The effect of Permethrin in the levels of ll..-2 and IL-4 in mice

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Abstract

The effect of permethrin was examined in three groups of mice which were orally administered with three different doses 1/5, 1/0 and 1/20 of median lethal dose (LD50) (400, 200 and 100 mg/kg, respectively). Cytokines, IL-2 and IL-4, were used to assess the effect of permethrin in inoculated mice. The level of IL-4 in all groups showed some resistance, while that of IL-2 appeared to be more affected. The level of IL-2 in mice that received 1/20 LD50 was less than that of IL-2 in those mice which received 1/5 LD50. It could be concluded that T- helperl (Thl) cells might be more vulnerable to the effect of permethrin than T - helper2 (Th2).

Introduction

Toxicity of the different pyrethroid products to the immune system have been produced in different animals. The immunosuppressive effect of cypermethrin and deltamethrine, in mice, cypermethrin, fenevalerate in goats and supercypermethrin in Wistar rats were documented (Lukowiczet-Ratajczak et al., 1992; Singh and Jha, 1996; Singh et al., 1993; Tulinska et al., 1995 and Tamang et al., 1988).

Assessment of the status of the immune system by monitoring the level of suspected cytokines has been employed overwhelmingly. For instance, Robinson et al. (1993) have monitored the level of interleukin-6 (IL-6) in serum of foals infused with endotoxins. The study aimed to evaluate the efficiency of 2 colostrum in protecting neonatal foals given sublethal dose of lipopoly saccharides (LPS). The efficacy of colostrum in protecting the neonates was tested by monitoring the concentration of serum IL-6. On the other hand, Jia- ma et al., (1995) monitored the tumor necrosis factor-a. (TNF-a.) and (IL-6) in cats infected with feline immunodeficiency virus (FIV). A depression in TNF-a. level was considered a characteristic feature of FIV.

However, the use of cytokines as criteria in assessing the immune system of animals intoxicated with pyrethroid is meager. Lukowiczet-Ratajczak et al., (1992) reported a decrease in interleukin-l (IL-l) in mice which had received deltamethrin. Interleukin-4 (IL-4) is a pleiotropic cytokine produced mainly by activated Th2 cells. Cells like basophils and mast cells could also produce IL-4. IL-4 plays a key role in differentiation of helper cells precursors to Th2 cells. IL-4 is considered prohumoral immunity cytokine due to the modulation of antibody production and regulation of isotype switching, particularly IgE (Howard and Harada, 1994; Banchereu and Rybak, 1994).

Interleukin-2 (IL-2) is also a pleiotropic cytokine produced by Tlymphocytes activated with mitogen or antigen. IL-2 is a vital cytokine in mediating a variety of immune responses. It stimulates the proliferation and differentiation of activated B lymphocytes. IL-2 is also an important growth promoter to a variety of cells like monocytes, natural killer cells and thymocytes. It also enhances the production of lymphocyte-activated killer (LAK) cells (Goldsmith and Green, 1994; Hatukeyama and Taniguchi, 1990).

The present study was aimed to examine the immunotoxicity of ermethrin. It is a member of synthetic pyrethroids, the newest major lass of inscticides. It is commonly used in Saudi Arabia or insect control. Pyrethroids are accounting for approximately 30 percent of the worldwide insecticide usage 3 (Vijverberg and van den Bercken, 1982). In addition to the scarce studies on immunotoxicity of permethrin, this study was also sought to employ cytokines, as a new criterion in evaluating the immune response of intoxicated mice.

Materials and Methods

Animals and Treatment

Twenty (6 weeks old) adult male BALB/C mice, weighing 25-30 9 each were divided into four equal groups. The Ist, 2nd and 3rd groups; were administered orally with 1/5,1/10 and 1/20 the LD50 (400,200 and 100 mg/kg, respectively) of aqueous solution of permethrin (Sharkesuper 10 EC) for 5 successive days, respectively. The 4th group was kept as a control and administered by water as a vehicle.. All mice were provided with food and

water ad-libitum. At the end of the experiment (5 days), mice were allowed to rest for further 3 days after which (8 days) they were killed by cervical dislocation and the spleens were removed aseptically and collected.

Preparation of the spleen cells

Spleen cells from all mice were harvested by squeezing the cells out by repeated injection of minimum essential medium (MEM) in the spleen. The cells were then washed once with MEM. The pool of the spleen cells of each group was diluted to a final dilution of 9x106 cells/mI in growth medium [Medium 199 (Flow Lab, Scotl and) containing 5% foetal calf serum (Sigma) and antibiotics (final concentration of 10 unit/ml penicillin G Sodium, 10 g/ml streptomycin sulfate and 25 g/ ml amphotericin B as fungizon) (Life Technology, USA)] and a final concentration of 10-12 g/ml of phytohaemagglutnin (Sigma) were added. The tubes were incubated at 37°C in the presence of 50% CO2 for 48 hours.

Determination of the IL-2 and IL-4 level

The level of IL-2 and IL-4 was determined using polyclonal anti-mouse IL-2 and anti- IL-4 cytokine ELISA kits (Amersham Life Sciences, UK). Supernatents 4 from each group were tested according to the standard procedure. The samples were left overnight before the conjugated antibodies were added.

Results

The levels of IL-4 and IL-2 in spleenocytes of mice treated with different doses of permethrin are shown in Table 1. The significant differences expressed in Table 1 are analysed by MSU Stat Program.

Table 1. The effect of permethrin on levels of IL-2 and IL-4 concentration in spleenocytes of mice

Groups	IL-4 (Picograms(PG/m1)	IL-2 (Picograms (PG/m1)
Control	1.0 a	6.3 bc
1/5	3.17 b	7.3 c
1/10	3.53 b	4.8 ab
1/20	2.8 ab	3.2 a

Means in two above columns followed by different letters are significantly different at the 0.05 level by least significant differences (LSD)

The level of IL-2 showed a slight increase with group 1/5, however a decrease in its level was more significant with the group which received lower doses, especially 1/20. On the other hand, the level of IL-4 showed a significnat increase with the groups 1/5 and 1/10.

Discussion

The effect of certain pyrethroid products have been reported in the immune response of mice, goats, Wistar rats and human (Klucinski et al., 1996; Luckowicz-Ratajczak et al., 1992; Singh and Jha, 1996, Singh et al., 1993; Tulinska et al., 1995 and Tamang et al., 1988). Luckowicz-Ratajczak et al. (1992) reported significant adverse effects of deltamethrin on the humoral immune response and cell mediated immunity. On the other hand Singh and Jha (1996) reported that fenvalerate was found to exert a significant reduction in the humoral and cell mediated immunity of intoxicated goats.

The results indicated that monitoring the level of cytokines in intoxicated animals could be useful in understanding the fine changes in the immune system, namely the T -helper-1 (Th1) and T -helper-2 (Th2) .The distinct decrease, that have been shown in the level of IL-2 in the doses (1/10 and 1/20) LD50 could indicate the adverse effect of permethrin on the Th1. On the other hand, IL-4 level showed a significant resistance to permethrin intoxication especially in groups that received 1/5 and 1/10 LD50. This might reflect the low vulnerability of Th2 cells to permethrin.

The decrease in the level of IL-2 in the group which received dose 1/20 LD50 (3.2 PG/ml) as compared to the group that received 1/5 (7.3 PG/ml) is of interest. It was reported that some chemicals with low acute toxicity may have carcinogenic or teratogenic effects at doses that produce no evidence of acute toxicity. Variation in the doses in whole organism is often complicated due to the multiple sites or mechanisms of toxicity, each with its own "dose response" relationship and subsequent effect (Eaton and Klaassen, 1996).

However, it could be concluded that permethrin treatment of 5 days duration in mice causes a significant immunosuppressive effect. Further studies on the effect of permethrin on Thl by incorporation of other cytokines, like interferon-'Y (IFN-'Y) could be useful in increasing the insight on this aspect. Furthermore, the use of broader sets of cytokines are necessary for understandig the effect of permethrin int he immune system of animals.

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تأثير مادة البيرمثرين
في مستوى الأنترولوكين - ٢ والأنترولوكين - ٤ في الفئران

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تم دراسة تأثير مادة البيرمثرين في ثلاثة مجاميع من الفئران، حيث حقنت المجموعة الأولى عن طريق الفم ب_ ١ : ٥ والثانية بـ ١ : ١٠ والثالثة بـ ١ : ٢٠ من الجرعة المتوسطة السمية (LD50) (ما يعادل ٤٠٠ ، ٢٠٠ ، ١٠٠ ملغم/كغم من وزن الجسم على التوالي)

وقد اخذ كل من السايتوكاينز (الأنترولوكين - ٢ والأنترلوكين - ٤) كمقياس لمعرفة حجم التسمم المناعي في الفئران المحقونة، وقد أظهرت النتائج مقاومة ملحوظة لمستوى الأنتررووكين - ٤ في كافة المجاميع المحقونة خصوصا المجموعتين بـ ١ : ٥ و ١ : ١٠ ، إلا أن الأنترلوكين - ٢ ابدى انخفاضا ملحوظا في المجموعة المحقونة بـ ١ : ٢٠ قياسا بالمجموعتين الأخريتين (١ : ٥ ، ١ : ١٠).

إن النتائج قد تشير الى احتمال حساسية الخلايا المساعدة - ١ للتسمم المناعي بالبيرمثرين أكثر من خلايا المساعدة - ٢ .