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Immunotoxicity effects of carbaryl in vivo and in vitro

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ABSTRACT

Carbaryl is a pesticide for controlling pests in agricultural industry. To determine of immunotoxicity effects of carbaryl, rats were exposure with carbaryl for 28 days. The lymphoid organ weight, lymphocyte proliferation, IL-2, IFN-γ, IL-4, IL-10, IL-1β and TNF-α cytokines level were measured, respectively. Exposure with carbaryl significantly reduced both thymus and spleen weight and also suppressed lymphocyte proliferation. In addition, carbaryl significantly decreased IL-2, IFN-γ, IL-1β and TNF-α and also increased IL-4, IL-10 cytokines. These findings suggest that exposure to carbaryl can induce immunotoxicity effects on lymphoid organ weight, suppresses the functions of lymphocyte and macrophage, Th2 polarization in Th1/Th2 balance by reducing of IFN-γ and increasing of IL-4 and IL-10 cytokines. Therefore, carbaryl can contribute to the development of allergic, autoimmune, cancer or infection diseases through immunotoxicity effects and unbalancing of Th1/Th2 immune response however, further study is necessary.

1. Introduction

Pesticides such as insecticides, herbicides and fungicides are widely used in agriculture industry to eliminate all types of pests (Cohen, 2007). Among the various agricultural pesticides, Carbaryl is one of the most powerful pesticides used to control and exterminate pests. Carbaryl is still considered as one of the most effective pesticides for plant protection by a great number of farmers for agricultural efficiency and production (Aktar et al., 2009).

On the other hand, Carbaryl is a derivative of carbamic acid and considered to be a member of a family of chemicals known as carbamate. This toxin inhibits acetylcholine esterase and acetylcholine neurotransmitter activity and also its mode of action is almost similar to that of organophosphate insecticides (Mdegela et al., 2010). The destructive rate of pesticides on body organs and tissues depends on the mode of contact, dosage, biological changes and the associated metabolites supply (Das Gupta et al., 2010). Several studies showed that carbaryl can induce toxicity in the humans, mammals and birds through dermal contact as well as respiratory

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and digestive systems (Cranmer, 1986). Although, humans have important role in the production and using of toxins, they are unintentionally exposed by the relevant effects. Several studies have shown that pesticides such as carbaryl can be absorbed through the skin and/or mucous membranes (Bouchard et al., 2008; Baynes and Riviere, 1998).

In addition, immunotoxicity-related epidemiological studies in human have indicated the association between environmental toxin contamination and immune-related diseases (Duramad and Holland, 2011; Chang et al., 2014). Changes in the immune response balance play an important role in the pathophysiology of a number of diseases such as asthma, autoimmunity and cancer. Lack of a proper immune response due to immunotoxicity can result in the development of chronic infection, tumor growth and cancer. Moreover, immunotoxicity-associated stimulation of the immune system may play a major role in the development of immune-related diseases such as allergy and autoimmunity (Corsini et al., 2013; Takahashi, 2010). In this regard, numerous studies have been shown on the effects of different pesticides on immune system (Vial et al., 1996; Voccia et al., 1999; Corsini et al., 2008). Moreover, in vivo and in vitro studies have shown that, pesticides can affect immune responses including antibody production, IL-2 production and T-cell proliferation, autoantibody production, changes in Th1 and Th2 cytokine production, NK, LAK and TC cells inhibition via different mechanisms (Li, 2007; Li et al., 2002; Badesha et al., 1995). On the other hand, systemic poisoning with carbaryl can result immune suppression, increasing the risk of allergic responses against allergens such as dust mites coming from domestic waste due to inappropriate immune responses (Dong et al., 1998). Therefore, in the present study we evaluated the immunotoxicity effects of carbaryl in male rats.

2. Materials and methods

2.1. Animals and treatment with carbaryl pesticide

In this study, experiments were carried out on male rats and were randomly divided into four groups. Two doses of 10 and 30 mg/kg of carbaryl pesticide in sterile, pyrogen-free 0.9% saline were administered by i.p. injection to male rat for 28 days (five consecutive days a week and two days off). Control animals received volume-equivalent injections of almond oil (vehicle), as a pesticide solvent, and sterile saline to rule out effects based upon the stress of the injection procedure itself. Moreover, body and lymphoid organs including spleen and thymus in all groups were measured. All experimental protocols in animals were approved by Babol University of Medical Sciences animal care committee and rats were kept under the same condition of feeding, and an optimum condition of 12:12 light-dark cycle.

2.2. Lymphocyte proliferation assay

The rats were sacrificed by cervical dislocation. The spleens were minced into small pieces and then were passed through 100 μm cell mesh to obtain a single cell suspension and erythrocytes were removed through Ficoll-Hypaque density gradient centrifugation. The splenic lymphocytes were washed and resuspended in RPMI1640 supplemented with 10% FCS. The lymphoproliferative response of splenic lymphocyte to 5 μg/ml of Concanavalin A mitogen was determined in three days cultures grown in complete medium and 5% CO_2 by measuring [^{3}H]thymindine (0.5 μci/well), 18 h prior to harvest on a cell harvester. Radioactivity [count per minute (cpm)] was measured by liquid scintillation counting. Moreover, the supernatant was assayed for IL-4, IL-10, IFN-γ (Bender Med System, Austria) and IL-2 (R&D system) cytokines using ELISA kits according to the manufacturer's instructions.

2.3. Measurement of pro-inflammatory cytokines of peritoneal macrophages

Rats were killed by cervical dislocation and then, peritoneal macrophages were harvested immediately by lavaging with ice cold sterile phosphate buffer saline (PBS). The cells were washed twice and plated in RPMI 1640 medium (Sigma Chemical Co.) containing 10% fetal bovine serum (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma Chemical Co.), and incubated for 2 h at 37 °C in 5% CO_2 humidified incubator. Non-adherent cells were removed by gently washing with PBS and freshly prepared medium was added. Cell viability was checked by trypan blue exclusion technique. An aliquot of the cell suspension was mixed with an equal volume of 0.4%w/v trypan blue in PBS and incubated for 10 min. The viability of the macrophages was >97%. The supernatant of LPS (1 μg/ml) stimulated peritoneal macrophages was used for determining of TNF-α and IL-1β cytokines using ELISA kits (Koma, Korea) according to the manufacturer's instructions.

2.4. Serum cytokines assay using ELISA method

One week after the last injection, blood samples were collected. The serum was isolated after centrifugation and kept in −80 °C until cytokines assessment. Serum samples were assayed by commercial IL-1β and TNF-α (Koma, Korea), IL-4, IL-10, IFN-γ (Bender MedSyste, Austria) and IL-2 (R&D system) ELISA kits according to the procedures supplied by the manufacturer.

2.5. Statistical analysis

Data are shown as mean ± SEM. One way ANOVA test was used for comparisons between control and experimental groups by SPSS 16.0. Statistical differences were regarded as significant with a value of $P < 0.05$.

3. Results

3.1. Toxicity effect of carbaryl on lymphoid organs

We evaluated of immuotoxicity effect of carbaryl on the body and lymphoid organs weight including spleen and thymus after a 28 day exposure with carbaryl. As shown in Table 1, exposure with 30 mg/kg carbaryl comparing control group significantly reduced mean spleen weight (486 mg vs 562 mg of control) and spleen cellularity of $42 \pm 8 \times 10^7$ vs $57 \pm 12 \times 10^7$