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IMMUNOTOXIC RESPONSE OF FEMALE BALB/C MICE TO DIAZIONE; CARBOFURAN AND CYPERMETHRINE INSECTICIDES

(With 2 Tables)

By

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التسمم المناعى فى اناث الفئران البيضاء المتعرضه للمبيدات الحشريه ديازينون كاربوفيوران والسيبرميثرين

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تم فى هذا البحث تقييم رد الفعل لدى الجهاز المناعى لفئران ال BALB/C الاناث عند تعرضها للمبيدات الحشريه . تم تعرض الفئران البيضاء إلى ثلاث جرعات من المبيدات الحشريه الديازينون – الكاربوفيوران والسيبرميثرين على مدى عشرة أيام .

كانت الجرعات المستخدمه من كل من المبيدات الحشريه عباره عن ١٪ – ١ر٠٪ و ١٠ ر٠٪ من الجرعات السامه نصف المميته على مدى التجربه . أوضحت النتائج عدم تأثر وزن الجسم بالجرعات المستخدمه ماعدا فى حالة الجرعه العاليه من السيبرميثرين (١٪ من الجرعه السامه نصف المميته / كيلو جرام / ١٠ أيام). بينما حدث إنخفاض فى وزن الطحال المطلق والنسبى وكذلك إنخفاض فى عدد الخلايا الموجوده بالطحال فى كل من الفئران المعرضه للمبيدات الحشريه بالمقارنه بالمجموعه الضابطه . أوضحت النتائج أيضا تأثر الجهاز المناعى للفئران بالجرعه العاليه وهى عباره عن ١٪ من الجرعه السامه نصف المميته وكذلك بالجرعه المتوسطه وهى عباره عن ١ ر٠٪ من الجرعه السامه نصف المميته ولكن بدرجات متفاوته بينما تحملت الأجهزه المناعيه للفئران المستخدمه الجرعه الصغيره وهى عباره عن ١٠ ر٠٪ من الجرعه السامه نصف المميته والتى لم تحدث أى تأثير فى المؤثرات المناعيه المستخدمه لدراسة التأثر المناعى بهذه المبيدات .

أثبت البحث إمكانية استخدام الحث المناعى سواء عن طريق استخدام ال Con. A أو ال LPS لقياس مدى تعرض الحيوانات للعديد من المبيدات الحشريه المستخدمه وكذلك إستخدام التفاعل الخلطى للخلايا الليمفاويه MLR لتحديد درجة التعرض لهذه المبيدات .

SUMMARY

The immunotoxic effect of diazinon, carbofuran and cypermethrin was evaluated in female BALB/C mice. Mice were dosed by gavage with 0.01, 0.001 and 0.0001 of the oral LDso of each technical insecticide. The lymphocyte proliferation assays were used to measure the functional activity of thymus-dependent (T-cells) and bursal equivalent (B-cells) lymphocytes by concanavalin A (con A) or lipopolysacharide (LPS). Mitogenesis or cell proliferation was measured by tritiated thymidine (^{3}H) incorporation. Results reveal, no changes in body weight except with the high dose of cypermethrin. Reduction in spleen cellularity and weight of intoxicated mice were recorded. In addition the mixed lymphocyte reaction (MLR) of the spleenic cells was changed. Lymphocyte proliferative reponses to Concanavalin A (con A) and Lipoplysaccharide (LPS) were also reduced in spleenocytes.

Keywords: Immunotoxic response, female balb/C mice, diazinon, carbofuran, cypermethrine, insecticides.

INTRODUCTION

The immune system as a target of chemical toxicants has only recently gained concern and importance. Recently there has been interest in developing assays that can be used as indicators of exposure to toxic agents *(SNYDER and VALLE, 1991).*

Two types of immunotoxic effects are possible; immunosuppression, and/or devlopment of cancer, and hypersensitivity, manifested as allergy or as autoimmunity *(BOTHAM, 1990).*

Organophosphate insecticides have been shown to be immunosuppressive in certain species *(STREET* and *SHARMA, 1974* and *STREET, 1981).* The investigators observed that a 28 day oral exposure of rabbits to methyl parathion dosed (1.5 mg/Kg/day) produced a marked reduction in splenic germinal centers following antigenic stimulation, as well as thymus cortical atrophy and reduced respone to tuberculin. Increasing evidence suggests that certain pesticides or formulations contaminants can alter immune functions in rodents, although studies in humans are limited and ambiguous *(STREET, 1981, THOMAS* et al., *1990 a).*

OLSON et al., *(1987)* reported an unusual inverse dose-related suppression of antibody response in mice following

exposure to aldicarb at concetration of 1 ppb in drinking water for 34 days. In contrast, *THOMAS* et al., *(1987, 1990 b)*, using similar exposure conditions that encompassed and exceeded earlier concentrations were unable to substantiate immune modulation or altered, susceptibility to challenge with infective agents in aldicarb-treated mice.

The newest major class of insecticides is the synthetic pyrethroids; a group of chemicals just entering the marketplace in 1980, but by 1982, accounting for approximately 30 percent of the worldwide insecticide usage *(VIJVERBERG and VANDEN BERCKEN 1982)*. Recent reports have appeared in the literature from the people's Republic of China, where synthetic pyrethrodis have been used on a large scale on cotton crops since 1982 *(STUART-HARLE, 1988 and HE* et al *1988 and 1989)*, there has been speculations about the relationship between these insecticides and clinical manifestations appeared on subjects engaged in packaging of them.

The measured parameters were restricted to factors such as serum immunoglobulins levels or T-lyphocyte numbers and gross observable toxicity. Recently there has been interest in developing assays that can be used as indicators of exposure to toxic agents. The aim of this work was to evaluate the use of spleenocytes proliferation assays as a tool for detection of pesticide exposure. In addition assessment of the immunotoxic effect of diazinon, carnofuran and cypermethrin on female BALB/C mice was also our goal.

MATERIALS AND METHODS

Animals and Treatments:

Female BALB/C mice, 6 weeks old and 20 to 23 g weight were classified into three groups, 10 each. For each of the selected pesticides 0.01%, 0.1% and 1% of the oral LD50 were given daily for 10 days dissolved in corn oil, one group kept as control and dosed with corn oil.

C57 BL/6 mice were the source of stimulator cells for the mixed lymphocyte reaction (MLR). All mice were purchased from Harlan Sprague Dawley, Inc., Indianapolis, USA. The animals were maintained on a 12/12-light and dark cycle and were provided with food and water ad libitum.

The insecticides were of technical qrades (98-99% active ingredients) supplied from Chem Service Company, West Chester, PA, USA.

Immune Parameters:

At the end of the experiment, mice were allowed to rest for 3 days after which they were wighed and killed by cervical

dislocation and then the spleen was removed aseptically and weighed. Then placed in petri dish and mashed in RPMI-1640 medium with a sterile syringe barrel, and made into one cell suspension by passing through a 25 G needle and adjusted to the needed concentration.

Mitogen-stimulated lymphoproliferative responses of spleen cell suspensions were measured by tritited thymidine (^{3}H-TdR) incorporation *(SMIALOWICZ, et al, 1985).*

Spleen cells were suspended in suplemented RPMI-1640 medium, containing 25 mM HEPES, 2 mM L-glutamine, 50 µg/ml gentamycin and 5% calf bovine serum.

The 72-h lymphoroliferative responses of spleen cells from individual control and treated mice were determined in triplicate at several concentrations of each mitogen for counting the radioactivity in radioactively-tagged samples and determination of total counts and counts per minutes (cpm) of each sample, using Beckman LS-3801 Liquid Scintillation Counter.

Harvesting of cells usunig cell harvester supplied from Skatron Co., then the samples, in multi-purpose premixed liquid scintillation cocktails, were placed in special vials. The vials were placed into the sample changer of the Liquid Scintillation Counter to be measured.

The one way mixed lymphocyte reaction (MLR) was performed using responder (Pesticed-dosed BALB/C mice) splenic lymphocytes according to the nethod of *BRADLY (1980).*

Stimulator cells were treated with mitomycin C at 50 µg/ml for 40 minutes at 37°C and 5% Co2, washed three times and resuspended at $4X10^6$ cells/ml in supplemented RPMI-1640 medium containing $5X10^{-5}$ M 2-mercaptoethanol (2-ME). Responder cells were resuspended in the same medium at $2X10^6$ cells/ml. To triplicate wells of round-bottom microtiter plates containig $2X10^5$ responder cells were added complete medium or $2X10^5$ stimulator cells in a total volume of 200 µl. In addition, wells containig stimulator cells plus Con A set up as an internal control for the efficacy of the mitomcin-C treatment of stimulator cells. MLR cultures were incubated for 96 h at 37°C and 5% CO2. Four hours prior to harvest, cultures were labeled with 1.00 µci/well ^{3}H-TdR (specific activity 6.7 ci/mM) in 10 µl/well. The results are expressed as the net counts/min., subtracting the counts/min. of responder only cultures from the counts/min. of responder plus stimulator cultures, according to *SMIALOWICZ et al., (1992).*

RESULTS

The effect of different doses of diazinon, carbofuran and cypermethrin insecticides on spleen and body weight of BALB/C mice is summerized in table 1. Body weight was not affected by the sued doses except in case of 0.025 mg/kg/day cypermethrin. Spleen weght was reduced and this reduction was dose dependant. The relative spleen weight was also reduced in relation to body weight. The cellularity of the spleen was affected in comparison to the control group of mice. Table 2, shows the effect of the selected doses of the pesticides on the proliferative responses of spleenocytes taken from female BALB/C mice. The lymphproliferative response to mitogen-stimulation and to the MLR of spleenocytes from mice exposed to the dose 0.01% of LD_{50} of the three pesticides were unaltered. However, spleenocytes from mice dosed 0.1% and 1.0% of LD_{50} when cultured with Con A or LPS, had reduced proliferation responses compared with control but at different levels.

DISCUSSION

Pesticides constitute a very important group of chemicals released into the environment. The influence on any of pesticides on the immune system or immune response has been nearly totally ignored despite obvious health implications. Our study was performed to determine whether the selected pesticides alter the immune function of BALB/C mice. Exposure of mice to 1.0% of oral LD_{50} of pesticides (0.3 mg/kg/day diazinone; 0.008 mg/kg/day carbofuran and 0.025 mg/kg/day cypermethrin) resulted in a significant reduction in immune functions. The dose of pesticide along with the 10 days do not exceed 1.0, 0.1 and 0.01% of the oral LD_{50}. A dose of 0.01% of LD_{50} along with 10 days found to be tolerated by female BALB/C mice. The moderate dose of 0.1% of LD_{50} gave a moderate immunotoxic effect in the treated mice.

Body weight was unaltered at any of the exposed mice or any dose level of the three pesticides except those exposed to 0.025 mg/kg/day of cypermethrin. Spleen weight was reduced with the exposure of pesticides, and spleenocyte number was also decreased in a dose related manner. Spleenocyte proliferative responses to Con A, LPS and MLR were significantly reduced and this reduction seems to be dose-dependant. Accordeing to *LUSTER, et al., (1993)*, a good correlation exists between changes in the immune tests and host resistance in that there were no instances where host resistances was altered without affecting an immune test or tests.

Certain xenobiotics or their metabolites can damage immunocomptence by directy interacting with one or more of the cells of the immue system and adversely affecting its function. It has also been proposed that xenobiotics may indirectly affect immune function by affecting other organ systems that will in return affect immunocompetence *(FUCHS and SANDERS, 1994)*. Organophosphate as well as carbamte pesticides have been reported to alter normal immune function *(RODGERS, et al 1986 and DEAN et al 1990)*. Cypermethrin as a member of the new generation of insecticides, pyrethroids, has been shown to reduce both humoral and cellular immune respone *(DESI et al 1985 and TAMANG et al 1988)*.

The results of this study indicated that spleenocyte proliferation assays may be applicable as biomarkers of exposure for a wide variety of pesticides as diazinon, carbofuran and cypermethrin.

These assays are also applicable for the detection of immuntoxic effects of pesticides. Finally many further investigations are needed to obtain a more detailed picture of pesticide immunotoxicity.

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Table 1. The effect of pesticides on body and spleen weights and cellularity in female BALB/C mice.

Pesticides	Dose (mg/Kg/day)	Parameters				
		Body weight (g)		spleen weight (mg)	Relative spleen weight (%)	spleenocytes $X10^7$/ml
		A	B			
Diazinon	0.3	21.60 ±1.75	21.03 ±1.85	98.75 ± 5.33**	0.470	3.55 ± 0.21*
	0.03	22.40 ±2.20	22.23 ±2.21	106.25 ±7.33**	0.478	3.78 ± 0.25
	0.003	21.56 ±2.20	21.37 ±1.99	95.00 ± 5.11**	0.445	3.25 ± 0.31*
Carbofuran	0.008	21.00 ±1.55	21.40 ±2.01	97.50 ± 6.15**	0.456	2.75 ± 0.33**
	0.0008	23.00 ±1.89	22.49 ±2.21	102.50 ± 5.65*	0.456	3.03 ± 0.28**
	0.00008	22.00 ±2.05	22.17 ±1.57	100.11 ± 6.75*	0.487	3.37 ± 0.24*
Cypermethrin	0.025	22.21 ±1.98	18.77 ±1.57**	98.50 ± 7.65**	0.495	3.01 ± 0.19**
	0.0025	22.11 ±1.87	21.82 ±1.76	98.75 ± 6.88**	0.456	3.62 ± 0.15*
	0.00025	22.22 ±2.08	21.33 ±1.96	97.98 ± 5.33**	0.431	3.66 ± 0.21
Control	------	22.45 ±1.65	22.35 ±2.15	118.75 ± 5.88	0.594	3.98 ± 0.22

A: Weight befor dosing.
B: Weight after the end of the experiment (13 day).
* Significant at $P< 0.05$.
** Significant at $P< 0.01$.

Table 2. The effect of pesticides on the lymphproliferative response of spleenocytes from female BALB/C mice.

Pesticides	Dose (mg/Kg/day)	Spleenocyte response (counts/min.) X10^4		
		Con A	LPS	MLR
Diazinon	0.3	24.54 ± 3.13**	18.65 ± 2.15	28.33 ± 1.13**
	0.03	29.44 ± 2.53**	19.88 ± 1.38	33.21 ± 2.05**
	0.003	35.01 ± 1.64*	20.58 ± 1.38	40.36 ± 2.51
Carbofuran	0.008	29.90 ± 3.56**	10.66 ± 1.55**	37.87 ± 3.04**
	0.0008	28.67 ± 2.03**	13.54 ± 0.98**	41.86 ± 2.12
	0.00008	33.62 ± 2.25*	16.71 ± 2.35*	41.77 ± 3.25
Cypermethrin	0.025	29.76 ± 1.98**	15.53 ± 1.75	37.53 ± 2.78**
	0.0025	29.82 ± 2.33**	17.31 ± 1.73	36.85 ± 3.64*
	0.00025	34.56 ± 2.84	17.53 ± 1.11	41.75 ± 3.56
Control	------	36.83 ± 3.15	18.90 ± 1.85	42.66 ± 3.41

Con A: Concanavalin A.
LPS: Lipopolysaccharide.
MLR: Mixed lymphocyte reaction.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.