SEM study on cytotoxic effect of monocrotophos (MCP) on lungs of rat

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Monocrotophos (MCP) on oral administration (0.28 mg/100 g of body wt. i.e. 1/5th of LD50) to female rats for 15 and 30 days damaged alveolar walls lined by type II cells (great alveolar cells); clara cells (non-ciliated cells) lining bronchiolar epithelium; and emphysematous lesions due to loss of inter-alveolar walls. This led to increase in surface tension in lung due to decrease in secretion of surfactant as a result of necrosis of great alveolar cells and Clara cells resulting in hypoxia. This effect was time dependent. In R group (15 days without pesticide after 30 days daily oral treatment), the toxic effects mentioned above still persisted which revealed non-repair of necrosis caused by MCP.

Monocrotophos (3-hydroxy-N-methyl-cis crotonamide dimethyl phosphate), an organophosphorus insecticide, has both systemic and contact actions and has been used against a wide range of insects including mites, soil worms, sucking insects, leaf eating beetles and other larvae of variety of crops. Its residual effect on different vegetable crops and on soil microflora has been studied. Lung is an important organ that helps in respiration and is most vulnerable organ for pesticide toxicity. Some workers have reported effect of some pesticides on the lungs of vertebrates. None of these workers have reported the recovery of toxicity after withdrawal of the organophosphate. Organophosphates are known to have anticholinesterase activity through phosphorylation of active serine hydroxyl group in the enzyme molecule. Furthermore, it is known that immediate cause of death is asphyxia in almost all cases related to toxicity of organophosphates. Histopathological and histochemical studies have been carried out on lungs of monocrotophos treated rats and reported that it causes necrosis in lungs. Clara cells lining bronchiolar epithelium cannot be observed with light microscope. In the present report, SEM study was carried out to understand the mode of action of monocrotophos on cells lining the lungs of rat.

Adult female albino rats of Wistar strain weighing 100-150 g were obtained from the Central Animal House, Panjab University, Chandigarh (Ministry Registration Number is CPCSEA 45/1999 dated 10.2.2000). These were divided into four groups T1, TII, R and C. Each group constituted at least 3-5 animals. These were provided with rat chow and water ad libitum. Each group was administered MCP (technical grade, purchased from Montari Agro, Industrial Area, Phase II, Chandigarh) through gavage at the rate of 0.28 mg/100 g of body wt. i.e. 1/5th of LD50 value of MCP as calculated by Janardhan et al. Rats (T1 group) were administered MCP for 15 days, TII for 30 days, R group animals were given a daily oral dose for 30 days and then these were allowed to recover for 15 days on normal diet. After treating for a given period, the overnight fasted rats from each group were anaesthetized. The chest was opened to expose lungs, trachea was cannulated and lungs were inflated through tracheal cannula with chilled glutaraldehyde (3%) prepared in 0.2 M of phosphate buffer (pH 7.2). Pressure was maintained for 5 min after which trachea was ligated, lungs were removed, cut into 2-3 small pieces and again fixed in fresh glutaraldehyde (3%) in phosphate buffer for one and a half hour in each of the four groups. These were washed 2-3 times in phosphate buffer and then dehydrated in various ascending grades of acetone (30 to 100%) for 30 min each. The tissues were then subjected to critical point drying followed by mounting on metallic stubs with the help of adhesive tape. The tissue was further trimmed as desired before pasting on stub prior to sputtering with gold for 15 min and viewed under SEM (Jeol 2601; at Central Institute Laboratory of Panjab University, Chandigarh).

Pulmonary alveoli—In control group (C group) under low magnification, the alveolar spaces surrounded by alveolar walls of lungs could be observed. Alveolar walls were lined by alveolar cells (Fig. 1). In
TI group, number and diameter of alveolar spaces increased. At some places necrotic changes were observed showing clumped mass of necrotic alveolar walls and cells (Fig. 2). In TII group, alveolar cells lining the walls of alveolar spaces showed severe necrosis. Area of the spaces was increased. Emphysematous lesions were observed which could be due to enlargement and coalescence of adjacent alveoli due to partial or complete loss of inter-alveolar septa (Fig. 3). R group showed slight recovery in topography of lung as compared to TII group because the damaged alveolar sacs and emphysematous lesions could still be observed (Fig. 4).

*Respiratory bronchiole.*—In C group rats, clara cells, lining the bronchiolar epithelium, exhibited characteristically rough and knobby surfaces. In between clara cells, some ciliated cells were present (Fig. 5). In group T1, these clara cells showed necrotic changes and thus a decrease in number. Their shape also changed (Fig. 6). In TII group, the number of clara cells decreased, their contour also changed and some of the cells appeared flat (Fig. 7). In R group, there did not appear much recovery as the damage persisted. Moreover, clara cells showed deformed surfaces revealing non-recovery after withdrawal of pesticide for 15 days from TII group (Fig. 8).

The present SEM studies on lungs after oral administration of MCP exhibited damages to alveolar walls and clara cells (non-ciliated cells) of bronchiolar epithelium; and emphysematous lesions. Alveolar spaces are lined by two types of cells—type I cells

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**Figs 1-4**—Effect of MCP on alveolar walls. (1) Alveolar spaces (AS) in surface view of control lung; (2) Necrosis to alveolar spaces (AS) and inter-alveolar walls in surface view of lung of T1 group (arrow); (3) Damaged alveolar spaces (AS) and inter-alveolar walls. Alveolar spaces increase in size. Arrow shows aggregation of necrotic tissue in lung of TII group; and (4) Damage to alveolar spaces (AS) and inter-alveolar walls. Arrow shows emphysematous lesion in R group lung.
(squamous epithelial cells) and type II cells (giant alveolar cells) as described earlier. Type I cells provide an intact surface of minimum thickness for readily permeable gases. They also ingest small amount of inhaled particulate matter that reaches the alveolar surface, whereas, the type II cells secrete surfactant-a surface active material rich in phospholipid dipalmitoyl lecithin, apoproteins and calcium ions, that on spreading on lung surface reduces surface tension and hence prevents collapsing of lungs. Thus, the damage to alveolar walls and type II cells would result in reduced secretion of surfactant leading to an increase in the surface tension. A decrease in phospholipids and proteins after paraquat and monocrotophos treatment has also been reported by earlier workers.

Bronchiolar epithelium is lined by Clara cells and ciliated cells. Clara cells can be best observed in SEM. Clara cells protrude considerably into lumen and exhibit characteristically rough and knobby surfaces. Their functional significance is subject to speculation, but the evidence suggests that these are secretory in nature and produce surfactant (phospholipids, apoproteins and calcium ions) present in alveoli. Damage to Clara cells was observed in the present observations. Such changes in bronchiolar epithelium have also been reported earlier after OOS-TMP treatment using SEM. These effects have been correlated with changes in bronchopulmonary lavage lactate dehydrogenase (LDH). Emphysematous lesions, observed during present study, were characterized by destruction of alveolar walls and consequently, marked enlargement of alveolar air spaces and loss of pulmonary capillaries, which might impair gas exchange.
Some of these changes like emphysema, enlargement of alveolar air spaces, a qualitative decrease of phospholipids and proteins have also been reported by us with light microscopic and histochemical studies of lungs of rat after MCP treatment. Erythema may lead to hypoxia i.e. deficiency of oxygen at tissue level. It has been concluded that MCP after oral administration caused necrotic effects on lungs in the form of emphysematous lesions, damage to epithelial cells of bronchioles and alveoli, and hence loss of inter-alveolar septa. Damage to type II cells of alveolar epithelium and Clara cells of bronchioles may have caused decrease in phospholipids and proteins which may be responsible for an increase in surface tension at respiratory surface. Necrotic effect of MCP treatment for 30 days could not recover after 15 days of withdrawal of pesticide indicated highly toxic nature of the pesticide in rats.

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References