Cadmium Accumulation and Detoxification by Alveolar Macrophages of Cigarette Smokers*

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Study objectives: Cadmium (Cd) is a toxic metal associated with emphysema and lung cancer, which is present in both air pollution and cigarette smoke. Metallothionein (MT) is an inducible protein that binds and detoxifies cellular Cd. The goals of this study were to determine whether increased concentrations of Cd are present in alveolar macrophages (AMs) of cigarette smokers (CSMs) and to determine whether MT accumulated in response to the presence of Cd.

Design: AMs were recovered by BAL from 10 healthy nonsmokers (NSMs) and 10 CSMs. The Cd content of the AMs was determined by inductively coupled plasma-mass spectrometry, and the MT content was determined using a Cd/hemoglobin radioassay (with 109Cd).

Measurements: Cd was detected in AMs recovered from all subjects, with higher mean (± SEM) concentrations in CSMs compared with those in NSMs (3.4 ± 0.5 vs 1.3 ± 0.2 ng/10⁶ cells; p < 0.005). There was a correlation between current smoking history (cigarettes per day) and the AM content of Cd (r = 0.74; p < 0.05). The mean AM content of MT was similar in NSMs (1.2 ± 0.2 μg/10⁷ cells) and CSMs (1.0 ± 0.2 μg/10⁷ cells).

Conclusions: AMs in CSMs accumulate significant amounts of Cd without a concurrent increase in MT content, indicating greater saturation of MT. Increased Cd burden in alveolar cells could contribute to the development of lung diseases in CSMs. (CHEST 2003; 124:1924–1928)

Key words: alveolar macrophage; cigarette smoking; cadmium; metallothionein

Abbreviations: AM = alveolar macrophage; Cd = cadmium; CdCl₂ = cadmium chloride; CSM = cigarette smoker; MT = metallothionein; NSM = nonsmoker

The inhalation of cadmium (Cd) can cause multiple respiratory disorders, including acute pneumonitis and emphysema, and exposure to Cd is associated with an increased risk of developing lung cancer.¹,² Cd is present in air pollution, although concentrations vary significantly in different geographic regions, and in cigarette smoke.³ Some studies⁴,⁵ have suggested that cigarette smokers (CSMs) have increased concentrations of Cd in lung tissue. However, an autopsy study⁶ has suggested that human lung Cd content is influenced more by exposure to air pollution than by smoking history. Therefore, the relative importance of air pollution and cigarette smoking in influencing lung Cd content is uncertain.

Cd is present in tobacco with concentrations determined by soil Cd concentrations in the region where tobacco is grown. Cd content in cigarettes of 1.8 to 2.8 μg per cigarette have been reported in European cigarettes⁷ and Mexican cigarettes.⁸ Approximately 10 to 20% of cigarette Cd content is inhaled in mainstream smoke, with 50% transferred into sidestream smoke, so that exposure to environmental cigarette smoke may be a significant source of inhaled Cd.⁹

The exposure of experimental animals to inhaled Cd particles leads to Cd accumulation in alveolar macrophages (AMs).¹⁰ It is uncertain whether AMs accumulate the Cd that is present in cigarette smoke, although there is significant accumulation of iron, which may be derived in part from cigarette smoke.¹¹ The Cd content of human AMs has not been previously reported.
Metallothionein (MT) is a cysteine-rich protein that binds Cd as well as other metals, including copper and zinc, and limits Cd-induced toxicity.\textsuperscript{12} AMs in experimental animals synthesize MT in response to inhalation exposures to Cd dusts; however, it is not known whether the Cd present in cigarette smoke induces MT accumulation.\textsuperscript{10,13}

Epidemiologic data\textsuperscript{14} have suggested that the Cd present in cigarette smoke contributes to the risk of lung cancer in CSMs, although the specific role of Cd in carcinogenesis is not certain. The presence of significant amounts of Cd accumulating in respiratory cells also could be a factor contributing to other smoking-induced diseases such as emphysema. The capacity of the lung to detoxify Cd by synthesizing MT may be important in limiting potential lung toxicity from smoking-induced Cd accumulation. However, studies\textsuperscript{15} have suggested that Cd-adapted cells still may demonstrate functional differences that could increase the risk of neoplastic transformation. The goals of the current study were to determine whether Cd present in cigarette smoke accumulated in AMs or induced these cells to accumulate MT.

**Materials and Methods**

*Study Subjects*

There were 20 male subjects recruited for these studies. The nonsmokers (NSMs) included 10 healthy volunteers who were life-long NSMs. Smoking subjects included five healthy volunteer smokers and five subjects who were undergoing diagnostic bronchoscopy for an abnormal chest radiograph finding. Subjects with chronic airflow obstruction (ie, \( FEV_1 < 70\% \)), significant sputum production, or a diagnosis of lung cancer were excluded from the study. Although the exact time interval between the last cigarette smoked and the performance of the BAL was not recorded, the majority of smoking subjects indicated that they had consumed at least one cigarette on the morning of the bronchoscopy, so this was a short interval.

CSMs undergoing diagnostic bronchoscopy were thought to have benign lung disorders based on radiographic improvement or stability over at least 1 year, or by findings on lung culture of an infectious agent. Of the five smokers with abnormal chest radiograph findings, one subject was determined to have a pulmonary infection with *Coccidioides immitis*, and in the other four subjects there was stability or improvement of the radiographic abnormality over the subsequent year. There were no known occupational exposures to heavy metals in any subject. Further characterization of study subjects is provided in Table 1.

*Chemicals, Supplies, and Radiochemicals*

All chemicals, including Cd chloride (CdCl\(_2\); Fisher Scientific; Pittsburgh, PA), \(^{109}\)CdCl\(_2\) (New England Nuclear; Boston, MA), bovine hemoglobin, and others (Sigma; St. Louis, MO), were obtained from well-known manufacturers.

*Recovery of AMs*

BAL was performed in the right middle lobe of all healthy volunteers, and in the right middle lobe or the lingula in the uninvolved lungs of the four smokers with abnormal chest radiographic findings. After sedation, the oropharynx was anesthetized with aerosolized lidocaine (2%), and the fiberoptic bronchoscope was inserted orally and wedged into the right middle lobe. This lobe was lavaged with four to five aliquots of normal saline solution (30 mL). The recovered BAL fluid was passed through sterile gauze to remove mucus. Cells were recovered by centrifugation, were washed twice, then were resuspended in saline solution. Total cell recovery was determined with a hemocytometer, and a cell differential was determined by counting 200 cells on a Wright-Giemsa-stained cyt centrifuge preparation.

In all cases, AMs comprised >90% of the recovered cells, and neutrophils comprised <5%. In some studies, cells recovered by BAL were allowed to adhere in tissue culture plates (Costar; Cambridge, MA) in the presence of RPMI-1640 medium. Non-adherent cells then were removed by gentle washing. The population of adherent cells was utilized as an enriched AM population for some studies.

*Cd Assay*

The Cd content of AMs was determined using inductively coupled plasma mass spectroscopy (Elan 6100; Perkin-Elmer; Foster City, CA). Cell samples were diluted in 1% nitric acid, and internal standards were used. The sensitivity of this assay for Cd was 0.2 ng/mL.

*MT Assay*

MT protein was quantified by the \(^{109}\)Cd-hemoglobin saturation assay as described by Eaton and Toal\textsuperscript{16} and as modified in our laboratory.\textsuperscript{17} This assay uses \(^{109}\)CdCl\(_2\) (solution, 22 \( \mu \)L concentration, 9.82 mCi/mL, specific activity, 1.97 mCi/mg Cd) added to a 100-mL solution of Tris-Hcl buffer (10 mmol/L) containing 2 \( \mu \)g Cd per millilitre. The final concentration was 0.2 \( \mu \)g Cd and 100,000 counts per minute per 100 \( \mu \)L solution. In polyethylene tubes, 100 \( \mu \)L sample (sonicated AMs) and 100 \( \mu \)L \(^{109}\)CdCl\(_2\) solution were combined, vortexed, and incubated for 10 min at room temperature. A 50-\( \mu \)L aliquot of 2% bovine hemoglobin (Sigma) was added to each tube, the tubes were vortexed, and then they were placed in boiling water for 1 min. The tubes were cooled on ice and then centrifuged at 10,000g for 3 min at room temperature. An additional 50 \( \mu \)L hemoglobin solution was added to each tube, the tubes were vortexed, and then they were placed in boiling water for 1 min. The tubes were cooled on ice and then centrifuged at 10,000g for 3 min at room temperature. An additional 50 \( \mu \)L hemoglobin solution was added to each tube, and the procedures were repeated. Subsequently, a 100-\( \mu \)L aliquot of supernate was removed from each sample and the amount of \(^{109}\)Cd was counted. Counts for background blanks and total blanks also were determined. The calculation of MT content was determined using the assumption of a formula weight for MT of 6,000 and with 6 g atoms Cd bound per mol MT.

All subjects gave informed consent, and the experimental protocol was approved by the institutional human subjects committee.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age, yr</th>
<th>Smoking History, pack-ys</th>
<th>FEV(_1), % predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSMs (n = 10)</td>
<td>45 ± 4</td>
<td>0</td>
<td>102 ± 7</td>
</tr>
<tr>
<td>CSMs (n = 10)</td>
<td>50 ± 6</td>
<td>28 ± 4</td>
<td>86 ± 9</td>
</tr>
</tbody>
</table>

*Values given as mean ± SEM.*

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Statistical Analysis

The data are expressed as the mean \pm SEM. Differences between groups were analyzed using the t test with Mann-Whitney rank sum test. In all tests, statistical significance was identified at the p < 0.05 level.

RESULTS

Cd Content of AMs

Cd was present in detectable amounts in the alveolar cell populations recovered from all subjects. In preliminary studies comparing the Cd content of adherent cells (ie, AMs) and nonadherent cells, we determined that > 95% of the Cd present in recovered alveolar cells was present in AMs. The percentage of neutrophils in recovered cells was < 5% in our study subjects; however, there likely was some Cd present in this component of the alveolar cell population. Since the primary Cd-containing cell in the recovered BAL fluid was the AM, the Cd content of alveolar cells was expressed based on the number of AMs present in the recovered fluid, as determined by total and differential cell counts.

The Cd contents of AMs recovered from study subjects are presented in Figure 1 and indicate that more Cd was present in AMs recovered from CSMs compared with NSMs (p < 0.005). There was a similar significant increase in Cd content of the AMs in CSMs, whether or not the values were expressed as nanograms per milligram of protein. Therefore, values were expressed as nanograms per 10^6 cells, as this had also allowed comparisons with Cd content in studies of AMs in prior animal studies. The overall increase in the mean Cd content in smokers was approximately threefold, although there was overlap in values between smokers and NSMs.

There was a correlation between the Cd content of AMs and smoking history expressed as the number of cigarettes smoked per day (Fig 2). The correlation between cumulative smoking history (pack-years) and AM Cd content was less than the correlation with the number of cigarettes smoked per day and was not significant.

MT Content of AMs

MT was detected in all AMs, with similar values found in NSMs and CSMs (Fig 3). Since higher concentrations of Cd were present in the AMs of CSMs, the Cd/MT ratio was significantly higher in the AMs of CSMs compared with that in NSMs (Table 2).

Figure 1. The Cd content in AMs recovered from NSMs and CSMs. The mean concentration of Cd in AMs was significantly higher in CSMs compared with NSMs. * = p < 0.005.

Figure 2. The Cd content in AMs recovered from CSMs is plotted as a function of current smoking (cigarettes/day). There was a significant correlation between Cd content and current smoking history.

Figure 3. The MT content in AMs recovered from CSMs and NSMs. There were no significant differences between the mean values determined in each group.
the Cd in the AMs of NSMs may be derived from air region from which subjects were recruited, so that on lung Cd content. Airborne Cd is present in the ing a cumulative effect of environmental exposures content increases with age, even in NSMs, suggest-

derived in part from other sources, such as second-
hand cigarette smoke.

Table 2—Cd and MT Content of AMs*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cd, ng/10^6 AMs</th>
<th>MT, µg/10^7 AMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSMs (n = 10)</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>CSMs (n = 10)</td>
<td>3.4 ± 0.5†</td>
<td>1.0 ± 0.2</td>
</tr>
</tbody>
</table>

*Values given as mean ± given SE.
†p < 0.005 compared with NSMs.

**DISCUSSION**

The main finding of this study was that significant amounts of Cd accumulate in the AMs of CSMs. There was a much smaller amount of Cd in other bronchoalveolar cells, including neutrophils. However, the primary cell containing Cd was the AM. The finding of increased AM content of Cd extends to prior reports of increased Cd content in the lung tissue of CSMs obtained at autopsy and indicates a possible method of assessing pulmonary Cd accumulation in vivo.4,5 In addition, we found similar amounts of MT in AMs recovered from CSMs and NSMs, indicating greater saturation with Cd.

To our knowledge, our study is the first to report Cd concentrations in human AMs. We found an approximately threefold increase in Cd content of AMs in CSMs compared with that in NSMs, suggesting a significant accumulation of Cd in these cells. A prior study5 reported an approximately sevenfold increase in the Cd content of lung tissue obtained from CSMs compared with that in NSMs. The total increase in Cd content of alveolar structures that was noted in our study actually may be similar to that reported in this prior study, since there were several-fold more AMs recovered by BAL from the alveolar structures of CSMs compared with those from NSMs. However, the results of our study are in contrast to those of a prior study6 that did not find a correlation between smoking history and lung Cd content.

Cd present in air pollution may contribute significantly to lung Cd content. A prior study6 noted that concentrations of Cd present in lung tissue obtained at autopsy increased approximately 20-fold in the Mexico City area from the 1950s to the 1980s, which was attributed to increases in the Cd content of air pollution. Another study18 indicated that lung Cd content increases with age, even in NSMs, suggesting a cumulative effect of environmental exposures on lung Cd content. Airborne Cd is present in the region from which subjects were recruited, so that the Cd in the AMs of NSMs may be derived from air pollution.19 However, it is also possible that Cd is derived in part from other sources, such as second-hand cigarette smoke.

Cd deposited in the lungs may persist for many years, as indicated in animal models.20 Data from human autopsies suggest that it may take > 20 years for lung-tissue Cd concentrations in ex-smokers to return to values present in NSMs.5 It is not known whether AM Cd persists within the lung for this extended period of time. Our finding that AM Cd content correlates better with recent smoking history compared with life-long cumulative smoking history suggests that Cd accumulation in these cells may change over shorter time periods.

Prior studies have not reported the Cd concentrations of human AMs, although a prior study10 measured the Cd content of rat AMs following multiple inhalations of Cd dust. After 22 inhalational exposures to Cd, rat AMs contained approximately 60 ng Cd per 10^n AMs, which is a substantially higher concentration than the 3.4 ng per 10^n AMs that we noted in the AMs of CSMs. This prior study also noted increased MT content of AMs following Cd exposure, as noted in other studies, indicating that these cells can accumulate MT in response to Cd.10–13 We found similar MT content in the AMs of smokers compared with NSMs, despite the higher Cd content. The reasons for this lack of MT accumulation in vivo are uncertain, although it may be that Cd concentrations in cigarette smoke are insufficient to induce MT synthesis. However, in a prior study,21 the lung concentrations of MT in experimental animals increased only transiently after exposure to cigarette smoke, and after 1 week levels had returned to baseline despite continued exposure to cigarette smoke. These findings suggest that cigarette smoking may have effects that limit the pulmonary accumulation of MT in response to Cd.

MT-bound Cd likely has limited cellular toxicity, however, unbound Cd may interact with vital cell structures and cause acute or chronic toxicity. Our finding that smoking increases the Cd content in AMs, but not the MT content, suggests MT is more saturated with Cd in the alveolar cells of CSMs, although there would be sufficient MT to bind the excess Cd accumulating in these cells. Since we did not specifically measure unbound Cd, it is uncertain whether there is also an increase in this more toxic form of Cd. The presence of unbound Cd could alter function or cause injury to AMs in CSMs.22,23

The accumulation of Cd within alveolar cells may have negative effects, even in the presence of an appropriate adaptive response. As reviewed by Hart and colleagues,15 the Cd-adaptive phenotype in cells may result in a reduced ability to repair DNA damage due to the inhibition of certain repair enzymes, as well as the inhibition of apoptotic cell death. The promotion of genetic damage in cells, together with the suppression of apoptosis, could
have negative results, possibly including the promotion of tumor development.

The contribution of inhaled Cd to respiratory diseases associated with cigarette smoking, such as emphysema and lung cancer, is uncertain. An epidemiologic study has suggested that inhaled Cd causes up to 9% of the lung cancer found in CSMs, and extrapolations from animal studies suggest that the value may be > 40%. However, it is difficult to extrapolate the findings of Cd toxicity in animal studies to humans, as there are substantial interspecies differences in the ability to detoxify Cd.

In summary, our findings indicate that Cd from cigarette smoke accumulates in AMs. Cd is also present in the AMs of NSMs, although at lower concentrations, which may be attributable to exposure to air pollution or to second-hand cigarette smoke. We also found that MT content in AMs is not increased in CSMs, indicating greater saturation of this important protective protein. Cd accumulation in respiratory cells, either with or without an adaptive response, may be a contributing factor to the development of smoking-induced lung diseases.

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