Investigation of the relative contributions of cigarette smoking and mineral dust exposure to activation of circulating phagocytes, alterations in plasma concentrations of vitamin C, vitamin E, and β carotene, and pulmonary dysfunction in South African gold miners

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Abstract

Objectives—To determine the relative effects of cigarette smoking and mineral dust exposure on numbers and activity of circulating phagocytes, plasma nutritional antioxidant state, and pulmonary function in South African gold miners.

Methods—Pulmonary function was assessed spirometrically, whereas reactive oxidant generation by circulating phagocytes, and plasma concentrations of the nutritional antioxidant nutrients vitamin C and vitamin E and β carotene were measured with chemiluminescence, spectrophotometry, or high performance liquid chromatography respectively.

Results—Cigarette smoking, but not mineral dust exposure, was associated with increased numbers and pro-oxidative activity of circulating neutrophils and monocytes, decreased plasma concentrations of vitamin C, and pulmonary dysfunction.

Discussion—In this study group occupational exposure to mineral dust has not been found to promote increases in the numbers or reactivity of circulating phagocytes or to be a significant cause of pulmonary dysfunction, the changes found being due primarily to cigarette smoking.

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Although chronic airflow obstruction is an important cause of mortality and morbidity among South African gold miners, controversy exists as to the relative contributions of cigarette smoke and dust.¹ Some sources regard cigarette smoke as the only established cause of airflow limitation,² but evidence is accumulating that seems to implicate occupational exposure to certain dusts such as fibrous particulates,^{3,4} and that associated with hard rock mining.⁵⁻⁷

In South African gold mines 30% of respirable dust is composed of free silica,⁸ and this has been shown to have an additive effect on mortality in miners who smoke.⁹ Also, morphological studies have documented effects in both miners^{10,11} and animals,¹² and the pathological

lesions in the bronchioles have been well described. Functionally, it is recognised that both airflow limitation and restriction occur, and it is postulated that these effects may be different manifestations of the same underlying inflammatory process.^{12,13} Macrophages ingest silica particles and in response release chemotactic cytokines, which attract hyperactive polymorphonuclear leucocytes (PMNLs) to the lungs. This process is perpetuated by the death of the macrophage and repetitive rephagocytosis of the silica particles.¹⁴⁻¹⁷ This cycle accounts for the high output of pulmonary macrophages and PMNLs even after brief exposure.¹⁵

Cigarette smokers likewise have excessive numbers of hyperactive PMNLs in the pulmonary parenchyma,¹⁸ and it is probable that both smoking and mining related diseases are mediated by the release of PMNLs and reactive oxidants derived from macrophages in the pulmonary interstitium.¹⁹⁻²¹ Release of reactive oxidants in excess of physiological protective mechanisms results in chronic elastolysis as a consequence of the functional inactivation of α -1-antiprotease²² and the autoactivation of PMNL derived metalloproteinases.²³ Also, lipid peroxidation,²⁴ DNA injury,²⁵ and cellular energy depletion²⁶ contribute to direct cellular injury. Consequently, intact antioxidant defences are of critical importance and may determine susceptibility to disease.²⁷

In this study we have measured and compared pulmonary function, reactive oxidant generation, total leucocyte count, and nutritional antioxidant state in the blood of gold miners who were smokers, non-smokers, and ex-smokers. These were also compared with similar variables from non-occupationally exposed non-smokers as controls.

Methods

STUDY POPULATION

Three hundred and eleven male white gold miners who presented for their annual medical examination at the Medical Bureau for Occupational Diseases in Johannesburg were randomly recruited to the study. Their mean (SEM) age was 39(1) years and cumulative dust exposure was 10 407 (1385)

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Table 1 Smoking and mining histories of participants

Variables	Mineworkers			Non-exposed non-smoker controls
	Smokers	Non-smokers	Ex-smokers	
No	169	98	44	33
Age (range) (y)	40(1) (21–62)	35(1) (21–55)	45(2) (25–62)	36(3)(19–58)
Smoking history:				
Pack-years	17.1 (1.0)	0	17.0 (2.4)	0
Current cigarettes/day	15.8 (0.5)	0	0	0
Urinary cotinine (μ M)	72 (0.1)	2.8 (0.1)	3.6 (0.3)	3.3 (0.4)
Mining history:				
Cumulative dust exposure (particle-years)	9658 (753)	9958 (1087)	14282 (2201)	0
Time interval since last exposure (h)	28 (1.2)	27 (1.7)	29 (2.3)	0

Results are expressed as mean (SEM).

particle-years. Table 1 shows a descriptive profile of these men classified by smoking. A non-occupationally exposed, control group of non-smokers comprising 33 white men with a mean (SEM) age of 36 (3) years, was also included. None of the men tested had clinical evidence of serious respiratory, cardiac, or systemic disease, and they all tested negatively for C reactive protein. This study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand.

CALCULATION OF EXPOSURE TO DUST AND SMOKE

The cumulative dust exposure was calculated as described previously.⁹ Mining activity was classified on the basis of measured dust counts into 11 groups for which the mean respirable dust count was calculated.²⁸ The number of shifts was multiplied by the mean dust count as defined for each activity and the results were expressed as particle-years. A smoking history was obtained and urinary cotinine concentrations were determined in all the participants.

LABORATORY REAGENTS

Unless indicated, these were obtained from Sigma Chemicals (St Louis, MO, USA). Hydroxyethylpiperazine-ethanesulphonic acid (HEPES) (5 mM) buffered indicator-free Hanks' balanced salt solution (HBSS) (Highveld Biologicals, Johannesburg) was used throughout as the neutrophil suspending medium.

Table 2 Comparison of spirometric variables

Spirometric variables (% of predicted value)	Mineworkers			Non-exposed non-smoker controls (n = 21) D	P values
	Smokers (n = 137) A	Non-smokers (n = 82) B	Ex-smokers (n = 39) C		
FEV ₁	98 (2)	107 (2)	99 (3)	112 (3)	A v B 0.0008 A v D 0.0016 B v C 0.0443 C v D 0.0139
FVC	101 (1)	105 (1)	101 (2)	106 (2)	A v B 0.0383
FEV ₁ /FVC	76 (1)	80 (1)	77 (2)	83 (2)	A v B 0.0043 A v D 0.0079 B v C 0.0376 C v D 0.0041
FEF ₅₀	71 (3)	83 (3)	78 (7)	92 (7)	A v B 0.0050 A v D 0.0044
FEF ₂₅₋₇₅	74 (3)	94 (3)	80 (5)	112 (8)	A v B/D 0.0001 B v C 0.0245 B v D 0.0245 C v D 0.0003
FEF ₅₀ /FVC	70 (3)	79 (3)	76 (6)	82 (6)	A v B 0.0173 A v D 0.0168

Results are expressed as mean (SEM).

ESTIMATION OF REACTIVE OXIDANTS

Generation of reactive oxidants by circulating neutrophils was measured with a previously described, whole blood luminol enhanced chemiluminescence (LECL) method.²⁹ Briefly, 50 μ l of heparinised whole blood was preincubated for 60 minutes at 37°C with 0.1 mM luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione) in 900 μ l of HBSS, pH 7.4. Blood neutrophils were then activated by the addition of phorbol-12-myristate-13-acetate (PMA; 100 ng/ml final concentration), a potent activator of NADPH oxidase. Responses of blood phagocytes to PMA activated LECL were then measured with an LKB Wallac 1251 luminometer (Turku, Finland). Peak LECL readings were integrated for 5 s intervals and recorded as mV.s⁻¹. With this system LECL has been reported to originate almost exclusively from blood neutrophils.²¹ An LECL value corrected for circulating leucocyte count (corrected LECL) was calculated by multiplying the uncorrected LECL value by the blood dilution factor in the assay system (50 μ l blood in a final assay volume of 1 ml = a dilution factor of 20) divided by the leucocyte count, which gives a leucocyte standardised LECL value per 10⁶ leucocytes.

OTHER LABORATORY INVESTIGATIONS

Total leucocyte counts and differential counts were performed with standard haematological methods. Plasma concentrations of vitamin E and β carotene were measured in hexane derivatised plasma samples with high performance liquid chromatography (HPLC).^{30,31} Plasma vitamin C was determined by colorimetry that used the 2,4 dinitrophenylhydrazine reaction.³² Urinary cotinine was measured with the direct barbituric assay.³³

LUNG FUNCTION

Spirometry was performed with a Vitalograph-S wedge bellows spirometer according to the American Thoracic Society's criteria³⁴ on 98 miners who smoked, 107 non-smokers, 39 ex-smokers, and 21 controls. Predicted values were calculated for the rest according to European Community for Coal and Steel (ECCS) (new version) standards,³⁵ and the following variables were obtained: forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁/FVC, forced expiratory flow between 25% and 75% of the vital capacity (FEF₂₅₋₇₅), FEF₅₀/FVC, and FEF₅₀.

STATISTICAL ANALYSES

Analyses of covariance, with age as covariant was used for comparisons between the different groups. Least squares means and the standard error of the least square mean are quoted where the effect of age was significant. Otherwise the arithmetic mean was used.

Results

LUNG FUNCTIONS

Table 2 shows a comparison of the spirometric

variables in the non-smokers, smokers, and ex-smokers among mineworkers and in the non-smoker, non-occupationally exposed controls. The values for smokers and ex-smokers were lower than those of the non-smokers in both the mining and control groups. Values for smokers and ex-smokers, however, were not significantly different. Importantly, most variables for miners who were non-smokers were lower than those of controls with FEF₂₅₋₇₅ reaching significance ($P < 0.02$).

PMA ACTIVATED WHOLE BLOOD LECL, NUMBERS OF CIRCULATING LEUCOCYTES AND NEUTROPHILS, AND PLASMA CONCENTRATIONS OF VITAMINS C AND E AND β CAROTENE

Table 3 shows these comparisons. Analysis of co-variance showed that vitamin E was affected by age and as significant age differences existed between the four groups, comparative data were adjusted for age.

LECL responses were significantly increased in the mineworkers who smoked compared with all other groups (all comparisons $P = 0.0001$). The LECL values corrected for leucocyte count, as well as the total leucocyte and neutrophil counts were also significantly higher in the smokers (all comparisons $P = 0.0001$). The numbers of circulating monocytes too were significantly higher in the mineworkers who smoked ($0.56 (0.01)$ v $0.43 (0.01)$ in non-smokers, $0.48 (0.03)$ in ex-smokers, and $0.41 (0.03) \times 10^6/\text{ml}$ in controls; the P values for comparison of smokers with non-smokers, ex-smokers, and controls were 0.001 , 0.02 , and 0.0001 respectively). There were, however, no significant differences in the numbers of circulating lymphocytes, eosinophils, and basophils between the four study groups (data not shown). Plasma concentrations of vitamin C were decreased by 11% ($P = 0.0106$), 12% ($P = 0.0120$), and 24% ($P = 0.0001$) in the non-smokers, ex-smokers, and smokers respectively in miners when compared with the control group. In the mineworkers, the smokers had less vitamin C in their plasma than the non-smokers ($P = 0.0001$) or ex-smokers ($P = 0.0016$). Vitamin E and β carotene concentrations were not significantly different between the four

groups, although mean concentrations of these were lower in smokers.

Discussion

Mineral dust exposure and cigarette smoking can independently lead to respiratory impairment.²⁷⁻³⁶ Pulmonary dysfunction in cigarette smokers is probably due to the chronic destructive inflammatory events¹⁸⁻²¹ described previously. To further characterise these events in miners, and to determine whether occupation and cigarette smoking have interactive proinflammatory effects, leucocyte count, generation of reactive oxidants by activated blood phagocytes, plasma concentrations of the antioxidant nutrients vitamins C and E and β carotene, and spirometric variables were investigated in goldminers who smoked, were ex-smokers, and non-smokers as well as in a group of controls.

Spirometric values for non-smokers in the mining and control groups were not significantly different, although those of the controls tended to be higher, particularly the maximal mid-expiratory flow. These slight differences may be due to the small numbers in the control group, or to relatively low levels of dust exposure in the mining group ($\pm 10\ 000$ particle-years). The spirometric values, particularly small airway functions, were significantly lower in the smokers than in the two non-smoker groups. Although the values for smokers and ex-smokers did not differ significantly, those of the ex-smokers tended to be intermediate between those of the smokers and non-smokers.

The total leucocyte and neutrophil counts, as well as generation of reactive oxidants by blood phagocytes, did not differ significantly between the non or ex-smokers and the non-mining control group. This indicates the absence of a detectable systemic inflammatory process due to inhalation of mineral dust in this study group, but does not exclude the possibility that these events occur locally in the lung. In miners who smoked, pronounced increases in the leucocyte count and generation of reactive oxidants were noted, a finding in agreement with other studies.³⁷⁻³⁸ The magnitudes of these differences were almost

Table 3 Comparison of PMA activated whole blood LECL responses, leucocyte counts, and plasma concentrations of vitamin C, vitamin E, and β -carotene in mineworkers who were smokers, non-smokers, and ex-smokers, and controls who were non-exposed and non-smokers

Variables	Mineworkers			Non-exposed Non-smoker controls D	P values
	Smokers A	Non-smokers B	Ex-smokers C		
LECL*(mV.s ⁻¹)	456 (13)	297 (17)	320 (25)	295 (29)	A v B/C/D 0.0001
Corrected LECL†	1078 (29)	887 (39)	893 (58)	881 (66)	A v B 0.0001 A v C 0.0045 A v D 0.0067 A v B/C/D 0.0001
Total leucocyte count ($\times 10^6/\text{ml}$)	8.6 (0.1)	6.7 (0.2)	7.0 (0.3)	6.8 (0.3)	A v B/C/D 0.0001
Neutrophil count ($\times 10^6/\text{ml}$)	5.2 (0.1)	3.9 (0.2)	4.2 (0.2)	4.0 (0.3)	A v B/C/D 0.0001
Vitamin C ($\mu\text{g}/\text{ml}$)	7.6 (0.2)	8.9 (0.2)	8.8 (0.3)	10.0 (0.4)	A v B/D 0.0001 A v C 0.0016 B v D 0.0106 C v D 0.0120
Vitamin E ($\mu\text{g}/\text{ml}$)	7.0 (0.2)	7.5 (0.2)	7.3 (0.3)	7.4 (0.4)	—
β -carotene (ng/ml)	130 (10)	140 (10)	150 (20)	150 (20)	—

Results are expressed as mean (SEM) except for vitamin E where the least square mean was used.

*PMA activated whole blood luminol enhanced chemiluminescence.

†Corrected for leucocyte count and volume used in assay.

identical to those previously reported for cigarette smokers who were not exposed to mineral dusts in the workplace.³⁸ These findings indicate that enhancement of proinflammatory indices and pulmonary dysfunction in this study group of miners are due primarily to cigarette smoking.

Although plasma concentrations of vitamin C were significantly lower in all three groups of miners compared with the control group, this may be attributed to differences in the quality of diet between the miners and controls.³⁹ The controls were university students and members of staff. Oxidative stress related to physical activity within a dusty environment may also contribute to the decreases in plasma vitamin C found.⁴⁰ Concentrations of the lipid soluble antioxidants vitamin E and β carotene, on the other hand, were not significantly different in the mineworkers compared with the controls.

In conclusion, occupational exposure to mineral dust has not, in this study group, been shown to promote increases in the numbers or reactivity of circulating phagocytes or to be a significant cause of pulmonary dysfunction, most of the changes found being due primarily to cigarette smoking. Although mining in poorly controlled environments cannot be excluded as a risk factor for health, our findings support the contention that where exposure is not excessive, more benefit may be achieved by dissuading mine workers from smoking than by strategies aimed at achieving further reductions in dust levels.⁴¹

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