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the Swedish short term exposure limit of 13 mg SO₂/m³ air (5 ppm), have been frequently found¹ (and N Stjernberg et al, unpublished data).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.²³

Pulmonary effects of SO₂ in man have mainly been studied indirectly by lung function tests,¹³ tests of hyperreactivity,⁴ 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 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.  

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. 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. 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°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-Grünwald-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 Similtest 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

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

<table>
<thead>
<tr>
<th></th>
<th>Total cell count × 10⁹/l</th>
<th>Lymphocytes × 10⁹/l</th>
<th>%</th>
<th>Macrophages/monocytes × 10⁹/l</th>
<th>%</th>
<th>Lysozyme positive macrophages/monocytes × 10⁹/l</th>
<th>%</th>
<th>% of macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before exposure (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>20</td>
<td></td>
</tr>
<tr>
<td>24 h after 10 mg 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>
<td></td>
</tr>
<tr>
<td>24 h after 20 mg SO₂/m³ (n = 10)</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>
<td></td>
</tr>
<tr>
<td>72 h after 20 mg 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>
<td></td>
</tr>
</tbody>
</table>
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 macro-
phages (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 lympho-
cytes 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 pollutant1
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.3

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 coun-
tries12 (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. This
workload is mainly light to moderately heavy which is
why we chose 75 W on the ergometer bicycle. This
workload also means that the workers are not forced to
take a deep breath much through the mouth.1 This is ben-
eficial, since the exposure of lower airways to SO2 can
be 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 breath-
ing.1314 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
and 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 stimu-
lizing factors.1617 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 Inter-
leukin-1 and could thus induce the lymphocytosis
observed after exposure to 20 mg SO2/m3. Inter-
estingly, this lymphocytosis was not accompanied by
any change in the ratio between Leu 2/Leu 3 positive
lymphocytes. This may indicate that the lympho-
cytosis was mainly due to lymphocyte chemotaxis and
not primarily to a proliferative response.

BAL after controlled chamber exposure with gas-
eous 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
(ppm) and 20 mg SO2/m3 (8 ppm), showed increased
alveolar macrophage activity and at the higher doses
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


