ORIGINAL ARTICLE

Increase in interleukin-6 and fibrinogen after exposure to dust in tunnel construction workers

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Objectives: To see if there is any change in blood concentrations of interleukin-6 (IL-6) and fibrinogen during a working shift in tunnel construction workers.

Methods: 12 Tunnel construction workers were followed up during a 24 hours period after returning from a 9 day work free period. The first blood sample was taken on Monday afternoon before starting the shift. Another was taken around midnight after 8 hours of work, and another the next afternoon after about 12 hours of rest. Exposure to respirable dust was measured by personal samplers.

Results: The exposure of the workers to respirable dust, in terms of an 8 hour time weighted average, varied between 0.3 and 1.9 mg/m³. For IL-6, there was an increase in the median serum concentration from 1.14 ng/l before starting the shift to 4.86 ng/l after 8 hours of work ($p=0.002$). For fibrinogen, there was an increase in the median concentration from 3.40 g/l before entering the shift to 3.70 g/l 24 hours later ($p=0.044$). There was a positive correlation between values of IL-6 at the end of the working shift and the fibrinogen concentrations the next afternoon (Pearson's $R=0.73$, $p=0.007$). The observed increase in IL-6 was significant for both smokers and non-smokers.

Conclusion: The study shows an increase in both IL-6 and fibrinogen concentrations during a working shift for both smoking and non-smoking tunnel construction workers.

Based on previous epidemiological studies, a hypothesis was launched in 1995 that inhalation of very fine dust from air pollution induces inflammatory reactions in the lungs with release of mediators which may influence blood coagulation.¹ Two years later it was proposed that one pathogenic pathway for this may be that the release of interleukin-6 (IL-6) from cells in the bronchial mucosa in turn stimulates the production of fibrinogen from hepatocytes.² In 1998 it was concluded in a meta-analysis of more than 18 studies that an association between fibrinogen concentration and the occurrence of coronary heart diseases is probably causal.³ As an increased blood concentration of fibrinogen increases the blood viscosity, this feature may be part of the pathogenic explanation as to why workers in several occupations with exposure to fine dust and other lung irritants have an increased risk of ischaemic heart diseases (IHDs).^{2,4} Heavy construction workers constitute one group who are exposed to dust and who have been found to have an increased risk of IHD. When compared with all other construction workers, a significantly increased cardiovascular mortality was found for drivers, machine operators, and rock workers⁵—that is, among workers usually found in tunnel construction work.

In an attempt to test the hypothesis that an increased risk of IHD could be explained by dust inhalation, IL-6 and fibrinogen were measured before and after exposure in 8 volunteers who were exposed for 4 hours to a median of 19.3 mg/m³ of organic dust inside a piggery. The results showed an increase in the blood concentrations of both IL-6 and fibrinogen 6 and 24 hours after the exposure had started.⁶

We wanted to see in this study if there is any change in the blood concentrations of IL-6 and fibrinogen in tunnel construction workers during a working shift.

SUBJECTS AND METHODS

Tunnel construction work is heavy labour, which involves operating huge machines and explosives in a limited space in a polluted atmosphere deep inside a mountain. Figure 1 is a photograph taken in the tunnel where the study was

conducted. There is no tradition for wearing any respiratory protection. The work consists of continuous 10 hour shifts with four men on each shift. After a working period of 2 weeks while living on the construction site, the workers have 1 week off when they usually go home.

For the purpose of this study three shift teams with four men in each were followed up during their first working shift after returning from 9 days of leave. The first shift always started on Monday afternoon at 1600. Before they entered the tunnel, blood samples were taken and personal dust samplers were mounted on each man. As well as a mixed stone dust, we presumed that the workers were also exposed to irritating blasting fumes from explosives and to diesel exhaust gases and particles, but no qualitative analysis of the exposure was made. Before entering the tunnel, the participants were also interviewed and they filled in a small questionnaire about smoking, height, weight, and current or chronic inflammatory diseases. Blood samples were taken for the analysis of IL-6, fibrinogen, C-reactive protein (CRP), cholesterol (total and HDL), albumin, and α -1-antitrypsin. Blood samples were kept at room temperature for about 20 minutes before they were centrifuged at 1500 g for 10 minutes. Blood plasma for the analysis of fibrinogen, and blood serum for the other analyses were then frozen in cryotubes at -25°C no more than 2 hours later. The personal sampling was performed using cyclones (37 mm SKC aluminium cyclone) with a cellulose acetate filter (Millipore type AA) and 0.8 μm pore size in a cassette connected to a pump (SKC 224-PCFX). The pumps were calibrated at 1.9 l/min, which gives a cut size at 5 μm . The samplers were mounted in the breathing zone of each man. The sampling started when the men entered the tunnel and continued for 8 hours until about midnight. Then the cyclones, cassettes, and pumps were dismantled and the filters secured

Abbreviations: IL-6, interleukin-6; IHD, ischaemic heart diseases; CRP, C-reactive protein; HDL, high density lipoprotein; ELISA, enzyme-linked immunosorbent assay; OD, optical density

Figure 1 Photograph taken of the construction workers working in the tunnel where the study was conducted.

in the cassettes for gravimetric analysis at a later stage. At the same time, new blood samples for the analysis of IL-6, fibrinogen, and α -1-antitrypsin were taken, and were treated and frozen using the same procedures as in the afternoon. New blood samples were taken from the same men for the last analyses of IL-6, fibrinogen, and α -1-antitrypsin after another 16 hours, before they started the next shift at about 1600 on the next afternoon when they had had about 12 hours rest.

The frozen (-20°C) serum samples were analysed for the concentration of IL-6 within 1 month of the sampling. The IL-6 was measured with the commercial enzyme linked immunosorbent assay (ELISA) kit Quantikine HS (R & D systems, UK). The lowest detectable concentration of IL-6 was

0.156 pg/ml. The Quantikine HS human IL-6 immunoassay uses the quantitative sandwich enzyme immunoassay technique where a monoclonal antibody specific for IL-6 has been precoated on to a microplate. A diluent (HD1D) containing a precipitate and IL-6 standard or a serum sample were added to each well on the microplate, and then the plate was incubated for 16 hours. Our serum samples and the standard were all added in duplicate. The IL-6 standard was reconstituted before use with a calibrator diluent HD6F. This reconstitution produced a stock solution of 10 pg/ml. The stock solution was used to produce a dilution series where the undiluted standard served as the high standard (10 pg/ml), and the calibrator diluent as the zero standard (0 pg/ml). The IL-6 conjugate was added to each well after the incubation, and the plate was incubated for another 6 hours. Substrate solution was then added to the wells, and the plate was incubated for 1 more hour. Amplifier solution was added to each well, and the colour developed in proportion to the amount of IL-6 which had been bound in the initial step. Stop solution was added to each well after incubation for 30 minutes and the intensity of the colour was measured as optical density (OD) at 492 nm (Titertek Multiscan Plus MKII, Elfab, Finland). All the incubations were at room temperature. The wells were washed with wash buffer only before adding IL-6 conjugate and substrate solution. The OD for each standard and sample were measured by taking the mean of the duplicate readings and subtracting the OD of the average zero standard. The IL-6 concentration of each sample was calculated with regression analysis for log transformation on the OD results.

All the other blood analyses were performed at the Department of Clinical Chemistry at the University Hospital of Trondheim, Norway. The fibrinogen concentration in plasma was measured by a Fibri-Prest automate by the clotting method of Clauss.⁷ The other blood analyses were done according to the standard procedures of the laboratory.

Statistical analyses

All data were analysed by the program SPSS, version 10.0 for windows, Illinois, USA. We used the Wilcoxon signed rank test for paired samples for the comparisons of blood concentrations at different times.

The ethics committee for medical research in mid-Norway approved the protocol of the study.

RESULTS

All the 12 workers who were asked to participate in the study agreed to do so. Table 1 shows some of their background characteristics. Half of the men stated having some degree of an acute inflammatory disease, mostly common cold, but none of them had increased CRP values. None of the men stated having any chronic inflammatory disease.

Table 1 Background characteristics of the 12 tunnel construction workers who participated in the study

Participant number	Age	Smoking*	Acute inflammatory disease	CRP (mg/l)	Total cholesterol (mmol/l)	HDL cholesterol (mmol/l)	Body mass index†
1	46	+	+	7.0	5.0	1.1	19.5
2	45	x	–	5.0	10.1	1.1	21.0
3	48	+	–	5.0	6.4	1.1	17.4
4	21	–	+	5.0	4.2	1.0	16.0
5	35	+	–	5.0	8.1	1.3	31.9
6	47	–	+	5.0	6.3	0.9	20.6
7	20	–	+	5.0	4.7	1.5	18.4
8	20	–	+	5.0	4.6	1.4	18.1
9	51	+	+	5.0	5.9	1.2	15.0
10	48	+	–	5.0	7.9	1.2	22.6
11	35	–	–	6.0	4.7	1.5	23.7
12	35	–	–	5.0	6.1	1.3	18.0
Median	40			5.0	6.0	1.2	18.9
Range	31			2.0	5.9	0.6	16.9

*+, current smoker; x, ex-smoker; –, non-smoker; †Weight (kg) divided by the square of height (m).

Table 2 Eight hour time weighted average exposure to respirable dust of the study participant, serum concentrations of interleukin-6, fibrinogen, and α -1-antitrypsin in 12 tunnel construction workers

Participant number	Exposure to dust (mg/m ³)	Interleukin-6 (ng/l)			Fibrinogen (g/l)			α -1-Antitrypsin (g/l)		
		‡	§	¶	‡	§	¶	‡	§	¶
1	1.20	1.17	6.43	0.89	4.10	4.10	4.10	1.40	1.40	1.20
2	1.10	1.22	8.28	1.27	3.60	3.30	3.90	1.80	1.30	1.40
3	1.30	0.71	2.93	0.71	3.00	2.90	3.20	1.30	1.40	1.30
4	1.20	0.44	1.54	0.40	2.60	2.60	2.60	1.00	1.20	0.80
5	0.50	1.79	6.63	3.29	3.40	3.90	4.10	1.70	2.00	1.60
6	0.30	0.74	1.83	0.51	3.40	3.20	3.50	1.50	1.70	1.30
7	1.00	0.61	1.39	1.14	3.20	2.80	3.00	1.30	1.10	0.90
8	1.90	4.47	9.23	5.57	3.90	3.80	4.50	1.20	1.20	1.20
9	0.70	0.75	4.14	0.81	3.40	3.70	4.00	1.60	1.70	1.80
10	1.20	1.63	4.40	1.15	3.40	3.40	3.30	0.50	1.60	1.30
11	1.00	3.72	6.76	4.21	4.40	4.20	4.90	1.60	1.30	1.70
12	1.50	1.10	5.31	1.27	2.80	2.70	2.80	1.10	1.00	1.20
Median	1.15	1.14	4.86	1.15	3.40	3.35	3.70	1.35	1.35	1.30
Range	1.60	4.03	7.84*	5.17	1.80	1.60	2.30†	1.30	1.00	1.00

* $p=0.02$ v ‡; † $p=0.044$ v ‡; ‡Monday afternoon before the shift after a 9 day work free period; §after an 8 hour working period in the tunnel; ¶before entering the tunnel on Tuesday afternoon.

Table 2 presents the exposure to respirable dust in terms of the 8 hour time weighted average respirable dust concentration of each worker, and their serum concentrations of IL-6, fibrinogen, and α -1-antitrypsin before the shift on Monday afternoon when they returned from a 9 day work free period, after an 8 hour working period in the tunnel, and before entering the tunnel again on Tuesday afternoon. Median values and ranges at each point in time are also given. Nine of the men had dust exposures between 1.0 and 1.5 mg/m³. For IL-6 there was a more than threefold increase in the concentration during the shift, which was significant ($p=0.002$). There was also a significant increase in the concentration of fibrinogen during the 24 hour period from Monday afternoon to Tuesday afternoon ($p=0.044$). The difference between the concentration of α -1-antitrypsin before and after the shift on Monday was not significant ($p=0.68$).

Four of the men (subjects 5–8) also had their fibrinogen measured on Friday afternoon after a 12 day working period. The median value for these four men was 3.25 (range 1.3) g/l on that occasion compared with 3.40 (range 1.8) g/l in the first measurement.

Figure 2 shows a scatterplot with the regression line drawn for a significant positive correlation (Pearson's $R=0.73$, $p=0.007$) between the concentration of IL-6 on Monday night after the shift and fibrinogen 24 hours after the start of work on Tuesday afternoon. In a bivariate linear regression analysis of the same relation, R^2 was 0.538.

Median 8 hour exposure to respirable dust, and median serum concentrations of IL-6, fibrinogen, and α -1-antitrypsin before starting the shift after a 9 day work free period on Monday afternoon, after an 8 hour working period in the tunnel, and before entering the tunnel on Tuesday afternoon in smoking and non-smoking study participants is shown in table 3. The percentage increase in IL-6 during the work shift

was 330.9 ($p=0.043$) for the five study participants who smoked, and 239.3 ($p=0.018$) for those who did not. None of the changes in the concentrations of fibrinogen or α -1-antitrypsin was significant when the study group was stratified according to current smoking habits.

DISCUSSION

The increases found in both IL-6 and fibrinogen after exposure to dust during tunnel construction work is, to our knowledge, the first observation of this type in a working population. The observation is in accordance with the results of two previous studies, which show such reactions in more experimental settings.^{6,8}

As well as a mixed stone dust, tunnel construction workers are also exposed to irritating blasting fumes from explosives

Figure 2 Scatterplot with the regression line drawn for the correlation (Pearson's $R=0.73$, $p=0.007$) between the concentration of IL-6 on Monday night after the shift and fibrinogen 76 hours later at the start of work on Tuesday afternoon.

Table 3 Median (range) 8 hour exposure to dust, and median serum concentrations of interleukin-6 (ng/l), fibrinogen (g/l), and α -1-antitrypsin

Smoking habits	Exposure to dust (mg/m ³)	Interleukin-6 (ng/l)			Fibrinogen (g/l)			α -1-Antitrypsin (g/l)		
		‡	§	¶	‡	§	¶	‡	§	¶
Current smokers (n=5)	1.2 (0.8)	1.2 (1.1)	4.4* (3.7)	0.9 (2.6)	3.4 (1.1)	3.7 (1.2)	4.0 (0.9)	1.4 (1.2)	1.6 (0.6)	1.3 (0.6)
Non-smokers (n=7)	1.1 (1.6)	1.1 (4.0)	5.3* (7.8)	1.3 (5.2)	3.4 (1.8)	3.2 (1.6)	3.5 (2.3)	1.3 (0.8)	1.2 (0.7)	1.2 (0.9)

* $p<0.05$ v ‡ before the shift on Monday afternoon after a 9 day work free period; § after an 8 hour working period in the tunnel; ¶ before entering the tunnel on Tuesday afternoon in smoking and non-smoking study participants.

and to diesel exhaust gases and particles. Our personal measurements of dust exposure of the study participants showed moderate levels of exposure to dust, with only small variations. It is remarkable that the observed reactions could occur at these exposures that were far below those used in the two experimental studies that have been cited.^{6,8} Regrettably, the narrow distribution of the exposures prohibited the analysis of the relations between level of exposure and the effects. In a previous study, we failed to show any reactions for IL-6 or fibrinogen in stainless steel welders (personal communication). This was probably because the participants were protected by adequate protective equipment.

The effects in the present study seem very clear, at least for the changes in IL-6. It is also remarkable that there is such a clear correlation between the increased concentrations of IL-6 found on Monday night and the concentrations of fibrinogen 15 hours later on Tuesday afternoon.

Circadian variations have been found for the blood concentrations of both fibrinogen and IL-6.¹⁰⁻¹³ In this study, the two samples that showed significant differences for fibrinogen were taken with a 24 hour interval. Thus diurnal variation was probably not had any influence on these results. For IL-6, the normal serum concentrations seem to vary between about 1 ng/l during the day from 0800 to 1400 and 2.5–5 ng/l during the night from midnight to 0500.¹² Although our two points of measurements, before entering the shift at 1600 and at the end of it at midnight, do not quite coincide with the presumed zenith and nadir values of IL-6, it cannot fully be ruled out that at least some of the variation found in IL-6 is due to the normal circadian rhythm of IL-6 concentrations. There is also some evidence that serum concentrations of IL-6 increase with physical exercise.^{14,15} As tunnel construction work is heavy labour, there is also a possibility that the physical activity of the participants may have influenced the differences found. In future studies on the variations of IL-6 both the circadian rhythm and possible changes due to physical activity have to be more carefully considered.

Half of the study participants stated that they had had an acute inflammatory disease at the start of the study. As this was not reflected in their CRP values or the basis concentrations of other acute phase proteins, we think that these infections must have been mild. Also, as the outcomes were observed within subjects with measurements before and after an exposure, we do not think that problems with infectious diseases have had any decisive influence on the results.

As 41% of the study participants were smokers, this could have brought about problems in the analyses. Non-smokers had higher basic values of IL-6 whereas the smokers had a slightly greater increase in their values during exposure. As these findings may be due to chance, we tend not to ascribe too much meaning to them. The important point is that the results, despite the small numbers, show a clear variation in IL-6 for both smokers and non-smokers. There was also an increase in fibrinogen concentrations in both smokers and non-smokers, but this was not significant ($p=0.154$ and 0.142 respectively). The concentrations of fibrinogen found in smokers and non-smokers were almost the same. As these results do not agree with previous studies,¹⁶ this finding might as well be due to chance.

It is also remarkable that the mean concentration of fibrinogen after 2 weeks of work in the four subjects from whom samples were taken, was lower at the end of the working period than at the start. When looking at this feature, together with the lower basic concentrations of IL-6 in smokers, it could be speculated that there might be some type of an adaptation of the pathophysiological pathway that gives stronger reactions once the exposure changes.

This study does not answer the question of whether the reactions found lead to an increased risk of IHD. We therefore think that exposure to dust should be considered as one of

many possible risk factors for IHD. It seems evident that increased concentrations of fibrinogen in blood may be a risk factor for IHD.^{3,8} To answer the question of a possible increased risk of IHD from exposure to dust, exposed groups of workers with positive IL-6 reactions have to be followed up in epidemiological studies, which also take other risk factors into account. It is remarkable that several previous studies have shown increased risks of IHD in workers exposed to dust without being able to explain the underlying pathogenic mechanisms.

This study, which shows an increase in both IL-6 and fibrinogen concentrations after exposure to dust in both smokers and non-smokers, may contribute to new and necessary knowledge linking occupational factors that have not been recognised so far with the epidemiology of cardiovascular diseases.

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