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Effects of ω-Chloroacetophenone (CN) Vapor Inhalation on Pulmonary Immune System of Mice

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Lacrimating agents like ω-chloroacetophenone (CN) are used to restore law and order during civil unrest, riots etc. These agents incapacitate by irritating the eyes, nose and respiratory tract with consequent production of profuse tears and mucus (Malhotra and Kumar 1987). Earlier reports have indicated that CN can cause primary contact dermatitis, erythema and necrosis (Schwarts et al., 1957; Pennys 1971; Holland and White 1972). Several investigators have confirmed allergic contact dermatitis using conventional skin patch test in man (Pennys 1971; Malhotra and Kumar 1987). Earlier studies have indicated lung damage due to inhalation of high concentration of CN from CN grenades (Lopez et al., 1987; Malhotra and Kumar 1987). Although CN is an alkylating agent, its toxic effects are attributed to alkylation of -SH containing macromolecules (Macworth, 1948).

Though, acute mammalian toxicity studies have indicated possible involvement of immune system but the precise early effects of this compound on the immune system have been poorly studied and therefore it is important to study the consequences of its effects on immune system. In this study early effects of CN vapor inhalation on the pulmonary immune system were studied. Mice were exposed to a single sublethal concentration of CN in a static exposure chamber. The parameters studied are; vascular permeability, cell damage and immune alteration based on increased total cell count, decreased viability and diminished phagocytic potential as indicators.

MATERIALS AND METHODS

Randomly bred swiss albino male mice (20-25g) were obtained from the animal house of our establishment. They were acclimatized for 3-4 d in polypropylene cages

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on dust free rice husk, fed pellet diet (Lipton India Ltd.) and given water **ad libitum**. The animals were exposed to CN vapors in groups of 6 per batch (Loading complement 0.71%). CN vapors were generated by heating a one tenth LC_{50} dose of the substance (0.469 mg/L) to complete vaporization in a static whole body all glass inhalation exposure chamber of 21-L capacity. Each batch of animals was allowed to inhale CN vapors for 15 min in this exposure system. The control animals were exposed to similar conditions without CN. The animals were sacrificed 2, 4 and 6 hr post exposure by cervical dislocation. Lungs were lavaged and the viability of cells recovered from an individual animal was determined by trypan blue dye exclusion test. The suspension was then spun at 400 xG for 10 min. and the supernatant was used for the estimation of the extra cellular protein content of the pulmonary fluid. The pellet was resuspended in Hanks balanced salt solution (HBSS) Ca^{++}, Mg^{++} free, and differential determinations for macrophages (MO), Polymorphonuclear cells (PMNs) and lymphocytes were performed on giemsa stained preparations. Cells were adjusted to contain 10^6 cells/ml in complete RPMI-1640 medium containing 10% Fetal calf serum (FCS) and antibiotics. This suspension was used to determine the phagocytic potential of cells of the bronchoalveolar lavage fluid. Cellular protein content was determined (Oren et al., 1963) and expressed as microgram of protein per million of cells (ug/10^6 cells). Extracellular pulmonary lavage protein was estimated (Lowry et al ., 1951) and expressed as ug protein total in the lavage collected. Bovine albumin (BSA Fr V, Sigma Chemical Co., USA) was used as protein standard.

Phagocytosis assay was performed using heat inactivated, fluorescein isothiocyanate (FITC) stained **Saccharomyces cerviceae** and opsonized with homologous heat inactivated (56^oC) mouse serum (Romert et al., 1983). Phagocytosis was measured according to a modified method (Hed, 1977). The cells and particle mixture (yeast-FITC:Mo,4:1) were incubated for 30 min at 37^oC in a humidified candle jar in an atmosphere of 5% carbon dioxide in air. After incubation the phagocytosis was stopped by transferring the cell particle suspension to a test tube kept at 0^oC in an ice water bath. The cold cell-particle suspension was mixed with equal volume of trypan blue (10mg/mL in phosphate buffer, 0.02M, p^H 7.2) to stain uningested particles with trypan blue, thereby to quench their fluorescence. A total of 300 cells were counted under the fluorescence microscope equipped with a blue excitation filter. The ingested particles were distinguished by their bright fluorescence. The phagocytic index (P.I.) was determined as follows:

$$P.I. = \frac{\text{Number of phagocytic cells}}{\text{Total number of cells counted}} \times 100$$

The results are expressed as Mean ± S.E. and were compared in CN exposed and control animals by Students `t' test (Fisher 1950).

RESULTS AND DISCUSSION

Exposure to 0.469 mg/L of CN vapors for 15 min, resulted in significant increase ($p<0.001$) in total leucocyte count and decreased viability in the pulmonary cavity of the experimentally exposed mice than control. The total cell count recorded was ten times greater than that of control mice within 2 hr post CN exposure (Table.1). This increase in cell numbers and reduced viability was evident as early as 2 hr post exposure. However, further increase was only marginal. In subsequent 4 to 6 hr the percent viability returned nearly to that observed in control animals (Table 1). Among the cell types recovered from the pulmonary cavity of CN exposed mice, the differential percentage of each cell type remained almost same following the first two hours, excepting PMNs. The significant increase in PMNs ($p<0.001$) was evident 2 and 4 hr post exposure, later this was found reduced to numbers akin to that observed in normal control mice (Table 1). The protein content of cells and cell free lavage increased four fold ($p<0.001$) within 2 hr and six fold ($p<0.001$) than the control in the subsequent 4 and 6 hr post exposure (Table 2).

The effect of CN vapor inhalation on phagocytic potential was studied as an indicator of pulmonary immune alteration. Phagocytosis of **S.cerviceae** by pulmonary phagocytes decreased significantly ($p <0.001$) as compared to control within 2-4 hr post exposure, later within 6 hr this diminished phagocytic potential returned to that observed in control animals (Table 3).

The inhalation of CN vapors has resulted in infiltration of cells representing an inflammatory reaction in the pulmonary cavity. Damaging effects presumably on resident cells are indicated by reduced viability percentage in the first two hours post exposure. Several previous investigators (Schwarts et al., 1957; Pennys 1971; Holland and White 1972) have reported inflammatory and allergic effects of this compound. The inflammatory response critically depends upon both intact blood vessels of pulmonary system and circulating cells. Following CN exposure, significant increase in cell count of the pulmonary cavity is due to an inflammatory reaction, where blood vessels loose

Table 1. Total and differential count of cells obtained from pulmonary lavage of control and CN exposed mice.

Group	No. of animals	Total leucocyte count (10^5)	Viability %	PMNs %	Macrophages %	Lymphocytes %
Control	9	6.42 ± 0.43	89.0 ± 1.3	0.526 ± 0.1	89.1 ± 0.9	10.4 ± 0.9
CN exposed:						
2 hr	6	63.5 ± 0.55[a]	79.5 ± 1.1[a]	1.410 ± 0.2[a]	87.9 ± 0.4	10.6 ± 0.2
4 hr	6	71.2 ± 0.47[a]	82.3 ± 1.5[b]	1.530 ± 0.2[a]	88.1 ± 0.3	10.4 ± 0.2
6 hr	5	69.2 ± 0.79	85.9 ± 1.8	0.381 ± 0.1	88.1 ± 1.3	11.5 ± 1.3

Values are mean ± S.E., CN exposure 0.469 mg/L for 15 min, [a] Significant from control : $p < 0.001$. [b] Significant from control : $p < 0.01$

Table 2. Cellular and extracellular protein content of pulmonary lavage of control and CN exposed mice.

Group	No. of animals	Protein content	
		ug/10^6 cells	ug total in cell free lavage
Control	9	64.15 ± 7.03	117.26 ± 4.76
CN exposed:			
2 hr	6	285.08 ± 20.02[a]	411.06 ± 25.79[a]
4 hr	6	373.88 ± 18.77[a]	666.29 ± 25.43[a]
6 hr	5	368.00 ± 12.08[a]	640.75 ± 33.69[a]

Values are mean ± S.E., CN exposure 0.469 mg/L for 15 min, [a] Significant from control: $p < 0.001$

Table 3. Phagocytosis of *Saccharomyces cerviceae* by macrophages obtained from control and CN exposed mice.

Group	No. of animals	Period post exposure	Phagocytic Index
Control	9	-	44.03 ± 0.88
CN exposed:			
	6	2 hr	33.28 ± 0.60[a]
	6	4 hr	32.16 ± 0.58[a]
	5	6 hr	45.20 ± 0.76

Values are mean ± S.E., CN exposure 0.469 mg/L for 15 min, [a] Significant from control: a: $p < 0.001$

their normal capacity to retain cells and fluid within the vasculature. As first line of defence PMNs and macrophages play an important role in the pulmonary immune system. In this study, significant rise in PMN count 2 and 4 hr post exposure further substantiates an inflammatory reaction. These observations are in agreement with Bach (1975). This increase in PMN counts is assumed to be due to a brief permeability phase, as

indicated by the relatively constant percentage of each cell type excepting PMNs,even after a significant increase in total cell count. Since the study is performed using a single sub acute exposure of CN for a short period of 15 min the brief permeability phase is conceivable. Ward, (1985) has described a histamine dependent permeability phase lasting only a few minutes. Brief permeability phase followed by appearance of mononuclear cells accompanied with death and disintegration of cells at the site of inflammation brought PMN percentage to levels similar to that observed in control animals. CN exposure results in destruction of resident type of cells initially within 2 hrs later freshly arrived immature as well as mature cells incriminated the cell count, percent viability and cellular protein content. The cell damage as also indicated by increased extra cellular protein content is considered to be due to sulfhydryl inhibition and cell lysis. Macworth (1948), has also reported sulfhydryl inhibition by CN in lymphocytes. Increased extra cellular protein content is the consequence of cell damage and increased permeability as also described by Lopez et al., (1987). Malhotra and Kumar (1987), reported lung damage at a high concentration of CN via inhalation route. Recently Husain et al., (1991), reported tissue damage by inhalation of CN aerosols in rats. Henderson et al., (1985), has described PMNs and protein content of bronchoalveolar lavage as most sensitive indicator of pulmonary damage. Our results also indicate similar effects due to CN vapor inhalation in experimentally exposed mice. Another important parameter of pulmonary defence system is phagocytosis, which is found significantly suppressed for 2-4 hrs post exposure. The damage and dysfunction of phagocytes can lead to indirect tissue destruction through altered host resistance to infectious agents (Luster and Blank, 1987). The data of this study indicated a short lived PMN infiltration accompanied with cell damage and suppressed phagocytic capability of pulmonary immunocompetant cells. These early alterations in normal functioning of pulmonary immune system may lead to increased susceptibility to infections. Data on these early indicators of pulmonary immune alteration in mice will incriminate our understanding of the immunotoxic potential of this chemical in human beings during occupational or intentional exposure. Though these effects in pulmonary system are not found long lasting, whether this situation is further flared up on repeated exposure needs to be analysed.

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