

Cell response in bronchoalveolar lavage fluid after sulfur dioxide exposure

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SANDSTRÖM T, STJERNBERG N, ANDERSSON M-C, KOLMODIN-HEDMAN B, LINDSTRÖM K, ROSENHALL L. Cell response in bronchoalveolar lavage fluid after sulfur dioxide exposure. *Scand J Work Environ Health* 1989;15:142-6. Environmental chamber exposure and bronchoalveolar lavage (BAL) were used to study the dose-response relationship between short-term exposure to sulfur dioxide (SO₂) and inflammatory reactions in the human lung as reflected in BAL fluid. Healthy subjects were exposed to 10, 13, 20, or 30 mg/m³ for 20 min. BAL was performed several weeks preexposure and 24 h postexposure. Mast cells, lymphocytes, lysozyme positive macrophages, and the total number of macrophages were significantly increased after SO₂ exposure. A dose-dependent increase in the cell response in BAL fluid was observed after exposure to 10-20 mg/m³, but no further increase was detected after 30 mg/m³. Inflammatory cell response was found in BAL fluid at SO₂ levels that occur in industrial indoor environments worldwide, and cell response to SO₂ was also seen below the short-term exposure limit of Sweden and many other countries (13 mg/m³).

Key terms: lymphocytes, lysozyme, macrophages, mast cells.

Long-term exposure to sulfur dioxide (SO₂) is known to increase the morbidity of chronic bronchitis and obstructive airway diseases (1-5). The increased morbidity may occur after long-term exposure both to polluted outdoor ambient air of industrialized areas and to indoor air in certain kinds of industries. The highest SO₂ concentrations are found in indoor environments in, eg, paper pulp industries, smelteries, and petroleum refineries. The morbidity of lung diseases among workers in these industries is often markedly increased even when compared with that of residents in the surrounding areas (4, 6, 7).

Even though several studies on the lung effects of SO₂ have appeared in the literature (8-12), the basal and primary mechanisms that cause the noxious effects have not been clarified. In a recent study we have demonstrated the usefulness of combining controlled chamber exposure and bronchoalveolar lavage (BAL) in the investigation of these mechanisms (13). We have recently studied the time course of the inflammatory reaction in human lung after SO₂ exposure and have found that inflammatory cell response in BAL fluid occurs after moderately high concentrations of SO₂ with peak effects 24 h after exposure (to be published). These results have raised questions about the safety of the short-term exposure limits set for SO₂ in industrialized countries.

Using these observations as a starting point, we have performed the present study to determine the exposure dose-response relationship between short-term exposure to SO₂ and the inflammatory response in human lung.

Subjects and methods

Subjects

Twenty-two healthy nonsmoking male volunteers between the ages of 22 and 37 years (mean 27 years) gave their informed consent to participate in this investigation. None of them had a history of bronchial hyperactivity or a history of airway infection during at least six weeks prior to the BAL. The preexposure dynamic spirometry values were normal for all the subjects.

Design of the study

The subjects were exposed to SO₂ concentrations of 10, 13, 20, or 30 mg/m³ (4, 5, 8, or 11 ppm, respectively) for 20 min. Ten subjects were exposed to 10 mg/m³ and eight subjects to each of the other concentrations. Flexible fiberoptic bronchoscopy with BAL was performed on all of the subjects at least two weeks before SO₂ exposure in order to obtain reference values for the BAL fluid. BAL was then performed 24 h after the end of the exposures. All the lavages were performed within 20 min of the scheduled time. Each volunteer was exposed one to three times. The period between the SO₂ exposures was at least three weeks. Immediately before and after the exposures the subjects' lung function values were recorded with a

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Vitalograph^(®) spirometer. Spirometry was also performed before the postexposure BAL. Before, during, and at the end of the exposure, the subjects were asked about symptoms from their eyes, nose, and airways according to a standardized questionnaire (14).

Sulfur dioxide exposure

The chamber exposure was designed to simulate the work conditions that have previously been identified in, eg, pulp mills and smelters with respect to the exposure concentration, work load, breathing pattern, and exposure time (4, 5). The exposure method has previously been described in detail (14). The exposure chamber measured $3.20 \times 2.00 \times 2.20$ m with an air volume of 14.1 m^3 . It was constructed of anodized aluminum with windows in one wall. Ambient air was drawn continuously through the chamber at $150 \text{ m}^3/\text{h}$, the result being one air exchange approximately every 5 min. Preexposure measurements showed very low levels of particulate matter in the chamber. During the exposures, the chamber air temperature was kept at 20°C , and the relative humidity at around 50 %. The SO_2 concentration in the exposure chamber was controlled by the addition of a gas stream from a 1 % SO_2 gas tube attached to the chamber air inlet. The chamber air was continuously analyzed with an electrochemical method, with which SO_2 is oxidized to sulfate and the current is registered on a recorder. The method has previously been described in detail (15). The subjects were exposed to SO_2 in the chamber for 20 min. During the last 15 min of the exposure the subjects worked on a bicycle ergometer with a work load of 75 W and were therefore mainly breathing through the nose.

Bronchoalveolar lavage

The subjects were given atropine subcutaneously before the examination. No complementary medication was given other than lidocaine, which was administered according to a standardized scheme for topical anesthesia. A flexible fiberoptic bronchoscope (Olympus BF 1T or BF 1T10) was inserted through the mouth with the patient in the supine position. After careful wedging of the bronchoscope tip in the middle lobe bronchus, sterile phosphate buffered saline pH 7.3 (PBS-A) at 37°C was infused in four aliquots of 60 ml and gently suctioned back after each infusion to a siliconized container placed in ice water. Informed consent was obtained from the subjects, and the study was approved by the Ethics Committee of the University of Umeå. The investigator who performed all the bronchoscopies had knowledge of the exposure concentrations to which the subjects had been exposed before the bronchoscopy.

The chilled recovered BAL fluid was immediately filtered through a nylon filter (pore diameter $100 \mu\text{m}$, Syntab Product AB, Malmö, Sweden) at the laboratory and centrifuged at $400 g$ for 15 min. The cell pellet

was resuspended in balanced salt solution to a concentration of 10^6 cells per milliliter. The total number of cells in the lavage fluid was counted in a Bürker chamber.

Cycentrifugal smears were prepared with 5×10^4 nonepithelial cells per slide with the use of a Cytospin 2^(®) (Shandon Southern Instruments Inc, Sewickly, Pennsylvania, United States). The slides were stained according to May-Grünwald-Giemsa for standard cell differential counts, and 200 cells per slide were counted. Mast cells were counted on slides stained with acid toluidine blue and counterstained with Mayer's acid hematoxylin (16). Lysozyme positive macrophages were demonstrated with Lysozyme^(®) antibody with the use of an immunoperoxidase technique (Dakopatts A/S, Copenhagen, Denmark). The ratio T helper/cytotoxic-suppressor cells was determined with the Simultest T Helper/Suppressor Test^(®) (Becton Dickinson AB, Stockholm, Sweden). Albumin was measured by electroimmunoassay (17).

Statistics

Wilcoxon's nonparametric signed rank test for paired observations was used for comparing the BAL parameters of the same individual before and after exposure. A P-value of <0.05 was considered significant.

Results

Bronchoscopy

All the subjects had normal endobronchial findings in the reference bronchoscopy before exposure and after exposure to SO_2 concentrations of 10 and 13 mg/m^3 (4 and 5 ppm, respectively). Twenty-four hours after exposure to 20 mg/m^3 (8 ppm) mucosal erythema was found in the distal half of the trachea and proximal main bronchi of all the subjects. The erythema was even more pronounced 24 h after exposure to 30 mg/m^3 (11 ppm).

The median amount of BAL fluid recovered after the reference BAL, before exposure, was 75 % of the instilled volume (interquartile range 66–75 %). The recovery did not significantly differ after exposure to different concentrations. The counts for lymphocytes, alveolar macrophages/monocytes, and mast cells are given in table 1. A significant increase in the total number of cells was found in the BAL fluid 24 h after exposure to 20 and 30 mg/m^3 (8 and 11 ppm, respectively). The increase consisted of lymphocytes, alveolar macrophages/monocytes, and mast cells, while the eosinophil and neutrophil levels were unaffected. Epithelial cells were few.

Lysozyme positive macrophages/monocytes were significantly increased at all the examined concentrations. The total number of alveolar macrophages/monocytes was significantly increased 24 h after exposure to 20 and 30 mg/m^3 (8 and 11 ppm, respective-

Table 1. Cell numbers and the albumin concentration in bronchoalveolar lavage fluid before and 24 h after chamber exposure to sulfur dioxide concentrations of 10, 13, 20, and 30 mg/m³.

Time of measurement	Total number of cells ($\times 10^7/l$)	Lymphocytes		Macrophages/ monocytes		Lysozyme positive macrophages/ monocytes		Mast cells		Albumin (mg/ml)
		N ($\times 10^7/l$)	%	N ($\times 10^7/l$)	%	N ($\times 10^7/l$)	% of macro- phages	N ($\times 10^4$)	%	
Before exposure (N = 22)										
Median	7.4	0.5	6	6.3	92	0.6	12.5	2.7	0.28	41
Interquartile range	5.4—10.2	0.3—0.7	5—9	4.9—7.9	89—93	0.3—1.6	5—18	0.6—6.3	0.28—1.39	29—46
24 h after exposure										
10 mg/m ³ (N = 10)										
Median	6.0	0.5	7	4.9	91	0.8***	14***	5.6	0.83	29
Interquartile range	5.0—9.6	0.3—1.1	6—9	4.4—8.7	88—91	0.5—1.4	10—17	3.0—15.7	0.56—1.94	21—33
13 mg/m ³ (N = 8)										
Median	9.1	0.8	13	7.8	85	1.4*	19*	9.9*	1.11**	52
Interquartile range	7.6—10.3	0.5—1.3	6—15	6.1—9.1	83—94	1.2—1.6	16—24	5.9—14.4	0.56—1.94	42—58
20 mg/m ³ (N = 8)										
Median	14.5**	1.4**	13*	12.4**	82*	1.6**	17*	10.8	0.83	44
Interquartile range	8.0—19.8	0.5—4.0	8—21	6.8—14.7	76—90	0.9—2.6	15—18	7.8—24.1	0.56—2.22	34—53
30 mg/m ³ (N = 8)										
Median	11.2*	1.2*	14*	10.4*	85*	1.7*	19*	17.3**	1.67**	39
Interquartile range	9.5—16.3	0.8—1.6	8—16	8.4—13.0	81—97	1.3—2.4	15—25	9.2—30.6	1.11—2.64	30—51

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ (postexposure values compared with preexposure values).

ly). The increase was the most pronounced in the population of macrophages/monocytes that was stained positive for lysozyme.

The total and relative numbers of mast cells were significantly increased 24 h after exposure to 13 mg/m³ (5 ppm). Twenty-four hours after exposure to 20 mg/m³ there were indications of an increase in the total number of mast cells ($P = 0.058$). The increase in mast cells reached a peak value after exposure to 30 mg/m³, the median value of the total mast cell numbers being more than five times higher than before the exposure.

The relative and total amount of lymphocytes was significantly increased 24 h after exposure to 20 and 30 mg/m³ (8 and 11 ppm, respectively). The lymphocytosis was moderate with median values of 13 and 14 % after the respective concentrations compared with a median of 6 % before exposure. The median value of the total number of lymphocytes was more than doubled after exposure to 13, 20, and 30 mg/m³ (5, 8, and 11 ppm, respectively). The median T helper/cytotoxic-suppressor cell ratio and albumin concentrations in BAL fluid were not significantly changed after any exposure to SO₂ concentration.

Spirometry

The lung function recordings with the Vitalograph^(a) immediately after the exposure and before the BAL showed small but nonsignificant decreases in the forced expiratory volume in 1 s (FEV_{1.0}), <5 % of the preexposure values.

Symptoms

Very mild symptoms from the eyes, nose, and throat were reported during the exposures without any sig-

nificant difference between the exposure concentrations.

Discussion

The cytologic examination of the recovered bronchoalveolar lavage fluid showed that SO₂ exposure gives an inflammatory cell reaction involving mast cells and alveolar macrophages even at the relatively low SO₂ concentrations of 10–13 mg/m³ (4–5 ppm). The macrophage reaction was more pronounced in terms of increased lysozyme production in the cells, visible by specific staining, than the increase in the total number of macrophages. An increased number of lysozyme positive macrophages is considered a marker for macrophage activation (18, 19). It should be noted that an inflammatory cell reaction occurred at levels of SO₂ that were previously regarded as safe and were below the short-term exposure limit for Sweden and several other countries, 13 mg/m³ (5 ppm) (5, 20). The exposure limits are mainly based on studies with indirect methods to evaluate noxious lung effects, methods that are far less sensitive than those employed in this investigation. The cellular reactions that were found after the SO₂ exposure may seem moderately pronounced when determined as the percentage of the total cell number and as the total cell numbers. It should be noted that these clearly defined reactive changes occurred after one single exposure of healthy nonsmoking subjects, while workers in some industries may be repeatedly exposed to similar doses in their indoor work environment (2, 4, 5). The demonstrated inflammatory response was not associated with any symptoms or clinical airway obstruction that could

serve as a warning signal of a noxious response in the lung to the inhaled SO₂.

The inflammatory cellular reaction increased as the dose increased from 10 to 13 mg/m³ (4 to 5 ppm), and also to 20 mg SO₂/m³ (8 ppm), when a significant lymphocytosis accompanied a further enhanced macrophage and mast cell response. There was, however, no significantly further increased cell reaction in the recovered BAL fluid at 30 mg/m³ (11 ppm), apart from an increase in the number of mast cells. These results could indicate that the dose-response curve for macrophage and lymphocyte response to SO₂ exposure is not linear but has a declining slope above 20 mg SO₂/m³ (8 ppm). We believe that concentrations above 30 mg/m³ (11 ppm) must be examined before any definite conclusions can be drawn about the dose-response relationship. In a recent study, a very pronounced increase of neutrophils was found in the BAL fluid of mongrel dogs 2 h after exposure through tracheostomy with an SO₂ concentration of 52 mg/m³ (20 ppm) (21). After exposure to 133 and 520 mg/m³ (50 and 200 ppm) the neutrophils were accompanied by extremely high numbers of epithelial cells. These results contrast to ours and reflect a severe toxic damage to the airway and alveolar epithelium by high exposure concentrations. The absence of lymphocytes and mast cells in BAL fluid in the mentioned report (21) is still somewhat surprising and highlights the difficulties in extrapolating results from animal experiments to man.

We have recently demonstrated that alveolar macrophages, mast cells, and lymphocytes are involved in the early response to SO₂ exposure (13). The exact mechanisms of the inflammatory response to SO₂ in the human lung has, however, not been clarified. It may be that one or several of these cell types are involved in the primary mechanism by which SO₂ triggers the inflammatory cell response. It could also be that SO₂ alters the adherence of these cells to the airway epithelium or mucous layer and that this phenomenon makes them more easily lavaged. There are many other possible mechanisms behind the reactions, and the vast epithelial surfaces exposed to SO₂ have immunologic capabilities that have, so far, not been extensively studied.

According to current concepts, the highly water-soluble gas SO₂ is mainly absorbed in the nasopharynx during nasal breathing (22, 23) and most of the SO₂ that enters the lung is believed to be absorbed in the trachea and proximal bronchi. The prominent erythema that was seen in these areas in our study during bronchoscopy indicate that a mucosal effect of SO₂ had indeed occurred in these areas. It should be emphasized that it cannot be concluded from which airway levels (from the alveoli to the tip of the wedged fiberoptic bronchoscope) the different cell populations in the BAL fluid have been collected after the SO₂ exposure. If the direct effects of SO₂ on the lung mainly occur in the proximal regions, it would be logical

to believe that the majority of the cells in the recovered lavage fluid is lavaged from the bronchi and bronchioli rather than from the alveolar region. We did not find any indication of increased alveolar permeability after exposure by measuring albumin in the BAL fluid. We believe that the pulmonary response to SO₂ is more pronounced in terms of acute bronchitis/bronchiolitis than alveolitis.

We conclude that the inflammatory cell response that was found in the BAL fluid after SO₂ exposure increased as the dose increased from 10 to 20 mg/m³, but that no further amplification of the reaction could be detected after 30 mg/m³. An inflammatory cell response in the BAL fluid was observed at and below the short-term exposure limit of Sweden and several other countries, 13 mg/m³. Well-defined cell reactions were found after one single exposure to SO₂ concentrations that occur in peak exposures in occupational indoor environments worldwide. We find it possible that repeated exposures to these SO₂ levels may cause persisting inflammation in the lungs of workers and that this phenomenon may be an important factor behind the increased incidence and morbidity of chronic lung diseases in workers exposed to SO₂. The safety of the short-term exposure limit for SO₂ is thus questioned.

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