Inflammatory cell response in bronchoalveolar lavage fluid after nitrogen dioxide exposure of healthy subjects: a dose-response study

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ABSTRACT: The combination of environmental chamber exposure and bronchoalveolar lavage (BAL) was used to study the effects of the common air pollutant nitrogen dioxide (NO_2). Eighteen healthy nonsmokers were exposed to NO_2 during 20 min in an exposure chamber during light bicycle ergometer work. All subjects were examined with BAL at least 3 wks before exposure, as a reference. The subjects were re-examined with BAL, in groups of eight, 24 h after exposure to 4, 7 and 10 mg $NO_2 \cdot m^{-3}$ (2.25, 4.0 and 5.5 ppm), respectively. An inflammatory cell response was found after exposure to all concentrations. An increase in the number of lymphocytes in BAL fluid was observed after 7 and 10 mg·m⁻³ ($p < 0.05$ and 0.02, respectively). An increase in the number of mast cells, that appears to be dose-dependent, was found after exposure to all concentrations. The proportion of lysozyme positive alveolar macrophages was elevated after exposure to 7 mg·m⁻³. The inflammatory mediators fibronectin, hyaluronan, angiotensin converting enzyme (ACE) and beta₂-microglobulin were unchanged by exposure. Due to the findings of inflammatory cell changes far below the peak exposure limits for work places in industrialized countries, 9–18 mg·m⁻³, the safety of these limits is questioned. Studies are in progress in our laboratory using BAL to evaluate the effects of repeated NO_2 exposure.
Eur Respir J., 1991, 4, 332–339.

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Nitrogen dioxide (NO_2) is a common air pollutant both in community air in urban areas and in the indoor environment in industries using combustion processes. Due to its poor solubility it is to a large extent deposited in the peripheral air spaces [1, 2] where it acts on cells according to its oxidant and free radical properties [3]. Inhalation of relatively low concentrations of NO_2 may increase airway resistance in healthy subjects and repeated exposure may give enhanced susceptibility to airway infections [4, 5]. Reduced diffusion capacity for carbon monoxide has been reported after a single exposure to 9 mg $NO_2 \cdot m^{-3}$ [6]. Exposure to high concentrations may result in chronic obstructive lung disease, emphysema or bronchiolitis obliterans [7].

Morphological studies in animals have shown that the most sensitive part of the lung to NO_2 exposure is the transitional zone between the terminal bronchiole and the alveolar duct [8, 9]. Alveolar effects mainly

occur after high NO_2 levels or long-term exposure [10, 11]. In animal studies increased numbers of neutrophils and macrophages [12–14], and in some investigations also lymphocytes and mast cells [10, 15], have been found in histological specimens and bronchoalveolar lavage fluid (BALF).

In man bronchoalveolar lavage (BAL) has only recently been employed in investigating the effects of NO_2 inhalation. Recently, we showed an altered composition of cells in BALF as the time-course was investigated for the inflammatory cell response induced by 7 mg $NO_2 \cdot m^{-3}$ healthy subjects [16]. NO_2 exposure has also been shown to cause an altered antiprotease activity, alpha2-macroglobulin increase in BALF and impaired virus inactivation by human alveolar macrophages (AM) [17–19].

This investigation was undertaken to examine the dose-response relationship between NO_2 exposure in man during light bicycle ergometer work and changes in the

BALF content of cells with regard to their concentration and function. This was measured as phagocytic ability and release of soluble components considered as a reflection of cellular activity.

Subjects and methods

Subjects

Eighteen healthy nonsmoking male volunteers with a mean age of 26 yrs (range 22–32 yrs) participated. None of them had experience symptoms of airway infection for at least six weeks prior to the study or had a history of asthma. Informed consent was obtained and the study was approved by the local Ethics Committee.

Design of the study

Flexible fibreoptic bronchoscopy with BAL was performed in all subjects in order to obtained reference BALF. In the following exposure series, each subject was exposed to at least one NO_2 concentrations. Six of the subjects were exposed to two NO_2 concentrations, with a time interval of at least 3 wks. The environmental chamber exposure was performed with 4, 7 and 10 $mg \cdot m^{-3}$ (2.25, 4.0 and 5.5 ppm) for 20 min. Eight subjects were studied at each concentration, according to a standardized protocol [20]. During the last 15 min of the exposure the subjects were working on a bicycle ergometer with a work load of 75 W. A second BAL was performed 24 h after the end of NO_2 exposure, which was at least 4 wks after the first lavage. Immediately before and after exposure, and before the post-exposure BAL, forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were recorded using a Vitalograph® spirometer (Vitalograph Ltd, Buckingham, UK).

Nitrogen dioxide exposure

The exposure chamber measured 3.20 × 2.00 × 2.20 m with an air volume of 14.1 m^3. It was built with anodized aluminium, with windows in one wall. Ambient air was drawn continuously through the chamber at 150 $m^3 \cdot h^{-1}$, resulting in one air exchange approximately every 6 min. During the exposures, the chamber air temperature was kept at 21°C and the relative humidity at 45%. The desired NO_2 concentrations in the exposure chamber were achieved by adding a gas stream from a gas tube containing 1% NO_2 gas into the chamber air inlet. The gas flow was maintained evenly by a Mass Flowmeter, Brooks 5850 Tr, Brooks Instruments, Vendeen, The Netherlands. The chamber air was continuously analysed with a direct indicating equipment (Nitrogen oxides analyzer 8440 B, Monitor Labs, San Diego, Ca, USA).

Bronchoalveolar lavage

All bronchoscopies were performed by the same investigator. Atropine was given *s.c.* prior to the examination and lidocaine 200 mg was used for topical anaesthesia. No sedative or other complementary medication was given. The flexible fibreoptic bronchoscope (Olympus BF 1T or BF 1T10, Japan) was inserted through the mouth with the subject in the supine position. After careful wedging of the bronchoscope tip in a 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.

Lavage fluid analysis

Cell counts. The chilled BALF was filtered through a nylon filter (pore diameter 100 μm, Syntab Product AB, Malmö, Sweden) 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 ml. The total number of cells in the lavage fluid was counted in a Bürker chamber. Cytocentrifugal specimens with 5×10^4 non-epithelial cells per slide were prepared using a Cytospin 2® (Shandon Southern Instruments Inc., Sewickly, PA, USA) 100 rpm (96 G) for 15 min. 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 10 visual fields at 16× magnification on slides stained with acid toluidine blue and counterstained with Mayer's acid haematoxylin [21]. Lysozyme positive AM were demonstrated with Lysozyme® antibody using an immunoperoxidase technique (Dakopatts A/S, Copenhagen, Denmark). Two hundred alveolar macrophages (AM) were counted. The ratio helper-inducer/cytotoxic-suppressor (CD8⁺/CD4⁺) T-cells was counted on 200 lymphocytes using light microscopy using the Simultest T Helper/Suppressor Test® (Becton Dickinsson AB, Stockholm, Sweden).

Non-cellular components. Albumin was measured with rocket electroimmunoassay according to LAURELL [22]. Concentrations were expressed in $mg \cdot l^{-1}$.

Fibronectin was analysed by a double-sandwich enzyme-linked immunosorbent assay (ELISA) developed by BLASCHKE *et al.* [23]. The aliquots of BAL fluid were defrozen at 37°C. Briefly, microtitre plates (NUNC, Denmark) were coated with rabbit-antihuman fibronectin antibodies (Dakopatts, Denmark) in phosphate-saline buffer, pH 7.2. After addition of unconcentrated BAL fluid samples the plates were incubated at room temperature for 2 h. Horse radish peroxidase-labelled antihuman fibronectin (Dakopatts) was added as second antibody and the plates were incubated for 1 h. The amount of bound peroxidase, which is proportional to the amount of fibronectin in the sample, was measured by analysing the enzymatic activity on

orthophenylendiamine. Serum fibronectin of nephelometric quality from Behringer-Hoechst (Frankfurt am Main, GFR) was used as standard. Concentrations of fibronectin were expressed in $\mu g \cdot l^{-1}$. The detection limit was 10 $\mu g \cdot l^{-1}$. Intra- and interassay variability were 3.7% and 6.4%, respectively.

Angiotension-converting enzyme (ACE) activity was analysed in principle according to RYAN et al. [24]. It was measured in unconcentrated fluid with a commercial radioassay (Ventrex Lab Inc; Portland, Maine, USA) using ^{3}H-benzoylphenylalanyl-alanyl-proline as substrate. Aliquots of the subcellular fractions containing 1–4 μg protein were mixed with the substrate in hydroxyethylpiperazine ethanesulphonic acid (HEPES)-NaCl buffer pH 7.4 and incubated for 15 min at 37°C. The amount of ACE was measured after extraction of the acidified reaction mixtures with Ventrex scintillation fluid. The analysis was also performed in the presence of the ACE inhibitor captopril and the difference in enzyme activity was calculated. The enzyme activity (U) was expressed as that quantity of enzyme that hydrolyses the substrate at an initial rate of 1% per min at 37°C.

Hyaluronan was analysed in duplicate in unconcentrated BALF by a radiometric assay using the Pharmacia Research Kit according to principles outlined previously [25, 26]. Concentrations were expressed in $\mu g \cdot l^{-1}$. The detection limit was 10 $\mu g \cdot l^{-1}$. Intra- and interassay coefficients of variation were 4.0% and 6.4%, respectively.

Beta$_2$-microglobulin was measured in duplicate, in unconcentrated fluid with a radioimmunoassay (Pharmacia Diagnostics AB, Sweden). The detection limit was 40 $\mu g \cdot l^{-1}$. Concentrations were expressed in $\mu g \cdot l^{-1}$ and the detection limit was 40 $\mu g \cdot l^{-1}$. The intra- and interassay variability was 6.2% and 7.0%, respectively.

Macrophage phagocytosis was measured as percentage engulfment positive cells, using a glass surface adherence method as described previously [27]. The method was modified according to the use of bronchoalveolar lavage cells. Briefly, 2×10^5 cells in 200 μl medium containing 10% pooled human AB$^+$ serum were allowed to adhere to a glass surface for 30 min in cell culture conditions. After rinsing the non-adherent cells away, yeast cells labelled with FITC and opsonized with human serum where added to the slides (2.5×10^7 yeast cells in PBS buffer solution, pH 7.4). After 30 min the phagocytosis was stopped by dipping the slides into ice-cold PBS containing 1 mM edetic acid (EDTA). The fluorescence of non-ingested yeast cells were quenched by dripping toluidine blue in saline (1 $mg \cdot m^{-1}$, pH 4.7) onto the slides. Yeast cell adherence (attachment) to a macrophage was defined as visible contact between a brown yeast cell and a macrophage. Engulfment was defined as the presence of fluorescent yeast cells within a macrophage.

Statistics

Values are given as medians and interquartile ranges (Q1–Q3) since the observed values were not normally distributed. Wilcoxon's non-parametric signed rank test

Table 1. – Cell numbers and alveolar macrophage phagocytic capacity in BALF 24 h after controlled chamber exposure with 4, 7 and 10 mg $NO_2 \cdot m^{-3}$

	Total cells $\times 10^7 \cdot l^{-1}$	Lymphocytes $10^7 \cdot l^{-1}$	%	Mast cells $\times 10^4 \cdot l^{-1}$	%	AM $\times 10^7 \cdot l^{-1}$	%	LZM+AM $\times 10^7 \cdot l^{-1}$	% of macrophages	AM % engulfment positive cells
Before exposure (n=18)	7.4 6.4–12	0.4 0.3–0.7	6 5–9	1.9 0–4.8	0.05 0–0.03	6.8 5.6–11.5	92 89–92	0.8 0.3–1.5	8 6–18	88 83–92
24 h after 4 mg $NO_2 \cdot m^{-3}$ (n=8)	9.6 8.2–15.2	1.0 0.5–1.7	10 5–13	12.5* 5–17.2	0.11* 0.07–0.11	8.4 7.1–13.9	88 83–90	1.0 0.7–1.7	10 8–12	87 83–92
24 h after 7 mg $NO_2 \cdot m^{-3}$ (n=8)	7.9 6.7–9.0	1.4* 0.7–2.0	16* 12–24	11.7* 6.9–13.6	0.14*** 0.11–0.22	6.4 5.0–7.6	79* 68–85	0.8 0.7–1.1	14* 11–18	87 85–89
24 h after 10 mg $NO_2 \cdot m^{-3}$ (n=8)	9.6 5.6–15.4	1.4** 0.7–2.1	12* 8–20	19.7* 12.2–27.8	0.17** 0.07–0.27	7.8 5.2–14	85* 78–90	1.0 0.7–1.9	12 8–18	87 83–89

Values are expressed as medians with interquartile ragnes. *: p<0.05; **: p<0.02; ***: p<0.01. Significant differences after exposure compared with before exposure. Wilcoxon's rank sum test for paired observations. BAL: bronchoalveolar lavage fluid; AM: alveolar macrophages; LZM+: lysozyme positive.

for paired observations was used for comparison of BAL parameters before and after exposure in the same individual. A p value of <0.05 was considered significantly.

Results

Symptoms

The subjects reported a mild metallic smell and teste in the mouth during exposure. A very mild irritation in the throat was reported by a few subjects.

Spirometry

Before exposure FEV_1 and FVC were $104\pm12\%$ (SD) and $99\pm10\%$ (SD) of predicted values, respectively [28]. No significant changes were found immediately after exposure or prior to the second lavage.
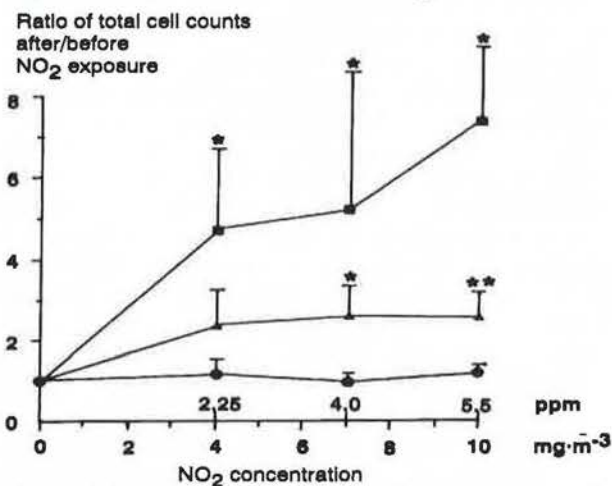

Fig. 1. – The relative changes in total cell counts for mast cells, lymphocytes and alveolar macrophages 24 h after exposure to 4, 7 and 10 mg $NO_2 \cdot m^{-3}$ (2.25, 4.0 and 5.5 ppm). The values are given as median ratio of cell counts after/before exposure±SEM. *: $p<0.05$; **: $p<0.02$; ■: mast cells; ▲: lymphocytes; ●: macrophages.

Bronchoscopic findings

All subjects has macroscopically normal endobronchial findings at the bronchscopy before exposure. After exposure to 7 mg $NO_2 \cdot m^{-3}$ a mild mucosal erythema was noticed in the distal trachea and main bronchi of four subjects. Four subjects examined after 10 mg $\cdot m^{-3}$ displayed a mild erythema and the remaining four showed a moderate erythema in the areas mentioned above.

BAL fluid finding

Cells. The recovered amount of fluid at the pre-exposure BAL was highly constant between examinations. The interquartile range was 75–75% of the instilled volume. The recovery after exposure to the various NO_2 concentrations did not differ from the pre-exposure values. The cell counts for total cells, lymphocytes, AM and mast cells are given in table 1.

When the concentration of lymphocytes was compared before and after exposure to 7 and 10 mg $\cdot m^{-3}$ significant increases were found ($p<0.05$ and $p<0.02$, respectively). A significant ($p<0.05$) increase in the relative amount of AM that stained positive for lysozyme was also seen 24 h after exposure to 7 mg $\cdot m^{-3}$. This increase was not found at the highest concentration of NO_2. The total number of mast cells was significantly increased in BALF after all exposure concentrations. Also, the percentage of mast cells increased significantly at all NO_2 concentrations ($p<0.05$, $p<0.01$ and $p<0.02$, respectively). The response increased by the dose as seen in figure 1. The relative changes in cell numbers after exposure are given in figure 1, calculated as ratio after/before exposure. Total cell number, neutrophils, eosinophils, epithelial cells, and T-helper-inducer/supressor-cytotoxic cell ratio were unaffected after all exposure concentrations (data not shown). The phagocytic activity of AM *in vitro* was 88% (83–92%) engulfment positive cells before and the activity was not significantly changed after exposure.

Table 2. – Soluble components in BALF before and 24 h after NO_2 exposure

		Before exposure n=18	NO₂ concentrations		
			4 mg·m⁻³ n=8	7 mg·m⁻³ n=8	10 mg·m⁻³ n=8
Fibronectin μg·l⁻¹	Median	74	76	70	74
	Q₁–Q₃	59–101	55–80	58–108	55–112
Hyaluronan μg·l⁻¹	Median	15	17	18	14
	Q₁–Q₃	11–25	14–24	13–26	12–27
β₂-micro-globulin μg·l⁻¹	Median	138	182	176	141
	Q₁–Q₃	105–165	148–214	166–215	129–175
ACE U	Median	9066[†]	4885[††]	9999	15544
	Q₁–Q₃	5783–17136	4464–5706	6064–18379	10636–17695
Albumin mg·l⁻¹	Median	46	48	48	54
	Q₁–Q₃	31–50	33–55	42–55	47–63

Q₁–Q₃: first–third quartile; [†]: n=16; [††]: n=6. BALF: bronchoalveolar lavage fluid; ACE: angiotensin converting enzyme.

Non-cellular components. The albumin, hyaluronan, fibronectin, beta$_2$-microglobulin concentrations and ACE activity are shown in table 2. No significant changes were detected after exposure.

Discussion

In the present study the NO$_2$ concentration range, 4–10 mg NO$_2$·m^{-3}, chosen to reflect conditions encountered mainly in industrial work places. We found an altered composition of the population of cells recovered in the lavage fluid all through the investigated concentration interval, *i.e.* even far below the peak exposure limits for NO$_2$ in work places, 9–8 mg·m^{-3} (5–10 ppm) in different countries. The increase in cell numbers appears to be dose-dependent for mast cells, but cannot be determined conclusively for lymphocytes. The pattern for AM stained positive for lysozyme is not conclusive in the present material. The latter may be a consequence of the relatively low number of subjects investigated at each concentration. Involvement of all three cell types in the toxicological reaction in the human lung to NO$_2$ is further supported by a parallel study in our laboratory investigating the time-kinetics of the inflammatory response in BALF after exposure to 7 mg NO$_2$·m^{-3} [16]. In the earliest NO$_2$ study using BALF analyses in man. Frampton and co-workers [19] reported no changes in cell numbers after 1.1 mg·m^{-3}. MOSHENIN and GEE [17] were also unable to detect any significant changes in the cell populations recovered in BALF after 7 mg·m^{-3}. However, in that study the NO$_2$ exposed individuals were cod to a small separate control group. Consequently, interindividual differences in cell numbers may well have influenced the results. It should, furthermore, be noted that mast cell and lysozyme stains were not applied in any of these studies.

In the present study an increase in the lymphocyte population, expressed as total lymphocyte number and percentage of recovered cells was a significant response to NO$_2$ exposure. However, no change in the ration of T helper-inducer/suppressor-cytotoxic cells was seen, in agreement with previous BAL studies with NO$_2$ and SO$_2$ in man [16, 29, 30]. The concentrations of beta$_2$-microglobulin, a potential marker of T-cell activation, was also unaffected after the exposures. Thus, the expansion of the lymphocyte population does not seem to be accompanied by a simultaneously enhanced activity of the cells.

Mast cells constitute 2% of the bronchial epithelial cells in human lung [31] and have previously been associated mainly with immunoglobulin (IgE) mediated allergic reactions and asthma [32, 33]. Recent studies have focused attention on other properties of this cell, which appears to be involved in conditions with alveolitis tissue damage and development of fibrosis [34, 35]. The observed increase in the number of mast cells is consistent with our earlier findings after exposure to both NO$_2$ and SO$_2$ [16, 29, 30]. It remains to be established what significance this has and its is

presently unclear whether these mast cells are active in the immune response or not. Support for an active role has previously been presented after observation of antihistamine inhibition of increased airway resistance after NO$_2$ exposure [36]. Furthermore, NO$_2$ induced degranulation of mast cells has been observed in a morphological study in rodents [15].

Considering the present and previous investigations, we can conclude that short-term exposure to moderately high NO$_2$ concentrations in man does not induced increase in the number of neutrophils, as reflected in BALF. This is surprising considering that neutrophilia has been a consistent finding in rodents after NO$_2$ exposure [12, 13] and increased neutrophil chemotactic activity has been demonstrated after *in vitro* challenge with NO$_2$ of human AM [37]. Varying susceptibility in different species, differences in exposure duration and concentration, and various conditions in *in vitro* test situations may account for the diverging results.

In addition to neutrophilia, increase in AM numbers has been the most pronounced inflammatory cell response to NO$_2$ in BALF and lung tissue in animal studies [12, 13]. We were unable to detect any significant increase in the total AM number, but after exposure to 7 mg NO$_2$·m^{-3} a larger proportion of the AM were lysozyme positive. This increase in the intracellular concentration of lysozyme has previously been shown to occur after a variety of stimuli in man including SO$_2$ exposure [29, 30, 38, 39]. It is consistent with recent results from a time-kinetic study, where the increased proportion of lysozyme positive AM persisted 72 h after exposure [16]. The findings could be caused by an enhanced production of lysozyme in the AM or be due to an inhibited secretory process after exposure.

Increased susceptibility to viral infections following NO$_2$ exposure has been proposed in epidemiological studies [4, 5]. This corresponds with in *in vitro* tests with animal [40, 41] and human AM [19] demonstrating NO$_2$ induced decrease in phagocytosis and inactivation of microorganisms. FRAMPTON and co-workers [19] found this to occur after an NO$_2$ concentration as low as 1.1 mg·m^{-3}. In this study we investigated whether *in vivo* exposure to NO$_2$ would impair phagocytosis *in vitro* by human AM, but were unable to detect any significant effect. This could have different explanations. Firstly, human AM phagocytosis may not be affected by NO$_2$ exposure *in vivo*, at least not in the investigated concentration interval. Secondly, impaired phagocytosis possibly occurs only after repeated exposures. Thirdly, the *in vitro* test situation *per se* may have stimulated the macrophages so that an impairment in phagocytosis present in vivo was masked *in vitro*.

Other signs of altered AM activity were also lacking as the concentrations of ACE and fibronectin in the lavage fluid were normal. ACE has been demonstrated to be release by AM in an activated state [42, 43] and an increased concentration in BALF has been reported in conditions with alveolitis [44]. Fibronectin is also known to be produced by AM in various lung disorders *e.g.* in association with fibrosing processes [26, 45].

It has, furthermore, been shown to be a sensitive marker of irradiation induced damage to the respiratory epithelium [46]. The discrepancy in the findings of an increased portion of lysozyme positive AM and unaffected phagocytic capacity and mediator production does not support the hypothesis that positive lysozyme staining of AM reflects a state of activation.

Hyaluronan is a glycosaminoglucan with a pronounced ability to immobilize water [47]. It has been demonstrated to correlated with reduced alveolar-capillary diffusion capacity for carbon monoxide in alveolitis [48, 49]. Interestingly, a decrease diffusion capacity for carbon monoxide has been reported in subjects exposed to similar NO₂ concentrations as in our study [6]. Hypothetically, in those subjects a hyaluronan-induced immobilization of water in the lungs could account for the decreased diffusion capacity. We were unable to detect any increase in hyaluronan and there was no increase in the BALF concentration of albumin after gas exposure suggesting that these was no pronounced damage of the alveolar-capillary membrane [43].

Despite the differences in deposition and chemical actions of nitrogen dioxide and sulphur dioxide there are interesting similarities between their effects in the human lung. Both may induced an increase in the numbers of lymphocytes and mast cells, and to some extent lysozyme positive macrophages, but only SO₂ gives a pronounced increase in the number of AM [16, 29, 30].

It is concluded that a single brief exposure to NO₂ in man, in concentrations that occur in peak exposures in work places, causes an inflammatory cell reaction in the lung as demonstrated by an increase in the number of lymphocytes and mast cells and in the proportion of LZM+AM. However, a single exposure does not seem to induced any significant changes in the alveolar-capillary permeability or cause any pronounced release of inflammatory markers. Whether this is also true for repeated exposures within the same concentration interval needs to be elucidated. The observed inflammatory cell reactions are caused by NO₂ concentrations below the peak exposure limits for indoor work in industrialized countries. Consequently, the safety of these exposure limits can be questioned. Studies are currently under progress in our laboratory using BAL examinations to clarify the effects of repeated NO₂ exposure.

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Réponse des cellules inflammatoires du liquide de lavage broncho-alvéolaire après exposition de sujets sains au bioxyde d'azote. T. Sandström, N. Stjernberg, A. Eklund, M-C. Ledin, L. Bjermer, B. Kolmodin-Hedman, K. Lindström, L. Rosenhall, T. Ångström.

RÉSUMÉ: Les effets de polluant très commun de l'air ambiant, le NO_2, ont été étudiés par la combinaison de l'exposition dans une chambre environnementale et le lavage broncho-alvéolaire (BAL). Dix-huit sujets sains non-fumeurs furent exposés à NO_2 pendant 20 minutes dans une chambre d'exposition au cours d'un travail léger à la bicyclette ergométrique. Tout les sujets ont été examinés par lavage au moins trois semaines avant l'exposition, à titre de référence. Les sujets ont été réexaminés par lavage broncho-alvéolaire dans des groupes de 8×24 heures après exposition à 4, 7 et

10 mg NO$_2$·m^{-3} (2.25, 4.0 et 5.5 ppm), respectivement. Une réponse cellulaire inflammatoire a été observée après exposition à toutes les concentrations. Une augmentation du nombre de lymphocytes dans le liquide de BAL a été observée après 7 et 10 mg·m^{-3} (p<0.05 et 0.02, respectivement). Une augmentation du nombre de mastocytes, qui s'avère dose-dépendante, est observée après exposition à toutes les concentrations. La proportion de macrophages alvéolaires lysozome positifs augmente après exposition à 7 mg·m^{-3}. Les médiateurs inflammatoires que sont le fibronectine, l'hyaluronan, l'enzyme de conversion de l'angiotensine (ACE) et la bêta$_2$-microglobuline, restent inchangés après exposition. Vu nos observations concernant les modifications des cellules inflammatories observées en dessous des limites tolérées pour les pointes d'exposition dans les lieux de travail des pays industrialisés (9–18 mg·m^{-3}), l'ont met en question la sécurité de ces limites. Des études sont poursuivies dans notre laboratoire au moyen du BAL, pour évaluer les effets de l'exposition répétée au NO$_2$.

Eur Respir J., 1991, 4, 332–339.