Biopersistence of the Mineral Matter of Coal Mine Dusts in Silicotic Human Lungs: Is There a Preferential Release of Iron?

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Toxic potency of quartz-containing dusts, including coal mine dusts, is usually inhibited by protective clay mineral layers on the surface of quartz particles. This investigation of 11 dusts recovered from lungs of coal miners with different silicosis grade shows that such layers persist during long-term contact with human lung tissues. On the other hand, the results suggest that an apparently preferential release of iron occurred in lungs with massive fibrosis. These preliminary results support the hypothesis of an iron-related harmfulness of coal mine dusts. — Environ Health Perspect 102(Suppl 5):265-268 (1994)

Key words: coal workers pneumoconiosis, lung dusts, biopersistence, quartz particles, iron-release

Introduction

Coal workers' pneumoconiosis usually is thought to relate to the presence of quartz in the respirable fraction of coal mine dusts. However, epidemiological and experimental evidence indicate that factors modulating the harmfulness of quartz-related or nonquartz-related factors must be involved (1,2). Comparative studies of the toxic potency of authentic coal mine dusts and artificial quartz/coal mixtures have suggested that quartz toxicity in authentic coal mine dusts may be inhibited by protective impurities on the quartz surface (3). This became convincingly apparent after laser microprobe mass spectrometric (LAMMS) studies of natural silica dusts and authentic coal mine dusts were performed (4-6). These analyses at the level of individual particles have revealed that only a minute fraction of "pure" quartz particles (typically about 1% of the particle population) may be detected in coal mine dusts. Most of the quartz surface in such highly heterogeneous particulate matter appeared to be covered by aluminosilicates.

To determine the long-term in vivo stability of these protective mineral layers, we performed this LAMMS study of coal mine dusts recovered by post mortem formamide digestion of coal workers' lungs featuring different silicosis grades. Especially for lungs with a high silicosis grade, recovered dusts exhibiting a high fraction of pure quartz particles were expected to be found as compared to authentic coal mine dusts and dusts recovered from lungs with low silicosis grade.

Material and Methods

Lung Digestion

Lung digestion and dust recovery were performed by H.J. Einbrodt (Institute of Hygiene and Occupational Medicine, University of Aachen, Germany). Lung digestion in a water-free and neutral milieu such as formamide avoids any alteration of the mineral matter and organic fraction of coal (7). Formamide digestion was performed at 135°C for 72 hr, followed by filtration and washing with distilled water (8). Since regional lymph nodes were isolated before digestion of the lungs, mainly intraalveolar and interstitially accumulated dusts were recovered.

Recovered Lung Dusts

Eleven samples were investigated, recovered from lungs of 11 coal miners from the Ruhr district (Germany) who died during the 1960s with low (+), medium (++), or high (+++) silicosis grades. Silicosis grades relate to pathological criteria: (+), for mainly diffuse or micronodular silicotic lung lesions of simple pneumoconiosis,

| Sample | Silicosis grade | Years underground | Ash content \(^a\) | Gross quartz content \(^a\) |
|--------|----------------|-------------------|-----------------|-------------------|
| L38    | +              | 16.0              | 16.0-24.0       | 4.3               |
| L42    | +              | 18.0              | 17.0            | 3.6               |
| L118   | +              | 25.0              | 33.0-38.0       | 5.0               |
| L214   | +              | 25.0              | 15.0            | 1.8-3.4           |
| L215   | +              | 19.5              | 25.0-30.0       | 2.5-4.2           |
| L52    | ++             | 30.0              | 38.0            | 6.2               |
| L246   | ++             | 30.0              | 10.0-12.0       | 0.5-2.0           |
| L233   | ++             | 43.0              | 54.0            | 1.0-9.0           |
| L236   | ++             | 23.5              | 24.0-27.0       | 3.4-5.4           |
| L237   | ++             | 23.5              | 16.0-18.0       | 2.0-3.0           |
| L239   | ++             | 22                | 65.0            | 33.0              |

\(^a\)Wt-%
(++, for nodular fibrosis, and (+++), for typical massive and nodular lesions of progressive and massive fibrosis ([9]; HJ Einbrodt, personal communication). Silicosis grade of the lungs, duration of miner’s underground activities, ash content, and gross quartz content (in wt-%) of the 11 investigated samples are given in Table 1 (after Einbrodt).

Reference Dusts
Since the occupational history of some miners was difficult to reconstruct, 10 reference samples of the respirable fraction of coal mine dusts were selected according to two criteria: underground sampling during the 1960s in five stratigraphic horizons of the Ruhr coal field (ranging from the low-ranking Horstler- to the high-ranking Bochumer-coal strata); and choice of two samples per stratum featuring major differences (up to 20 wt-%) in their ash contents. In this way, reference dusts with widely differing ash contents (between 10 and 60 wt-%) were analyzed to provide comparative data on the dusts to which the miners were exposed (especially the elements that were present).

Laser Microprobe Mass Spectrometry (LAMMS)
Gross chemical and mineralogical analyses of coal mine dusts (using, for instance, infrared-spectroscopy) take no account of the heterogeneity of individual particles. For instance, a dust sample containing 10 wt-% of quartz (as determined by conventional bulk analyses) could mean either that 10% of the particle population consists exclusively of quartz or, at the other extreme, that all particles have an admixed fraction of 10 wt-% quartz. Therefore, single particle analysis becomes a mandatory approach in a strategy to correlate toxic potency with physicochemical parameters of dust samples.

The present paper deals with such a single particle approach using LAMMS. A LAMMS instrument basically consists of three components: a light microscope used to select a point of analytical interest in the sample; a laser source used to vaporize and ionize microwevels of samples; and a time-of-flight (TOF) mass spectrometer to identify ions generated by the laser source. With the LAMMS technique it is possible to analyze atomic constituents and, to a certain extent, molecular constituents of samples, with a spatial resolution in the 1-μm range and detection limits down to the (sub)ppm range (10). LAMMS may be considered a surface analysis, especially when the instrument is operated with low laser irradiation to concentrate the analysis on the outer shells rather than on the bulk volume of individual particles. Results of single particle analyses are given in the form of chemical histograms characterizing the different specimens according to selected group parameters. Such incidence data (in particle-%) give the percentage of particles featuring certain elements or combinations of elements.

In the present study, LAMMS analyses were performed on dust samples deposited by adhesion on a formvar-coated electron microscope grid. A modified version of the LAMMA 500 instrument (Leybold-Heraeus, Cologne, Germany) was used to analyze dusts using the positive-ion mode and operated with low laser irradiation. Incidence data for pure quartz particles and typical major elements of the minerals present in coal mine dusts (i.e., Si, Na, Mg, Al, K, Ti, Ca, and Fe) were obtained and compared with average data of 10 reference dusts. Signal intensities of each element were semiquantitatively weighted by normalization to the 40K and/or 44Ca isotopic signals used as internal standards and detection limits. Analyses were performed on 400 and 100 particles per sample for both the recovered and reference dusts. Size of analyzed particles was within the maximum size distribution for each sample (between 0.5 and 1.5 μm).

Results
A previous study of silica reference dusts (natural quartz sands and rock crystals) showed that LAMMS could discriminate between pure quartz particles and an admixture of quartz and clay particles, based on the Si/Al signal intensity ratios in the mass spectrum of individual particles (4). Other LAMMS investigations of various coal mine dusts showed that the fibrogenic potency of some coal quartz-rich samples correlated better with the incidence of pure quartz particles than with the gross quartz content (5.6). In the present study of recovered lung dusts we hoped, therefore, to find a positive correlation with silicosis grade.

However, no relationship was demonstrated between silicosis grade and incidence of pure quartz particles (Figure 1). Moreover, maximum incidence of pure quartz particles was about 1.3% of the particle population for the 11 recovered dusts (Figure 1). Even for sample L239, which had an extremely high gross quartz content (33 wt-%, Table 1), only a minute fraction of pure quartz particles could be detected. By comparison, incidence of such particles in authentic coal mine dusts usually represent up to 1.5% of the particle population (even for quartz-rich samples with gross quartz content up to 25 wt-%) (5.6). It may, therefore, be assumed that long-term contact between the mineral matter of coal mine dusts and human lung tissues does not disclose additional pure quartz particles.

Since pathological findings apparently do not relate to the presence of pure quartz particles or in vivo instabilities of protective clay mineral layers, it appears mandatory to search for other dust parameters that might correlate with silicosis grade. Incidence data for some typical elements of the mineral matter of coal mine dusts (mainly aluminosilicates) are presented in chemical histograms for Si (Figure 2), for Mg (Figure 3), for Na (Figure 4) and for Ti (Figure 5). No positive correlation with silicosis grade exists. It merely appears that both mineral-rich samples L223 and L239 with high ash content (Table 1) had a high incidence of Mg-containing particles compared to the other lung dusts or reference dusts.

Incidence data for marker elements of clay minerals such as Al (Figure 6), K (Figure 7), and Ca (Figure 8), showed no positive correlation with silicosis grade. However, dusts recovered from silicotic lungs had higher Ca-incidence, compared to reference dusts (Figure 8). Additional calcium relates to well known microlithifications of endogeneous origin, occurring in response to the presence of dusts in lungs.

Dust recovered from lungs with nodular (+++) and massive (++++) fibrosis had lower Fe-incidence compared to lungs with lower silicosis grade (+) (Figure 9). Average incidence data are about 58 particle-% for dusts recovered from lungs with low (+) silicosis grade and 17 particle-% for higher (+++ and ++++) silicosis grades. From these data, we can conclude that nodular and massive fibrosis related to in vivo release of

![Figure 1. Incidence of pure quartz particles for the 11 lung dust samples.](image-url)
exogeneous iron, although iron in lungs with simple pneumococosis might also relate to deposition of endogeneous iron (II). Release of iron is more convincingly revealed by comparison between average incidence data for lungs with high silicosis grade (17 particle-%), and reference dusts (40 ± 8 particle-%) which roughly correspond to the average incidence data for dusts recovered from lungs with low silicosis grade (58 particle-%).

Discussion

There are many reports of the inhibition of quartz toxicity in various quartz-containing dusts (J–6). Protective effects of aluminosilicates layers and other surface impurities, which hinder the interaction between quartz surfaces and biological cells or tissues, have been considered. LAMMS investigations at the level of individual particles have demonstrated that most of the quartz in coal mine dusts is hidden within clay mineral particles, since the maximal incidence for pure quartz particles is about 1.5 particle-%. Removal of impurities from silica particles by chemical treatment leads to an increase of the cytotoxicity of natural silica dusts (J2). One might assume that dusts undergo similar removal of protective surface contaminations during a long-term contact with biological tissues. It might have been expected that instabilities of the surface layers on quartz would result in an increased incidence of pure quartz particles higher than 1.5 particle-%, or in a positive correlation with silicosis grade.

The results showed, however, that most of the quartz of the dusts recovered from silicotic lungs still was contaminated by clay minerals. Neither increased incidence of pure quartz particles nor correlation with silicosis grade was observed. Special attention was paid to sample L239 with its unusually high gross quartz content (33 wt-%; Table 1). We could have expected that this dust would have a high incidence of pure quartz particles, coming from an authentic dust originally containing a high incidence of pure quartz particles. However, incidence of such particles was similar for sample L239 and for quartz-rich authentic coal mine dusts (25 wt-%), containing only 1.5 particle-% of pure quartz particles. Moreover, sample L239 did not exhibit more surface-available quartz than the other lung dusts. Therefore, it appears that aluminosilicate layers on quartz do not dissolve completely and consequently do not reveal new quartz particles with free surfaces. Protective layers persist, even during long-term contact with human lung tissues (up to 40–50 years).

However, such surface layers do undergo partial dissolution and apparently preferential leaching out, especially of iron. Comparison between incidence of Fe-containing particles of reference dusts and of dusts recovered from lungs with various silicosis grades demonstrates that release of iron occurred in lungs with nodular and massive fibrosis (Figure 9). Fe-containing
particles consist basically of a matrix of clay minerals (mainly illite and mica). Their typical mass spectra are Al, K-dominated. The presence of Fe-signals in the mass spectra of reference dust particles is usually associated with simultaneous Mg-signals. In the present study, the recovered dusts L223 and L239 showed high incidence of magnesium (Figure 3) and might have been expected simultaneously to demonstrate higher incidence for iron. This was not the case, however, since iron apparently is released preferentially compared to other elements present in the mineral matter of coal mine dusts (especially Mg). Similar selective in vivo release of elements, including Fe, from illite, in parallel with progressive transformation of the crystalline structure, have been observed in animal experiments (13).

Earlier LAMMS investigations also showed positive correlations between incidence of Fe-containing particles and cytotoxicity of quartz-poor coal mine dusts (6) first evidence about the fibrogenic potency of iron-bearing minerals (most probably ferrous iron) in coal mine dusts have also been published (14). These dusts induced nodular fibrotic reactions, but a recent study has been made of two quartz-rich coal mine dusts with equal gross quartz content (approximately 7 wt-%) that produced widely different pulmonary reactions in animal lungs (15). Inhalation of one dust caused fibrotic nodules, the other dust did not. LAMMS investigation revealed that fibrogenicity of both samples was negatively correlated with the incidence of pure quartz particles but positively correlated with the incidence of Mg-containing particles, suggesting that the quartz-related harmfulness of coal mine dusts is most probably potentiated by Fe-containing mineral particles.

These observations are in line with those on the catalytic role of iron in the Fenton reaction, which occurs at the surface of both fibrous and nonfibrous minerals leading to an active release of toxic hydroxyl free radicals (16). Moreover, iron leaches from asbestos both in vivo and in vitro (17,18), and when mobilized from asbestos minerals, may have a stronger oxidation efficiency than iron that remain associated with the fibers (19,20).

**Conclusion**

Impurities on the surface of quartz particles strongly inhibit the quartz-related harmfulness of various quartz-containing dusts. Previously published LAMMS investigations of coal mine dusts have clearly demonstrated that most of the quartz of such mixed dusts is hidden within protective clay mineral particles. The present study indicates that long-term contact between the mineral matter of coal mine dusts and human lung tissues does not affect the persistence of the protective layers on quartz. These preliminary results suggest also that there is a relationship between massive nodular fibrosis and iron leached from the dusts, although a dose effect or individual susceptibilities might also play a role in the development of this pathology. This and other published LAMMS investigations are part of a growing body of evidence indicating that coal workers' pneumoconiosis relates to the presence in coal mine dusts, not only of surface-available quartz but also of Fe-containing minerals. Future investigations also will concentrate on the biological fate of iron released from mineral particles.

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