IMMUNOTOXIC EFFECTS OF LAMBDA-CYHALOTHRIN
IN RABBITS

Ashraf M. Morgan* and Ahmed H. Osman **

WWW.estoxicology.org

* Department of Toxicology and Forensic Medicine, ** Department of Pathology, Faculty of Veterinary Medicine, Cairo University

ABSTRACT

The effect of Lambda-cyhalothrin on the immune response was studied in rabbits exposed to the insecticide via food at concentrations of 1373.23, 686.76 or 343.38 ppm (equivalent to 1/10th, 1/20th and 1/40th oral LD50, respectively) for 8 weeks. The humoral immune response was measured by determination of the antibody titre against sheep red blood cells (SRBC), a T - cell dependent antigen. Moreover, the cell-mediated immune response was evaluated by the delayed - type hypersensitivity reaction to tuberculin. The chemical treatment resulted in a dose - dependent suppression of both humoral and cell-mediated immune responses as evidenced by decreased serum hemolysin titres and inhibition of the delayed-type hypersensitivity reaction to tuberculin, respectively. In addition, leucopenia, lymphopenia, depletion of lymphoid cells in the white pulps of spleen, mesentric lymph nodes and Peyer's patches and severe atrophy of thymus cortex were recorded. The serum total protein, albumin, globulin (specially gamma globulin), albumin/globulin (A/G) ratio and the organ to body weight ratios for spleen and thymus were significantly decreased with increasing the insecticide dosage. Moreover, different pathological alterations in liver, and brain were also observed. In conclusion, Lambda-cyhalothrin exposure suppressed both humoral and cell-mediated immune responses in rabbits at the tested concentrations in a dose - dependent manner.

Key words: Lambda-cyhalothrin –humoral - cellular - immunity – rabbits.

INTRODUCTION

The ability of natural or synthetic agro-chemicals and other environmental chemicals to affect immune responses has aroused considerable interest. Subtle immunological alterations which may be associated with low level pesticide exposure have not been completely evaluated and might cause break down to vaccination (Varshneya et al. 1988) and increased susceptibility to infection (Muneer et al., 1988). Therefore, immunosuppression by pesticides and other chemicals of environmental importance is a developing concern in toxicity assessment (Santoni et al.,1998; 1999 and Tryphonasa et al., 2004).

Cyhalothrin is a type II pyrethroid used predominantly on cattle and sheep and to a lesser extent on pigs and goats for the control of a broad range of ectoparasites, including flies, lice, and ticks (Abbud Righi and Palermo-Neto, 2003). Lambda-cyhalothrin has the same spectrum of insecticidal activity as cyhalothrin, but it is more active (U.S. EPA, 1988). The acute oral toxicity of lambda-cyhalothrin is higher than that of cyhalothrin. LD50 values are as follows: rat, 144-243 mg/kg (cyhalothrin); 56-79 mg/kg (lambda-cyhalothrin) and mice, 37-62 mg/kg (cyhalothrin), 20 mg/kg (lambda-cyhalothrin). Clinical signs of cyhalothrin and lambda-cyhalothrin toxicity include ataxia, unsteady gait, hyperexcitability, piloerection, subdued behaviour, salivation, incontinence, scouring, and chromodacryorrhoea (WHO, 1990).

Previous findings indicated that most type II pyrethroids such as cypermethrin, supercypermethrin forte, fenvalerate and deltamethrin, display immuno-suppressive effects on humoral and cell mediated immune response in adult mice, rats, rabbits and goats (Desi et al.,1986; Tamag et al., 1988; Lukowicz-Rotajczak and Krechniak, 1992; Singh and Jha, 1996 and Insititoris et al., 1999; 2002). Some pyrethroids have also been reported to cause lymph node and splenic damage as well as carcinogenesis and mutagenesis (Hallenbeck and Cunningham-Burns, 1985 and Amer et al., 1993).

Subacute to chronic oral exposure to permethrin and its analogs cypermethrin, bioallethrin and deltamethrin have been reported to cause local and systemic immunomodulation in mice. These immune effects include reduced size and cellularity of the spleen and thymus, and inhibited antibody production and contact hypersensitivity responses with diminished natural killer cell cytotoxicity in mice and rats (Blaylock et al., 1995; Santoni et al., 1998; Punareewattana et al., 2000 and 2001 and Pratera et al., 2002). In addition to oral exposure, preliminary observations in humans exposed by the topical route to permethrin, as occurs with insecticide use or treatment for
lice or mites, suggested that these individuals may suffer immune modulation, specifically, T-cell and antibody-mediated immunomodulation, alterations in class II MHC cells, and modified cytokine levels (Puig et al., 1989 and Zhang et al., 1999).

This study was undertaken because there are no available information on the immunotoxicological effects of lambda-cyhalothrin exposure, although toxicity data on its acute, subacute and other toxicological effects are available. Also, pyrethroids are neurotoxic and a close relationship between the nervous system and the immune system exists (Hori et al., 1995 and Straub et al., 1996). Therefore, we have investigated the effects of the type II pyrethroid insecticide lambda-cyhalothrin on humoral and cellular immune responses of rabbits exposed via food.

MATERIALS AND METHODS

1. Chemicals

Lambda-cyhalothrin, [1α(S*),3α(Z)] - (±) - cyano (3-phenoxypyphenyl) methyl3- (2-chloro-3,3,3-trifluoro-1-propenyl) -2, 2-dimethyl cyclopropane carboxylate; CAS RN/91465 – 08 – 6. official code OMS 3021, is the most commonly and profusely used pyrethroid pesticide in Egypt. The commercial formulation, lambda-cyhalothrin (provided by Zeneca Co., USA) (Worthing and Walker 1994) was used in this study. All test solutions were freshly prepared before each experiment.

2. Animals:

Male New Zealand white rabbits 1.5 months of age, weighing 1.75–2.10 kg were purchased from the National Institute of Ophthalmology Research, Giza. They were provided with food and water ad libitum for 10 days prior to the experimental work for acclimatization.

3. Experimental design:

3.1. Determination of acute oral LD50:

LD50 of test insecticide was determined according to the method described by Weil (1952) owing to its simplicity and the few number of experimental animals required.

Twelve male rabbits were used. They were divided into 4 equal groups and dosed with 1400, 700, 350 and 175 mg lambda-cyhalothrin/kg b. wt., respectively, in a single oral doses. The number of dead animals and symptoms of acute toxicity in the treated groups were recorded postadministration and the LD50 was then calculated.

3.2. Chemical treatment for immunotoxicity investigation:

Fourty male New Zealand white rabbits were weighed and classified into 4 equal groups. The first group received no chemical treatment and served as control. The other 3 groups received the insecticide in their food for 8 weeks (Street and Sharma, 1975) at concentrations of 1373.23, 686.76 or 343.38 ppm (equivalent to 1/10th, 1/20th and 1/40th oral LD50, respectively.

3.2.1. Humoral immune response: Both control and treated animals were immunized after 4 weeks of experiment by intraperitoneal injection with 0.5 ml of a 20% SRBC suspension in Alsever’s solution. Ten days after immunization, blood samples were collected from the ear vein and the hemolysin antibody titres were determined by the complement fixation test according to Seinen et al. (1977). Also, serum total protein (using test kits) and the electrophoretic pattern of serum protein fractions were estimated according to King and Wooton (1959) and Chin (1970), respectively.

3.2.2. Cell-mediated immune response: It was evaluated in the same animals used for humoral immunity by measuring the delayed-type hypersensitivity reaction to tuberculin (Park - Davis, Deteroit, MI) according to the method adopted by Street and Sharma (1975). Tuberculin solution was intradermally injected at 0.1 ml in the clipped flank on days 43 and 56 of experiment. The diameter and thickness of skin reactions were measured with a caliper 24 hours after tuberculin injection.

3.2.3. Haematology: Total and differential leucocytic counts were determined on days 0, 38 and 56 of experiment according to Schalm et al. (1975).

3.2.4. Body and organ weight: Body weight and organ to body weight ratios for spleen and thymus were determined at the end of exposure period.

3.2.5. Histopathology: Histological changes in lymphoid tissues (spleen, thymus, mesenteric lymph node and ileum Peyer’s patches), liver and brain were examined at the end of experiment according to Durry and Wallington (1980).

4. Statistical analysis: The comparison between control and treated groups was made using the student's "t" test to measure the statistical significance of treatment effects (Snedecor and Cochran, 1980).

RESULTS

1. The median lethal dose (LD50):

The mortality data in rabbits given oral successive dose levels of lambda cyhalothrin were recorded in table (1).

The observed toxic symptoms following lambda cyhalothrin administration in high doses to male rabbits were hypersensitivity, profuse salivation, course tremor progressing to choreoathetosis and paralysis. These symptoms appeared after 2-3 hrs of administration and most mortalities occurred within 4-6 hrs postadministration.
Log LD50 = Log Da + d (F + 1), Where:

-significant changes.

Rabbits exposed to lambda cyhalothrin in their food at concentrations of 1373.23, 686.76 or 343.38 ppm (equivalent to 1/10th, 1/20th and 1/40th oral LD50, respectively) did not show any toxic symptoms or behavioural changes throughout the test period.

The results given in Table (2) showed that serum hemolysin antibody titres, serum total protein, albumin, globulin (specially gamma globulin) and albumin/globulin (A/G) ratio were significantly decreased by lambda cyhalothrin exposure with increasing the insecticide level. In addition, the insecticide decreased the organ to body weight ratios for spleen and thymus in a dose-dependent manner.

The oral LD50 of lambda cyhalothrin was calculated as follow:

Log LD50 = Log D0 + d (F + 1), Where:

- D = Log constant ratio between doses.
- F = a value obtained from Weil table based on the number of animals used/dose level as well as number of dead animals/dose level.

LD50 = 416.222443 mg /Kg body weight.

Morgan & Osman (2007) Immunotoxic Effects of Lambda-Cyhalothrin in Rabbits

Table (1): Mortality data of the acute oral doses of cyhalothrin in male New Zealand white rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>No. of rabbits/group</th>
<th>No. of dead rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>175</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>350</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>700</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1400</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

The oral LD50 of lambda cyhalothrin was calculated as follow:

Log LD50 = 2.243038 + 0.376287494 = 2.619325494

2. Effects on humoral immune response, serum proteins and organ to body weight ratios for spleen and thymus:

Rabbits exposed to lambda cyhalothrin in their food at concentrations of 1373.23, 686.76 or 343.38 ppm (equivalent to 1/10th, 1/20th and 1/40th oral LD50, respectively) did not show any toxic symptoms or behavioural changes throughout the test period.

The mesenteric lymph nodes of exposed rabbits at 1373.23 ppm showed severe lymphoid depletion and atrophy. Also, it revealed decreased size and number of lymphoid follicles with little or no reactive centers. The lymphoid sinuses were distended with lymph, few lymphocytes, macrophages and erythrocytes. Apoptosis of lymphocytes was also clearly defined as apoptotic bodies inside the macrophages (Fig.9-10). Moderate depletion and atrophy of lymphoid follicles was observed in exposed rabbits at 686.76 and 343.38 ppm, apoptosis and nuclear fragmentation of lymphocytes were also seen (Fig.11-12).

Ileum Peyer's patches of the two highest doses- treated animals showed severe atrophy and depletion of its lymphoid follicles. Focal necrosis of lymphoid cells with cellular infiltration mainly macrophages and plasma cells were noticed (Fig.13-14). Moderate depletion of Peyer's patches lymphoid follicles was noticed in the lowest dose- treated rabbits. Apoptosis of lymphocytes was also seen in the form of clumping of cellular debris (Fig.15-16).

Liver examination of exposed rabbits at 1373.23 ppm revealed disorganization and degeneration of hepatocytes. Biliary cirrhosis was also observed in all examined cases which characterized by hyperplasia of bile ducts, fibrous connective tissue proliferation of portal triads and leukocytic infiltration mainly lymphocytes, macrophages and plasma cells. Fibrous septa were joined up adjacent portal tracts but leaving the centrilobular area unaffected to produce a pattern of monolobular fibrosis (Fig.17). On the other side, liver of exposed rabbits at 686.76 and 343.38 ppm showed mild degenerative changes of hepatocytes with marked cholangiofibrosis. The bile ducts revealed

Microscopically, spleen of the two highest doses (1373.23 and 686.76 ppm)-treated animals showed atrophic changes in the white pulps characterized by decreased size of lymphoid follicles and periarlierolar lymphoid sheath (PALS) in comparison with the control group (Fig.1-2). Shrunken red pulp components with marked hemorrhages and hemosiderosis with a relatively increased collagenous connective tissue stroma between the narrow compressed vascular spaces were also seen (Fig.3). However, spleen of the lowest dose (343.38 ppm)-treated rabbits revealed thickening of its capsule, moderate atrophy and depletion of lymphoid follicles with small sized PALS (Fig.4).

Thymus gland of the two highest doses- treated groups showed severe cortical atrophy, characterized by necrosis of cortical lymphocytes with large clumps of nuclear debris to form "starry sky" appearance of cortex. Subsequent to thymic necrosis, the cortex appeared thin compared to that in agematched control (Fig.5-6). Tubular structures lined by flattened epithelial cells were associated with the progressive atrophic lobules of thymus gland. These tubules were dilated and contained a homogenous eosinophilic material (Fig.7). On the other hand, moderate cortical atrophy was observed in the lowest dose-treated group. Also, Individual cell necrosis of cortical lymphocytes was seen in form of clumping of cellular debris (Fig.8).

25

2. Effects on humoral immune response, serum proteins and organ to body weight ratios for spleen and thymus:

The insecticide affected the delayed-type hypersensitivity response to tuberculin as shown in Table (3), where the diameter and thickness of skin reactions were decreased at the two highest doses used, both at 44th and 57th days of the exposure period.

3. Effects on cell-mediated immune response:

The insecticide affected the delayed-type hypersensitivity response to tuberculin as shown in Table (3), where the diameter and thickness of skin reactions were decreased at the two highest doses used, both at 44th and 57th days of the exposure period.

4. Effects on haematology:

The total and differential leucocytic counts in control and treated rabbits are summarized in Table (4). Leucopenia, lymphopenia and neutrophilia were observed in treated animals in a dose and time-dependent manner especially at the end of exposure period.

5. Effects on histological structures of lymphoid tissues, liver and brain:

Grossly, the lymphoid tissues, liver and brain of control and lambda cyhalothrin treated rabbits did not show any significant changes.
hyperplasia with massive proliferation of portal triad fibrous connective tissue. The hepatic lobules appeared with normal archetcher in comparison with the highest dose – treated group (Fig.18).

Brain tissues of the two highest doses- treated rabbits showed focal areas of neuronal necrosis in the external pyramidal cell layer of cerebral cortex. The neuronal cells showed different degrees of damage including: shrinkage, angular shape, eosinophilic cytoplasm with pyknosis and karyorrhexis of its nuclei (Fig.19). Also, the brain of the lowest dose -exposed animals did not differ clearly from the two highest doses treated ones. The pyramidal neuronal cells showed degenerative changes characterized by cellular swelling in which the nuclei were surrounded by a hallow zone (Fig.20).

DISCUSSION

Lambda-cyhalothrin, a type II pyrethroid, is widely used for numerous applications, varying from plant protection to general pest control (Anadon et al., 2006). Studying its effect on the immune system is of a great ecotoxicological importance in the protection of human health and animal wealth.

The humoral immune response was evaluated by measuring the serum hemolysin titres against sheep red blood cells (SRBC) a T-cell dependent antigen which needs cooperation of T-helper cells, B-cells and macrophages (Veit and Michael, 1972 and Blakley, 1997). The recorded reduction of humoral immune response confirmed the immunossupression occurred in rats and mice (Desi et al., 1986; Łukowicz-Ratajczak and Krechniak, 1992 and
The suppression of humoral immunity by lambda cyhalothrin exposure was sustained by the observed lymphopenia, decreased spleen to body weight ratio and depletion of lymphoid cells in the white pulps of the spleen. The reduced humoral immunity can be attributed to inhibition of antibody production by plasma cells and/or inhibition of differentiation of B-Lymphocytes to plasma cells (Phillip and Munson, 1997 and (Punareewattana, 2001). In addition, T-helper cells may be affected as SRBC is a T-cell dependent antigen (Seinen et al., 1977). Among the factors that may be involved in lambda cyhalothrin-induced alterations of splenic T and B cell distribution and proliferation, the pesticide ability to interfere with the release and/or production of some cytokines (Lukowicz-Ratajczak and Krechniak, 1992).

In the applied dose range, lambda cyhalothrin induced also a dose-dependent decrease in the delayed type-hypersensitivity (DTH) reaction which mean inhibition of the cell-mediated immune response. This finding is in line with several results in the literature. Varshneya et al. (1992) demonstrated a dose-dependent decrease of DTH in rats and rabbits following a two month oral exposure to cypermethrin. Similar results in which marked thymocyte depletion were previously obtained in rats exposed to cypermethrin (Desi et al., 1986). Moreover, high doses of cypermethrin, supercypermethrin forte and deltamethrin, displayed an immunosuppressive effect on cell-mediated immune response in adult mice, rats and goats (Desi et al., 1986; Tamag et al., 1988; Lukowicz-Ratajczak and Krechniak, 1992; Varshneya et al., 1992; and Tulinska et al., 1995). Also, a marked lymphocyte depletion was observed in the thymus and lymph nodes of cypermethrin -treated rats (Desi et al., 1986 and Tamag et al., 1988). A significant decrease in the total leucocyte count was also reported after cypermethrin exposure of rats (Varshneya et al., 1992).

The suppression of cell-mediated immune response means inhibition of another subpopulation of T-lymphocyte, the so-called T-effector cells (Seinen et al., 1977) and/or inhibition of lymphokines production by activated T-cells (Santoni et al., 1998). The inhibited cell-mediated immune response is supported by the recorded lymphopenia, decreased thymus to body weight ratio and the histopathological changes in thymus, mesenteric lymph nodes and ileum Peyer's patches with depletion of lymphoid cells in their tissues as sensitized lymphocytes originate in the thymus and are formed in the active centers of spleen and lymph nodes (Sell, 1972).

It has been explained that most type II pyrethroids exert a direct effect on leucocytes, through an action on Na⁺-membrane channels and/or by an indirect action on macrophage (key elements in cellular immune responses) activity via hypothalamic pituitary adrenals axis activation (Righi and Palermo-Neto, 2005). The suppressed cellular immune response observed in our study could be attributed to these effects. It has been reported also that the cytotoxic effects are based on the specific genotoxicity of pyrethroids which causes the immunosuppression (Puig et al., 1989 and Dianovsky and Sivikova, 1997). Jamil and Naravaneeni (2005) confirmed the genotoxicity of lambda-cyhalothrin on human lymphocytes cultured in vitro. Synergistic effects may aggravate immunotoxicity and allergotoxicity (Didi et al., 1998). Moreover, Fuchs and Sanders (1994) stated that any xenobiotic capable of inducing peripheral neurotoxicity, could potentially be capable of indirectly inducing immune alterations, by affecting the signals from nerve terminals to lymphocytes. The interactions of pyrethroids with neuronal receptors may be an important regulator of immunomodulation (Vivjerberg and Van den Bercken, 1990). This was sustained by the recorded brain lesions in our results.

Several lines of evidence indicate that type II pyrethroids are strong inducers of both adrenaline (A) and noradrenaline (NA) release (Cremer and Seville, 1982 and Bradburry et al., 1983); neurotransmitter release seems to be secondary to the increased Na⁺ entry (Eells and Dubocovich, 1988), and depression of the resting chloride conductance, which amplifies sodium effects (Burr and Ray, 2005). Noradrenaline is also synthesized and stored in the splenic and thymic nerve terminals (Leposavic et al., 1992 and Straub et al., 1998). In addition, alpha-and beta-adrenergic receptors have been demonstrated on several immune cell type, including different subpopulations of T lymphocytes, granulocytes, monocytes, macrophages, and NK cells (Jetschmann et al., 1997). Output of neuroendocrine pathways has a modulatory effect on the migratory behaviour of lymphocyte in vivo. Thus, it can lead to rapid changes in the specific phenotype of lymphocytes accumulating in tissues and organs undergoing immune challenge (Ottaway and Hushand, 1994). Therefore, the reduced proliferative response observed in the spleen and thymus from lambda cyhalothrin exposed rabbits, could be the result of pyrethroid-induced catecholamine release. These catecholamine-mediated suppressive effects have been partially attributed to NA-induced apoptosis (Joseffson et al., 1996).

In agreement with our results regarding liver pathology, Institoris et al. (1999) found a dose-dependent increase in the liver weight after permethrin exposure of rats. Also, Luty et al. (1998 and 2000) confirmed the presence of parenchymatous degeneration with lymphoid infiltrations in the liver of rats and mice exposed to alpha-cypermethrin. Moreover, the brain of exposed rats showed focal concentration of the neurocytes' cytoplasm with pyknosis and disappearance of some Purkinje cells (Luty et al., 1998). Toukhy and Girgis (1993) described an inhibitory effect of pyrethrin on the activity of the total ATPase in rat liver, which may disturb the active transport of Na⁺, K⁺ and Mg²⁺ ions and result in pathological changes in liver cells. The recorded tissue damage in the liver and brain could be attributed also to the effect of reactive oxygen species (ROS) generated during this pyrethroid insecticide metabolism (Kale et al., 1999).
It has been reported that pyrethroids induce hepatotoxic effects with a consequent suppressive effect on plasma protein production and/or albumin globulin ratio (Aldana et al., 1998). The pathological changes in liver may affect serum total protein and/or albumin globulin ratio and consequently considered as one of the contributing causes of immunosuppression in our study.

Rivarola and Balegno (1991) reported that the reduction in plasma protein, particularly albumin, in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Furthermore, the recorded reduction in gamma globulin concentrations in sera of rabbits following lambda-cyhalothrin exposure can be interpreted through the adverse cytotoxic effect of the insecticide on the immunocompetent cells; more definitely the B-lymphocytes and plasma cells engaged in the production of various kinds of immunoglobulins. This assumption was ascertained by the previously established lowering effect of the insecticide on the haemolysin antibody titers in sera of treated rabbits as well as the lymphocytic depletion recorded in spleen of those rabbits.

In conclusion, the presence of immune alterations subsequent to lambda cyhalothrin exposure at the tested doses suggests that subacute and/or chronic exposure to lambda cyhalothrin in the environment has the potential to alter immune function. Therefore, we advise to use this insecticide at the recommended field application levels away from vegetation to be eaten by animals and to minimize the direct exposure to it as much as possible in order to avoid its immunosuppressive effect.

REFERENCES


Josefsson, E.; J. Bergquist; R. Ekman and A. Tarkowski (1996): Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. Immunology, 88: 140–146.


