinergic system may not be involved in the depressant-like effect of malathion in the FST. The pro-oxidant effect of malathion was also attenuated by ZnCl₂, restoring TBA二十年, the hippocampus. The morphological alterations in the dentate gyrus were attenuated by ZnCl₂. Based on these findings, it is tempting to speculate that zinc may be a useful agent in the neuroprotection against brain damage caused by malathion.

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