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EDITORIAL

Experimental exposures to nitrogen dioxide

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Nitrogen dioxide (NO_2) produced by combustion processes, is a major constituent of ambient air pollution in urban and industrial areas. It also plays a role as an indoor air pollutant and in the workplace. Indeed acute inhalation accidents in the workplace with high concentrations of NO_2 provided the first evidence of its adverse respiratory effects.

Epidemiological studies provided evidence of an association between indoor as well as outdoor NO_2 exposure and the frequency or duration of respiratory illness and lung function impairment [1, 2]. However, it is well-known that interpretation of epidemiological studies in terms of causal relationships poses many problems, due to confounding variables and the complexity of air pollutant mixtures. Therefore, controlled exposure studies in human subjects are essential to the understanding of the health effects of NO_2.

Effects on lung function and airway responsiveness

The first experimental studies were performed during the early seventies, mainly by German investigators focusing on the relevance of NO_2 in the workplace, using concentrations which were high compared to ambient levels. These studies demonstrated that the threshold NO_2 concentrations necessary to increase airway resistance during short-term exposure were about 2.5 ppm in healthy subjects and 1.5 ppm in subjects with chronic bronchitis [3, 4]. NO_2 exposure at levels of 5–7.5 ppm caused an increase of airway responsiveness to acetylcholine in healthy subjects [4]. Patients with chronic bronchitis showed an impairment of gas exchange after breathing 4–5 ppm NO_2 [5]. Antihistaminic agents, but not atropine or beta$_2$-agonists, were able to attenuate the NO_2 response [6]. The data suggested increased susceptibility to NO_2 in patients with chronic bronchitis compared to healthy subjects, a result which is in accordance with a recent study on patients with chronic obstructive pulmonary disease (COPD) [7].

Subjects with bronchial asthma also form a group sensitive to NO_2. Even at concentrations of 0.1 ppm NO_2, a proportion of those investigated by OREHEK *et al.* [8] demonstrated an increase of airway responsiveness to carbachol. Subsequent studies have shown that short-term exposure to low concentrations of NO_2 (0.1–0.5 ppm) enhanced airway responsiveness to histamine [9], methacholine [10, 11] or carbachol [12] in at least some of the asthmatic subjects studied. In addition, bronchoconstriction induced by exercise and hyperventilation of cold air [13], or air with 0.75 ppm sulphur dioxide [14], was increased after breathing 0.25–0.3 ppm NO_2. However, other studies in subjects with bronchial asthma failed to demonstrate changes of bronchial responsiveness after exposure to 0.1–0.3 ppm NO_2 [15–17]. In summary, data confirm that asthmatic subjects are more susceptible to NO_2 than healthy subjects but the functional changes induced by NO_2 depend on the severity of the asthmatic disease and the type of exposure protocol.

Effects on cellular and non-cellular components of bronchoalveolar lavage fluid

The application of flexible bronchoscopy and bronchoalveolar lavage (BAL) has provided a unique opportunity to study events within the distal airways and alveoli at a cellular and biochemical level. Several authors have utilized this technique to elucidate the effects of controlled NO_2 exposures.

In healthy individuals the functional activity of the α_1-proteinase inhibitor in BAL fluid was reduced by 45% after breathing 3–4 ppm NO_2 [18] but not after exposure to 1.5 ppm [19], whereas the level of the antiprotease α_2-macroglobulin in BAL fluid was elevated after continuous exposure to 0.6 ppm NO_2 [20]. None of these studies revealed alterations of BAL cell counts or non-cellular constituents, such as total protein, albumin, leucocyte elastase and lactate dehydrogenase.

SANDSTRÖM and co-workers [21] investigated the dose-response characteristics of the NO_2 effect in BAL. Their data indicate a dose-dependent increase of BAL mast cells and lymphocytes 24 h after short-term exposures to 2.25, 4.0 and 5.5 ppm NO_2. The percentage of lysozyme-positive alveolar macrophages was significantly elevated after 4.0 ppm NO_2, whereas the levels of fibronectin, hyaluronan, angiotensin converting enzyme and β_2-microglobulin in BAL fluid were not altered after any concentration. Using the same exposure protocol with 4 ppm NO_2, these authors also investigated the time course of the NO_2 response [22]. The number of mast cells and lymphocytes was increased 4, 8 and 24 h after exposure but had returned

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to normal after 72 h as compared to control lavage, whereas a significant increase in the percentage of lysozyme positive macrophages was seen 24 and 72 h after exposure.

Recently, we performed bronchoscopy and BAL in subjects with mild bronchial asthma and healthy controls one hour after a 3 h exposure to 1 ppm NO_2 [23]. We could not substantiate significant changes in the cellular composition of BAL fluid and bronchial biopsies as compared to a control day. However, in the asthmatic subjects, NO_2 exposure induced slight changes in the BAL levels of prostanoid mediators. Thus, the enhanced susceptibility to NO_2 in asthmatics, shown by lung function measurements, was reflected in changes of inflammatory mediators.

In this issue of the Journal, SANDSTRÖM and co-workers [24] present a study on the effect of repeated exposures to 4 ppm NO_2 on BAL cell populations in 10 healthy subjects. Subjects were exposed over 20 min every second day, six times, and BAL was performed 24 h after the last exposure. The authors found no change in the numbers of lymphocytes and mast cells, in contrast to the single exposure studies [21, 22]. The numbers of macrophages, B-cells and natural killer cells were slightly decreased. The absolute number of T-suppressor cells was reduced and consequently the helper/suppressor ratio was elevated, again in contrast to single exposures [22]. In addition, repeated exposures caused a reduction of the total number of lymphocytes in the peripheral blood. It is interesting to compare these results with those of RUBINSTEIN *et al.* [25]. In five healthy volunteers, who underwent four repeated 2 h exposures to 0.6 ppm NO_2 within 6 days, they found, 2 h after exposure, a greater number of lymphocytes in peripheral blood, no change of T-cell helper/suppressor ratio in BAL and an increase in the proportion of natural killer cells in BAL. The total dose of NO_2 inhaled in both studies [24, 25] was in the same range.

These discrepancies highlight the problems posed by the attempts to elucidate the mechanism of action of NO_2 using bronchoalveolar lavage. Most of the observed effects are small and seem to be heavily dependent on experimental conditions. Obviously, NO_2 exhibits a complex and time-dependent interference with the regulatory network which governs function and integrity of the airways. This phenomenon may be partially due to the fact that not NO_2 itself but rather a variety of products of reactions with epithelial surface constituents mediate the cellular response [26, 27]. The details of the *in vivo* kinetics of NO_2 are largely unknown. Furthermore, the data of SANDSTRÖM and co-workers [22] on the time course of the NO_2 effect suggest that time dependence may differ between parameters and that the effects of NO_2 may shift from fast effects, detectable in cell numbers, to slow effects, related to the functional state of cells, indicating a cascade of events within the cellular control mechanisms. It is conceivable that these phenomena should be most prominent in repeated or prolonged exposures.

Since little can be inferred from the literature on the reproducibility of BAL parameters after NO_2 exposures [21, 22] and the effects demonstrated so far have been only small, conclusions drawn from the available studies should be considered with some caution. For example, in the paper of SANDSTRÖM and co-workers [24], the problem of weighing the scatter of responses against the magnitude of the NO_2 effect is reflected by the fact that the mean value of T-cell helper/suppressor ratio was 2.3 before and 2.8 after exposure, with ranges increasing from 2.1–2.8 (before) to 2.4–4.0 (after exposure). Essentially, the biological and clinical significance of the experimental exposure results is unclear. Nevertheless, the findings of SANDSTRÖM and co-workers [24] regarding immunologically relevant cells are compatible with animal data on enhanced susceptibility to respiratory infection during NO_2 exposure [28]. They can also be reconciled with a study in humans, which demonstrated impairment of alveolar macrophage inactivation of influenza *in vitro* in half of the subjects exposed to 0.6 ppm NO_2 *in vivo* [29]. Furthermore, from epidemiological evidence, increased rates of acute respiratory illness in children exposed to high indoor levels of NO_2 [1] may well be interpreted as the result of recurrent episodes of respiratory infection.

Although the NO_2 concentration used in the study of SANDSTRÖM and co-workers [24] was similar to peak values which may be observed in the workplace, the relevance of these data to understanding of the effects at lower concentrations remains to be established. This will hold, especially with regard to epidemiological data, since 4 ppm NO_2 is far beyond the levels measured even under the worst conditions in the ambient air of industrial areas and in traffic. We have to face the possibility that the pathophysiological mechanisms relevant to real-life exposure conditions cannot be simply extrapolated from any feasible controlled exposure experiment in human subjects. Nevertheless, despite these difficulties, even the interpretation of the epidemiological data on NO_2 may benefit from the findings that the NO_2 response may differ between single and repeated exposures and may involve time-dependent changes of cellular immune defence mechanisms.

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