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Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice

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ABSTRACT

The present study was undertaken to determine the effects of malathion exposure on oxidative stress, functional and metabolic parameters in kidney and liver of prepubertal male mice. For this reason, two separated groups of prepubertal male mice were used in this experiment. Animals were divided into two groups, group 1 served as a control and received the corn oil and group 2 was treated with 200 mg/kg body weight (b.w.) of malathion for 30 days. In result, we found that the malathion administration led to the perturbation of biochemical markers and histopathological as well as molecular damages. These changes were accompanied by an oxidative alternation which was evaluated by lipoperoxidation process and MDA production, a diminution of sulfhydril groups (−SH) content and an antioxidant enzyme activities depletion such as total superoxide dismutase (SOD) and its isoforms, catalase (CAT) and glutathione peroxidase (GPx) in both kidney and liver tissues. These changes were related with many histopathological lesions in the liver and kidney tissues. More importantly, this insecticide clearly caused a decline in the GPx-4 expression in liver as well as GPx-3 in kidney. These data suggest that prepubertal male mice exposure to malathion showed a marked deregulation of liver and kidney functions.

1. Introduction

Extensively using of organophosphorus pesticides in different fields such as agriculture, medicine and industry can cause many disturbances in human and wildlife. These organophosphorus (OP) compounds are immediately degraded in the environment. Their concept was introduced following the ban on organochlorines which can bioaccumulate and biomagnify, which results in ecotoxicological effects [1,2]. Particularly, malathion [*O*,*O*-dimethyl-*S*-(1,2-dicarcethoxyethyl) phosphorodithioate] is an OP pesticide habitually used to eradicate ectoparasites, household insects, to conserve stored grain and to eliminate disease-inducing arthropods [3,4,5]. On the negative side, it is one of OPs agents that exerts diverse toxic effects throught the inactivation of serine esterases [6], mostly acetylcholinesterase (AChE) and butyrylcholinesterase which leads to an overstimulation of the cholinergic pathways [7,8]. The OPs can achieve all the tissues leading eventually to several pathological difficulties, this is due to their lipophilic nature and their simple and rapid intestinal assimilation, including a insufficiency of the immune system [9,10] pancreatitis [11], liver disease [12,13] hematological pathosis disorder [14], kidney injury [15], decrease fertility and reproduction capability [16]. Many studies have reported toxic effects of this OPs in both humans [17,18] and animals [19,20]. Being the main actors of xenobiotic biotransformation, regulation of hepatic gene expression may play a central role in the adaptive response to altered metabolism by changing the capacity of enzymes in relevant metabolic pathways [21]. Hence, liver is the principal metabolizing site for mediating biotransformation of thionoorganophosphates and with kidney contributing to the elimination of toxic products [22]. These tissues are considered among the main targets of malathion toxicity which is mediated through oxidative stress generated by reactive oxygen species (ROS) [23,24,25]. ROS such as superoxide anion, peroxyl radicals, hydroperoxyl radical, hydrogen peroxide are produced from the molecular oxygen as a consequence of normal cellular metabolism [26]. At low or moderate concentrations, ROS are considered as part of normal oxidative metabolism, but at elevated concentrations, they cause tissue injuries, including lipids, proteins oxidation, DNA damage [27], and enzyme inactivation. They are also implicated in many pathological conditions such as cancer, diabetes, cardiovascular, pulmonary and autoimmune diseases, neurological disorders and aging, among others. The main objective of this

Abbreviations: MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; OPs, organophosphorus

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study was therefore to highlight the role of oxidative stress as a precursor of molecular and histopathological complications following subacute exposure of prepubescent mice to malathion.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide, eosin stain, 5.5′-dithiobis 2-nitrobenzoic acid (DTNB), Malathion (98% purity) (fyfanon 50 EC 500 g/l), RPMI (Roswell Park Memorial Institute) and triton X-100 were purchased from SIGMA and Invitrogen.

2.2. Animals and experimental fields

Female and male mice were purchased from Pasteur Institute of Tunis. All experiments were performed according with the local ethics committee of Tunis University for the use and care of animals in accordance with the NIH recommendations. The animals were provided with food (standard pellet diet- Badr Utique-TN) and water *ad libitum* and maintained in animal house at controlled conditions: temperature (22 ± 2 °C) and 12 h light-dark cycle. Primiparous females were placed three per cage with one male breeder and vaginal smear was examined daily in the evening. At the weaning age (21days), after the lactational period of their offspring (prepubertal male mice) were separated and then randomly divided into two groups of 16 animals each: Group 1 was served as control and received the corn oil. Group 2 received by intragastrique gavage, the malathion in corn oil at the dose of 200 mg/kg, b.w. during 30 days. The age of animals and the used malathion-dose as well as the treatment duration were chosen based on previous work [16].

On the last day of experiment, animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg) and sacrificed by decapitation. The blood was collected in heparinized tubes and the plasma was obtained after centrifugation at 3000*g* for 15 min. The tissue specimens were removed and placed in a phosphate buffered saline (PBS) solution, homogenized and centrifuged for 15 min at 9000*g*. Organs supernatants and plasma were stored at −80 °C for biochemical parameters determination.

2.3. Evaluation of body organ weights

The initial and final body weights were recorded. Mice in each group were euthanized and their organs were stripped from fatty tissues and blood vessels. Then, these organs were blotted, and their absolute weights were measured. Clinical signs of body and organs were evaluated for toxicological criteria and organ weights were expressed per 100 g body weight to normalize the data for statistical analysis.

2.4. Functional and metabolic parameters determination

To assess the liver function disorders, plasma alanine aminotransferase (ALT), phosphatase alkaline (PAL), aspartate aminotransferase (AST), total and direct bilirubin were measured using commercially available diagnostic kits (Biomaghreb, Ariana, Tunisia).

Concerning renal function damages, plasma urea, creatinine, uric acid and albumin analyses were also performed using commercially available diagnostic kits (Biomaghreb, Ariana, Tunisia).

2.5. Oxidative deterioration of lipids and protein thiol groups determination

Final products of lipiperoxidation, malondialdehyde (MDA) were determined using the method of Buege and Aust [28] and total thiol groups (−SH) concentration was performed according to Hu and Dillard [29].

2.6. Antioxidant activities determination

The activity of superoxide dismutase (SOD) was determined by using modified epinephrine assays and characterization of SOD isoforms was performed using KCN (2 mM), which inhibits Cu/Zn-SOD or H_2O_2 (5 mM), affecting both Cu/Zn-SOD and Fe-SOD whereas Mn-SOD was insensitive to both inhibitor [30,31].

The activity of CAT was assessed by measuring the initial rate of H_2O_2 disappearance at 240 nm [32] and GPx activity was quantified by the procedure of Flohé and Günzler [33].

2.7. Total RNA isolation and RT-PCR analysis

Total RNA was prepared using Trizol reagent according to the manufacturer's instructions. Total RNA (1 µg) reverse was transcribed using MMLV reverse transcriptase (Invitrogen, Tunis, Tunisia) by incubation at 25 °C for 10 min, at 42 °C for 60 min and at 99 °C for 5 min. The synthesized cDNA was amplified using Taq DNA polymerase (Invitrogen, Tunis, Tunisia) and the following specific primers:

GPx-4: **F: 5′-AGTACAGGGGTTTCGTGTGC-3′**
R: 5′-CGGCAGGTCCTTCTCTATCA-3′
GAPDH: **F: 5′-GTGGATATTGTTGCCATCA-3′**,
R: 5′-ACTCATACAGCACCTCAG-3′.

PCR conditions were 30 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s, followed by 5 min incubation at 72 °C. PCR products were run on 1.5% agarose gel and then stained with ethidium bromide.

2.8. Histopathological examination

Immediately after the euthanasia, small pieces of both tissues were harvested and washed with ice cold saline, fixed in a 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. These pieces were cut into 5 µm thick, deparaffinized, hydrated and stained sections with hematoxylin and eosin (HE). The liver and kidney sections were examined in control and malathion treatment.

2.9. Protein determination

Protein concentration was determined according to Bradford method using bovine serum albumin (BSA) as standard [34].

2.10. Statistical analysis

Statistical significance was determined by one-way ANOVA using Statview statistical software. Results were expressed as means ± standard error of the mean (S.E.M.). The data are repre-sentative of 16 independent experiments. All statistical tests were two-tailed, and a p value of 0.05 or less was considered significant.

3. Results

3.1. Body weight, liver and kidney relative weights

As shown in Table 1, the sub-acute exposure of prepubertal male mice to malathion reduced the body weight and mass gain of prepubertal male mice. In contrast, a significant increase in the relative weights of both liver and kidney was observed in malathion-treated mice.

3.2. Liver and kidney functions

According to Table 2, the malathion (200 mg/kg) exposure was associated with liver and kidney dysfunctions in prepubertal male mice, while, a significant increase in some liver biochemical parameters including ALT, AST, PAL and LDH was observed in malathion-treated

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