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Toxic effects of cadmium microparticles on the respiratory system

An experimental study on rats and mice

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NICOLE DESPAUX, Pharm.D., and CLAUDE BOUDENE, D.Sc.¹

BOULEY, G., DUBREUIL, A., DESPAUX, N. and BOUDENE, C. Toxic effects of cadmium microparticles on the respiratory system: An experimental study on rats and mice. *Scand. j. work environ. & health* 3 (1977) 116—121. Experiments on male, specific pathogen-free rats (193) and mice (66) are reported. The test and control animals received a single 15-min exposure to cadmium (cadmium oxide) and aluminum (alumina) microparticles, respectively. The air cadmium content was 10 mg/m³, and 4 µg of this metal was retained by the lungs of the rats. The animals were observed for 24 days. The following significant differences appeared between the control and test groups: in the cadmium exposed rats the relative lung weight (percentage of body weight) became temporarily higher; the absolute number of alveolar macrophages decreased at first and then increased; numerous polymorphonuclear and lymphocytic cells appeared in the alveoli. According to these inflammatory phenomena, at the 48th h after exposure, there was an alteration in the clearance kinetics of inhaled bacteria and an increase in the death rate of cadmium exposed animals following a test infection with *Salmonella enteritidis* (rats) or *Pasteurella multocida* (mice) aerosols. The mechanism of cadmium toxic action on the respiratory system is discussed.

Key words: air pollution, airborne infection, cadmium, microparticles, respiratory system.

The cadmium level of urban environments is growing because of the increasing use of this metal in industry. In nature, it is closely related to zinc and therefore appears in zinc metallurgical areas. Cadmium is used in storage batteries, plastics, paints, and even in oral dentures; it is also present in tobacco smoke (4, 8, 9, 16, 17, 29).

In toxicology, the oral route is the most

important. But the respiratory route must not be neglected, particularly in some work areas. The target organs of this poison are the liver, kidneys, lungs, and sex glands. Cadmium can indirectly cause osteomalacia and can cross placental membranes.

In the acute respiratory intoxication of man, cadmium induces pulmonary edema and bronchopneumonia. In less severe or chronic cases, it primarily causes emphysema and respiratory obstruction, with subsequent pulmonary fibrosis.

We have studied the toxic effect of cadmium on rats and mice subjected to a single, short exposure to cadmium fumes.

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MATERIAL AND METHODS

Microparticles of cadmium oxide (for the test animals) and alumina (for the controls) were produced by the pyrolysis, at 650°C, of droplet nuclei generated from cadmium acetate or aluminum lactate aqueous solutions (equal molarities). This method is similar to that of Horstman et al. (13). The atmosphere was cooled before being introduced into the exposure chambers of the animals. On the way out of these enclosures the microparticles were collected on cellulose acetate filters with a porosity of 0.45 μm . The chemical determination of the pollutant levels was made by flame atomic absorption spectrophotometry. The determination of the microparticle size was performed with electronic photomicrography.

In our experiments the rodents inhaled 10 ± 2 mg (mean $\pm$ SD) of cadmium per cubic meter of air. The diameter of 93 to 100 % of both the cadmium oxide and alumina particles was less or equal to 3 μm (95 % confidence limits). The animals were exposed in two enclosures which could hold 11 rats or 22 mice at the same time. Only the noses of the rodents were exposed to the chamber atmosphere. Except for the chemical composition of the particles, the physical and chemical conditions of the two enclosures did not significantly differ (temperature, relative humidity, carbon monoxide and carbon dioxide levels). There was only one constant 15-min exposure for each animal.

We used 193 male Sprague-Dawley rats (body weight 190 ± 25 g) and 66 male OF1 mice (body weight 30 ± 2 g). These animals were specific pathogen-free (S.P.F.); the control and cadmium exposed groups were composed of rodents of equal ages and body weights that did not significantly differ at the beginning of each experiment.

The comparative study of the control and test animals was made according to the following conditions:

1. *Immediately after exposure* (0 time): Determination of the cadmium contained in the area of the trachea-bronchi-lungs.

2. *At the 48th h after exposure*: (a) Susceptibility to infection by one 25 % lethal

dose (LD 25)², predetermined with non-exposed animals, of *Salmonella enteritidis* for the rats and *Pasteurella multocida* for the mice. These test germs were inhaled (infectious aerosols) in a Henderson apparatus (11). (b) Clearance kinetics of *S. enteritidis* inhaled by rats in the same dosage and under the same conditions as for infection. We determined the evolution with time (hours) of the number of viable bacteria harvested in the area of the trachea-bronchi-lungs (26).

3. *From the 1st to the 24th day after exposure*: (a) Evolution with time of the relative fresh weight (percentage of body weight) of the rat lungs. (b) Absolute and relative number (percentage of free cells) of rat alveolar macrophages harvested with seven endopulmonary washings (3). (c) Death rate of the macrophages, measured with the trypan blue exclusion test for cell viability (23). (d) Measurement of macrophage size in an attempt to determine their replacement rate in the alveoli (2). (e) Ability to adhere to glass in Leighton's tubes (number of alveolar macrophages stuck to cover-slip per microscopic field). (f) Ability of these cells to phagocytose latex particles in vitro (6).

The data were statistically analyzed, according to the number of measures and experimental conditions, by the following tests: Pearson's chi square, Student-Fisher's t and paired t, and Wilcoxon's W and T tests (28).

RESULTS

Only the data indicating significant differences between the control and cadmium exposed groups are reported.

Cadmium content of the lungs immediately after exposure

In 11 control rats, the cadmium content of the lungs was less than the sensitivity level of our method. In 11 cadmium exposed rats, it was 3.651 ± 0.411 $\mu\text{g/g}$ of lung or 3.754 ± 0.455 μg when expressed per pair.

We performed only one measurement on the test mice. It indicated a cadmium content of 1.4 μg in the lungs.

² Dose killing 25 % of infected animals.

Table 1. Death rates of rats and mice either exposed to cadmium or infected by airborne bacteria or both (dead/total tested and % mortality).

Group	Rats	Mice
I- Cadmium	0/11 (0 %)	0/20 (0 %)
II- Infection	5/28 (18 %)	9/22 (41 %)
III- Cadmium + infection	18/28 (64 %)	22/22 (100 %)
Statistical test and p value	χ^2 I-II-III = 20.27 $p = 4 \times 10^{-5}$ χ^2 II-III = 12.47 $p = 4 \times 10^{-4}$	χ^2 I-II-III = 42.71 $p = 7 \times 10^{-10}$ χ^2 II-III = 18.45 $p = 2 \times 10^{-5}$

Fig. 1. Pulmonary clearance. Evolution with time of the number of viable *S. enteritidis* harvested from the rat lungs.

Susceptibility to respiratory infections at the 48th hour

Rats and mice were randomly placed in three groups of each species: group I was the cadmium exposed group (Cd controls); group II, the nonexposed but infected group (infection controls); group III, the cadmium exposed infected group (test rodents). We observed these animals for three weeks.

The results given in table 1 show that, in our experiments, cadmium alone was not lethal (group I). However, when cadmium was associated with airborne infection, the death rate of the animals significantly increased (group III) in comparison to the corresponding rate of the rodents in group II (infected only).

Clearance kinetics of *S. enteritidis* in the rat respiratory system

The results of the clearance kinetics of *S. enteritidis* are expressed in fig. 1 as the

logarithms of viable bacteria percentages (based on 0 time of infection), according to time (hours after infection).

From 0 to 1 h there was no significant difference between the control and cadmium exposed rats. Later we observed an important alteration in clearance kinetics, i.e., between the first and sixth hour, the angular coefficient was -0.101 ± 0.032 for the controls and $+0.082 \pm 0.030$ for the cadmium exposed animals ($t = 4.158$, $p = 1 \times 10^{-3}$).

Time evolution of the relative weight of the rat lungs

The results of the time evolution of the relative weight of the rat lungs are presented in fig. 2. Between the 1st and 24th day the angular coefficient was -0.009 ± 0.001 for the controls and -0.015 ± 0.001 for the exposed animals. The difference was significant ($t = 3.378$, $p = 0.001$).

Fig. 2. Evolution with time of the relative weight of the rat lungs.

Absolute and relative number of harvested alveolar macrophages

Seven washings of 5 ml per rat were performed to harvest the alveolar macrophages. The absolute number was expressed per animal rather than per gram of lung because of weight differences observed between the controls and cadmium exposed animals.

At the 48th h after exposure the absolute number of harvested macrophages did not significantly differ between the groups, but the relative number of these cells showed the appearance of numerous polymorphonuclear and lymphocytic cells in the lungs of cadmium exposed rats. In six controls we counted 86.17 ± 8.40 % macrophages and in six exposed 43.33 ± 17.13 % ($T = 0$, $p = 0.05$).

The evolution of the absolute and relative number of macrophages, according to time after exposure, is given in fig. 3. It can be seen that the corresponding mathematic expressions of this evolution are $y = a + bx$ for the controls and $y = ax^b$ for the test rats, as determined by the best fit to equations $y = a + bx$, $y = ae^{bx}$ and $y = ax^b$ (comparative values of the correlation coefficients).

Size of alveolar macrophages harvested in rats

Forty-eight hours after exposure the number of macrophages with a diameter equal or inferior to $13 \mu\text{m}$ was significantly lower in six controls than in six test rats, $345/600$ (58 %) for the former and $388/600$ (65 %) for the latter ($\chi^2 = 6.482$, $p = 0.01$).

DISCUSSION

We have exposed rodents to cadmium oxide microparticles because they are the most frequent chemical and physical form in which this toxic substance can be found in polluted urban atmospheres (9). We chose the particular dose of cadmium used (1/4 to 1/5 of the lethal dose, under our experimental conditions) because it induces disturbances in the respiratory system of the test animals but does not modify

Fig. 3. Absolute (left ordinate) and relative (right ordinate) number of alveolar macrophages harvested (seven lavages).

body weight evolution. We performed a short, single exposure because a long or repeated one could cause modifications in the evolution of respiratory disturbances. In fact we have previously observed a lower pulmonary retention of cadmium after several exposures when compared with the theoretical dose inhaled by the rats according to cadmium air content, exposure length, and respiratory flow (10). Besides, Yoshikawa showed the preventive effect of pretreatment with a low dose of cadmium in the acute toxicity of this metal on other organs or tissues than the respiratory system in mice (35), and Rugstad and Norseth (25) observed an increased synthesis of metallothionein, a cadmium binding protein (1, 18, 33), in cultured human epithelial cells preexposed to cadmium. Lastly male animals were chosen for the experiment because they seem more susceptible to the studied pollutant than females (27).

In our experiments we observed a cadmium pulmonary retention of 25 % of the amount theoretically inhaled by the rats. This percentage fits within the range of results already published (19, 34, 36).

We found inflammatory phenomena demonstrated by a temporary increase in lung weight and by the presence of numerous macrophages in the alveolar-free cells harvested. At the same time, the number of pulmonary macrophages was first lowered. In addition we observed an afflux of probably neoformed macrophages, as deduced from the size reparti-

tion of these cells. Our statements confirm the results reported by Paterson (22) for rats exposed to larger doses, i.e., pulmonary edema appears, possibly followed by centrilobular emphysema (31) and by fibrosis.

The mechanism of these disturbances can be explained by the cytotoxic (20, 21, 30) and enzyme impairment (4, 12, 32) action of cadmium. At the cellular stage, cadmium inhibits respiration and causes cytoplasmic necrosis, an alteration of the nucleus membrane, and nuclear inclusions. The alveolar macrophages altered by the phagocytosis of cadmium microparticles can release their proteolytic enzymes *in situ*, and, since cadmium can inhibit the activity of such enzymes as α 1-antitrypsin, as shown *in vitro* by Chowdhury and Louria (5), we think the observed lesions could be self-maintained with the afflux of neoformed macrophages and microphages. Another cause of disturbance is the increase in surface tension of the membrane lining pulmonary alveoli (24) after cadmium intoxication.

The produced lesions impair the clearance of bacteria introduced into the respiratory system and therefore increase the susceptibility of cadmium intoxicated rats and mice to experimental airborne infection. This phenomenon explains the enhancement of respiratory infections after cadmium exposure via the respiratory route. As for other routes, Cook et al. (7) found that the intravenous administration of an acute dose of cadmium acetate enhances the susceptibility of rats to an intravenous challenge of *Escherichia coli*. In addition, in the case of experimental intoxication by soluble cadmium, the antibody immune response is impaired in both rats and mice (14, 15).

The biochemical action mechanism of cadmium is not yet completely known, but the metal seems to act in competition with other cations such as zinc (8, 9).

In our laboratory we are extending our studies in the following two ways: (a) *in vitro*, i.e., we are investigating the cytotoxic mechanisms of cadmium oxide microparticles upon cells (alveolar macrophages, fibroblastic cells), and (b) *in vivo*, i.e., we are studying the distribution of inhaled cadmium oxide microparticles (insoluble form) in the whole rat organism

in comparison to inhaled droplet nuclei of cadmium acetate (soluble form).

NOTE

As we were finishing this paper, we discovered Gardner et al.'s work with soluble cadmium aerosol. Our results are a confirmation of theirs.

[GARDNER, D. E., MILLER, F. J., ILLING, J. W. and KIRTZ, J. M. Alterations in bacterial defense mechanisms of the lung, induced by inhalation of cadmium. *Bull. eur. physiopathol. respir.* 13 (1977) 157—174.]

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