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The effect of ethephon on immune system in male offspring of mice

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ABSTRACT

Ethephon can liberate ethylene which could interfere the plant growth process. The aim of the present study was to determine the effect of ethephon on developing immune system of male offspring. Ethephon could enhance NK cell activity in male mice. For 4-week-old male mice, lymphocytes of peripheral blood increased while the hemolytic plaque number decreased. Delayed type hypersensitivity(DTH) was inhibited in all groups. The expression of protein Bcl11b and p-p38 in thymus of treatment groups were lower than control group. Our results indicated that cellular immunity of male offspring is more sensitive to ethephon when exposed in pregnancy and lactation period. It should be emphasized that exposure to ethephon during the in utero stage and lactation stage still could damage the immune function of animal in the period before fully mature even in the dosage that could not influence the immune function of adult animal.

1. Introduction

Ethephon can liberate ethylene which is absorbed by the plant and eventually interfere the process of plant growth. It has been reported that ethephon has a role in improving fruit quality and facilitating the harvest of fruits (Mahajan et al., 2010). Ethephon poisoning is mainly caused by oral and rarely by percutaneous absorption from productive contact. The tissue is obviously irritated and corroded when contacting ethephon which is strong acidic solution. It can be decomposed into ethylene in the gut and lead strong narcotic effect after absorbed to the central nervous system (Jin, 2006).

Fetal and early postnatal life represent critical periods in the development of the immune system. Developmental immunotoxicity has gained increasing recognition as a significant factor for influencing the risk of disease in later life (Dietert and Holsapple, 2007). The development of the immune system during fetal and early postnatal life include a series of programmed cell and organ events in the bone marrow and thymus microenvironment. It needs to be occured in womb, otherwise immune function would be abnormal after birth. The developing immune system can be considered to be more sensitive to xenobiotic insults than the adult immune system (Di Gioacchino et al., 2011).

Developmental immunotoxicity is associated with infectious diseases and cancer. It also increases the risk of autoimmune, inflammatory or allergic response. With the social developing, more and more new chemicals are produced and applied in the daily life. Some chemicals in the environment can change the health status of the body after birth by influencing the early development of the body. Fetal and early neonatal development are critical periods during which environmental factors can alter the trajectory of physiological system maturation thereby affecting the risk of chronic diseases in both children and adults (Dietert, 2011a, 2011b). Juvenile onset diseases such as allergic, inflammatory and autoimmune diseases have shown increasing prevalences in the last decades. The role of chemical exposures in these phenomena is unclear. It is thought that the developmental immune system is more susceptible to toxicants than the mature situation (Hessel et al., 2015). The prevalence in children of early onset diseases related to malfunctioning of the immune system, such as asthma, allergies and a host of autoimmune diseases, including inflammatory bowel disease and diabetes, are steadily increasing in western societies (Dietert, 2011a, 2011b). Among a wide variety of suggested causes are pre- and early postnatal developmental exposures to chemicals (Tonk et al., 2015). Immune-based diseases impact in excess of 25% of the pediatric population in some countries (Dietert and Zelikoff, 2009). These diseases and associ-

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ated immune-based conditions that arise later in life may cause a significant lifetime burden (Tonk et al., 2012).

More and more human immune system dysfunctions are caused by environmental factors currently. Organic phosphorus is a kind of pesticides and plant growth regulators which is an important category of chemical substances and could enter body through food. Learning the damage on immune system caused by plant growth regulator of organophosphate class and exploring the mechanism of injury is helpful to clarify the way of damaging the immune system by chemicals furtherly and provide the basis of food safety management.

Currently, there were few researches about ethephon immunotoxicity. The literature suggests that ethephon group at dosage of 67–268 mg/kg bw could lead footpad swelling and hemolytic plaque logarithm significantly lower than that in control group, phagocytic index was significantly reduced at 268 mg/kg bw dose. It shows that ethephon has a certain degree of inhibitionon on immune function (Liu et al., 2009). It also suggests the need of furtre study about ethephon immunotoxicity. Therefore, it is necessary to confirm the immune toxicity especially developmental immunotoxicity of ethephon and its mechanism. This study tends to find the effects of ethephon on the developing immune system of the BALB/c male offspring mice and explore the related mechanism.

2. Materials and methods

2.1. Animals

Parental F0 SPF BALB/c (40 males and 80 females) whose weight ranged from 18 to 20 g were obtained from the Vitalriver. F0 animals were mated at a ratio of 2 females:1 male to obtain the offspring. The day when the plug was found in the vaginal was regarded as day 0 of gestation (GD0). The morning after birth was considered postnatal day 1 (PND1). Litters were not standardized and pups were weaned on PND 21. All animals were housed in a room at a controlled temperature (22 ± 2 °C) and humidity 50%–60%, with a 12 h:12 h light:dark cycle. Animals were supplied with standard diet and tap water. F_0 females were housed individually for the birth and rearing their young (F_1). All the experiments were approved by the institutional committee of animal research.

2.2. Test compound and exposure

Ethephon with a labeled purity of 98% or higher was purchased from China Okay Plant & Chemical Technology Co. Ltd. At 23 °C, the weight of ethephon dissolving in 100 ml water is 123.9 g at most. Pregnant mice were randomly assigned to the treatment groups or the control group, and they were gavaged by ethephon or distilled water from the 6th day of gestation (GD6) to weaning at dosage of 15.9, 47.7, 143 mg/kg/day.

2.3. Effects assessment

2.3.1. Assessment of general toxicity

Subsets of F_1 male mice (n = 10/dose group) were evaluated for the effect of ethephon. They were weighed on PNDs 1, 21, 28 and 42. On PND 21, remove two male pups from every litter to raise. On PNDs 28 and 42, one male pup from every litter was sacrificed by cervical dislocation. Blood was collected by enucleation. The serum was separated and detected with hemocytometer. During necropsy, spleen and thymus were weighed for the calculation of organ weight factor. The organ weight factor was calculated to determine the effect of ethephon on general condition of pups immune organs. Organ weight factor was calculated as follows: Organ Weight Factor (%) = Organ weight (g)/Live body weight (g × 100. Other related immune assessments were also performed on PND 28 and 42.

2.3.2. Natural killer cell activity

Splenic natural killer (NK) cell activity was assessed by using an invitro LDH assay (Li et al., 2006). YAC-1 cells were subcultured 24 h before the experiment start and washed three times by Hanks solution. RPMI 1640 complete culture medium was used to adjust the concentration of YAC-1 cell to 1×10^5/ml and the spleen cell to 5×10^6/ml. 100 μl of YAC-1 cells and spleen cells was added to each well of U-shaped 96-well plate. Target cell spontaneous release control were 100 μl YAC-1 cells mixed with 100 μl culture medium. Target cell maximum release control were set as YAC-1 cells and 5% triton 100 μl. Each mouse has triplicate parallel samples. The samples were mixed thoroughly and incubated for 4 h in 37 °C, 5% CO_2 incubator. Then all samples were centrifuged for 5 min at a speed of 1500 r/min. 100 μl supernatant from each well and 100 μl LDH substrate solution were mixed in 96-well plate. After 2 min, 30 μl HCl (1 mol/L) was added into each hole for 3 min. OD value was measured at 490 nm in a microplate reader. NK cell activity = (OD of reaction hole–OD of spontaneous release hole)/(OD of maximum release hole– OD of spontaneous release hole) × 100%

2.3.3. T lymphocyte proliferation

T lymphocyte proliferations were determined by MTT assays. Spleen cells were seeded in 24-well culture plates, with 2×10^6 cells in each well. Cells were cultured with RPMI-1640 medium containing 10% fetal bovine serum in the presence or absence of Con A (5 μg/ml). After the cultivation for 68 h, 50 μl MTT (5 mg/ml) was put into every well and cultured for additional 4 h in the 37 °C, 5% CO_2 incubator. Then after discarding the supernatant, the violet crystals were dissolved in acidified isopropanol that the volume ratio of hydrochloric acid (1 mol/L) and isopropanol was 1:24. The dissolved mitochondrial formazan sediments were quantified with a microplate reader by measuring the absorbance at 570 nm wavelength. The optical density was directly proportional to the number of living cells.

2.3.4. Humoral immunity

Changes in humoral immunity were detected by hemolytic plaque assay (Li et al., 2006). Five days after immunization with sheep red blood cell (about 1×10^8 SRBC) mice were sacrificed and the spleen of mice were dispatched sterilely. Adjusting the concentration of single spleen cell suspension to 1×10^5/ml. The surface medium (1% agarose) was melted and kept in 45 °C water bath. Mixed with an equal amount of 2-fold concentration Hanks solution whose pH was 7.2–7.4. It was divided into small tubes that contain 50 μl of 10% SRBC (dispersed by SA). Added spleen cell suspension and quickly poured it on the slide which coated with agarose. Put the slide into the cassette after the agarose solidified. Incubated it for 1.5 h at 37 °C. Added complement that was diluted with SA (1:10) to the slide rack groove and incubated it for another 1.5 h. Counted the number of plaques.

2.3.5. Cell-mediated immunity

Delayed type hypersensitivity reaction was detected by plantar thickening method, reflecting the changes of immune function (Zhou, 2006). Mice were immunized by intraperitoneal injection with 0.2 ml sensitization sheep red blood cell (about 1×10^8 SRBC). The thickness of plantar was measured by vernier caliper on 4th day after immunization. Then 20 μl SRBC (about 1×10^8 cells) were injected into the measurement site subcutaneously. After 24 and 48 h, measured the same site for three times and recorded the results.