Mediators of inflammation in response to air pollution: a focus on ozone and nitrogen dioxide

A 'CONCERTED ACTION' PROJECT IN THE EU BIOMED2 PROGRAMME FOR PROMOTING RESEARCH THAT MEETS THE PUBLIC HEALTH NEEDS OF EUROPEAN CITIZENS

ABSTRACT—Recent epidemiological and environmental chamber studies have strengthened the link between air pollution and respiratory disease. Acute exposure to ozone and nitrogen dioxide (NO_2) for short periods results in both upper and lower airway inflammation and patients suffering from asthma and allergic rhinitis are particularly at risk. Neutrophils are involved in the acute inflammatory reaction following ozone exposure whereas NO_2 poses a more complex response involving neutrophils, mast cells and lymphocytes. The prostanoids play an important role in early symptomatic and functional responses, although their source is still unclear.

Air pollution has become a matter of growing public and scientific concern as increasing motor vehicle traffic has brought a new spectrum of oxidant pollutants which include ozone and oxides of nitrogen (NO_x) [1]. They have been incriminated as factors contributing to the increasing morbidity of asthma [2–6]. We review here the role of various inflammatory mediators that contribute to airway inflammation and the current understanding of the various mechanisms that underlie airway injury following exposure to ozone and nitrogen dioxide (NO_2).

Airways inflammatory response to ozone

Cellular response

Ozone at ambient levels evokes symptomatic and functional responses [7–14] (Table 1) and inflammation both in the upper and lower airways [14–16], with marked variation between individuals; the inflammatory response is greater in the proximal portion of the lower airways than in the distal portion. Concentrations of ozone as low as 0.08 ppm [16] induce neutrophilic bronchitis. Inflammation is seen as early as 3 hours and lasts for up to 24 hours following exposure to ozone [17]. Because there is no direct relationship between inflammatory and functional responses following exposure to ambient levels of ozone [18], the mediators which attract neutrophils may not be the same as those producing the early symptomatic and functional responses. This also implies that it may be misleading to classify the population as 'responders' and 'non-responders' purely on the basis of lung function responses.

Prostanoids

Ozone is a powerful oxidising agent which damages tissue by generating free radicals and by peroxidation of lipids in cell membranes [19–22]. As a result, prostanoids such as PGE_2, $PGF_2\alpha$, 8-epi-$PGF_2\alpha$ and TxB_2 are released into the bronchoalveolar lavage (BAL) fluid [23,24]. They stimulate pulmonary neural afferents, producing some of the characteristic responses to ozone exposure. Administration of indomethacin, a cyclo-oxygenase inhibitor [25,26] (Fig 1), prior to ozone exposure abrogates the functional responses in normal subjects and asthmatics [27]. Surprisingly, administration of steroids [28] prior to ozone challenge resulted in a paradoxical response in forced expiratory variables and numbers of BAL neutrophils.

Cytokines and adhesion molecules

The concentration of cytokines, including IL (interleukin)-6, IL-8 and GM-CSF (granulocyte macrophage colony stimulating factor) rises in the bronchoalveolar lavage (BAL) fluid following ozone exposure. IL-8 is constitutively expressed in the bronchial epithelium, and exposure to ozone provokes further synthesis of this cytokine in the bronchial epithelium, although macrophages are also an imporant source of IL-8. Exposure of mouse airways to ozone increases the expression of macrophage inflammatory protein-2 (MIP-2) [29], a chemokine capable of inducing neutrophil chemotaxis. Exposure to ambient levels of ozone also results in an upregulation of intercellular adhesion molecule-1 (ICAM-1) [30] in the vascular

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Table 1. Symptomatic and functional responses to ambient ozone

Symptoms	Functional responses
• Shortness of breath	*Decrease in:*
• Inspiratory chest pain	• FEV1
• Tiredness	• FVC
• Eye irritation	• Inspiratory capacity
• Wheeze	• Total lung capacity
	Increase in:
	• Airway resistance

endothelium of the proximal airways mucosa associated with an increased expression of CD11b, a ligand for ICAM-1 on BAL neutrophils.

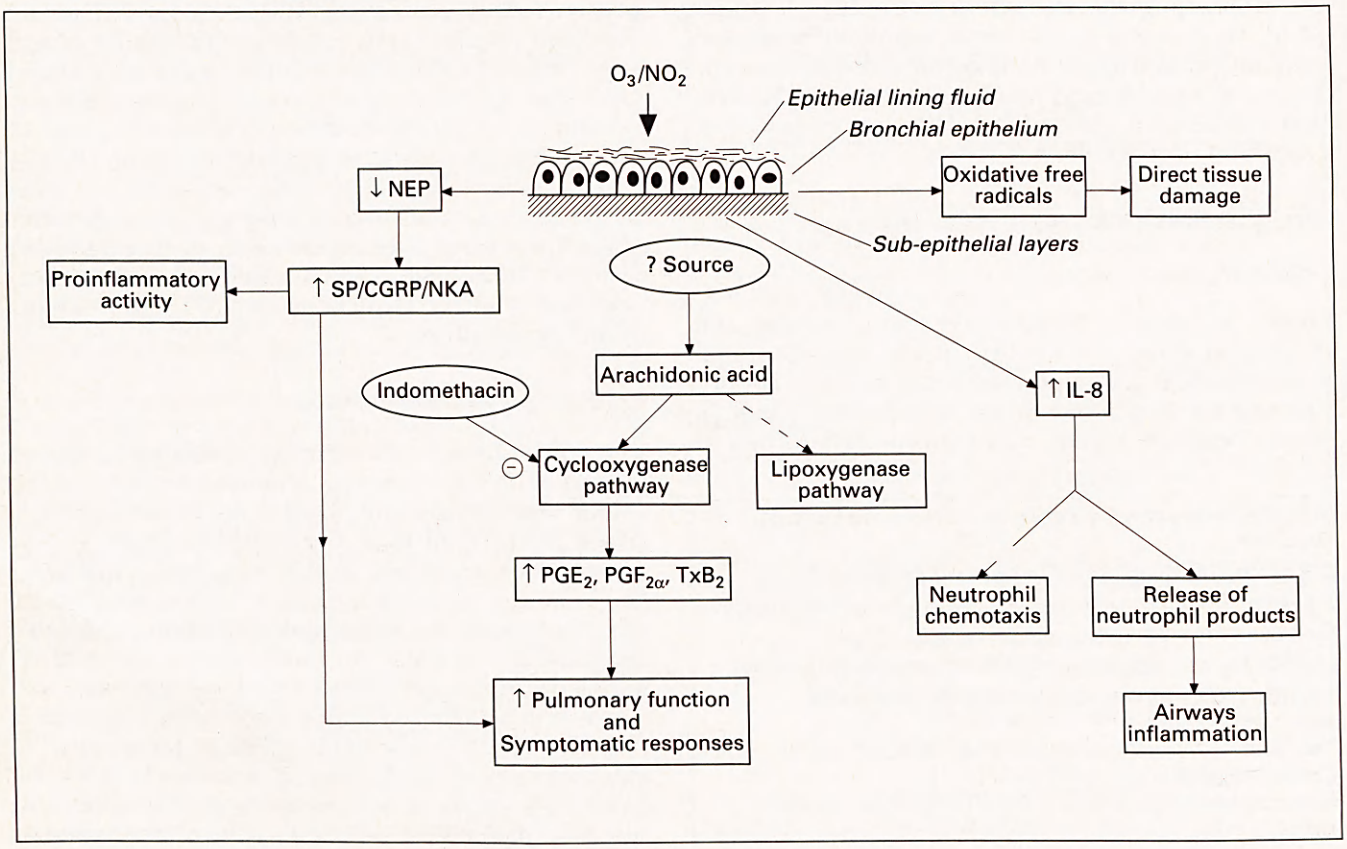
The nuclear transcription factor NF-κB induces the expression of several endothelial cell adhesion molecules—and of endothelially derived cytokines [31,32]. It is activated by a wide variety of extracellular stimuli including pro-inflammatory cytokines, bacterial and viral products and oxidative stress, and inhibited by treating endothelial cells with antioxidants [33]. It is therefore reasonable to speculate that 'oxidant stress' such as exposure to ozone and NO_2 will activate NF-κB and lead to upregulation of cell adhesion molecules and pro-inflammatory cytokines in the vascular endothelium.

In addition to producing airway inflammation, ozone can induce an acute-phase systemic response seen as an increase in the levels of C-reactive proteins in the plasma and of IL-6 in BAL and plasma [34]. The significance of these observations is not fully understood although animal experiments have shown that IL-6 could play an important role in inducing 'tolerance' [35] repeated exposure to ozone.

Effects of ozone in nonadrenergic noncholinergic (NANC) nerves

Increased levels of substance P (SP) have been found in BAL fluid immediately following exposure to 0.25 ppm ozone for one hour [24]. SP can induce bronchoconstriction, increase airway permeability, activate neutrophils and increase mucociliary transport [36–38]. These observations suggest that tachykinins such as SP and neurokinin A (NKA) could contribute to early functional and symptomatic responses, and with their immunomodulatory properties augment the airway inflammation. It is plausible that ozone diminishes the activity of neutral endo-

Fig 1. Probable inflammatory mechanisms involved in O_3/NO_2 induced airway injury (normal airways)



peptidase (NEP) an epithelial enzyme responsible for the metabolism of endogenous neuropeptides, with a resultant increase in the concentration of SP.

Effects of ozone in 'high risk groups'

Recent studies have focused on the 'high risk' groups such as asthmatics and patients suffering from allergic rhinitis. Ozone potentiates the response to inhaled allergens in patients with allergic airway disease [39], and epidemiological studies [40–42] have shown strong links between exacerbations of asthma symptoms and 'ozone episodes'. In patients suffering from allergic rhinitis, exposure to ozone can induce an influx of neutrophils and eosinophils in the nasal mucosa [43], and a significant correlation was detected between neutrophil numbers and levels of IL-8 in nasal lavage fluid of asthmatics [44] following exposure to 0.24 ppm ozone. IL-8 is principally secreted by mononuclear phagocytes, fibroblasts, endothelial and epithelial cells [45]. It is an important chemoattractant for neutrophils and induces release of granule products and free radicals from neutrophils [46,47] which cause epithelial injury, sloughing and tissue damage.

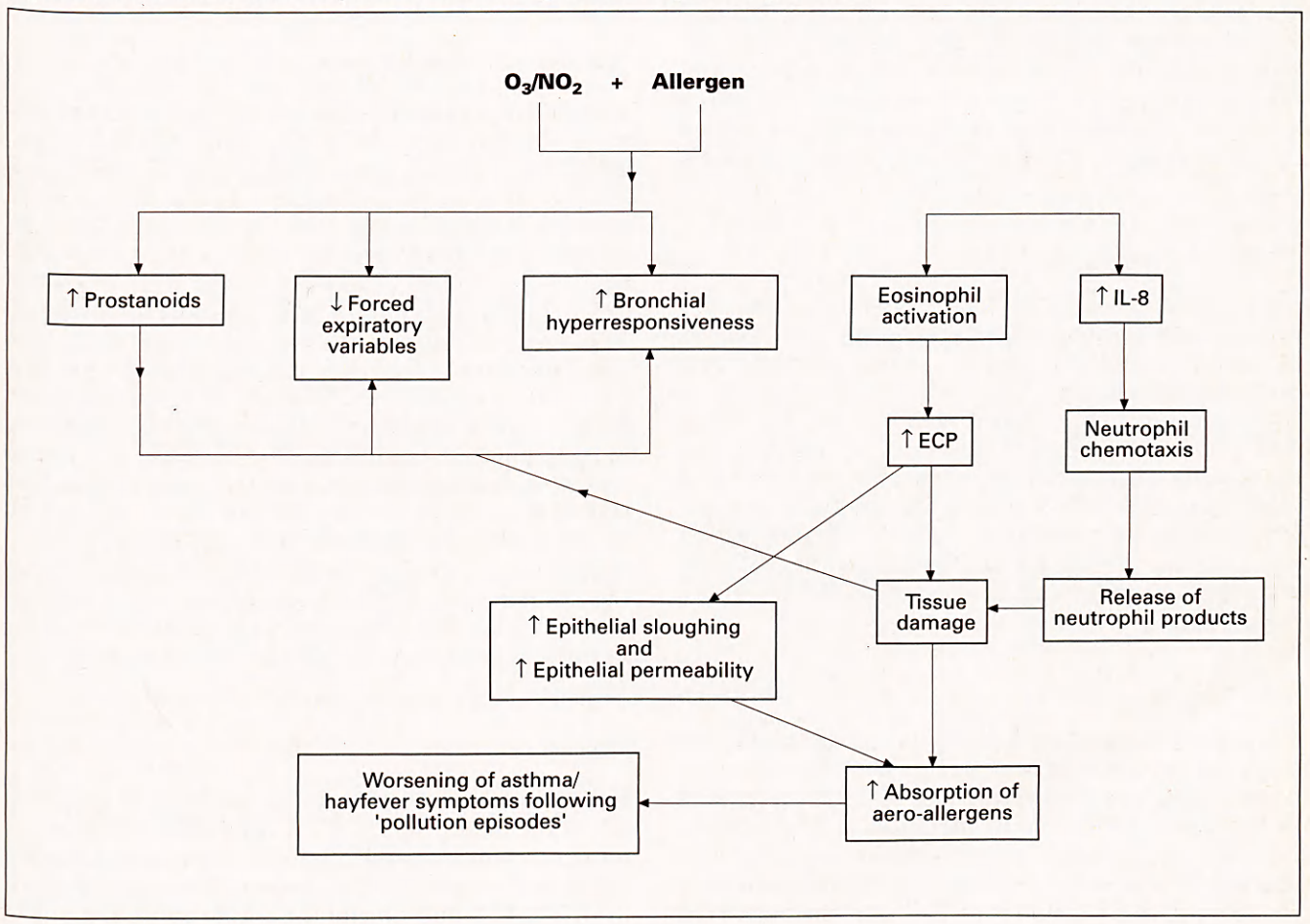
Ozone increases epithelial permeability [48] in normal human subjects and this phenomenon is likely to be exaggerated in asthmatics in whom the underlying airway inflammation and 'epithelial shedding' would result in a greater absorption of the inhaled aero-allergens. This could explain the exacerbations of asthma and hay fever symptoms immediately following 'ozone episodes'.

Figures 1 and 2 summarise the inflammatory effects of ozone in normal and asthmatic subjects. (See Table 2 for WHO guidelines for ozone.)

Table 2: WHO guidelines for ozone. These concentrations should not be exceeded during exposures over 1 or 8 hours

1 hour	8 hours
0.076-0.100 ppm	0.050-0.060 ppm

Fig 2. Effects of combination of pollutants and allergens on allergic airways disease



Airway inflammatory response to NO_2

Cellular response

Exposure to NO_2 increases the number of neutrophils in the bronchial portion of the lavage fluid (proximal airway lavage) [49], and the levels of neutrophil myeloperoxidase (MPO) [50].

Using concentrations of NO_2 between 2.25–5.0 ppm for 20 minutes in normal subjects revealed a dose dependent mastocytosis and lymphocytosis in BAL fluid 4 to 24 hours after exposure, and an increase in macrophages at concentrations greater than 4 ppm of NO_2 [51]. This effect was even more marked in smokers, suggesting that they are potentially at greater risk from the adverse effects of NO_2 [51].

Prostanoids

Healthy subjects exposed to 1 ppm NO_2 for three hours showed no significant changes in prostaglandins and histamine levels in BAL fluid [52], although TxB_2 levels were significantly higher. In asthmatics there was a decrease in the levels of 6-keto-PGF1α (a metabolite of PGI_2, a bronchodilator), and higher levels of TxB_2 (a metabolite of TxA_2, the bronchoconstrictor) and of PGD_2 (also a bronchoconstrictor), whereas levels of leukotrienes and histamine remained unchanged. These changes support the idea that asthmatics are more susceptible to the adverse effects of NO_2 than non-asthmatics. In contrast to the study described above [51] inflammatory cell numbers did not change in either group at this low (1 ppm) concentration of NO_2.

Protease and anti-protease balance

Alpha-1-protease inhibitor (α1-PI), inhibits plasma and lung elastase, an important factor in the pathogenesis of emphysema [53,54] and so protects the lung from proteolytic damage. Exposure to NO_2 (3 or 4 ppm) for 3 hours decreased the functional capacity of α1-PI in BAL by 45%, although this was not accompanied by neutrophil migration in the airways; furthermore, *in vitro* exposure of α1-PI to NO_2 did not affect its capacity to inactivate neutrophil elastase. This observation suggests that NO_2 exerts its effect indirectly, probably through the products of lipid peroxidation which are highly reactive with α1-PI.

Cytokines

It is logical to speculate that NO_2 being a powerful oxidising agent, could damage or stimulate the cell membrane of the bronchial epithelium through the process of lipid peroxidation and generation of free radicals. NO_2 at a concentration of 0.4 ppm significantly increases the release of the pro-inflammatory cytokines GM-CSF, IL-8 and TNF-α from bronchial epithelium *in vitro* and of IL-1α, IL-1β, IL-6, IL-8 and Groγ in response to 1.5 ppm NO_2 [55,56].

Effects on NANC nerves

The effect of NO_2 on the nonadrenergic noncholinergic (NANC) nerves has not been evaluated in humans. In guinea pigs, NO_2 increases bronchial responsiveness to acetylcholine and neurokinin [57] and lowers the volume density of nerves (ie the number of nerves per unit volume of tissue) which are immunoreactive to the sensory neuropeptides SP and calcitonin gene-related peptide (CGRP). Because the overall density of nerves remains unchanged, this finding indicates that some of the nerves lost their immunoactivity because they had released their sensory peptides into the lung by antidromic stimulations; such a local release can lead to neurogenic inflammation with bronchoconstriction, bronchial hyperresponsiveness and vasodilatation.

Although it is not possible to evaluate the clinical implications of these changes in inflammatory medicators, their presence suggests that NO_2 at levels encountered at the sites of heavy motor vehicle traffic, or in homes where gas is used as a source for cooking and heating appliances, could have adverse effects on lung function.

NO_2 and respiratory infections

Epidemiological studies [58–60] have shown an association between exposure to NO_2 and respiratory tract infections. Exposing normal subjects to 1.5 and 4 ppm NO_2 for 20 minutes on alternate days for 6 days causes a small but significant decrease in CD8+ cells, NK cells (CD16+ and CD56+) and B cells (CD19+) in the BAL fluid [61] but these changes are not seen at exposure to lower concentrations of NO_2. The adverse effects of NO_2 on the immune system are reflected in the impaired ability of alveolar macrophages, obtained by BAL after exposure to 0.6 ppm NO_2 to inactivate influenza virus *in vitro* [62]. Exposure of nine subjects to NO_2 impaired the inactivation of influenza virus in four whose cells produced more IL-1 than the alveolar macrophages from the five subjects whose cells could inactivate the virus. Damage of cell membrane by NO_2 via lipid peroxidation could alter the uptake of the virus by damaging cell surface receptors or decreasing endocytosis. The inflammatory effects of NO_2 in normal and asthmatic subjects are summarised in Figs 1 and 2.

Future work

This 'concerted action' (project leader: J M Polak) is our contribution to the EU's BIOMED2 programme for promoting research that meets the public health needs of European citizens. It includes participants from UK, Italy and Belgium. We will continue to study

the various mechanisms that cause airway inflammation following short-term exposures to ambient levels of ozone and high indoor levels of NO_2. Using immunohistochemical techniques we are exploring the roles of inflammatory cells, cytokines, adhesion molecules and sensory neuropeptides in airway inflammation. We are also studying the role of anti-oxidants by measuring the levels of vitamins A, C, E, glutathione, uric acid and the products of oxidative injury including protein carbonyls and lipid hydroperoxides. Results of these studies will shed more light on the current understanding of the health effects of air pollutants.

Exposure to air pollutants potentiates responses to aero-allergens. In future, studies should address the health effects of pollutants in a wider perspective, using a range of different concentrations of pollutants, as well as studying the effects of combinations of pollutants and allergens, and carrying out interventional studies by giving supplements of anti-oxidants and nonsteroidal anti-inflammatory drugs.

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