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Pulmonary Biochemical Assessment of Fenitrothion Toxicity in Rats

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Fenitrothion (0,0-dimethyl 0-4-nitro-m-tolyl phosphorothioate), an organophosphorus insecticide, is sprayed world-wide on large scale for the control of spruce budworm. These sprayings bring direct exposure of humans and domestic animals to the insecticide through inhalation and are considered a major concern in the assessment of environmental risks (McTaggart-Cowan 1977). Chevalier et al. (1981) observed a dose-dependent morphological changes in rat lungs after intratracheal instillation of fenitrothion. However, little efforts have been done to assess the pulmonary biochemical changes as a result of fenitrothion exposure. In recent years, bronchoalveolar lavage has been suggested as a means of early damage indicator of the lung due to a variety of environmental toxicants (Henderson 1984). Also, lipid peroxidation which is a degradation process, is generally considered as one of the mechanisms through which a variety of chemicals could cause tissue injury (Plaa and Witschi 1976). In order to identify the early biochemical changes which could reflect the initial damage caused by interaction of fenitrothion with lung tissue, the present study was undertaken on the alterations in the bronchoalveolar lavage fluid and lipid peroxidation of lung mitochondria.

MATERIALS AND METHODS

Fenitrothion was purchased from Bayer (India) Ltd., Bombay. Male Wistar strain rats (150-175 g) in groups of four to five were used throughout the study. The rats were bred in the ITRC animal colony and maintained at 25° C under a 12-hr light/dark schedule.

The LD_{50} of fenitrothion has been reported to be 300 mg/Kg if given intratracheally to rats (Chevalier et al. 1982). In this study, the animals received a single intratracheal (i.t.) injection of 30 mg/kg of fenitrothion dissolved in ground nut

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oil (in a final volume of 0.25 ml in each rat). The control animals received i.t. injection of equal volume of vehicle only. The experimental and their respective control animals were sacrificed at 1, 4, 7, 14, 21 and 30 days following the i.t. injection.

At various points in time, the lungs of the rats were lavaged using the standard technique of Brain (1970), with four 5-ml washings with PBS (pH 7.2) at room temperature. Total recovery of injected fluid was over 93% in each rat. The lavage fluid was separated into cell-free lavage and cell-pellet by centrifugation at 400xg for 10 min at 4°C. Suitable aliquots of cell-free lavage fluid from experimental and control rats were used and biochemical measurements were done for the following parameters. Lactate dehydrogenase (LDH) was assayed according to Henderson et al. (1978a) by measuring the decrease in O.D. at 340 nm. Ascorbic acid was estimated according to Skoza et al. (1983), total sialic acid according to Downs and Pigman (1976) and protein content according to Lowry et al. (1951). The phospholipids were extracted according to Bligh and Dyer (1959) and total inorganic phosphorus in the extracts was estimated according to Bartlett (1959).

Mitochondria from fenitrothion instilled and control lungs were isolated according to Mustafa et al. (1974). Mitochondrial lipid peroxidation was evaluated according to Wilbur et al. (1949). The incubation mixture in a total volume of 4.0 ml contained 200 μM ascorbic acid, 20 μM Fe^{+++}, 0.3-0.4 mg of mitochondrial protein and 0.15 M KCl and was incubated with vigorous shaking at 37°C for 1 hr and then the malonaldehyde formation was measured. An extinction coefficient of 1.56×10^5 M^{-1}/Cm was used for quantifying malonaldehyde (Wills, 1966).

Statistical significance was determined by Student's t test.

RESULTS AND DISCUSSION

The effect of fenitrothion exposure on the cell-free lavage LDH activity as well as non-enzymatic parameters is shown in Table 1. The LDH activity showed an increase at all the stages of exposure and at 1, 4 and 7 days, the increase was 320, 636 and 427%, respectively, when compared to controls. However, thereafter the activity declined but was still over 100% higher than controls at 30 days. Both protein and sialic acid contents increased at most of the stages with protein showing a maximum increase of 135% on day 1 whereas sialic acid showed a maximum increase of about 332% at 4 days. Thereafter, the increase in both cases was less pronounced. Ascorbic acid content in the cell-free lavage fluid decreased in the experimental rats up to 7 days. Thereafter, it increased and a significant increase of 38 and 33% was recorded at 14 and 21 days, respectively.

Table 1. Comparison of cell-free lavage LDH activity and contents of protein, sialic acid, phospholipids and ascorbic acid in control and fenitrothion instilled rats[a].

Period of exposure (days)	LDH activity (U/total lavage)	Protein (mg/total lavage)	Sialic acid (μg/total lavage)	Phospholipids (mg/total lavage)	Ascorbic acid (μg/total lavage)
1	4966.0 ± 1078.6* (1183.3 ± 225.5)	10.99 ± 2.91* (4.68 ± 0.86)	207.40 ± 30.57* (129.25 ± 34.36)	1.42 ± 0.24* (2.33 ± 0.57)	30.51 ± 3.39 (39.53 ± 3.53)
4	6500.0 ± 300.0* (883.3 ± 230.9)	6.87 ± 0.51* (4.24 ± 0.61)	457.48 ± 43.93* (106.00 ± 6.60)	1.43 ± 0.10* (1.94 ± 0.10)	39.55 ± 3.55 (41.80 ± 7.64)
7	3866.0 ± 665.8* (774.0 ± 57.7)	6.66 ± 0.76* (4.04 ± 0.57)	318.81 ± 29.00* (120.23 ± 25.25)	3.54 ± 2.21 (1.54 ± 0.68)	35.59 ± 16.16 (45.76 ± 8.90)
14	2533.3 ± 152.8* (832.0 ± 513.2)	7.92 ± 2.00* (4.44 ± 1.94)	361.49 ± 71.14* (164.91 ± 55.36)	3.74 ± 0.54* (1.67 ± 0.80)	59.88 ± 7.64* (43.54 ± 13.19)
21	2233.0 ± 152.8* (845.5 ± 57.7)	6.79 ± 0.49* (4.28 ± 0.55)	137.46 ± 24.66 (118.82 ± 8.59)	2.31 ± 0.15 (2.22 ± 0.60)	52.54 ± 6.76* (39.54 ± 8.01)
30	2075.0 ± 623.8* (950.0 ± 675.8)	5.21 ± 1.88 (4.40 ± 1.21)	157.50 ± 26.02 (134.05 ± 28.17)	2.43 ± 0.18 (2.11 ± 0.53)	56.77 ± 10.13 (44.06 ± 7.39)

[a] Values are arithmetic mean of four animals ± S.D. control values are given in parentheses.
* Significantly different from controls ($P < 0.05$).

Similarly, phospholipid profile showed a decrease initially but tended to increase subsequently and a maximum increase of 124% was observed at 14 days of fenitrothion exposure.

Lung mitochondrial lipid peroxidation, as induced by ascorbate and Fe^{+++}, showed a remarkable increase throughout the exposure period (Table 2) and an almost 9-fold increase in the malonaldehyde formation was observed at 7 days of fenitrothion instillation.

Table 2. Effect of intratracheal administration of fenitrothion on lipid peroxidation of lung mitochondria[a]

Period of exposure (days)	Lung weight (g)	Ascorbate-induced lipid peroxidation (n moles MDA formed/hr/mg of protein)
1	2.20 ± 0.22 (1.86 ± 0.18)	2.68 ± 0.27* (0.55 ± 0.05)
4	2.50 ± 0.16* (1.98 ± 0.20)	3.24 ± 0.48* (0.56 ± 0.05)
7	2.40 ± 0.30 (2.30 ± 0.16)	6.58 ± 0.68* (0.75 ± 0.09)
14	3.74 ± 0.27* (2.49 ± 0.47)	1.74 ± 0.07* (0.31 ± 0.02)
21	3.00 ± 0.40 (2.87 ± 0.27)	0.80 ± 0.04* (0.28 ± 0.05)
30	2.86 ± 0.50 (2.26 ± 0.48)	0.46 ± 0.06 (0.32 ± 0.02)

[a]Values are arithmetic mean of four rats ± S.D. Control values are given in parentheses.
*Significantly different from controls ($P < 0.05$).

The foregoing results clearly indicate that significant biochemical alterations take place as a result of acute fenitrothion exposure. One of the most significant changes observed in the bronchoalveolar lavage fluid was the increase in LDH activity which could be due to cell lysis or membrane damage and indicates significant pulmonary injury. Total soluble protein elevation observed in the present investigation, could be an indicator of the breakdown of the alveolar-capillary barrier as reported in other studies (Alpert et al. 1971). The increased sialic acid levels in the bronchoaleveolar lavage may be due to

transudation of serum proteins or increased secretion and/or even damage of alveolar cells (Reid 1977). However, the changes in the above parameters were less pronounced in the later stages of exposure indicating activation of local repair mechanisms which could ultimately lead to the healing of injury.

Pulmonary surfactant and antioxidants play a central role in preventing the structural and functional integrity of the lung tissue (George and Hook, 1984; Fridovich and Freeman, 1986). The changes in the phospholipid profiles reflect an altered type II metabolism, an initial inhibition and then activation. The role of airway ascorbic acid is not very well understood but it could play an important role in the protection of lung cells against various environmental toxicants (Skoza et al. 1983).

An interesting feature of the present investigation was that increased LDH activity in the lavage fluid was associated with significant increase in the ascorbate-induced mitochondrial lipid peroxidation which reflects destructive metabolic process (Mustafa et al, 1973). From the data obtained in the present experimental conditions, it is difficult to conclude if fenitrothion has the capability to induce lipid peroxidation in mitochondria directly. Our data, however, suggests that fenitrothion increases the susceptibility of mitochondria to lipid peroxidation induced by ascorbate and Fe^{+++} which in turn suggests that fenitrothion inhibits or alters the mitochondrial anti-oxidant system.

Thus, significant pulmonary biochemical alterations take place as a result of acute fenitrothion exposure in rats. These changes could be as a result of injury to lung tissue in the initial stages and defensive adaptations to meet the stress in the later stages. However, at this stage it is not clear whether these alterations are due to direct effect of parent substance or due to a metabolite and necessitates further studies. The present investigation also indicates that appearance of various biochemical entities in the lavage fluid is a useful and sensitive indicator of early damage to the lungs.

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