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Airway inflammation in iron ore miners exposed to dust and diesel exhaust

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ABSTRACT

Objective. To investigate if underground miners exposed to dust and diesel exhaust in an iron ore mine would show signs of airway inflammation as reflected in induced sputum.

Methods: Twenty-two miners were studied twice, after a holiday of at least two weeks and after three months of regular work. Twenty-one "white-collar" workers acted as controls. All subjects completed a questionnaire regarding medical and occupational history, and underwent lung function testing and induced sputum collection. Total and differential cell counts and analyses of the fluid phase of the induced sputum were performed. Sampling of personal exposure to elemental carbon, nitrogen dioxide, and inhalable dust were recorded.

Results: The average concentration of inhalable dust was 3.2 mg/m^3, nitrogen dioxide 0.28 mg/m^3 and elementary carbon 27µg/m^3. Miners had increased numbers of inflammatory cells, mainly alveolar macrophages and neutrophils, and increased concentrations of fibronectin, metalloproteinase-9 and IL-10 in induced sputum compared with controls.

Conclusion: Miners in an underground iron ore mine demonstrate persistent airway inflammation, that is as pronounced after a four week holiday period, as after a three months work period underground in the mine.

184 words

INTRODUCTION

Studies have shown increased occurrence of respiratory diseases such as silicosis and bronchitis in miners (1, 2). The air pollution in mines is complex and variable. The emissions come from a variety of sources, including dust from drilling, blasting and handling of the ore/rock and exhaust from vehicles. Silicosis has a well-defined causative agent, silica dust, but the cause of chronic bronchitis and chronic obstructive pulmonary disease in miners is less clear (3-5). Even if air pollution in mines may have decreased in recent years due to improved ventilation and new techniques, the levels might still be higher than in other occupational environments as the mining occurs in closed spaces. In the past, quartz dust was a serious hazard in Swedish iron mines, but after extensive improvements of the working environment since the beginning of the 1970-ies, the risk of developing silicosis in miners today is low (2). Studies of Swedish iron mines have indicated an increased risk of chronic bronchitis, however only in smoking underground miners (3-5).

The airway effects of high exposures to diesel exhaust have been investigated in controlled experimental studies (6-13). The methods for investigation have included lung function measurements (6, 9), bronchoscopy with bronchial biopsies and airway lavages (7,8,10) and, more recently, induced sputum (11-13). Study populations have included healthy control subjects as well as asthmatics with different disease severity (6, 10-13). The experimental diesel exhaust exposure studies have shown neutrophilic and lymphocytic inflammatory responses in the airway mucosa, bronchial lavage and sputum as well as markers of activation of various cell types (6-12). However, the experimental studies have only employed short-term exposure at high concentrations, and it is unclear if the results can be generalised to long-term occupational exposure at lower levels.

The aim of this study is to investigate if airway inflammation, reflected in induced sputum, is present in healthy non-smoking underground miners working in an iron mine with high levels of dust and diesel exhaust. To the best of our knowledge, this is the first study to investigate miners in a true occupational situation, using induced sputum to assess airway inflammatory responses.

METHODS

The mine environment

This study was undertaken in a Swedish iron ore mine, where all mining since the 1920-ies has been underground. The dominant mode of mining is sub-level caving. Diesel powered engines were introduced in the mine in the mid 1960-ies.

The air pollution in the investigated mine is complex. The most important sources include diesel exhaust and rock dust, which is being vortexed into the air by the traffic in the mine, and/or dust drilling, loading and crushing of the rock/ore. Blasting routinely happens at midnight. The mine is then ventilated until five o´clock (AM), at which point the miners can enter the mining areas if the levels of carbon monoxide and nitrogen dioxide are satisfactory. However, these blasting gases are also released to some extent during handling of the blasted rock and ore.

Subjects

Healthy underground miners were invited to take part in the study. Eligible subjects were identified from the company health-service registry according to the following entry criteria:

no history of respiratory disorder including bronchitis or any other respiratory symptoms, never-smokers or ex-smokers for five or more years, no history of eye and/or nose allergies, no treatment with anti-inflammatory drugs for any condition, underground work in the mine

for at least three years and, finally, a work site underground with exposure to diesel exhaust during loading, lorry driving, road maintenance or construction work.

Control subjects

Healthy non-smoking workers (research workers not affiliated with the study, technical staff and office workers), without a history of respiratory conditions, allergies and other disorders and without any relevant medications were invited to participate as control subjects.

All subjects received both verbal and written information about the study and consented to participation. The Ethics Committee of Umeå University approved the study.

Study design

The miners were investigated twice. The first examination took place after at least four weeks of summer holiday in August, and before returning to the mine. This was made to investigate subjects under conditions free of recent occupational exposure to diesel exhaust or dust. The second examination was performed in November after at least three months of regular work in the mine.

The investigation included a thorough occupational history pertaining to exposure to dust and diesel exhaust, as well as previous disorders especially focused on respiratory conditions and allergies. No respiratory infection within the last four weeks of each examination was allowed. Lung function measurements were performed prior to a sputum induction.

The control subjects were studied at a single time point during wintertime.

Lung function

Lung function measurements were made using a dry bellows spirometer (Vitalograph, Vitalograph Ltd, Buckingham, UK). Three reproducible measurements of forced vital capacity (FVC) and forced expiratory volumes in one second (FEV_1) were performed and the best value recorded. The values were expressed as percent of predicted normal using the reference values from the Coal and Steel Union (14).

Sputum induction

Sputum induction was performed by a method described by Pin et al (15) and slightly modified. Hypertonic saline (4.5%) was nebulized using an ultrasonic nebulizer (DeVilbis 2000, DeVilbis Co, Somerset, PA, USA) with an output of about 1.5 $mL \cdot min^{-1}$.

All subjects were pretreated with an inhaled β_2-agonist (0.2 mg salbutamol) before the induction. Inhalation of the hypertonic saline was made at 3 intervals of 5 minutes.

FEV_1 was monitored before and after each inhalation period. Following each inhalation period, subjects were advised to blow their nose and rinse their mouth with water before coughing sputum into a sterile container. The samples obtained were kept on ice up to 1 h before processing.

Sputum processing

Sputum was processed according to the method described by Pizzichini et al (16). At least 400 non-squamous cells were counted and differential cell counts were expressed as percentage of the total non-squamous cell count. The proportion of squamous cells was obtained by counting 400 additional cells and expressing this as a percentage of the total number of cells. Samples were considered adequate for analysis if the squamous cell

contamination was < 20 % and the viability > 50%. The total cell count was calculated by dividing the total number of cells by the volume of processed sputum (1mg=1μL).

Fluid phase measurements

Fluid phase measurements of matrix metalloproteinase 9 (MMP-9) and fibronectin reflect the activity of alveolar macrophages. Myeloperoxidase (MPO) is an oxidising enzyme and levels reflect neutrophil activation. Interleukin-10 is a inflammatory cytokine with predominantly inhibitory actions and is an important modulator of monocyte/macrophage function (17-20).

Myeloperoxidase (MPO)

Myeloperoxidase was analysed using a radioimmunoassay (Pharmacia MPO RIA, Pharmacia&Upjohn Diagnostics Sverige AB, Uppsala, Sweden. The detection limit of this assay was <8 μg/L.

Interleukin –10 (IL-10), metalloproteinase-9 (MMP-9), fibronectin

Interleukin-10, metalloproteinase-9 and fibronectin were measured by ELISA technique (IL-10 and MMP-9 kits from R&D systems Europe Ltd, Abingdon, UK and fibronectin from Pharmacia &Upjohn Diagnostics, Uppsala, Sweden). The minimum detection limit for IL-10 was < 0.5 pg/ml, MMP-9 < 0.156 ng/ml and fibronectin was 10 μg/L.

Air sampling

Elemental carbon, nitrogen dioxide and inhalable dust were measured with sampling equipment attached to the subjects and measured in the breathing zone of the workers. Two measurements of elemental carbon and nitrogen dioxide, and three measurements of inhalable

dust were obtained for each miner. Occupational exposures are often quasi-log-normally distributed, that is the log-transformed concentrations follow the normal distribution (21, 22). By multiple measurements of each worker the total variance in a group of workers can be divided by within-worker (day to day) and between-worker (within jobs) variance. A uniformly exposed group characterized by a small between-worker variance is sometimes measured by the ratio ($_BR_{95}$) of the 97.5th to the 2.5th percentiles of the means. A group with a ratio below 2 is often considered to be uniformly exposed (21).

Elemental carbon

Elemental carbon was sampled on a glass fibre filter (SIMPEDS) and the amount was determined by colorometric analysis. The colorometric analysis was performed according to standard VDI 2465 at a Swiss laboratory (Institute Universitaire Romand de Sante´au Travail, Lausanne, Switzerland).

Inhalable dust

The amount of inhalable dust was measured with an IOM personal sampler (SKC Inc, PA, USA) and sampled according to the standard EN 481 (23). This samples the fraction of particles that enters the nose and mouth with a 50% cut off point of particles with a diameter of 100 μm (24). The sampling flow was 1.7-2.1 L /min. The amount of dust was determined by weighing the filters.

Nitrogen dioxide.

Nitrogen dioxide was measured using personal diffusive samplers and ion chromatographic analysis (25).

Statistics.

Empirically, occupational exposures are usually log-normally distributed (21) and the Shapiro-Wilk test was used to test the hypothesis of lognormal distributions.

Paired t-tests were used to compare lung function measurements in miners between investigations. Independent sample t-tests were used for comparisons between subjects and controls. Sputum cell counts and fluid phase measurements, are given as median and interquartile range For the fluid phase measurements the Wilcoxon´s test was used for comparisons in miners between investigations, and a Mann-Whitney U test was used for comparisons between miners and controls. P- values < 0.05 were considered statistically significant.

RESULTS

Demographics and lung function

The initial study population comprised 29 male miners. However, six miners were excluded from all analyses due to inability to produce sputum of sufficient quality for the cell analyses, and one was unable to complete both investigations. Therefore, data is presented for a total of 22 miners from whom paired sputum samples were available. The mean age was 43 years (30-59 years), and whilst all were current non-smokers, 10 were never-smokers and 12 ex-smokers. Of the ex-smokers, six had been regular smokers (five had been smoke-free for 10 or more years, and one had been free of smoking for at least six years) and six were previous occasional smokers. Five of the regular ex-smokers had 10-12 pack-year and one had a six pack-year history of smoking. None of the subjects had a history of chronic bronchitis.
The average number of years spent working underground was 18 (3-39 years).

The control group comprised 24 men, researchers, technical staff and office workers without allergies or history of respiratory or other disorders. All but one were never-smokers. In the control group, the sputum samples were of insufficient quality to be analysed further in three cases. Thus, data from 21 control subjects are reported. The mean age was 40 years (25-63 years). All subjects, both miners and controls had normal lung function measurements. **(Table 1).**

No significant differences in lung function were seen between miners and controls. (Lung function measurements in Table 1 are from the first assessment of the miners after the holiday). There was no difference in the lung function of the miners between the two test occasions.

Sputum cell findings

Cell viability in sputum was good, with mean percent viability in miners 79 % and in control subjects 78%. There were statistically significant differences in the total number of cells/ml of sputum between miners and controls. This was mainly due to increased numbers of macrophages, and neutrophils in the miners. There were no significant differences in numbers of lymphocytes or eosinophils between groups. **Table 2**

The total cell numbers and the differential cell counts were not statistically different between the first and the second sputum induction in the miners. **Table 3**

Fluid phase measurements

Levels of MPO measured in sputum from miners on both occasions, and controls did not differ. Fibronectin levels and IL-10 were elevated in sputum from miners from both inductions, compared to control subjects, but there was no difference between assessments. MMP-9 levels were greater in miners than controls, but were reduced following a 3 months period at work.

Table 4

Exposure

Nitrogen dioxide

Twenty-nine samples from 18 individuals were collected. For 11 miners the measurements were made on two occasions. The mean value was 0.28 mg/m^3 (range 0.05-0.68mg/m^3).

Elemental carbon

In total 27 measurements were made for 18 miners and in 8 individuals the measurements were made on two separate occasions. The mean value for all measurements was 27 μg/m^3 (range 5-61μg/m^3).

Inhalable dust

Forty-one measurements in 14 miners were obtained. For all individuals two or three measurements were made on separate occasions. A single high value of 35 mg/ m^3 was included in the analysis. The second highest value was 9.3 mg/m^3. The mean dust level was 3.2 mg/m^3 (range 0.1– 35 mg/m^3).

For all exposures the hypothesis of lognormal distributions could not be rejected. For nitrogen dioxide and elemental carbon the variations within miners were higher than between miners. The ratios between the 97.5th and the 2.5th percentile of the log-normally distributed mean exposures of the miners (between-worker distribution), equivalent to a factor containing 95% of the individual mean exposures derived from lognormal distribution, were for nitrogen dioxide 1.2, elemental carbon 1.6, and inhalable dust 5.0.

Table 5

DISCUSSION

The air pollution in mines is complex. Particulates in this mine have two major sources, dust from the rock/ore and diesel exhaust. The concentration of inhalable dust was variable both between and within workers, but was much higher than the concentrations of elemental carbon (EC), measured to reflect diesel particles. The between worker variability, here measured as $_{B}R_{95}$, was different for inhalable dust and EC, but similar for nitrogen dioxide and EC. Nitrogen dioxide and EC are mainly measures of exposure to diesel exhaust, while inhalable dust reflects the contribution from rock and ore.

Elemental carbon concentrations of 27 μg/m^3 are higher than would be encountered in a busy street or road.. Zaebst and co-workers found average levels of around 5 μg/m^3 in truck drivers, measured inside the truck (26). However, our EC levels are considerably lower than the German maximum allowable concentration (MAC) values of 300 μg/m^3 during occupational underground work (27). The average concentration of nitrogen dioxide of 0.28 mg/m^3 (0.16 ppm) found in this mine, is well below the Swedish MAC of 2 mg/m^3 for nitrogen dioxide from diesel exhaust (28), but considerably higher than concentrations of nitrogen dioxide in some Swedish cities, where background levels below 0.03 mg/m^3 are common (29). For the general environment there is a limit of 0.09 mg/m^3 (30).

The level of inhalable dust in the mine, average 3.2 mg/m^3, was also considerably higher than dust levels in the general environment. The MAC for inorganic dust, sampled as total dust, in Sweden is 10 mg/m^3 (28). There is no direct conversion between dust sampled as total dust and that of inhalable dust, but it seems reasonable to assume that the level of total dust in the mine would be below the MAC.

The induced sputum measurements indicate an ongoing inflammatory process in the airways of miners, which was present even after four weeks of summer vacation. The number of inflammatory cells in induced sputum of miners was more than double that of controls with the increase driven by a three-fold increase in alveolar macrophages. Macrophages have an important role in the clearance of inhaled particles. Substantial increases in macrophage numbers have been discussed following occupational exposures to organic and inorganic dust, and following controlled exposure to diesel exhaust (8,31). Increased numbers and lifespan of alveolar macrophages have also been found in cigarette smokers (32). In the present study, however, most miners were ex-smokers for many years, had a limited number of pack-years of smoking, and had normal lung function and no respiratory symptoms. Therefore, the increase in macrophages is considered to be driven by the dust load from the underground mining and not by their previous smoking.

The macrophage is thought to be the main source of fibronectin, which in miners was more than double that of controls. Fibronectin is a proinflammatory glucoprotein that enhances phagocytosis and is involved in cell-cell interaction at sites of inflammation. Macrophages can furthermore be a source for matrix metalloproteinase-9 (MMP-9). This metalloproteinase is involved in airway remodelling due to its ability to cleave both structural proteins as well as regulatory proteins in the airways. It can also modify cellular function by interaction with cytokines and other factors. MMP-9 is also produced by neutrophils, which were similarily increased in the induced sputum of miners. Neutrophils might act synergistically with the macrophages in response to inhaled particles and potentially cause damage to the lung. Interestingly, despite the increased numbers of neutrophils, there was no increase in myeloperoxidase, an oxidative enzyme and marker of neutrophil activation. Increased neutrophil numbers have not been an omnipresent finding after particle exposures, but

consistently raised following exposure to diesel exhaust. While neutrophilic inflammation may be present in earlier stages of diesel exhaust induced inflammation, the activation of these cells appears to be a later event.

When the study was proposed it was assumed that miners would have an airway inflammatory response due to exposure in the mine during a work period. A period away from work, such as a holiday, was expected to allow recovery from a potential inflammatory state in the airways. Surprisingly, the (non-significant) trend was for a higher level of inflammatory cells after the holiday than after the 3 months period at work. This increase was most noticeable for alveolar macrophages. Interestingly, MMP-9, with its ability to induce airway remodelling as a result of a noxious challenge, was significantly higher in the miners after the holiday as compared to during the work period, and the level was increased significantly compared to the control subjects.

Interleukin 10 (IL-10) is an important regulatory cytokine which mainly has an inhibitory function. It originates from T-lymphocytes, epithelial cells and other cells. While this cytokine was undetectable in most control subjects, the miners had substantially elevated levels in induced sputum. The levels were considerably higher during the work period than after the summer vacation, in contrast to MMP-9, and cell numbers which tended to be higher after the summer holiday. The acute inflammatory events following air pollution particle exposure seems to be driven by a unique set of signal transduction pathways and kinases resulting in enhanced production of chemoattractants from epithelial and other cells and thus resulting in an influx of inflammatory cells such as neutrophils, macrophages and lymphocytes. IL-10 is one of the major inhibitory cytokines that acts to normalize acute phase responses and to prevent uncontrolled inflammation. The present data suggest that airway

cells, such as, bronchial epithelial cells, may secrete IL-10 to suppress the particle induced inflammation and prevent an uncontrolled response. The greatest requirement for this regulatory control would be during the work period (33)

In conclusion, the miners in an underground iron ore mine demonstrate an airway inflammatory response in induced sputum, with increased levels of macrophages, neutrophils, fibronectin and MMP-9. Interestingly, the inflammation was as pronounced after a four-week holiday period as after a three months work period underground in the mine. We suggest that interleukin-10, due to its ability to modulate monocyte/macrophage function and inflammatory events, may play a role in controlling airway inflammation caused by inhalation of rock/ore dust and diesel exhaust.

To the best of our knowledge this is the first study to address airway inflammation using induced sputum in a true occupational life situation in miners. Additional studies need to be performed to investigate the long-term risk of particle induced chronic airway inflammation.

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Table 1: Demographics and lung function

	Miners **n=22** **(all males)**	Control subjects **n=21** **(all males)**
Age (mean (range))	43 yrs (30-59)	40 yrs (25-63)
Smoking habits	10 never-smokers 12 ex-smokers	20 never-smokers 1 ex-smoker
Mean working time underground (mean (range))	18 yrs (3-39)	-
FEV_1 (% of predicted normal)	105 (11.6)	109 (11.4)
VC (% of predicted normal)	101 (9.9)	106 (10.2)
$FEV_{\%}$	102 (5.7)	101 (5.8)

FEV_1=forced expiratory volume during one second; VC=vital capacity; $FEV_{\%}$=FEV1/VC; SD=standard deviation

Table 2: Sputum cells, miners I vs control subjects

*Miners I=investigated during holiday **before** work period*

	Miners I **n=22** **median (IQR)**	Control subjects **n=21** **median (IQR)**	p-value
Total cells x 10^6/ml	4.4 (3.1-6.5)	1.8 (1.6-2.6)	0.0001
Macrophages x 10^6/ml	2.3 (0.9-3.7)	0.8 (0.5-1.3)	0.002
Neutrophils x 10^6/ml	1.5 (0.6-3.6)	0.5 (0.4-1.3)	0.025
Lymphocytes x 10^6/ml	0.1 (0.05-0.4)	0.06 (0.02-0.13)	0.056
Eosinophils x 10^6/ml	0.01 (0.00-0.02)	0.00 (0.00-0.01)	NS

IQR=interquartile range

Table 3: Sputum cells in miners at the end of the holiday (I) vs miners after at least 3 months work period (II)

	Miners I **n=22** **median (IQR)**	Miners II **n=21** **median (IQR)**	p-value
Total cells x 10^6/ml	4.4 (3.1-6.5)	3.0 (2.0-4.4)	NS
Macrophages x 10^6/ml	2.3 (0.9-3.7)	1.4 (0.8-2.5)	NS
Neutrophils x 10^6/ml	1.5 (0.6-3.6)	1.1 (0.4-2.0)	NS
Lymphocytes x 10^6/ml	0.1 (0.05-0.4)	0.07 (0.02-0.15)	NS
Eosinophils x 10^6/ml	0.01 (0.00-0.02)	0.01 (0.00-0.03)	NS

IQR=interquartile range

Table 4: Fluid phase measurements in miners at the end of the holiday vs. after at least 3 months at work.

	Miners I **n=22** **Median (IQR)**	Miners II **n=21** **Median (IQR)**	Control subjects **n=21** **Median (IQR)**
MPO (mg/mL)	312 (210-491)	244 (140-416)	185 (106-342)
Fibronectin (µg/L)	108 (56-124)*	120 (64-130)†	50 (28-92)
MMP-9 (ng/mL)	48 (32-120)	38 (17-64)‡	28 (13-46)§
IL-10 (pg/mL)	0.7 (0.0-1.4)¶	1.2 (0-4)**	0.0 (0.0-0.4)

MPO=myeloperoxidase; MMP-9=matrix metalloproteinase-9; IL-10=interleukin-10; IQR=interquartile range
*Miners I vs control subjects p<0.001;
†Miners II vs control subjects p=0.02
‡Miners I vs Miners II p=0.048
§Miners I vs control subjects p=0.03
¶Miners I vs control subjects p=0.02
** Miners II vs control subjects p=0.004

Table 5: Levels of pollutants in the mine and variability between subjects

	N	K	Concentration mean (range)	$_{B}R_{.95}$
NO_2 (mg/m^3)	29	18	0.28 (0.05-0.68)	1.2
Elemental carbon ($\mu g/m^3$)	27	18	27 (5-61)	1.6
Inhalable dust (mg/m^3)	41	14	3.2 (0.1-35)	5.0

N: number of measurements, K: number of subjects
$_{B}R_{.95}$: Ratio of the 97.5th and 2.5 percentile of the log-normally distributed mean of the workers (between worker variability)