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Effect of Tricresyl Phosphate on Humoral and Cell-Mediated Immune Responses in Albino Rats

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Tricresyl phosphate (TCP), a phosphate ester of methyl phenyl is a well known industrial additive. It is widely used in plasticizer, lacqures and varnishes, fire retardent, extreme pressure and gasoline additives, and sterilization. Most human intoxications of TCP occurred due to accidental ingestion of adulterated alcoholic bevarages, cooking oil or industrial inhalation of heated vapours. Prominent outbreaks occurred in United States in the early 1930's due to TCP contaminated alcoholic bevarages (Smith et al. 1930) and in Morocco in the year 1959 due to food oil adulteration (Smith and Spalding 1959). Since then an estimated 40,000 human cases of poisoning have been attributed to TCP (Abou-Donia 1981; Abou-Donia and Lapadula 1990). In India, TCP induced toxicity has been reported earlier in the year 1962 at Malda District (West Bengal) and recently at Behala, South Calcutta (Chaudhuri et al. 1962; Srivastava et al. 1990). Each time nearly 400 to 500 people were effected after consumption of mustard oil contaminated with TCP.

Studies with laboratory animals, case reports of accidental or acute poisoning and epidemiological studies have provided significant information about the neurotoxicity of TCP (Sandmeyer et al. 1981; Abou-Donia 1981; Abou-Donia and Lapadula 1990). The effect of TCP on the integrity of the immune system has recently drawn interest as an additional index to potential problem (Gleichmann et al. 1989). Althouth TCP has been in use since the nineteenth century, very few immunological studies have been performed on exposed animals (Sharma and Watnable 1981). We are unable to find any reports regarding the effect of TCP on immune system in mammalian species. This has therefore, prompted us to investigate the effect of TCP on immune system employing rats as the experimental animals. Included in this report are our preliminary findings on humoral and cell-mediated immune responses in rats exposed to sub-chronic doses of TCP.

MATERIALS AND METHODS

Technical grade TCP, 90% mixture of ortho, para and meta isomers, (Aldrich Chemical Co., Milwankee, WI), tetanus toxoid (Tetanus Vaccine,

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Bio Vaccine Pvt. Ltd., Hyderabad), Freund's complete adjuvant (Difco Laboratories, Detroit, MI) and tissue culture media RPMI-1640 (Hi-Media Laboratories Pvt. Ltd., Bombay) were used.

Wister male albino rats weighing 85-90 grams were used. These animals were housed four in a cage and individually labelled. The rats were randomly divided into four groups of 10 animals in each and fed standard laboratory diet containing 0 (control), 20, 50 or 100 ppm of TCP and water ad libitum for six weeks. The feeds were prepared by dissolving known concentration (20, 50 or 100 mg) of TCP in groundnut oil (30 ml) and incorporated into 1 kg of diet. The control received an equal volume of vehicle in an identical manner. Food consumption, general condition and any other clinical symptoms were looked for daily. Body weights were recorded weekly.

Animals were immunized subcutaneously with tetanus toxoid (0.2 ml) mixed with equal volume of Freund's complete adjuvant after 25 days of TCP exposure. Sterile liquid paraffin (5 ml) was injected intraperitoneally in these immunized rats 48 hours before terminating exposure. Blood samples were collected after six weeks of TCP exposure from chloroform anesthetized rats by cardiac puncture without opening the abdomen and serum was separated from the individual samples. Heparin was used in collecting whole blood for leucocyte migration inhibition (LMI) test. Peritoneal macrophages were collected by washing the peritoneal cavity with the media RPMI-1640 under aseptic condition for macrophage migration inhibition (MMI) test. The liver, spleen and thymus were removed immediately, blotted and weighed.

The serum antibody titer to tetanus toxoid was estimated by indirect haemagglutination techniques as described earlier using Lexbro microtiter plates (Banerjee and Hussain 1986) Quantitation of serum IgM and IgG was carried out by single radial immunodiffusion method (Banerjee and Hussain 1986).

LMI and MMI were assayed as described by us in detail earlier (Banerjee and Hussain 1986; Banerjee 1987b.) The final cell suspension was adjusted to contain 15 x 10^6 cell per ml. Cell viability (by trypen blue exclusion) was usually 99%. Concentrations of tetanus toxoid were adjusted to 25 µl/ml and 50 µl/ml for LMI and MMI tests respectively and percentage migration inhibition was calculated.

The results are expressed as mean and their standard deviation (SD). Comparisons were made with control group using students t test. A "p" value of 0.05 or less was considered to be significantly different from control.

RESULTS AND DISCUSSION

Effect of TCP on immune system of mammals has not been reported although their neurotoxic effects are well domonstrated (Abou-Donia 1981; Abou-Donia and Lapadula 1990; Somkuti and Abou-Donia 1990). Our interest in immunotoxic effect of TCP stemmed from neurotoxicity of this compound since a close dynamic relationship exists between nervous and immune system (Link 1979). Attempts were made to select

exposure levels which did not produce over toxicity. It was considered appropriate to incorporate TCP 20 to 100 ppm levels in the diet of experimental animals for the purpose of sub-toxic study (Sandmeyer et al. 1981, Somkuti and Abou-Donia 1990). Testing of sub-toxic effects upon immune responses is important in relation to human health aspects of TCP particularly due to widespread use of this chemical and several epidemic poisoning (Gleichmann et al. 1989). Results of a preliminary study on immunotoxicological evaluation of TCP in rats are presented in this communication.

Rats exposed to TCP at the test dose levels for six weeks did not show any signs and symptoms of cholinergic or delayed neurotoxic effects throughout the experiment. Consumption of feed and water was the same for treated and control rats. No significant difference was noted in body, liver, spleen and thymus weights between control and treated rats (Table 1), suggesting that TCP at the test dose levels did not produce any stress responsible for the observed immunosuppressive effect in the present study.

Serum antibody titer and immunoglobulin levels were studied for the estimation of humoral immune response. Rats exposed to TCP showed a significant decrease in serum antibody titer to tetanus toxoid in dose dependent pattern (Table 2). The serum immunoglobulin (IgG and IgM) concentrations were also significantly decreased in rats exposed to 100 ppm TCP (Table 2). These results indicate important changes in humoral immunity may occur after TCP exposure.

Table 1. Body and relative organ weights of tetanus toxoid stimulated rats exposed to different concentration of TCP for six weeks*

Exposure level (ppm)	Body Wt. (BW) (Gram)	Liver Wt/BW ratio x 10^{-3}	Spleen Wt/BW ratio x 10^{-3}	Thymus Wt/BW ratio x 10^{-3}
0	131.00 ± 7.20	36.00 ± 4.00	3.50 ± 0.34	1.68 ± 0.30
20	134.00 ± 8.00	35.00 ± 3.50	3.20 ± 0.40	1.78 ± 0.40
50	130.00 ± 6.20	38.00 ± 4.50	3.55 ± 0.35	1.80 ± 0.20
100	132.00 ± 5.00	36.00 ± 4.40	3.40 ± 0.25	1.72 ± 0.42

* Data presented as the mean ± SD of 10 rats in each group.

The effect of TCP on cell-mediated immune response was evaluated with the help of MMI and LMI test. Rats exposed to TCP and subsequently immunized with tetanus toxoid showed marked decrease in LMI and MMI responses in a dose related pattern (Table 3). Although it appears that the depression of cell-mediated immunity extends to the primary humoral response, however, more light can be thrown in this direction by studying the response to a thymus independent antigen (Banerjee 1987a).

Table 2. Effect of TCP on serum antibody titer and immunoglobulin concentrations in rats immunized with tetanus toxoid*

Exposure level (ppm)	Haemagglutination - $\log_2$ titer	IgM (mg/ml)	IgG (mg/ml)
0	13.60 ± 1.10	0.68 ± 0.12	15.40 ± 2.00
20	14.50 ± 1.40	0.65 ± 0.15	14.50 ± 1.75
50	11.60 ± 1.20[a]	0.70 ± 0.10	15.50 ± 1.80
100	9.20 ± 1.50[b]	0.54 ± 0.12[a]	12.00 ± 1.50[b]

*Value are the mean ± SD of 10 rats per group. a-significantly lower than control; $p<0.05$; b-$p<0.01$

The results of the present study reveal a suppression of humoral and cell-mediated immune responses in rats exposed to sub-toxic doses of TCP. This suppression was found to increase in a dose dependent pattern. Adverse effect of TCP on immune function could place the host in a more vulnerable position against various pathogens.

Table 3. Effect of TCP on cell-mediated immune response in rats immunized with tetanus toxoid*

Exposure level (ppm)	LMI Mean ± SD (%)	MMI Mean ± SD (%)
0	38.20 ± 6.40	44.00 ± 5.50
20	36.40 ± 5.50	46.50 ± 7.50
50	28.10 ± 7.25[a]	35.50 ± 6.50[a]
100	26.00 ± 5.60[b]	30.00 ± 7.05[b]

*Ten rats were used in each group. a-significantly lower than control; $p<0.05$; b-$p<0.01$

Immunotoxicity of TCP was observed at dose level (50 ppm) which has been reported not to cause any toxicological effects (Sandmeyer et al. 1981). In contrast to our observation, stimulation of the immunological response by an increase in diffuse lymphatic tissue and lymphatic infiltrations in spleen and liver respectively, and increase in plasma protein was observed in white Leghorn hens administered with tri-o-cresyl phosphate (0.78 gram/kg) (Watanable and Sharma 1981). However, none of the tests for humoral (complement-fixing antibodies against nervous tissue or liver) or cellular immunity (splenic migration inhibition and delayed hypersensitivity) indicated stimulation of either system in these treated hens. It is emphasized that the threshold level of the chemical below which no effect would be seen depends on the method of testing for immune responses, animal species, endocrine and nutritional status of the host and type of antigen against which the responses are studied (Vos 1977; Watanable and Sharma 1981; Banerjee 1984; Banerjee et al. 1986; Banerjee and Hussain 1986; Banerjee 1987a; Banerjee 1987b; Gleichmann et al. 1989). More extensive and systematic studies on

dose - time relationship in different experimental animals appear to be essential in order to evaluate the effect of TCP on immune system of mammalian host. Since there are numerous functions associated with the immune system it is necessary to study multiple parameters to properly evaluate the immune function.

It is clear from our preliminary study that the immune system may be a sensitive target for TCP. Suppression of immune responses by organophosphate compounds has been demonstrated by various workers (Vos 1977; Faith et al. 1980; Casale et al. 1983; Banerjee 1984; Gleichmann et al. 1989). The explanation for immuno-suppressive effect of TCP may lie at many levels. Like all other organophosphate compounds, TCP may influence physiological and pathological condition, hormonal function, nutritional status, hepatic metabolism of other endogenous and immunoregulatory substances (Vos 1977; Faith et al. 1980; Casale et al. 1983; Gleichmann et al. 1989). It may also act directly or indirectly on lymphoid cells, lymphoid cell distribution, immunoglobulin metabolism, T-cell/B-cell - macrophage cooperation and macromolecular biosynthesis (Vos 1977; Gleichmann et al. 1989). Furthermore, in a previous study serum carboxylesterase, neurotoxicesterase and acetylcholinesterase activity were significantly inhibited in rats administered with TCP (Carrington and Abou-Donia 1988). A correlation between the inhibition of these esterases and suppression of immune responses by organophosphate has been suggested earlier (Casale et al. 1983). Hence, immunosuppression by TCP may be a consequence of toxic chemical stress associated with organophosphate-induced cholinergic stimulation. The effects of organophosphate-induced cholinergic stimulation on the immune responses were well demonstrated by earlier workers (Casale et al. 1983). It is now important to elucidate the phenomenon in order to understand its mechanism of immunosuppression and the possible health hazards due to continued use of TCP for several purposes. Further investigations are in progress to study the nature of toxic effects of TCP on primary and secondary immune cytokinetics, lymphocyte-mediated cytotoxicity, lymphoid cell distribution, reticuloendothelial system, and relationship between the anti-cholinesterase toxicity and immune response. These studies would contribute to the understanding of the mechanism of action of TCP at the cellular level and could be utilized for a meangingful extrapolation of poisoning in humans.

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