Comparative pulmonary toxicity of cadmium and nickel: Histopathological and bronchoalveolar lavage analysis

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Pulmonary toxicity of cadmium and nickel was evaluated in rat lungs following intratracheal instillation of their chlorides. Concentration of both the metals varied from 0.2 - 5 mM. Both the metals increased total number of cells, number of polymorphonuclear neutrophils, total protein, sialic acid and the activity of lactate dehydrogenase and β-glucuronidase in bronchoalveolar lavage 3 days after exposure. Increase in the levels of the selected parameters was more following Cd exposure than in Ni exposed rats. Histologically there was an inflammatory response and interstitial fibroblastic proliferation in the lungs of Cd exposed animals. These changes were mild in Ni-exposed animals and higher concentrations of Ni were needed to produce changes similar to those produced by smaller concentrations of Cd.

Lung is directly exposed to mineral particles, fibres, bacteria, fungi, antigens and other materials contained in the inhaled air. Continuous exposure to fluctuating concentrations of metals in ambient air and work place environment increases metal pulmonary bioavailability and toxicity. Laboratory studies in animals have revealed that soluble NiCl₂ and NiSO₄ are more toxic to respiratory tract than insoluble NiO. Exposure through inhalation to higher concentrations of NiO resulted in an increase in the pulmonary inflammatory response. However, very soluble and poorly soluble chemical forms of Cd have both been found to be equally toxic to lungs. Earlier studies demonstrated significant differences in the reactivity of alveolar macrophages exposed in vitro to soluble salts of Ni and Cd. The present study has been aimed at comparing the relative pulmonary toxicity of these salts of Ni and Cd in vivo.

Materials and Methods

Animals—Male albino rats (150 ± 10 g body weight) of the Industrial Toxicology Research Centre, Lucknow, India were used. The animals were reared under normal conditions of husbandry and fed freely on a pellet diet supplied by Hindustan Lever Ltd., Bombay, India. The animals had free access to drinking water.

Exposure of animals—Rats were divided into 7 groups. The animals of groups 1 to 3 were inoculated intratracheally 0.2 ml of 3 graded concentrations of CdCl₂ (0.2, 1 and 5 mM corresponding to 7.3, 36.5 and 182.5 µg of Cd respectively). The lowest dose of Cd used corresponded to its 1/25 LD₅₀ value. The animals of groups 4 to 6 were similarly inoculated identical molar concentrations of NiCl₂ 6H₂O corresponding to 9.5, 47.5 and 238 µg of Ni respectively. Since the aim of the study was to compare the pulmonary response to equimolar concentrations of the two metals the dose of Ni was not correlated to its LD₅₀ value. Group 7 animals were inoculated 0.2 ml physiological saline vehicle alone to serve as controls. Because of higher mortality of CdCl₂ exposed animals, rats in batches of 4 to 6 animals were killed 3 and 15 days after exposure.

Collection and analysis of bronchoalveolar lavage (BAL)—Animals from each group were sacrificed by exsanguination. The trachea was exposed and the lungs rinsed in situ with 7 ml physiological saline in aliquots of 4 and 3 ml at a hydrostatic pressure of 30 cm of water. The recovery of BAL fluid was usually more than 90%. The aliquots of lavage from each animal were collected in precooled tubes, pooled and centrifuged at 200 g for 10 min at 4°C.

The cell-free supernatant was separated and used for the measurement of total protein, sialic acid, assay of LDH and β-glucuronidase enzyme activities.
The pellet was washed thrice with phosphate buffered saline (PBS, pH 7.2) and after the last washing resuspended in a known volume of PBS. After determination of total number of cells in a Neubaur counting chamber, the cell suspension was centrifuged. The supernatant was discarded, the sediment resuspended in rat serum and drop of cell suspension was smeared on a glass slide. After fixation with acetic acid-methanol (1:3) the smears were stained with Giemsa for enumeration of the relative proportion of different cell types.

**Histological methods**—The lavaged lungs were fixed in situ by injecting 10% formal-saline at a hydrostatic pressure of 30 cm via the trachea which was then tied before the thorax was opened. The lungs were removed intact and suspended in formal-saline before longitudinal blocks were taken for routine embedding. Representative 5 μm thick sections were cut and stained with hematoxylin and eosin, impregnated with silver for demonstration of reticulin and stained with Masson's trichrome for visualisation of collagen.

**Results and Discussion**

The average body weight of the animals was significantly decreased 15 days after exposure (Fig. 1). After 48 hr of exposure 50% animals inoculated with 5 mM CdCl₂ died. The body weight of NiCl₂ inoculated animals remained unaltered and all the animals survived throughout the experimental period. Even though solubility of CdCl₂ could be the cause of increased mortality, it has been reported that lung retention of bioavailable Cd could contribute significantly to lethality.

Animals inoculated with 0.2 mM CdCl₂ and sacrificed 3 days after exposure revealed thickening of the alveolar septa due to congestion of blood vessels and infiltration by mononuclear leucocytes. Many mononuclear cells, predominantly macrophages, were present in the alveolar lumen. Cellular infiltration of septa resulted in narrowing of the alveolar lumen. Appearance of polymorphonuclear cells and erythrocytes in alveolar lumen was accompanied by a fibrinous exudate, which at places was extensive. Many alveoli were filled with an exudate containing limited number of cellular elements. In such alveoli, nevertheless, the alveolar septa were thickened due to proliferation/infiltration by mononuclear cells (Fig. 2). At 15 days the histological changes were less marked, though hyperaemia and thickening of alveolar septa due to mononuclear cell infiltration were seen. When the animals were inoculated with 1 mM CdCl₂ and sacrificed 3 days after exposure the histopathological alterations were similar to those seen in animals inoculated with 0.2 mM CdCl₂ at the corresponding time interval. However, larger areas of lung tissue were involved and the inflammatory reaction was more intense resulting in consolidation of the exudate in the alveolar lumen. Animals exposed to 1 mM CdCl₂ and sacrificed 15 days later revealed profuse hemorrhages and thickening of the alveolar septa. The tissue reaction involved larger areas. In animals inoculated with 5.00 mM CdCl₂ and sacrificed 3 days later, there were areas in the lung tissue which exhibited proliferation of fibroblasts in the interstitium and large number of macrophages in the alveolar lumen (Fig. 3). The lungs of animals inoculated various concentration of NiCl₂ and sacrificed at different time intervals after exposure showed histopathological changes of a much lesser magnitude than seen following CdCl₂ exposure (Figs. 4 and 5).

Total number of cells in the bronchoalveolar lavage was significantly increased in rats exposed to either CdCl₂ or NiCl₂ (Fig. 6). Increase in the number of cells after 3 days was mainly due to an increase in the number of polymorphonuclear cells (P < 0.01; Fig 7). The number and type of cells recoverable in BAL is believed to be a reflection of the type of inflammatory reaction of the lower respiratory tract geared at
protecting the delicate alveolar epithelium from the harmf ul effects of chemicals that impinges on its surface\textsuperscript{11}. Neutrophils and macrophages have been demonstrated to be recruited following acute CdCl\textsubscript{2} induced lung injury through release of chemotactic agents\textsuperscript{12}. PMNS recruited into the interstitium and airways after Cd exposure may induce lung inflammation through degranulation with resultant release of elastase and oxygen radicals\textsuperscript{3}.

Protein content of the BAL was increased very significantly at day 3 in NiCl\textsubscript{2} and CdCl\textsubscript{2} exposed animals ($P<0.01$) and at day 15 in rats exposed to 5 mM NiCl\textsubscript{2} ($P<0.05$; Fig. 8). Sialic acid contents were significantly increased at both the time intervals in the

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Fig. 2—Section of lung of a rat 3 days after exposure to 0.2 mM CdCl\textsubscript{2} showing presence of erythrocytes and fibrinous exudate in the interstitium and alveolar lumen. H & E x 520.

Fig. 3—Section of lung of rat 3 days after exposure to 5 mM CdCl\textsubscript{2} showing interstitial proliferation of fibroblasts and presence of large number of alveolar macrophages in lumen of alveoli and alveolar ducts. H & E x 520.

Fig. 4—Section of lung of rat 3 days after exposure to 0.2 mM NiCl\textsubscript{2} showing patency of the alveolar lumen and increased cellularity of their walls. H & E x 520.

Fig. 5—Section of lung of a rat 3 days after exposure to 1 mM NiCl\textsubscript{2} showing polymorphonuclear cell infiltration in the peribronchiolar region. H & E x 520.
lungs of rats exposed to all the concentrations of the two salts (Fig. 9). An increase in the lavage soluble proteins and sialic acid could be due to increased transudation of serum proteins following injury to the alveolar capillary barrier, death of various cell types lining different regions of airways and presence of inflammatory cell.

The activity of LDH in BAL was increased maximally at day 3 after exposure to NiCl₂ (P < 0.01). At day 15 after exposure to NiCl₂, the activity of LDH was also increased (P < 0.01) but it was less than observed at day 3 (P < 0.01 with 1 and 5 mM concentration). In CdCl₂ exposed lungs, the increase in the activity of LDH was noticeable only at day 3 after exposure (P < 0.01 with 1 and 5 mM concentration) (Fig. 10). Alterations in the enzyme activities of β-glucuronidase in BAL were similar to those observed in the case of LDH. An increase in LDH activity in the BAL suggests increased permeability of the plasma membrane of the target cells resulting in leakage of the cytoplasmic enzyme.

Increase in the BAL cellular and biochemical constituents was more at 3 days than at 15 days. This change could be attributed to in vivo mechanisms geared to ameliorate the toxicity of metal by synthesis of metal binding proteins. Even though no attempts were made to estimate metallothioneins in the present investigations, decrease in various toxicity parameters with lapse in time, in spite of increasing metal concentration following prolonged inhalation exposure has been reported. More damaging potential of CdCl₂ than Cd-thionein also supports existence of metal-scavanging systems in lungs.

Greater toxicity of Cd in comparison to Ni is supported in a number of in vivo and in vitro experiments. In rabbits inhaling chlorides of Ni at levels close to occupational TLV there was a marked decrease in the lysozyme activity in BAL, alveolar macrophages and supernatant medium of cultured macrophages. Lysozyme levels were, however, either increased or remained unchanged following inhalation.
of soluble Cd\(^{18}\). These results were confirmed in alveolar macrophages exposed in vitro to graded overlapping concentrations of NiCl\(_2\) and CdCl\(_2\). Manifold lesser amounts of Cd than Ni were needed for detachment of cells from glass surface and causing a 50% decrease in viability\(^4\).

Despite the controversy concerning the mechanism of Ni and Cd toxicity there is an increasing evidence to suggest that lipid peroxidation plays an important role in the process. Increased formation of free radicals could result in a disbalance in the oxidant antioxidant capacities and thereby contribute significantly in an impairment in the normal functional integrity of lungs \(^{19}\). Whether Ni and Cd induced inhibition of Ca\(^{2+}\) activated K\(_{Ca}\) channels in vascular smooth muscles would contribute in pulmonary toxicity needs further investigation\(^{20}\).

References