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the Swedish short term exposure limit of 13 mg SO2/m3 air (5 ppm), have been frequently found (and N Stjernberg et al, unpublished data). Similar findings have been reported in other countries.

Pulmonary effects of SO2 in man have mainly been studied indirectly by lung function tests,11 tests of hyperreactivity,6 and in epidemiological surveys17 (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.4 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, concentrations 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 hyperreactiveness. Pre-exposure lung function and gas distribution tests 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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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 standarised questionnaire.

BAL

The method of BAL is slightly modified from previous studies by our group. All bronchoscopies were performed by the same investigator. A flexible fibreoptic bronchoscope, Olympus BF 1 T or BF 1 T10, was used with the subject in the supine position. The same instrument was used for all examinations in each subject. Lidocaine 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°C, 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.

Cytocentrifugal 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-Grünewald-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. 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 erythaema 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

<table>
<thead>
<tr>
<th>Time after Exposure</th>
<th>Total cell count x 10⁷/ml</th>
<th>Lymphocytes x 10⁶/ml</th>
<th>%</th>
<th>Macrophages/monocytes x 10⁶/ml</th>
<th>%</th>
<th>Lysozyme positive macrophages/monocytes x 10⁶/ml</th>
<th>% of macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n = 12</td>
<td>6·9 (2–17·4)</td>
<td>0·3 (0·1–2·6)</td>
<td>6</td>
<td>6·3 (11·8–14·3)</td>
<td>92</td>
<td>0·4 (0·1–3·4)</td>
<td>5</td>
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<tr>
<td>24 h after 10 mg</td>
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<td></td>
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<tr>
<td>SO₂/m³ (n = 10)</td>
<td>6·0 (1·4–14·0)</td>
<td>0·5 (0·1–1·3)</td>
<td>7</td>
<td>4·9 (1·3–12·3)</td>
<td>91</td>
<td>0·8 (0·1–1·5)</td>
<td>14</td>
</tr>
<tr>
<td>24 h after 20 mg</td>
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</tr>
<tr>
<td>SO₂/m³ (n = 4)</td>
<td>16·0 (8·6–21·0)</td>
<td>3·1 (1·2–5·9)</td>
<td>20</td>
<td>12·4 (6·8–14·3)</td>
<td>76</td>
<td>2·6 (1·5–4·6)</td>
<td>18</td>
</tr>
<tr>
<td>72 h after 20 mg</td>
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<tr>
<td>SO₂/m³ (n = 4)</td>
<td>8·2 (7·0–12·2)</td>
<td>1·0 (0·5–1·5)</td>
<td>12</td>
<td>7·1 (5·8–11·2)</td>
<td>88</td>
<td>0·5 (0·4–2·1)</td>
<td>7</td>
</tr>
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</table>
The workers' exposure to works and wide are on a term and frequent basis. Until now, the exposure to peaks of SO₂ was based on the Sulphur dioxide and phages and the cytotoxic effect. An increase in the ratio of macrophages was found to be associated with the exposure to SO₂. An increase in the ratio of macrophages was found to be associated with the exposure to SO₂. In the present study, the workers were exposed to SO₂, which resulted in a significant increase in the ratio of macrophages. The workers in these industries are mainly exposed to SO₂, which results in an increase in the ratio of macrophages. 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. 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. Even though the doses of SO₂ that reach the alveolus appear to be small, cell reactions in BAL fluid indicate noxious effects at this level of the airways, by contrast with the mild symptoms from 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. Lysozyme in macrophages is also believed to be a marker of cell activation and may be increased by a variety of stimuli. After the low exposure to SO₂, 10 mg/m³, the 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 that 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 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 lungs. BAL, 24 hours after short term exposure with 10 mg (0.01 ppm) and 20 mg SO₂/m³ (0.8 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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References


