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Effect of o-Chlorobenzylidenemalononitrile (CS) on Humoral Immune Response to Bacterial Lipopolysaccharide in Mice

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o-Chlorobenzylidene malononitrile (CS) is one of the most commonly employed sensory irritants used in the control of riots (BALLANTYNE 1977). The toxicity of CS in humans after inhalation and in animals after administration in lethal and sublethal doses through various routes has been extensively studied (BALLANTYNE 1977, BALLANTYNE & SWANSTON 1973, 1978, CHOWDHURY 1978, 1979). However, the effect of CS on the immune system has not been reported so far.

The necessity and importance of the inclusion of the immune system during regular toxicity testing of any chemical has been dealt extensively in an excellent review by VOS (1977). This report as also the fact that CS is an alkylating agent and many alkylating agents act as immunosuppressants (BACH 1975) have led us to undertake this study on the effect of CS on the immune response.

We have earlier observed that CS suppresses the immune response to a thymus dependent antigen, viz. sheep red blood cells in mice when they are subjected to short term toxicity with sublethal doses of CS (NAGARKATTI & NAGARKATTI 1979a). In the present study using similar doses, the effect of CS has been studied on the weight and cellularity of various lymphoid organs, and the humoral immune response to a thymus-independent antigen, viz. bacterial lypopolysaccharide (LPS).

MATERIALS AND METHODS

CS was prepared as described by CORSON & STOUGHTON (1928) and was tested for purity by gas-liquid chromatography and ultraviolet absorption spectroscopy. Male, Swiss albino mice, 4-weeks-old were injected with CS in olive oil intraperitoneally (i.p.) and the LD_{50} was determined by the method of KARBER (1931).

For short term toxicity studies, mice received 10 daily injections of CS in the doses of 8 and 16 mg/kg body weight i.p. while the controls received only olive oil.

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One week following the last injection of CS, the mice were weighed, sacrificed and liver, spleen, thymus and axillary lymph node were weighed. The cellularity of spleen and thymus was determined by preparing single cell suspension (NAGARKATTI et al. 1978).

LPS (Difco Laboratories, USA) was dissolved in PBS at a concentration of 1 mg/mL and 100 µg was injected i.p. in each mouse two days after the last injection with CS. Seven days after priming with LPS, blood was collected from each mouse and was processed for the determination of antibody titres and the spleen for specific antibody producing cells by Jerne's plaque technique described by us elsewhere (NAGARKATTI & NAGARKATTI 1979b, NAGARKATTI et al. 1980). In all the experiments, groups of 8-10 mice were used. The data were analysed by Student's t-test and P values <0.05 were considered significant.

RESULTS

LD_{50} of CS in mice was found to be 32 mg/kg body weight. There was no significant change in the body weights and relative organ weights of mice receiving 8 mg/kg dose of CS. At 16 mg/kg doses, there was no significant alteration in the body weights and the weights of spleen and lymph node while there was a significant increase in the weight of liver and a fall in the weight of thymus (Table 1).

Table 1. The effect of CS on body weights and relative organ weights.

Dose of CS	Body weight (g)	Relative organ weights (g of organ/100 g body wt.)			
		Liver	Spleen	Thymus	Lymph node
Control	23.0[a] ±3.6	5.0 ±0.5	0.46 ±0.10	0.24 ±0.05	0.06 ±0.05
8 mg/kg	24.2 ±4.5	5.5 ±0.5	0.35 ±0.06	0.22 ±0.10	0.03 ±0.01
16 mg/kg	20.9 ±2.7	6.5[b] ±1.0	0.41 ±0.08	0.13[c] ±0.08	0.02 ±0.01

[a] Mean ± S.D. [b] $P<0.05$ [c] $P<0.01$

The cellularity of spleen and thymus did not alter significantly in animals receiving lower doses of CS while at higher doses, the cellularity of only thymus showed a significant fall (Table 2).

Table 2. Effect of CS on cellularity of spleen and thymus.

Dose of CS	Body weight (g)	Cellularity (Cell count x 10^6)	
		Spleen	Thymus
Control	25 ± 3[a]	154 ± 64	54 ± 19
8 mg/kg	27 ± 4	115 ± 42	54 ± 23
16 mg/kg	25 ± 4	109 ± 39	34 ± 21[b]

[a]Values expressed as Mean ± S.D. [b]$P<0.05$.

The serum antibody titres and antibody producing or plaque forming cells (PFC) to LPS in spleen showed a significant decrease at 16 mg/kg doses while at the lower dosage the response was not altered significantly (Table 3).

Table 3. Effect of CS on antibody titres and PFC response to LPS.

Dose of CS	Immune response to LPS	
	Antibody titres (Log_2)	PFC/10^6 spleen cells
Control	4.30 ± 1.15[a]	124 ± 17
8 mg/kg	4.24 ± 0.90	135 ± 15
16 mg/kg	1.74 ± 1.13[b]	52 ± 13[b]

[a]Values expressed as Mean ± S.D.

[b]$P<0.01$ when compared to controls.

DISCUSSION

The toxicity of CS in humans and in experimental animals has been extensively studied although the effect on the immune system has not been reported so far. The present study deals with the effect of CS in sublethal doses on the humoral immunity in mice,

as it has been stressed that the toxicological evaluation of CS should be carried out in lethal and sublethal doses as this gives an insight into the basic mechanism of toxicity and the likely adverse effect in man (BALLANTYNE & SWANSTON 1978).

In the present study we have used sublethal doses of 8 and 16 mg/kg body weight. These doses were less than LD_1 (23 mg/kg), a dose often employed in similar studies (BALLANTYNE & BESWICK 1972). There was a decrease in the weight and cellularity of thymus in mice receiving only higher doses of CS. As we have observed earlier that there is a significant increase in corticosterone levels in mice treated with 16 mg/kg doses of CS but not in lower doses (NAGARKATTI & NAGARKATTI 1979a), the effect of CS on the thymus observed in the present study is probably due to this indirect effect caused by CS as high concentration of corticosterone is known to give rise to atrophy of the thymus (SPACKMAN & RILEY 1976).

We have observed earlier that the antibody response to thymus dependent antigen is suppressed in mice treated with CS (NAGARKATTI & NAGARKATTI 1979a). LPS, however, is a thymus independent antigen as the antibody response to this antigen is not controlled by T lymphocytes and is dependent only on B lymphocytes (ANDERSON & BLOMGREN 1971, BARTH et al. 1973). In the present study the humoral immune response to LPS was suppressed in CS treated mice thereby suggesting that CS affects the B lymphocyte functions too.

CB is an alkylating agent and many alkylating agents have been reported to be immunosuppressive due to their action on nucleic acids (BACH 1975). However CS differs from other alkylating agents in that it does not interact with nucleic acids in biological systems (H.M.S.O. 1971). In spite of this property, our studies establish that CS suppresses the humoral immunity when administered in sublethal doses in mice.

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