Is the short term limit value for sulphur dioxide exposure safe? Effects of controlled chamber exposure investigated with bronchoalveolar lavage

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ABSTRACT Bronchoalveolar lavage (BAL) which has not previously been used in investigating the effect of sulphur dioxide (SO₂) on the human lung was performed on 12 subjects before and after controlled chamber exposure with SO₂ for 20 minutes. BAL fluid 24 hours after exposure with 10 mg SO₂/m³ (4 ppm, 10 subjects) showed increased alveolar macrophage activity as judged by an increase in lysozyme positive macrophages. Twenty four hours after 20 mg/m³ (4 subjects) a further increase was seen, which was accompanied by an increase in total numbers of macrophages and lymphocytes. Seventy two hours after exposure (4 subjects) cell numbers had virtually returned to pre-exposure levels. These previously uninvestigated reactions indicate potentially noxious effects of SO₂ in the lungs at exposure levels that are regarded as relatively safe.

Sulphur dioxide (SO₂) is a gas that is rapidly oxidised to sulphuric acid in contact with moist epithelial surfaces in the eyes, nasopharynx, and lower airways, where it may cause damage. It is a major air pollutant in urban areas, particularly in the working environment of pulp industries and factories using various combustion and smelting processes. High peak exposures, many times exceeding the Swedish short term exposure limit of 13 mg SO₂/m³ air (5 ppm), have been frequently found¹ (and N Stjernberg *et al*, unpublished data). Similar findings have been reported in other countries.^{2,3}

Pulmonary effects of SO₂ in man have mainly been studied indirectly by lung function tests,^{4,5} tests of hyperreactivity,⁶ and in epidemiological surveys^{1,7} (and N Stjernberg *et al*). Direct investigation has been restricted to workers who have died of massive exposure to SO₂, in whom gross histopathological changes in the lung tissue with haemorrhagic alveolar oedema have been reported.⁸ Bronchoalveolar lavage (BAL), which is a commonly used method for investigating conditions at the alveolar level has not to our knowledge previously been used for studying SO₂ effects in man.

Our aim, using the BAL technique, was to determine if short term exposure to SO₂ in concentrations around the Swedish short term exposure limit, concen-

trations which are commonly found in industrial environment, causes potentially harmful effects on the alveolar cell population.

Subjects and methods

SUBJECTS

Twelve healthy, non-smoking subjects, aged 22-30 (mean 24) participated in this investigation. None had a history of airway infection for at least six weeks before BAL or a history of bronchial hyperresponsiveness. Pre-exposure lung function and gas distribution were normal in all subjects.

SULPHUR DIOXIDE EXPOSURE

The exposure chamber measured 3.20 × 2.00 × 2.20 m with an air volume of 14.1 m³. It was built of anodised aluminium with windows in one wall. Ambient air was drawn continuously through the chamber at 400 m³/h, resulting in one air exchange about every two minutes. Pre-exposure measurements have shown low levels of particulate matter in the chamber. During exposure, the chamber air temperature was kept at 20°C and the relative humidity around 50%. The concentration of SO₂ in the exposure chamber was controlled by adding a gas stream from a 1% SO₂ gas tube to the chamber air inlet. The chamber air was continuously analysed with an electrochemical method in which SO₂ is oxidised to

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sulphate and the current registered on a recorder.⁹

The subjects were exposed to 10 and 20 mg SO₂/m³ air (4 and 8 ppm respectively). The exposure time was 20 minutes and the test subjects were working on a bicycle ergometer with a work load of 75 W. Immediately before and after exposure, and 15 minutes after exposure, dynamic spirometry was recorded using a Vitalograph spirometer. Dynamic spirometry was also performed before the postexposure BAL. Before, during, and at the end of the exposure, the test subject was asked about symptoms using a standardised questionnaire.

BAL

The method of BAL is slightly modified from previous studies by our group.¹⁰ (All bronchoscopies were performed by the same investigator.) A flexible fibreoptic bronchoscope, Olympus BF 1 T or BF 1T10, was used with the subject in the supine position. The same instrument was used for all examinations in each subject. Lidocain was used for topical anaesthesia. Atropine 0.5–0.75 mg was given subcutaneously as premedication. The bronchoscope was inserted through the mouth and wedged in the middle lobe bronchus. Sterile phosphate buffered saline (PBS-A) at 37°, pH 7.3, was infused in four aliquots of 60 ml and gently suctioned back after each infusion into a siliconised container placed in ice water. The chilled lavage fluid was filtered through a nylon filter (pore diameter 100 µm, Syntab Product AB, Malmö, Sweden) at the laboratory and centrifuged at 400 G for 15 minutes. The cell pellet was resuspended in balanced salt solution to give a concentration of 10⁶ cells per ml. The total number of cells in the lavage fluid was counted in a Bürker chamber.

Cytoprifugal smears were prepared with 5 × 10⁴ non-epithelial cells per slide using a Cytospin 2 (Shandon Southern Instruments Inc, Sewickly, PA, USA). Slides were stained according to May-Grün-

wald-Giemsa for standard cell differential counts and two hundred cells per slide were counted. Mast cells were counted on slides stained with acid toluidine blue and counterstained with Mayer's acid haematoxylin.¹¹ Lysozyme positive macrophages were shown with lysozyme antibody using an immunoperoxidase technique (Dakopatts A/S, Copenhagen, Denmark). The ratio helper-inducer/suppressor-cytotoxic T cells was determined using the Simultest T Helper/Suppressor Test (Becton Dickinson AB, Stockholm, Sweden).

BAL was performed at least two weeks before exposure to SO₂ in all 12 subjects (table). Ten subjects underwent BAL 24 hours after exposure with 10 mg SO₂/m³. BAL was also performed in four subjects 24 hours after exposure with 20 mg SO₂/m³ and on four subjects 72 hours after exposure. The time between exposures to SO₂ varied between three and five months in the individuals who were exposed twice. Informed consent was obtained from the subjects and the study was approved by the ethical committee of the University of Umeå.

STATISTICS

Wilcoxon's non-parametric signed rank test was used.

Results**BRONCHOSCOPY**

Before exposure and after 10 mg SO₂/m³, all subjects had normal endobronchial findings. Twenty four hours after exposure to 20 mg SO₂/m³, all four subjects showed a mucosal erythema in the distal part of trachea and proximal main bronchi.

BAL

The median amount of BAL fluid recovered at the pre-exposure lavage was 69% (interquartile range 64–72%) and did not differ significantly after exposure. The number of neutrophils, eosinophils, and mast

Cell numbers in bronchoalveolar lavage fluid after controlled exposure to sulphur dioxide. Data are given as median with range

	Total cell count × 10 ³ /l	Lymphocytes		Macrophages/monocytes		Lysozyme positive macrophages/monocytes	
		× 10 ³ /l	%	× 10 ³ /l	%	× 10 ³ /l	% of macrophages
Before exposure (n = 12)	6.9 (2–17.4)	0.3 (0.1–2.6)	6 (2–18)	6.3 (11.8–14.3)	92 (81–97)	0.4 (0.1–3.4)	5 (3–20)
24 h after 10 mg SO ₂ /m ³ (n = 10)	6.0 (1.4–14.0)	0.5 (0.1–1.3)	7 (4–22)	4.9 (1.3–12.3)	91 (75–93)	0.8 (0.1–1.5)	14 (5–19)
24 h after 20 mg SO ₂ /m ³ (n = 4)	16.0 (8.6–21.0)	3.1 (1.2–5.9)	20 (12–28)	12.4 (6.8–14.3)	76 (68–84)	2.6 (1.5–4.6)	18 (15–22)
72 h after 20 mg SO ₂ /m ³ (n = 4)	8.2 (7.0–12.2)	1.0 (0.5–1.5)	12 (6–20)	7.1 (5.8–11.2)	88 (79–92)	0.5 (0.4–2.1)	7 (4–17)

cells, and the ratio of helper-inducer/suppressor-cytotoxic T cells, were within normal limits and were not significantly changed after exposure. The counts for lymphocytes and macrophages/monocytes are given in the table.

Twenty four hours after exposure to 10 mg SO₂/m³ there was a significant increase in lysozyme positive alveolar macrophages (Lys⁺MF) in total number and in per cent of the total amount of alveolar macrophages (Tot-MF) compared with values before exposure ($p < 0.01$ respectively).

Twenty four hours after exposure to 20 mg SO₂/m³ Lys⁺MF were further increased, both in per cent of Tot-MF and in total number. Tot-MF and the total cell number were also increased. A mild lymphocytosis was also seen, with a median value of 20% (range 12–28%) as compared with 6% (range 2–18%) before exposure. The total number of lymphocytes in BAL was two to four times higher than before exposure. An increase in the numbers of macrophages and lymphocytes was found in all four subjects.

Seventy two hours after exposure to 20 mg SO₂/m³ lymphocytes, Lys⁺MF, Tot-MF, and total cell numbers had virtually returned to pre-exposure levels.

LUNG FUNCTION

Vitalograph recordings before, immediately after, and 15 minutes after exposure to SO₂ showed no significant decrease in lung function. Similar results were obtained 24 and 72 hours after exposures immediately before BAL.

SYMPTOMS

The subjects reported mild symptoms from the eyes and nose during exposure. All denied symptoms from the eyes or airways after exposure.

Discussion

Sulphur dioxide is a common environmental pollutant³ and large numbers of factory workers world wide are frequently exposed to levels well exceeding the short term exposure limits, which vary between 10 and 20 mg SO₂/m³ in the industrialised countries.² Until now, the effects of short term exposure of SO₂ on the human lung have not been investigated by a direct technique such as BAL.

The SO₂ exposure levels in the present study are based on previous measurements in pulp industry works and are equal to the upper and lower range of short term exposure limits in industrialised countries^{1,2} (and N Stjernberg *et al*). The controlled chamber exposures were designed to simulate the work conditions in the type of industries in which exposure to SO₂ is frequent and particulate levels low. The workers in these industries are mainly occupied

with supervising chemical processes but are frequently exposed to peak levels of SO₂ during short periods when they check and adjust the machinery. The workload is mainly light to moderately heavy which is why we chose 75 W on the ergometer bicycle. The workload also means that the workers are not forced to breathe much through the mouth.¹ This is beneficial, since the exposure of lower airways to SO₂ is distinctly higher during oral breathing than during nasal breathing and rapidly increases with increased airflow. Approximately 98% of SO₂ had been found to be absorbed in the nasopharynx during nasal breathing.^{13,14} Even though the doses of SO₂ that reach the alveoli appear to be small the cell reactions in BAL fluid indicate noxious effects at this level of the airways, by contrast with the mild symptoms from the upper airways and absence of airflow obstruction.

Lysozyme positivity is a property of monocytes newly recruited to the alveoli and is usually lost when they have matured to macrophages.¹⁵ Lysozyme in macrophages is also believed to be a marker of cell activation and may be increased by a variety of stimuli.^{16,17} After the low exposure to SO₂, 10 mg/m³ Tot-MF was unchanged while the relative numbers of Lys⁺MF were increased. This indicates that the Lys⁺MF seen in BAL had not migrated from the blood stream after the exposure but were residing alveolar macrophages who had reacted to the SO₂ stimulus with lysozyme production. Twenty four hours after 20 mg SO₂/m³, however, a migration to the alveoli appears to have taken place. That that alveolar macrophage activation was the only observed cellular reaction after the low exposure level may indicate that this is a prime target cell for SO₂. Macrophages also have the ability to induce lymphocyte chemotaxis and proliferation by release of mediators such as Interleukin-1 and could thus induce the lymphocytosis observed after exposure to 20 mg SO₂/m³. Interestingly, this lymphocytosis was not accompanied by any change in the ratio between Leu 2/Leu 3 positive T lymphocytes. This may indicate that the lymphocytosis was mainly due to lymphocyte chemotaxis and not primarily to a proliferative response.

BAL after controlled chamber exposure with gaseous pollutants seems to be a useful method for investigation of effects at the alveolar level of the lung. BAL, 24 hours after short term exposure with 10 mg (4 ppm) and 20 mg SO₂/m³ (8 ppm), showed increased alveolar macrophage activity and at the higher dose also a mild lymphocytosis that had virtually returned to normal 72 hours after exposure. These previously uninvestigated reactions indicate potentially noxious effects of SO₂ in the lungs at exposure levels that are presently regarded as relatively safe. Further studies are in progress to evaluate the effects of SO₂ in the lungs of man.

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