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Inhalation of cadmium, lead or its mixture Effects on the bronchiolar structure and its relation with metal tissue concentrations

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Abstract

The human population in the industrialized world is constantly exposed to chemical mixtures of pollutants such as metals; information about the consequences of the interactions of these compounds on health is scarce. The current study examines the effects of the inhalation of lead (Pb), cadmium (Cd) and Pb–Cd mixture in mice models analyzing the metal concentrations in lung, and the morphological modifications in the bronchiolar epithelium identified by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) after 4 weeks of inhalation. Our results showed that metal concentrations in lung were higher compared to controls; however, Pb concentrations drastically decrease with the mixture. This reduction was also observed in the inhalation chamber. These data correlate with the morphological alterations observed, which consisted of flattened and decreased number of nonciliated bronchiolar cells (NCBC), bald ciliated cells and bundles of NCBC. These modifications were mainly given by Cd, alone or in combination with Pb. The clusters formed by NCBC cells suggest cell proliferation which probably means that after metal inhalation, the cells enhance their proliferative capacity in order to repopulate the bronchiolar wall.

Keywords: Bronchiole; Cadmium; Lead; Scanning electron microscopy; Mixtures; Metals; Inhalation exposure

1. Introduction

Air pollution is a major problem worldwide, and there has been a particular concern about metals such as lead (Pb) and cadmium (Cd) which are highly toxic and have increased in the atmosphere entering the lung through respirable size particles (diameter $\leq 2.5\,\mu m$) (Fortoul et al., 1996). Some reports have indicated that the average concentration of Pb in urban areas is from 5 to 10 $\mu g/m^3$, while for Cd is 0.06 $\mu g/m^3$ (Fortoul et al., 1996). Tobacco smoke is an important source of Cd exposure and it has been reported that one cigarette contains about 1–2 μg or more (Valverde et al., 2000), and this concentration is related to tobacco's leaf origin (Saldivar et al., 1991).

Previous reports from our group indicate that metals in the air are a problem in Mexico City, mentioning an increase in Pb, Cd, Ni and V (Fortoul et al., 1996, 1999, 2002). Several publications refer that metals are adsorbed on suspended particles and enter through the lung to the systemic circulation-inducing damage (Fortoul et al., 1999). The majority of the reports about the effects of chemicals on biologic systems are conducted on one chemical at a time. However, in the environment, people are exposed to mixtures, and not to single

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chemicals. Carpenter et al. (2002) reported that the number of chemicals to which humans are exposed has increased dramatically in the last 100 years; however, information about the inhalation of toxicant mixtures and its effects on health is limited (Mumtaz et al., 2002; Carpenter et al., 2002).

Chemicals interact in different fashions. If we consider two chemicals, they may act in a common site such as a receptor or an enzyme, and the effect might be additive, antagonic, synergistic or magnifying (Carpenter et al., 2002; Bae et al., 2001).

As a consequence of the information mentioned above, we decided to identify the effects that inhalation of Pb, Cd or Pb–Cd mixture have on mouse bronchiolar ultrastructure, as well as the relationship between exposure time, metal concetration and metal interaction.

2. Materials and methods

2.1. Animals

Seventy-two CD1 adult male mice (32 ± 2 g) were randomly divided in three groups of 24 animals each. Group I inhaled lead acetate 0.01 M (Sigma Chemical Co., St. Louis, MO, USA) for 1 h twice a week during 4 weeks; Group II inhaled cadmium chloride 0.006 M for the same period of time (Sigma Chemical Co.) and Group III was exposed to a mixture of cadmium chloride and lead acetate in the same concentrations indicated above. Each group had eight control animals that inhaled deionized water with the same protocol. Inhalations were performed in a closed acrylic box (31.47 cm^3) connected to an ultranebulizer (UltraNeb 99 DeVilbis), with 10 L/min continuous flux. The ultranebulizer is designed to produce droplets in a 0.5–5 μm range. A trap for the vapor was located in the opposite side with a solution of sodium bicarbonate to precipitate the remaining metals.

The concentrations of Cd, Pb and Cd–Pb mixture in the chamber were quantified as follows: a filter was positioned at the outlet of the ultranebulizer during the whole inhalation time at a flow rate of 10 L/min. After each exposure, the filters were removed and weighed; the metals were quantified following the same protocol as with tissue samples. Six filters for each metal were evaluated (Fortoul et al., 1999).

After inhalation, the animals (10 each time) were returned to their cages and fed with pelleted diet and tap water ad libitum (Fortoul et al., 1999). The exposure schedule was repeated for 1, 2, 3 and 4 weeks, and mice (four exposed, two controls from each group) were euthanized for ultrastructural analysis at the end of each treatment.

2.2. Electron microscopy analysis

Experimental animals were anesthetized with sodium pentobarbital, the trachea was exposed and lungs were fixed intratrachealy by instilation of 2.5% glutaraldehyde in cacodylate buffer, pH 7.4 (470 mmol) at total lung capacity (TLC). After instilation, the trachea was tied up and the cardiopulmonar block was removed from the chest cavity. The left lung was processed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (González del Pliego et al., 2001) and the right one for metal concentrations (Fortoul et al., 1996). Samples were analyzed in a Zeiss SEM DSM-950 electron microscope and a Zeiss EM-10 trasmission electron microscope. All the samples were evaluated by two blinded observers.

2.3. Metal concentrations in lung tissue

Lung samples and filters were processed as mentioned by Fortoul (Fortoul et al., 1996). Samples were analyzed using a graphite furnace Atomic Absorption Spectrometer (Perkin Elmer model 2380). The light source came from a hollow cathode lamp. Formaldehyde and the blanks were also analyzed to identify metal contamination from this source. Accuracy was assured by three random determinations of seven different standard solutions, prepared with the same reactives used during the metal analysis. For Pb, wavelength was 318.4 nm, the detection limit was 0.37 ppm, and the slit 0.7 nm, and for Cd, the wavelength was 228.2 nm, the detection limit 0.1 ppm and the slit 0.7 nm. Each sample was analyzed in triplicate (Fortoul et al., 1996).

2.4. Statistical analysis

ANOVA test was applied to analyze metal lung concentrations, and Mann–Whitney *U*-test for metal concentrations in the filters. Differences were considered at $p < 0.05$.

3. Results

3.1. Metal concentration in the inhalation chamber

The variations of metal concentrations in the filters are shown in Fig. 1; a drastic decrease of both metals with the mixture is observed.

3.2. Metal concentrations in lung tissue

The pattern of metal concentrations is observed in Fig. 2. The concentrations were expressed in micrograms per gram of dry weight tissue (DWT). Pb concentrations showed an evident increase since the first week of inhalation (1.01 μg/g), decreasing and stabilizing its values during the next 3 weeks. At all times, Pb concentrations were statistically different from controls ($p < 0.05$).

Cadmium increased drastically since the first week (2.7 μg/g), doubling its concentration on the third (5.1 μg/g), and decreased at the fourth week. All concentrations were statistically different from controls ($p < 0.05$).

The concentrations of Pb in the mixture notoriously decreased when compared with those found in the inhalation of

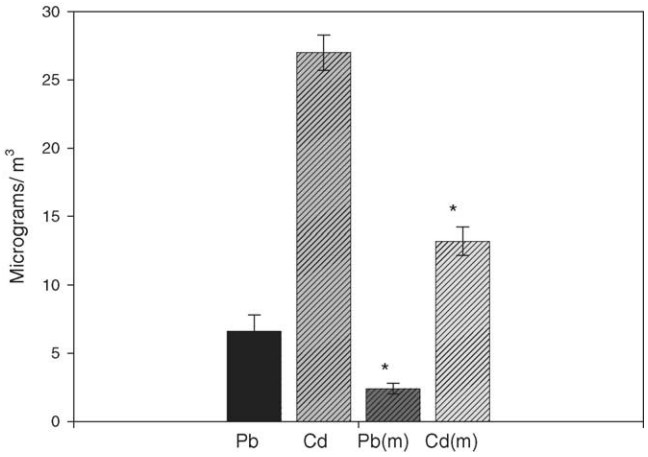

Fig. 1. Filters' concentrations of Cd, Pb or its mixture in μg/m^3 (n = 6). *p < 0.05 Pb/Cd vs. Pb or Cd (n = 6) Mann–Whitney U-test.

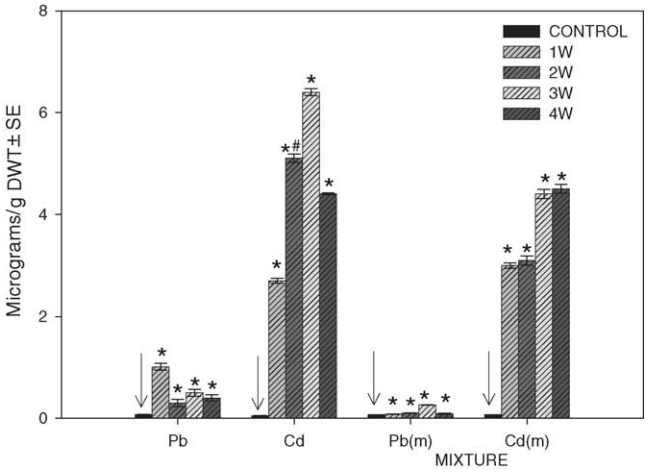

Fig. 2. Lung concentrations of Cd, Pb and its mixture in μg/g of dry weight tissue (DWT); n = 9 ANOVA test, p < 0.05; (*) statistically different from controls; (#) statistically different from W1 vs. W3; (↓) control.

Pb alone, while Cd increased its concentrations in the same way as when it was measured alone but in less proportion.

4. Terminal bronchiole morphology

Terminal bronchiole in control animals was identified as tubular structures covered with columnar or cubical cells with prominent apical surface that protrude to the lumen; these cells are the nonciliated bronchiolar cells or Clara cells (NCBC). On the cells' surface, some irregularities are identified, as well as some small blebs extruded from its apex. Some ciliated cells are identified between the nonciliated bronchiolar cells (Fig. 3A).

After 1 week of Pb inhalation, the surface landscape showed decreased volume of NCBC with an irregular surface due to the presence of multiple rounded protrusions; vesicles leaving the cell surface can also be identified. Ciliated cells were more evident and the cilia were overlapped (Fig. 3B). In contrast, the findings of 1 week of Cd inhalation revealed more NCBC as well as images of incipient dividing cells. An area without NCBC was evident, covered with ciliated cells and the cilia evidenced an irregular pattern (Fig. 3C).

After 3 weeks of Pb inhalation, the NCBC decreased in volume and in number, the remaining cells were in division process; additionally, some cells were flattened and exhibited secreted material on its surface (Fig. 3D).

When 1-week Pb–Cd-exposed cells were evaluated, the damage observed was overwhelming, identifying a decrease in number and volume of NCBC. Also, increased secretion vesicles were evident as well as bare ciliated cells. Bronchiole morphology was highly distorted as a consequence of the mixture inhalation (1 week). The leading changes were bald ciliated cells, completely shrunken NCBC and bundles of dividing cells (Fig. 3E).

After 4 weeks of mixture inhalation, bronchioles surface changes were striking with almost all NCBC collapsed (Fig. 3F).

The proliferation found in the exposed animals was more evident when samples were analyzed by transmission electron microscopy (Fig. 4A and B). In control bronchiole, less than two NCBC were observed, and more ciliated cells can be distinguished, while in exposed bronchioles, the identified conglomerated cells consisted of six NCBC, with increased rough endoplasmic reticulum compared with controls and nuclei contained more euchromatin.

5. Discussion

The study of chemical mixtures is scarce for different reasons, but essentially as a consequence of the difficulty to identify the mechanisms by which both metals generate damage (Bae et al., 2001). Although relative few studies have looked into the interactions of two chemicals in the environment, humans and animals are exposed to multiple substances at the same time. The rising importance for the study of mixtures is because every day, the number of chemicals at which living creatures are exposed is increasing drastically, and the expected effects are unpredictable because of the differences in their chemical interactions (Bae et al., 2001; Mumtaz et al., 2002; Carpenter et al., 2002).

In this report, we are exploring the distal airways, which are sites of epithelial injury because of several factors, including distribution of the toxicant through the branching airway structure, cellular population of the bronchiolar epithelium and the capability of the cells in this region to activate or detoxify xenobiotics. Although changes induced by gases and particle mixtures are reported to produce additive damage in the bronchiole (Mautz et al., 2001), no information is available about the effects that metal mixtures might induce on this site. The literature refers to the important role that the nonciliated bronchiolar cells have in the restoration of the distal airways (Boers et al., 1999). Here, we evidence the

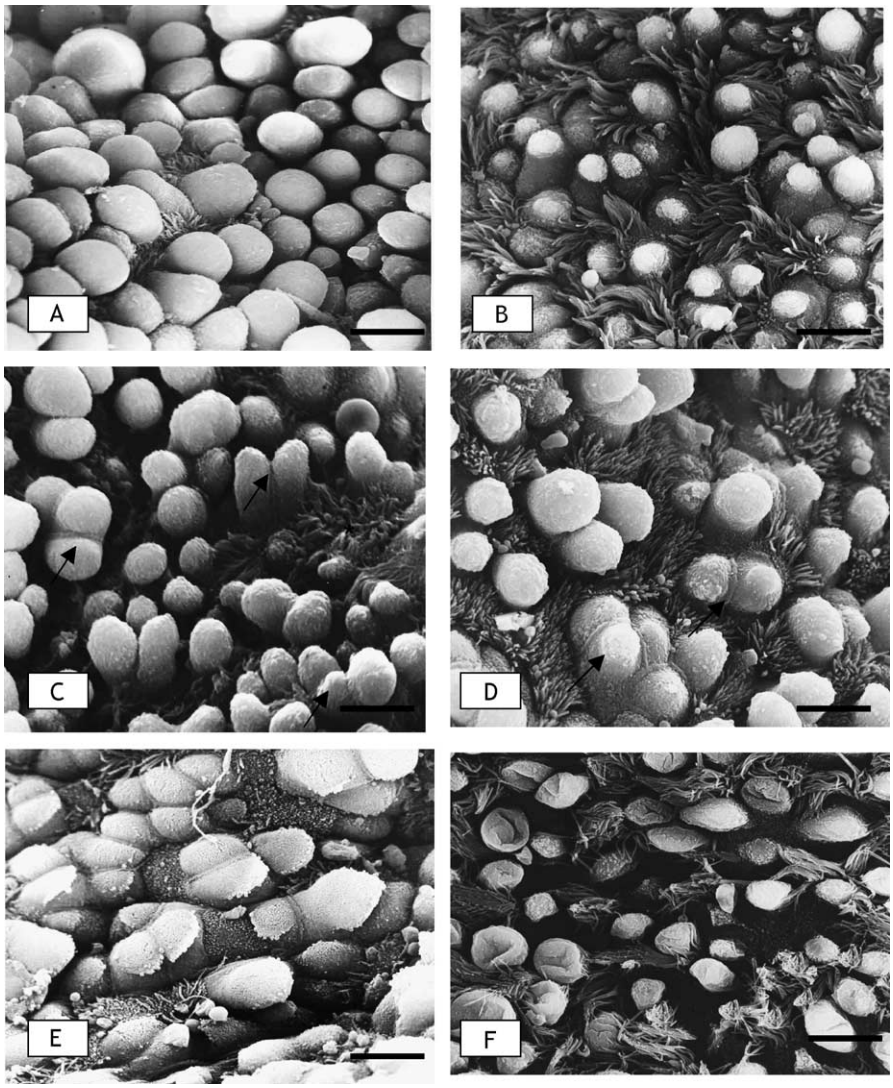

Fig. 3. (A) Control bronchiole with prominent NCBC; (B) 1-week Pb inhalation, with some dividing cells (arrows); (C) 1-week Cd inhalation with dividing cells (arrow) and areas devoid of NCBC (*); (D) 3-weeks Pb inhalation, with several clusters of dividing cells (arrows) and short cilia; (E) 1 week of Cd–Pb mixture inhalation, decreased volume nonciliated bronchiolar cells and ciliophoresis; (F) decreased in number and in volume NCBC are evident. Bar = 5 μm.

proliferative response of the NCBC after the injury evoked by Pb, Cd or its mixture, data which support the role of these cells on the bronchiolar cell repopulation.

As it is known, Pb exposition is given by inhalation, as a consequence of its use as an anti-knocking agent, and in ceramic folk art workers (Olaiz et al., 1997) or other industrial activities (Fortoul et al., 1999). Effects on central nervous system, kidney, lung and hematopoyesis, as well as its genotoxic effects, have been reported by other authors (Valverde et al., 2000, 2002; Hengstler et al., 2003).

Changes that Pb exposure induces in the bronchioles might be explained by the generation of reactive oxygen species (ROS), feature that has been reported in other organs (Yiin et al., 1999).

Cadmium exposure has been involved in a variety of pathological conditions, e.g. neurotoxicity, nephrotoxicity and carcinogenicity. Also, its competence to alter various cellular processes including metabolism, protein synthesis and cell proliferation has been documented (Sampson et al., 1984; Watjen et al., 2000). Its toxicity is related to the production of ROS and lipid peroxydation. Moreover, the exposure to this metal has been associated to emphysema and lung cancer (Fortoul et al., 1996; Murthy and Holovack, 1991).

In connection with the metals evaluated here, some reports pointed out a slight synergistic effect of Pb and Cd (Pohl et al., 2003), findings that contrast with our results, since here we identify a predominant Cd effect which is sustained with the lung metals' concentrations and the morphological alterations.

A possible explanation for the interaction that Pb and Cd have in the filters suggests that Cd decreases Pb dissolution, so less Pb is nebulized, and its concentration in the lung decreased compared with Cd. The increased effects observed by Cd alone or with the mixture could also be answered by

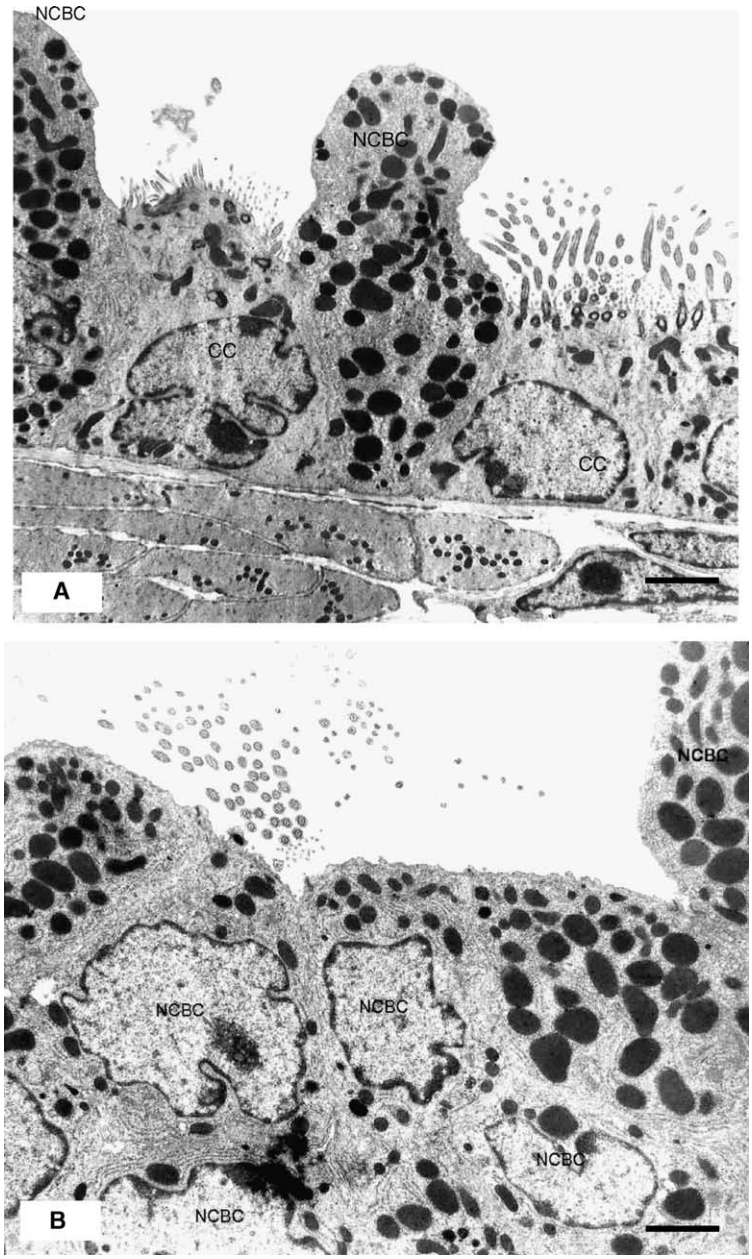

Fig. 4. (A) Bronchiolar epithelium from a control mouse. Two dome-shaped nonciliated bronchiolar cells (NCBC) are observed, with electron-dense granules in its cytoplasm. Three ciliated cells are also evidenced (CC). (B) At least six NCBC fill the bronchiolar surface. The nuclear size and the distribution of the chromatin differ from controls, suggesting highly metabolic active cells, supported by the hyperplasic rough endoplasmic reticulum (*). Bar = 3 μg.

the higher affinity of Cd to –SH groups which play an important role in the anti-oxidant function of proteins such as glutathione (Antonio et al., 2002).

There is difficulty in predicting the way in which the mixture will behave, since factors related to the interactions with enzymes, receptors, other metals, etc. could change the effects and should be taken in consideration. The main interest of this report resides in the fact that all living organisms are exposed to mixtures in the environment, not only binary mixtures, as we are reporting here, but also combinations of three or more metals, or even combinations of metals with organic pollutants. This approach becomes increasingly difficult because of the exponential multiplication of the number of test groups with the increasing number of chemicals in a mixture. Further research is needed to identify the potential interactions of complex mixtures and its consequences in living organisms.

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