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Cadmium-induced Pulmonary Injury in Mouse: A Relationship with Serum Antitrypsin Activity

Parimal Chowdhury,[1] Donald B. Louria,[2] Louis W. Chang,[3] and Phillip L. Rayford[1]

[1]Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205; [2]Department of Preventive Medicine and Community Health, New Jersey Medical School, Newark, NJ 07103; [3]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Investigations on the role of cadmium in influencing the protease-antiprotease balance in the sera of mice revealed that cadmium produces a significant reduction in serum trypsin inhibitory capacity (TIC) in mice within 4 h following an injection of cadmium chloride (13.8 mg Cd/kg). Such inhibitory effect, however, was only transient, and the TIC activity returns close to normal values by 24 h. Morphological analysis on lung tissues of mice exposed to cadmium for 4 h showed emphysematous-like lesions with distended alveoli. The morphologic changes in lung tissues was found to be in accord with the significant depression in serum antitrypsin activity. The transient serum TIC changes accompanied with the changes produced in lung tissue as a result of acute Cd exposure indicate that mice can be used as a viable animal model for future chronic studies and for the mechanism of Cd-induced pulmonary emphysema which frequently occurs in industrial workers exposed to cadmium.

Experimental or occupational exposure to Cd leading to severe pulmonary diseases has been reported by various investigators (BONNELL 1955; LANE & CAMPBELL 1954; FRIBERG *et al.* 1973; STRAUSS *et al.* 1976; BUS *et al.* 1978) and was outlined in a recent review (CHANG *et al.* 1981). Despite indications that a widespread form of centrilobular emphysema was described in high percentage of industrial workers exposed to Cd, controversy still exists on the mechanism of Cd-induced pulmonary emphysema. JANOFF & CARP (1978), CARP & JANOFF (1978), and OHLSSON *et al.* (1978), on separate studies demonstrated that the development of emphysematous lesions in lung could be induced both *in vito* and *in vitro* as a result of local inactivation of pulmonary antitrypsin. Reduction of antitrypsin activity in sera of heavy smokers has also been recently demonstrated (OHLSSON *et al.* 1978; CHOWDHURY *et al.* 1981). These results are consistent with the hypothesis that an imbalance in protease-antiprotease system may lead to lung tissue injury. Recent studies in our own laboratory with human α_1-antitrypsin *in vitro* and *in vivo* suggest that Cd alters trypsin inhibitory capacity (CHOWDHURY & LOURIA 1976; CHOWDHURY & RAYFORD 1981). In this communication, we present data on the *in vivo* experiments on mice exposed to Cd with the attempt to correlate serum TIC changes with morphologic alterations.

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METHODS

CFW mice weighing 20-22 g were obtained from Carworth Farms, Ann Arbor, MI. Animals were maintained with Purina Lab Chow and tap water for a week. Sixty mice were divided into 12 groups containing 5 mice per group. Each mouse received a single $CdCl_2$ injection at a dosage of 13.8 mg Cd/kg b.w. or with saline solution. Animals were sacrificed at 0, 1, 2, 4, and 24 h after injection. Blood samples were collected through axillary incisions and spun down immediately after collection. Serum was kept frozen at -20C before analysis. Lungs from all animals were perfused with saline and fixed in 10% formalin for light microscopic study.

Serum trypsin inhibitory capacity (µmol/ml/min) was measured following the method of DIETZ *et al.* (1974). The TIC (µmol/ml/min) represents the amount of substrate hydrolyzed by trypsin per min under the assay conditions and is expressed in international units. Cadmium concentration in whole blood was determined by the method of BOGDEN & JOSELOW (1974) using an atomic absorption spectrophotometer.

Tissue cadmium were analyzed from the extract of the digested tissue in a mixture of HNO_3 and $HClO_4$ (1:1), following the technique described by WESTERLAND & HAMMERSON (1970).

For light microscopic investigation, lung tissue was fixed in 10% buffered formalin, dehydrated with graded ethanol, and embedded in paraplast. Sections of 5-6 µm were cut and stained with hematoxylin and eosin. Detailed staining procedure was followed from the standard histotechnology manuals (CHANG 1979; AFIP MANUAL 1968).

Statistical analysis of the results were evaluated in Figure 1 as means ± 2 S.D. Student's t-test were applied (SNEDECOR & COCHRAN 1967).

RESULTS

The effect of an acute dose of cadmium (13.8 mg Cd/kg) on serum TIC is shown in Figure 1. It is noted that following cadmium injection, serum TIC declined rapidly within 1 h. This reduction in serum TIC continued at a steady rate up to 4 h. At every time point examined, the decline is significant as compared to the TIC value of the control animals. The TIC activity, however, restored to near normal within a 24-h period (Figure 1).

Light microscopy revealed no remarkable morphological changes in lungs exposed to 1 or 2 h of cadmium. However, significant distention of the alveoli occurred in animals exposed to 4 h of Cd (Figure 3) as compared to those from control animals (Figure 2). No necrotic change was observed in the alveolar lining cells. This distention of alveolar space was only a transient phenomenon as lung morphology returned to normal at 24 h. This transient morphological change correlated well with the biochemical (TIC) changes.

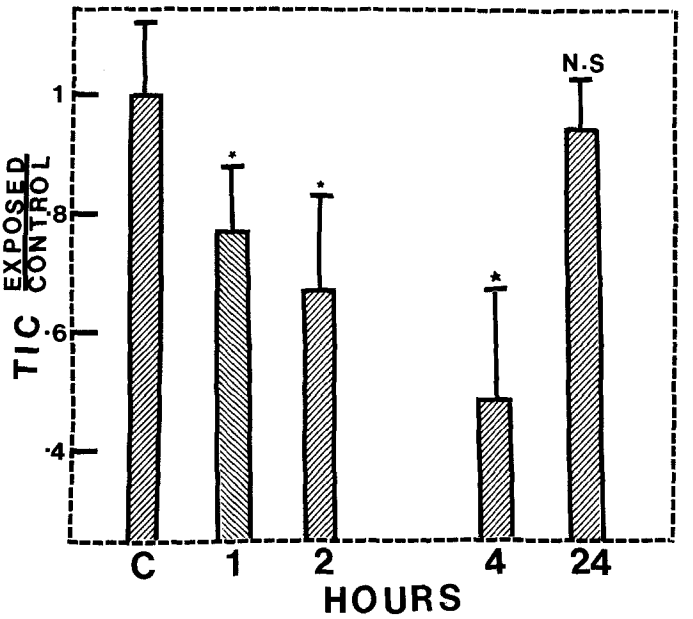

Figure 1. Influence of acute cadmium exposure (13.8 mg Cd/kg b.w.) on plasma TIC of mice. TIC is expressed as a ratio of the TIC values obtained with the sera of exposed animals over the control (saline treated). The results are mean ± S.D. (N = 12). The asterisk indicates the significant difference of $p < 0.05$, as compared to the control group. Obtained by Student's paired t-test.

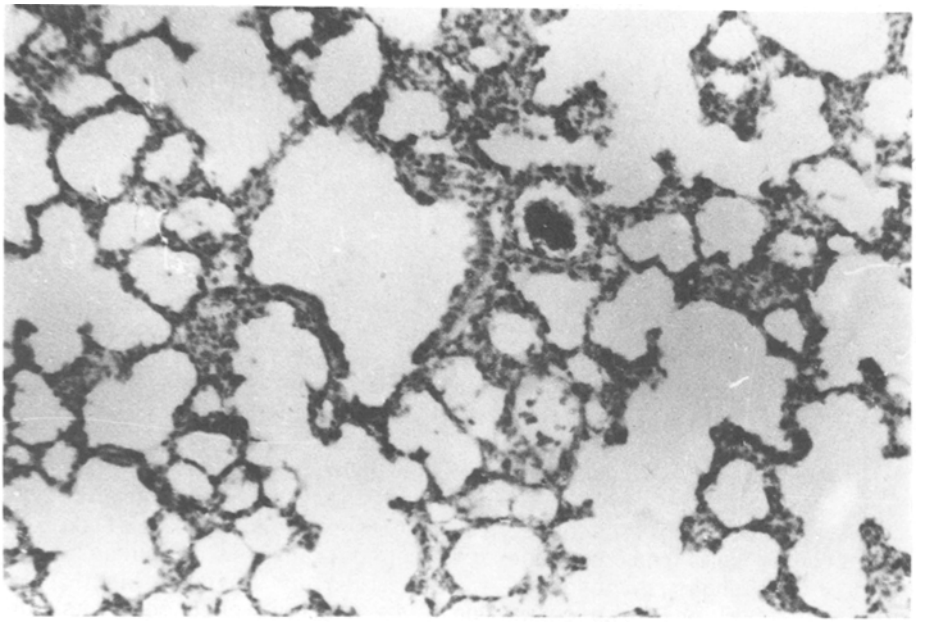

Figure 2. Lung, mice, saline control. Normal alveolar space is demonstrated. H & E, x 400.

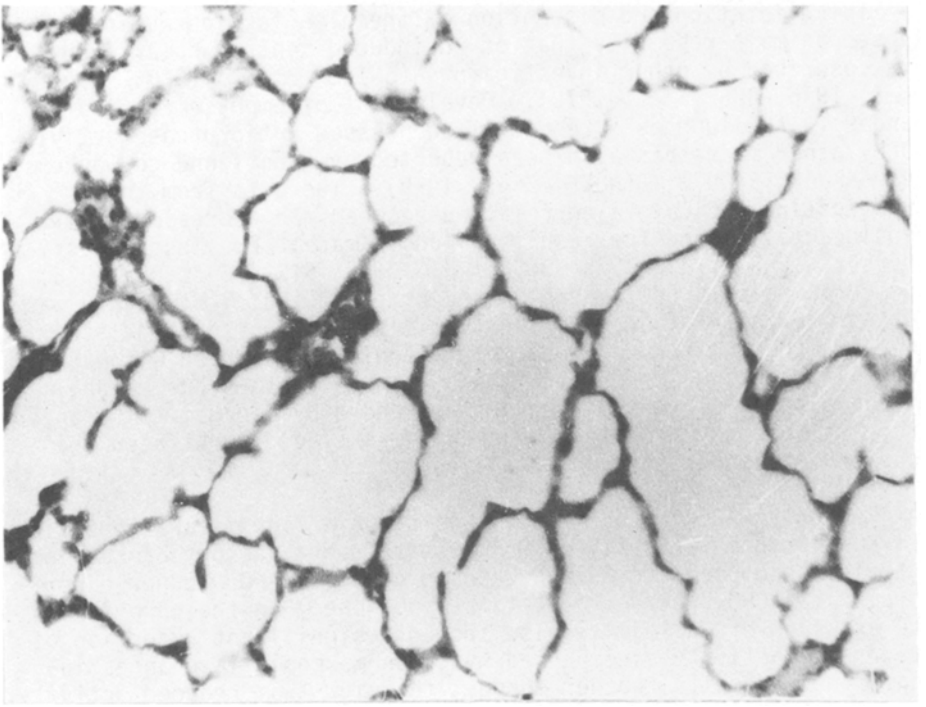

Figure 3. Lung, mice, $CdCl_2$ treated, 4 h (13.8 mg Cd/kg b.w.). Severely distended alveolar spaces are evident. No necrosis seen in alveolar lining of cells. H & E, x 400.

DISCUSSION

The experimental data obtained in the present investigation indicate that cadmium chloride produced a significant reduction in the serum antitrypsin activity in mice. The data further indicate that the dimunition of serum TIC due to acute Cd exposure is a transient phenomenon but appears to produce lung damage rapidly as shown by morphologic examination. It is a well established phenomenon that cadmium induces the production of metallothionein (MT), a Cd binding protein, by the liver (KAGI & VALEE 1961; KIMURA *et al.* 1974; WEBB 1973; WINGE *et al.* 1975), and it has been claimed that metallothionein will complex with cadmium leading to a reduction of Cd-toxicity (KIMURA *et al.* 1974; PISCATOR 1964). Based on our present data, it is not unreasonable to postulate that the rapid suppression of TIC represents a toxic influence on the α_1-antitrypsin by the injected Cd^{+2} ions and the gradual restoration of free Cd^{+2} ions in the circulation as a result of MT production in the liver to form MT-Cd complex yielding less Cd^{+2} to interfere with the TIC activity. It should, however, be noted that between 90-100% incorporation of cadmium to MT has been found to occur between 6-8 h of Cd injection (CAMPBELL & WEBB 1976; TANAKA *et al.* 1974).

The dilatation and distention of the alveolar spaces in Cd exposed animals resembles that of Cd induced centrilobular emphysema described by other investigators (SNIDER *et al.* 1973; STRAUSS *et al.* 1976; BUS *et al.* 1978). Development of emphysematous changes in the lung as a result of suppressed antiprotease activity and by other means has also been reported by Janoff and co-workers (CARP & JANOFF 1978; JANOFF *et al.* 1979). The data from this investigation indicate a correlation between the depressed antitrypsin activity and the resulting lung damage. Previous investigators (SNIDER *et al.* 1973; BUS *et al.* 1978; STRAUSS *et al.* 1976) exposed animals to $CdCl_2$ aerosol and proposed that lung injury was a direct toxicity of Cd^{+2} on the lung tissues. Our present study indicates that Cd induced lung injury could be produced without direct introduction of Cd into the pulmonary tissues and morphological alteration in the lung was preceeded by biochemical changes. A reversal of the morphological change was also demonstrated when the biochemical defect was normalized.

Recently MAREK *et al.* (1980) has shown a significant reduction in α_1-antitrypsin activity of 87 workers from a factory of cadmium batteries as opposed to 39 non-exposed controls. Our other experimental data (CHOWDHURY *et al.*, to be published) on the chronic low dose exposure of Cd in mice also showed a significant reduction of antitrypsin activity accompanied by gross morphologic changes in lung. These results further support the view that reduced activity of α_1-antitrypsin could be a factor in the pathogenesis of pulmonary emphysema caused by cadmium. It would be important to see whether or not chronic low dose exposure of cadmium from either occupational or environmental sources do produce a significant reduction of antiprotease activity.

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