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Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid

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ABSTRACT

Lambda-cyhalothrin is a synthetic pyrethroid insecticide used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control. The objective of this study was to investigate the propensity of lambda-cyhalothrin (LTC) to induce oxidative stress, changes in biochemical parameters and enzyme activities in the kidney of male rats and its possible attenuation by Vitamin C (vit C). Renal function, histopathology, tissue malondialdehyde (MDA), protein carbonyl (PCO) levels, antioxidant enzyme activities and reduced glutathione (GSH) levels were evaluated. Exposure of rats to lambda-cyhalothrin, during 3 weeks, caused a significant increase in kidney MDA and protein carbonyl levels ($p<0.01$) as compared to controls. Co-administration of vitamin C was effective in reducing MDA and PCO levels. The kidney of LTC-treated rats exhibited severe vacuolations, cells infiltration and widened tubular lumen. The activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-*S*-transferase (GST) were significantly decreased due to lambda-cyhalothrin exposure. Co-administration of vitamin C ameliorated the increase in enzymatic activities of aminotransferases (AST and ALT), lactate dehydrogenase (LDH), creatinine and urea levels and improved the antioxidant status.

These data indicated the protective role of ascorbic acid against lambda-cyhalothrin-induced nephrotoxicity and suggested a significant contribution of its antioxidant property to these beneficial effects.

1. Introduction

Large-scale application of pesticides to crops and forests may contribute to the presence of toxic substances in the environment. These chemical compounds can find their way into the water reservoirs, streams and rivers, thus producing an adverse impact on the aquatic biota, animals and human health (Husain et al., 1994, 1996; Handy et al., 2002; John and Prakash, 2003). Synthetic pyrethroids such as lambda-cyhalothrin (LTC) are the most widely used Type II pyrethroid pesticide. With regard to effectiveness and toxicity, this compound appears to be the first-choice insecticide used than organochlorines, organophosphates and carbamates (Pauluhn, 1999). It is highly used in the cotton plantation, in vegetable production and to control a wide range of insect pests in a variety of crops (Schenone and Rojas, 1992; Awumbila and Bokuma, 1994). Consistent with its lipophilic nature, LTC has been found to cause adverse effects on many tissues, chromosomal aberrations and micronucleus formation in mouse bone marrow (Campana et al., 1999; Fahmy and Abdallah, 2001; Celik et al., 2003, 2005). Recent studies performed in our laboratory (Fetoui et al., 2008, 2009) reported that reactive oxygen species (ROS) are involved in LTC-induced neurotoxicity, hepatotoxicity and oxidative damages. In our knowledge LTC-induced renal injury has not been yet explored.

Although several scavenging agents and antagonists are established to reduce pesticides toxicity (Kalender et al., 2004; Grajeda-Cota et al., 2004), some of them are burned with undesirable side effects. Vitamin C (L-ascorbic acid) is a well-known antioxidant, which functions as an electron donor to protect the body from radicals and pollutants (Iqbal et al., 2004; Banerjee et al., 2001). To date, mega doses of vitamin C have shown promise as an effective influence in the treating and preventing of allergic rhinitis (Thornhill and Kelly, 2000), diabetes (Anderson et al., 2006), heart disease (Liu et al., 2002) and cancer (Enwonwu and Meeks, 1995). Although the beneficial effects of vitamin C remained controversial, which might cause diarrhoea and abdominal distension in humans (Thornhill and Kelly, 2000) doctors have recommended patients to consume even higher doses of vitamin C at the level of grams per day (Johnston, 1999). However, this has not been proven conclusively and the underlying mechanism through which vitamin C functions is still unknown. Recently, Rai et al. (2009) showed that supplementation of ascorbic acid and alpha-tocopherol ameliorates carbofuran-induced erythrocytes damages. Interestingly, Fetoui et al.

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(2009) favoured strongly the ameliorative effects of ascorbic acid against lambda-cyhalothrin-induced hepatic injury in adult rats.

This study was interested to evaluate the nephrotoxicity effects of lambda-cyhalothrin probably through the generation of free radicals and the possible preventive effects of L-ascorbic acid. Markers of renal function (creatinine, uric acid and urea plasma levels), renal antioxidant enzyme activities and histopathological changes, oxidative stress (thiol containing biomolecules, lipid peroxidation, carbonyl groups and antioxidant enzymes in kidney) were evaluated as indicators of LTC nephrotoxicity.

2. Material and methods

2.1. Chemicals

Lambda-cyhalothrin is a synthetic pyrethroid insecticide ($C_{23}H_{19}ClF_3NO_3$). CAS chemical name [α-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclo-propanecarboxylate], CAS registry number 91465-08-6. A commercial formulation of lambda-cyhalothrin, named "KARATE® 5EC" (Syngenta agrochemicals, Greensboro, USA) was used in the experiments. All other chemical products used in this study were purchased from Sigma Chemical Co. (St. Louis, France).

2.2. Animals

The experimental protocol was approved by the Local Animal Care Committee, and all the experimental procedures were carried out in accordance with international guidelines for Care and use of laboratory animals.

Adult Wistar rats (aged 8–9 weeks; weighing 140–150 g) were obtained from the Central Pharmacy (SIPHAT, Tunis, Tunisia). The animals were housed at 22 ± 3 °C temperature, 45 ± 5% humidity, 12 h light–dark cycle, and left to acclimatize for 1 week before the experiments. They were fed standard laboratory chow (Fetoui et al., 2006, 2007) and provided with water *ad libitum*.

2.3. Experimental procedure

After 1 week of acclimatization, 28 male rats were randomly divided into four groups of seven animals each. The first group of rats served as the control, received *ad libitum* distillate water and 1 ml of saline solution given by intraperitoneal way. The second group (vitamin C) was given daily a single intraperitoneal (i.p.) dose of vitamin C in saline solution (200 mg/kg bw) while the third group (LTC) received through drinking water 668 ppm of LTC. Animals in the fourth group (LTC+vitamin C) were given a single i.p. injection of vitamin C (200 mg/kg bw) per day, 12 h after the onset of lambda-cyhalothrin administration. The dose of lambda-cyhalothrin used in this study represented 1/10 of LD_{50} (612 mg/kg bw). This dose was used by previous investigations since it is toxic but not lethal to rats (Celik et al., 2005; Fetoui et al., 2008). This dose of vitamin C (200 mg/kg bw) per day) gave good protection against nephrotoxicity (Appenroth et al., 1997; Gokalpa et al., 2005). Lower doses of vitamin C gave less protection while higher doses were not much more effective.

At the end of the experimental period, the animals in different groups were sacrificed by cervical decapitation to avoid stress conditions. Blood was collected into EDTA tubes and centrifuged (3000*g* for 15 min) for the separation of plasma. The kidney was dissected out, weighed and washed using saline solution. The renal tissue was minced and homogenized (10% w/v) in an appropriate phosphate buffer saline (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) and centrifuged at 10,000 *g* for 15 min at 4 °C. The resulting supernatant was used for various biochemical assays.

2.4. Biochemical assays

2.4.1. Estimation of urea, uric acid, creatinine and lactate dehydrogenase activity

Lactate dehydrogenase activity, urea, uric acid and creatinine levels were estimated spectrophotometrically using commercial reagent kits (refs. 20125, 20143, 20092, 20151 respectively. Biomaghreb Diagnostics, Ariana. Tunisia).

2.4.2. Lipid peroxidation assay

Lipid peroxidation in the renal tissue was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) which were expressed in terms of malondialdehyde content according to Draper and Hadley (1990) method. Briefly, Aliquots of kidney homogenates were mixed with 1 ml of 5% TCA and centrifuged at 4000*g* for 10 min. 1 ml of thiobarbituric acid reagent (TBA, 0.67%) was added to 500 ml of supernatant and heated at 95 °C for 15 min. The mixture was then cooled and was measured for absorbance at 532 nm. The MDA values were calculated using 1,1,3,3-tetraethoxypropane as the standard and expressed as nmoles of MDA/g of tissue.

2.4.3. Protein carbonyl assays

Protein oxidation was determined based on the reaction of the carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitrophenylhydrazone, as described by Reznick and Packer (1994). Samples were read at 370 nm and carbonyl content was calculated using the molar absorption coefficient for aliphatic hydrazones (22,000 $M^{-1}\,cm^{-1}$) and expressed as nmol carbonyl/mg protein.

2.4.4. Measurement of reduced glutathione (GSH)

Kidney GSH content was determined by Ellman's method (Ellman, 1959), modified by Jollow et al. (1974) based on the development of a yellow colour when DTNB is added to compounds containing sulfhydryl groups. Briefly, 3 ml of sulfosalicylic acid (4%) were added to 500 ml of homogenate tissues for deproteinisation. The mixture was centrifuged at 2500*g* for 15 min. Then Ellman's reagent was added to 500 ml of supernatant. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as mg/g of tissue.

2.4.5. Assay of antioxidant and glutathione metabolizing enzymes

Catalase (CAT) was assayed by the decomposition of hydrogen peroxide according to the method of Aebi (1984). Decrease in absorbance due to H_2O_2 degradations was monitored at 240 nm for 1 min and the enzyme activity was expressed as μmol H_2O_2 consumed/min/mg protein.

Total SOD activity was evaluated by measuring the inhibition of pyrogallol activity as described by Marklund and Marklund (1974). One unit (U) corresponds to the enzyme activity required to inhibit half of the oxidation of pyrogallol. SOD activity as expressed as U/mg of protein.

Glutathione peroxidase (GPX) activity was measured according to Flohe and Gunzler (1984). The enzyme activity was expressed as nmoles of GSH oxidized/min/mg protein.

Glutathione reductase (GR) activity was assayed by the method of Carlberg and Mannervik (1975) modified by Mohandas et al. (1984). The enzyme activity was quantified by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg of protein.