Oxidant Gases

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The acute and chronic action of the oxidant gases ozone, nitrogen dioxide and oxygen on the morphological appearance of cells of the alveolar and bronchiolar epithelium is reviewed. Type I cells of the alveolar and ciliated cells of the bronchiolar epithelium appear to be sensitive targets for the oxidant gases. The degree of damage is influenced by age, nutritional status and the development of tolerance.

Introduction

The airways of the lung are lined with a layer of ciliated and nonciliated cells (1). In the large airways (trachea, main bronchi, and bronchi), the nonciliated cell population comprises serous, goblet, intermediate, and basal cells. In the small airways (terminal and respiratory bronchioles), Clara cells make up the nonciliated cell population. Goblet, serous, and Clara cells usually protrude above adjacent ciliated cells and contain secretory granules. In the alveoli, large, flat squamous cells (Type I) cover about 97% of the alveolar surface (2). The remaining 3% of the gas-exchanging area is covered by cuboidal cells (Type II), which usually contain secretory granules (lamellar bodies). Beneath the epithelial surface is the interstitium, which is composed of acellular supporting materials (collagen and elastin), fibroblasts, and migratory cells. Within the interstitium there is a vast network of capillaries lined with endothelial cells. Together, the Type I cell, interstitium, and endothelial cell make up the air-blood barrier of the lung. Macrophages are present in the surface-active film covering the alveoli. This alveolar lining layer is continuous with a mucoid layer lining the airways.

The purpose of this paper is to describe the effects of oxidant gases on these structures. When animals are exposed to oxidant gases such as nitrogen dioxide (NO₂), ozone (O₃) or oxygen (O₂), the gas first contacts the acellular lining layer, then the macrophages, epithelial cells, and the interstitium, and finally the endothelium and capillary blood of the alveoli. The ability of these gases to affect cells of the lung is influenced by their concentration, aerodynamics, solubility, reactivity, and dilution as the air permeates the more distal alveoli (3–5). The ability of the animal to resist oxidant damage depends on its age and nutritional status and the degree to which it has adapted or become tolerant to the gas (6–9). Oxidant-induced injury to lung cells is discussed in this paper with respect to these factors.

Acute Lung Injury

When animals are exposed continuously to sublethal concentrations of NO₂ (2 to 17 ppm) or to O₃ (0.5 to 1.0 ppm), the epithelial cells lining the airways and alveoli are damaged (5,10–20). The primary anatomical site of cellular damage is the alveoli in the central region of the pulmonary acinus and the adjacent bronchioles (terminal or respiratory) (5,18). The peripheral portion of the pulmonary acinus and larger airways generally are not affected as severely (Figs. 1 and 2). Morphologically, greater injury is expressed as a large amount of alveolar damage and more involvement of the upper airways (3,21). These characteristics are thought to be due to a concentration gradient that exists during inhalation of NO₂ or O₃.

In the bronchiolar regions, the first morphological changes are observed in ciliated cells (5,14,17,19,22–24). By 4 hr of exposure, a loss of cilia begins. By 8 hr, the

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loss is obvious, and sloughing of dying and necrotic ciliated cells is seen (Figs. 3–5). After 16 hr of exposure, the ciliated and nonciliated cells of the bronchiolar epithelium become uniform in height. Although Clara cells are relatively resistant to injury, they lose secretory granules during the first 24 hr of exposure (25,26). By the second day of exposure, cellular hypertrophy and hyperplasia are evident in the bronchiolar epithelium and foci of multilayered cells become obvious (13,26–29). From day 2 through 15, the epithelium tends to maintain this cellular appearance, with a distinct absence of cilia (Fig. 6). During this period, numerous ciliated vacuoles are observed (Fig. 7), along with rodlike structures in nonciliated cells (Fig. 8). When the animals are allowed to recover in air, normal ciliated, and nonciliated cells appear again by day 7 of recovery (Fig. 9) (10,25,30–33).

In alveolar regions, the first morphological changes are observed also by the fourth hour of exposure...
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Figure 6. Bronchiolar epithelium after 15 days of exposure to 17 ppm NO₂. ×1250.

Figure 7. Ciliated vacuole in a ciliated cell after 15 days of exposure to 12 ppm NO₂. ×8710.

Figure 8. Rodlike structures in nonciliated cells after 5 days of exposure to 12 ppm NO₂. ×5822.

Figure 9. Bronchiolar epithelium in a rat exposed to 15 ppm NO₂ for 24 hr and allowed to recover in air for 7 days. Compare with Fig. 3 and note the return of a normal ciliary bed. ×1250.

(18,19,34,35). At this time, Type I cells near the opening of the terminal bronchioles exhibit swelling, and occasional areas of alveoli have lost the covering Type I cells, leaving basement membrane exposed (Figs. 10 and 11). These changes increase in intensity so that, after a day of continuous exposure, the larger areas of alveoli have lost their damaged Type I cells (Fig. 12). Cellular debris and some interstitial and alveolar edema become apparent (10,15–19,22,35). Type II cells appear to be relatively resistant to NO₂ or O₃, as do alveolar macrophages. Endothelial cells appear not to be affected by NO₂, but occasionally swelling has been reported during exposure to O₃.

After 48 hr of continuous exposure, areas previously devoid of Type I cells are covered by a low cuboidal epithelium (Figs. 2, 13, and 14). These cells are derived
from Type II cells but lack lamellar bodies (36–39). Cellular debris, mucoid material and macrophages are present near the openings of the terminal and respiratory bronchioles. By day 4 of exposure, the epithelium has assumed a more squamous appearance, and most of the cellular and amorphous debris has been eliminated (Fig. 15). However, the number of epithelial cells present has increased and the air-blood barrier is thicker (3,10,16,40,41).

Other cells in the alveoli also multiply during exposure to NO$_2$ or O$_3$ (38,39,42). The number of dividing macrophages increases by day 3 of exposure. Whether this is in response to the presence of cellular debris or to hormonal stimulation associated with Type II cell division is not clear. The number of interstitial cells and the thickness of the interstitial space also increased (20,33,43–47). In the capillaries, there is often an increase in the number of circulating mononuclear cells. The tissue remains in this condition for at least an additional 15 days (Fig. 16). If animals are allowed to recover in air, the epithelium soon assumes a normal appearance (10,26). These changes have been described in a variety of animals, including mice, rats, rabbits, cats, dogs and primates.

Figure 10. Example of injured alveoli near the opening of the terminal bronchiole after 12 hr of exposure to 15 ppm NO$_2$. ×555.

Figure 11. Swelling and sloughing of Type I cells after 12 hr exposure to 15 ppm NO$_2$. ×7000.
From these studies, three important conclusions were derived. First, the cells most vulnerable to NO₂ or O₃ (target cells) are the ciliated bronchiolar and Type I alveolar cells. Both cell types have a much larger surface area exposed to the NO₂ than do the more resistant types (Type II cells and Clara cells). Second, the epithelium can repair itself in the presence of sublethal levels of NO₂ or O₃. Third, the new cells are relatively resistant to continuous exposure to the same concentration of NO₂ or O₃. Because repair does occur and the new cells are resistant to injury, it is important to understand the time sequence of injury when studying the effects of NO₂ or O₃ on these target cells.

When animals are exposed continuously to lethal (100%) and sublethal (85%) concentrations of O₃, damage is observed in the airways and alveoli. However, there is no preferred anatomical site of damage, and damage is observed diffusely throughout the lung (48-57).

In the airways (bronchi and bronchioles), swelling of nonciliated cells and loss of cilia are observed after 2 days of exposure (58,59). By day 3, necrotic nonciliated cells are present and sloughing of ciliated cells is
observed. Proliferation of cells to repair such injury begins by day 4 if the animals survive the exposure (29,60).

In the alveoli, slight swelling is observed in endothelial cells on day 2 of exposure. By day 3 of exposure, individual endothelial cells undergo cytoplasmic changes and fragmentation, which can result in destruction of up to 50% of the capillary bed (Fig. 17) (54–56,61). These changes are accompanied by increased thickness of the interstitium and damage to Type I cells. Between day 3 and day 4, a variety of lesions—such as fibrinous exudation, hyaline membrane formation and capillary congestion—are observed. In animals surviving these effects, proliferation of Type II cells, similar to that described for NO₂ and O₂ exposure, begins between day 4 and day 5 (36,38,49,62). Surviving animals allowed to recover in air for 56 days have a nearly normal epithelial lining, but septal scars remain due to the increase in interstitial tissue (54).

If animals are exposed to sublethal concentrations of O₂ (85%), changes in the endothelium similar to those seen with 100% O₂ occur (3,61,63,64). However, very little
damage is observed in the Type I epithelium. There is a net increase in Type II cells and a large increase in interstitial cells between day 5 and day 14 of exposure. Some investigators consider these changes to be adaptive (61,65). Results similar to these have been described in many species of animals, including man (53).

These studies suggest that the morphological target cells during exposure to O₂ are ciliated and nonciliated cells in the airways and endothelial cells in the alveoli. Although other cells in the alveoli may be affected during exposure, it is not clear whether this is due to the oxygen or is a secondary response to the initial injury.

Factors That Affect the Amount of Tissue Injury

The target cells associated with damage to the airways and alveoli during exposure to NO₂, O₃ or O₂ were described in the previous section. The degree to which target cells in the tissue are affected is subject to several factors. Obviously, concentration affects the amount of tissue damage. However, age, nutritional status, and the development of tolerance are also important in this regard.

![Tissue architecture after 15 days of exposure to 17 ppm NO₂. ×46.](image1)

![Damage to endothelial cell in a rat after 2 days of exposure to 100% O₂. ×11,600.](image2)
A study of aging (10,30) revealed that relatively old rats exposed to 14 ppm NO₂ suffered greater injury than did young rats exposed to 17 ppm NO₂. Morphologically, the nature of the injury observed in the airways and alveoli was the same for both groups, but the area of damage in the alveoli was more extensive in the older animals and was associated with increased alveolar edema (10,30). Further studies showed that initiation of repair of damaged epithelium in older animals was delayed by approximately 1 day. Both factors—the greater extent of tissue damage and the retarded onset of cell proliferation—are likely to be responsible for the edema and hence the higher mortality rate in the older animals. After exposure to about 14 ppm NO₂, 12% of 11-month-old rats died, but 35% of the 19-month-old rats died. It is interesting, however, that older animals that survived the first 3 days of exposure were able to repair the damage, as young rats did, despite continuous exposure to NO₂. The reason for the difference in initial susceptibility to injury was not determined. Older animals exposed to O₃ or O₂ are also known to be more susceptible to tissue damage (61,66).

In contrast, Stephens et al. (67) observed resistance to high concentrations of NO₂ or O₃ in rats exposed from birth until they were about 30 days old. In this study, loss of cilia was reported in the terminal bronchioles of nursing rats from 8 to 20 days of age. In the alveoli, no evidence of damage to Type I cells was seen until the rats were 21 to 35 days old. The basis for the refractory nature of the alveolar epithelium up to 20 days was not apparent. Young animals also are more resistant to the toxic effects of O₂ (68–70).

Another factor that may affect oxidant damage is the nutritional status of the animal. For example, the tissue levels of the natural antioxidants vitamin E and selenium are important mediators of cell damage. In a series of studies with rats fed diets containing various amounts of vitamin E prior to exposure to NO₂, Fletcher and Tappel (71) found that vitamin E-supplemented animals died later than those fed vitamin E-deficient diets. Death was not averted, however, in animals supplemented with vitamin E during exposure to NO₂.

Recently, Evans et al. (72) demonstrated that rats depleted of vitamin E and selenium suffered more Type I cell damage in response to NO₂ exposure than did those fed adequate levels of these compounds. However, the supplemented groups were protected for only 6 to 12 hr. After this time, all groups incurred the same amount of damage. Chow et al. (73) reported more injury in rats depleted of vitamin E than in rats on supplemented diets when they were exposed to O₃. These studies demonstrate that biological antioxidants play a role in affecting the extent of damage sustained by alveolar epithelium during exposure to NO₂ or O₃.

Although NO₂ and O₃ injure pulmonary cells of rats upon initial exposure, the tissue becomes relatively tolerant to the same concentration during continuous exposure. If the animals are removed from the NO₂- or O₃-containing environment, the tolerance decreases. Using intermittent exposures, Creasia et al. (52) showed that tolerance that had developed to 10 ppm NO₂ persisted for about 7 days before re-exposure to the same concentration again caused significant injury. Similar results were obtained from rats exposed to ozone (38,74). Animals exposed to 85% O₂ also developed tolerance after 5 days of exposures (3,61,64,75–78).

Effects of Chronic Exposure

Early pathologic events during and after limited initial exposure of rats to NO₂ or O₃ are reparable, as described, but persistent exposure to subacute concentrations of at least 14 ppm NO₂ for several months leads to permanent distortion of the architecture of the rats' lungs (Figs. 18 and 19), with loss of alveolar tissue (27,28,31,43,79,80,81,82). Lower concentrations also affect the lungs, but 1 or 2 ppm—even for the lifetime of the rat—fails to induce parenchymal destruction (82), although respiratory rates and lung volumes become elevated and metabolic changes are seen in the bronchiolar epithelium (42).

Figure 18. Parenchyma of a 24-month-old control rat. ×20.

Figure 19. Parenchyma of a rat exposed for prolonged, varying periods to ~ 15 ppm NO₂ over a 24-month period, exhibiting a loss of alveolar surface area. ×20.
In chronically exposed animals, the characteristic paucity of cilia in the airways and the relatively uniform hypertrophy of the epithelium of the small airways, with their occasional foci of hyperplasia, indicate a much reduced—but continuing—response of the lining cells to the persistent presence of NO$_2$ or O$_3$. From the onset of exposure, the most severely affected tissues of the airways and parenchyma—mainly the terminal bronchioles, alveolar ducts and adjacent alveoli—are infiltrated with inflammatory cells. These are largely macrophages both within the alveoli and the small airways (where they often fill the former close to the small airways and ducts) and in the interstitial tissue. Other types of mononuclear cells, and occasionally granulocytic cells, are included. Mast cells are often seen dispersed throughout the lung.

The ubiquitous network of connective tissue elements that permeates the alveolar walls and small airways reveals changes in staining properties of both the elastic tissue and the collagen strands in long-exposed rats (48). Besides appearing to become thicker with time, the elastic strands tend to fracture and some of the collagen fibers appear as thick, ropelike structures (47). Simultaneously, the basement membranes supporting both the alveolar epithelium and the capillary endothelium become broader in proximal alveoli, in contrast with the unusually thin, attenuated alveolar walls of more peripheral alveoli. In general, pulmonary blood volume per gram of tissue is reduced in the heavy, voluminous, nonepithelated lungs that develop during exposure to high, subacute concentrations of about 15 ppm NO$_2$ (28).

The internal diameters of terminal bronchioles decrease during prolonged exposure as their walls (composed mainly of smooth muscle, connective tissue, and epithelial lining) become thicker (38). The diameters are reduced further because of an accumulation of mucous and serous secretions from the increased number of peripherally activated "goblet" and other secreting cells of the bronchial and bronchiolar mucosa; hence, airflow is correspondingly reduced. Cellular debris, fibrous exudate, and the attendant macrophages and other cells of the immune system are additional obstacles to flow.

Although these changes do not involve target cell toxicity, they do reflect changes in the tissue due to chronic exposure. Whether these changes reflect reparative or adaptive phenomena or the direct action of NO$_2$ or O$_3$ on the structural organization of the lung is not clear. However, because of the damage, long-term studies should be included in any research on oxidant-induced lung toxicity.

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