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A comparative study on toxicity induced by carbosulfan and malathion in Wistar rat liver and spleen

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ABSTRACT

Organophosphorus (OP) and carbamate (CM) pesticides are widely used in agriculture. These pesticides are highly toxic to humans and their residues in food pose potential threat to human health. In this comparative study, we investigated the effect of subchronic exposure of OPs (malathion, MAL) and CM (Carbosulfan, CB) on rat liver and spleen. Biochemical analysis showed that levels of hepatic enzymes (ALT, ALP, LDH and PAL) changed after exposure to the pesticides. In the liver extracts, lipid peroxidation index increased after the treatment by pesticides. Our results indicated that exposure to MAL and CB leads to alteration of liver redox status. Both pesticides induced focal inflammation and fibrosis in the liver. After subchronic administration of MAL (200 mg/kg) and CB (25 mg/kg), systemic inflammation, as depicted by the increase in IFN-δ activity in liver, was observed in both malathion and carbosulfan treated animals. In addition, the results showed that MAL significantly increased TCD4+ and TCD8+ lymphocyte number. It also decreased INF-δ and IL-4 production. However, CB induced a reduction of TCD8+ number and cytokine production in spleen cells. In conclusion, malathion and carbosulfan had significant immunomodulatory properties in the spleen with inflammation and oxidative stress induction in the liver.

1. Introduction

Organophosphorous pesticides are utilized the world over to improve crop productivity. Despite their availability, there was a constant search for newer and effective pesticides that led to the development of carbamates. Carbamates and Organophosphorous pesticides are anti-cholinesterase agents, also called acetylcholinesterase (AChE) inhibitors, which are widely used in agriculture and medical treatment. Anti-cholinesterase pesticides mainly include organophosphates such malathion (MAL) as well as carbamates such as carbosulfan (CB). The acute toxicity of these pesticides has been well documented [1–3]. The toxic acute effects of carbamates are very similar to the acute effects derived from the poisoning of OPs. Both groups of compounds are inhibitors of AChE and therefore cause very similar symptoms. Nevertheless, OPs and carbamates differ in the stability of the complex with AChE. Indeed, OPs are able to phosphorylate serine residues of AChE in a non-reversible way, whereas the carbamylation of the same serine residue is less stable, the typical decarbamylation time being between 30 and 40 min [4].

Malathion is an organophosphate insecticide of low mammalian toxicity which is used extensively throughout the world to control major arthropods in public health programs, animal ectoparasites, human head and body lice, household insects and to protect grain in storage [5]. It was described that malathion exposure increases lipid peroxidation in rodent erythrocytes, liver and brain [6,7]. Other studies indicate that the activities of antioxidant enzymes were increased, reduced or not changed in the liver, brain and erythrocytes of animals poisoned with this compound [8].

Carbosulfan has substituted carbofuran as insecticide, because of environmental reasons and its lower persistence. According to Marsden et al. and Umetsu and Fukuto, carbosulfan presents lower toxicity for the animals than carbofuran [4,9]. Hydrolysis is the main path of carbosulfan to carbofuran degradation, its first metabolite, but in some cases, this latter might present longer persistence in a low pH environment [10].

Liver, which is the main organ responsible for metabolism, is a common target of pesticide toxicity [11]. Chronic exposures to MAL or CB could induce toxicity with oxidative stress as the main mechanism [6,12,13]. Malathion and carbosulfan exposure has been also associated with metabolic disorders [3,13], oxidative stress [14], immunotoxicity [13,15], inflammation [16] and hepatotoxicity [17]. The pathological lesions caused by pesticides affect the liver structure

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and function leading to hepatosteatosis. A growing body of evidence supports the role of hepatic inflammation in the pathogenesis of chronic liver disease [18]. Indeed, liver inflammation leads to the secretion of pro-inflammatory cytokines which, in turn, contribute to a feed-forward amplification of inflammatory signaling and subsequent progression of hepatosteatosis [19].

With these considerations, the aim of the current work was to compare the effect of a subchronic flow experiment of two pesticides, MAL or CB, on male Wistar rats. Plasmatic enzyme activity was evaluated in order to determine their suitability as biomarkers of toxicity on liver. In addition, we evaluated the mechanism of pesticide hepatotoxicity.

2. Methods

2.1. Chemicals

Malathion and Carbosulfan were obtained from the Agricultural Struggle Center (Tunis, Tunisia). 1-Chloro-2,4-dinitrobenzene (CDNB); 2,6,-di-tert-butyl-4-methylphenol (BHT); 2-thiobarbituric acid (TBA); 5,5-dithio bis(2-nitrobenzoic acid) (DTNB); Ammonium chloride (NH_4Cl); Bovine serum albumin; Epinephrine; Glutathione reductase; Hydrogen peroxide (H_2O_2); Potassium hydroxide (KOH); Reduced glutathione (GSH); S-butyrylthiocholine iodide; Sodium azide; Sulfosalicylic acid; Trichloroacetic acid (TCA) and β-Nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma-Aldrich Co. (Germany). Deoxyribonucleotide (dNTP) mix; DNA ladder; DNase; Free RNase; Oligo-dT; $MgCl_2$; Random hexamer; RNAse inhibitor; RPMI-1640; Taq DNA polymerase; TRIzol reagent and Primers for INF-γ were obtained from Invitrogen (St Thomas Aubin, France). Alanine transaminase (ALT); Alkaline phosphatase (ALP); Aspartate transaminase (AST); Bilirubin (BLR); Glucose oxidase and Lactate deshydrogenase (LDH) kits were provided from Randox Laboratories Diagnostics, Ltd. (UK). The complementary DNA (cDNA) synthesis kit was provided from Invitrogen (St Thomas Aubin, France).

In all cellular experiments, cells were resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin and 2 mM glutamine (Invitrogen, Cergy Pontoise, France). Sandwich ELISA kits were used for quantitation of IFN-gamma and IL-4 (Biovendor, Karasek, Czech Republic). Monoclonal anti-rat antibodies were used for flow cytometry analysis: FITC-CD3, RPE-CD4, FITC-CD8-alpha and isotype control (AbCys, Paris, France).

2.2. Animals

Adult male Wistar rats weighting 200 ± 30 g (ten weeks old) were procured from Tunisian Society of Pharmaceutical Industries and housed two per clean plastic cage and allowed to acclimatize in the laboratory environment. Animals were maintained in a mass air displacement room with a 12-h light: 12-h dark cycle at 24 ± 2 °C with a relative humidity of 50 ± 10%. Balanced food and drinking water were given to the animals *ad libitum*.

2.3. Ethics

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal experiments were carried out under strict compliance with the Guidelines for Ethical Control and Supervision in the Care and Use of Animals. All efforts were taken to minimize suffering.

2.4. Determination of optimum dose

The choice of malathion and carbosulfan dose was based on previous works of our group [3,13] and corresponds to an acceptable dose that did not cause any sign of toxicity until the end of the experiment period.

2.5. Experimental design

After a pilot study and determination of optimum MAL dose (200 mg/kg) and CB dose (25 mg/kg), the rats were randomly divided into three groups of 12 animals. One group was orally given equal amount of vehicle (corn oil) and used as the control, the second group received a dose of 200 mg/kg of malathion and the third group received a dose of 25 mg/kg during 30 consecutive days and sacrificed 2 h after the last ingestion.

Fasted animals were decapitated without preliminary anesthesia, and arteriovenous blood was quickly collected in EDTA tubes for blood cellularity determination. Plasma was separated by centrifuging at 3000 × *g* for 15 min and used for AChE and liver enzyme dosage. At the same time, the liver was removed for the determination of oxidative stress and inflammation parameters and spleen for cytometric analysis and cytokine production.

2.6. Biochemical markers in plasma

2.6.1. Acetylcholinesterase

Plasma acetylcholinesterase (E.C.3.1.1.7) activity was determined at 25 °C in phosphate buffer tris (0.1 M; pH 7.4) with 0.3 mM DTNB using 1.0 mM ATCh by the Ellman spectrophotometric method [20]. Protein concentrations in plasma and spleen were determined by the Coomassie reagent using serum bovine albumin as a standard [21].

2.6.2. Marker enzymes

To assess the liver injury, activities of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Lactate deshydrogenase (LDH) in plasma were assayed using kits obtained from Randox Laboratories Diagnostics, Ltd. (UK).

2.7. Biochemical markers of liver injury

2.7.1. Lipid peroxidation

Lipid peroxidation was evidenced by measuring the + formation of thiobarbituric acid reactive substance (TBARS) hydroperoxides using the method of Ohkawa et al. [22]. Briefly, 0.1 ml of liver supernatant and 1.9 ml of 0.1 M sodium phosphate buffer (pH 7.4) was incubated at 37 °C for 1 h. After the incubation, the mixture was precipitated with 10% TCA and centrifuged (2300 × *g* for 15 min at room temperature) to collect supernatant. Then 1 ml of 1% TBA was added to the supernatant and placed in boiling water for 15 min. After cooling to room temperature absorbance of the mixture was taken at 532 nm and was converted to MDA and expressed in nmol per mg protein using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

The supernatant of liver homogenates homogenized in phosphate buffer (0.1 M; pH 7.4) was taken for the analysis of oxidative related parameters, Catalase (CAT), Superoxide dismutase (SOD), Glutathione S-transferase (GST) and Glutathione peroxidase (GPx). These parameters were measured by the methods as follows. All results were corrected using the level of protein contents in the samples.

2.7.2. Reduced glutathione

Reduced glutathione (GSH) level was determined in hepatic liver tissue (homogenized directly in 10 volumes of 5% sulfosalicylic acid) by the methods of Griffith [23] based on an enzyme recycling assay