EFFECTS OF 0.12 AND 0.80 PPM OZONE ON RAT NASAL AND NASOPHARYNGEAL EPITHELIAL MUCOSUBSTANCES: QUANTITATIVE HISTOCHEMISTRY

Abstract — The present study was designed to characterize changes in the quantity of mucosubstances in surface epithelia of rat nasal airways after short-term ozone exposure. Rats were exposed for 7 days (6 h/day) to 0.0, 0.12, or 0.8 ppm ozone, and killed immediately or 7 days after the last exposure. Nasal cavities were processed for morphometric analysis of intraepithelial mucosubstances. Compared to controls, rats exposed to 0.12 ppm ozone had increased amounts of stored mucosubstances within epithelium lining the medial aspect of the nasal turbinate immediately after exposure, but no change within epithelia of the nasopharynx. Rats exposed to 0.8 ppm ozone had increased quantities of stored mucosubstances within transitional and respiratory epithelium lining turbinates and lateral walls of the anterior nasal airway and significant decreases in stored mucosubstances within epithelia of the nasal septum at the end of exposure. Seven days after the end of exposure, the amounts of intraepithelial mucosubstances returned to control levels along the septum, but remained greater than those of controls along the turbinates. We demonstrated that exposures to ambient levels of ozone induce significant changes in the stored secretory product of nasal epithelium in the rat, and that these changes persist for at least 7 days post-exposure.

PRINCIPAL INVESTIGATORS

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Recently, it has been reported that ambient levels of ozone, the major oxidant pollutant in photochemical smog, can induce secretory cell hyperplasia and increases in epithelial mucosubstances in the nasal epithelium of monkeys.[1] These studies not only showed that secretory cell hyperplasia and increased intraepithelial mucosubstances can be induced in the primate nasal mucosa by low concentrations of ozone, but also that certain regions of the nasal airway and specific types of epithelium are particularly sensitive to ozone-induced secretory changes.

Many inhalation toxicology studies that are used for making risk assessments of environmental air pollutants are conducted with rodents as the animal models. There have been no reports of inhalation studies of rodents exposed to ozone in which the nasal airways have been histologically evaluated for toxicant-induced lesions.

The purposes of the present study were (1) to characterize possible quantitative changes in mucosubstances in the surface epithelia of rat nasal and nasopharyngeal airways after short-term exposures to ozone and (2) to compare our findings in the rat with those previously described in the monkey.

MATERIALS AND METHODS

Animals and Exposures

Thirty female, F344/N 12-14-wk old rats from the Institute's breeding colony were used in this study. Animals were divided into five experimental groups (N = 6/group). Rats were exposed for 7 days, 6 h/day to either 0.00, 0.12, or 0.80 ppm ozone in whole body chambers.

After the seventh day of exposure, 6 air control rats and 12 ozone-exposed rats were killed immediately, and the head of each rat was removed from the carcass, fixed, and decalcified. The remaining 12 ozone-exposed rats were kept in HC-1000 chambers supplied with filtered room air for 7 days after the end of the ozone exposure, prior to sacrifice.

The nasal cavity was transversely sectioned at two specific anatomic locations, as previously described by Young: (1) immediately posterior to the upper incisor teeth (section 1), and (2) at the level of the first molar teeth (section 2). These frontal tissue blocks were embedded in glycol methacrylate. One-micron-thick sections were cut from the anterior surface of glycol methacrylate-embedded blocks and stained for demonstration of intraepithelial mucosubstances.

Four specific areas of the nasal epithelium within section 1 were selected for morphometry. These regions included the surface epithelium lining (1) the nasal septum, (2) the medial aspect of the nasal turbinate, (3) the lateral aspect of the nasal turbinate, and (4) the medial and lateral aspects of the maxilloturbinate. Three regions of the surface epithelium lining the nasopharynx (section 2) were analyzed to estimate the amount of intraepithelial mucosubstances in the distal nasal airway. These regions included the lateral, dorsal, and ventral walls of the nasopharynx.

Three-to-six microscopic fields within a region were analyzed per slide. The volume of stored mucosubstance per surface area of epithelial basal lamina was determined with an image analyzer and calculated by methods previously described in detail by Harkema et al.[2]

RESULTS

Figure 1 compares the quantitative changes in volume density (nL/mm^2) of mucosubstances in the four different nasal surface epithelial regions in section 1 of the rat nasal cavity, after the 7 day exposures to either 0.0, 0.12, or 0.8 ppm ozone. Compared with air-exposed controls, there was a significant increase in total stained mucosubstance in the transitional, nonciliated, cuboidal epithelium lining the nasal and maxilloturbinates (NC/NT and NC/MT, respectively) immediately after the end of exposure to 0.8 ppm ozone. In addition, there was a significant increase (approximately 50%), compared to controls, in the quantity of mucosubstances in the

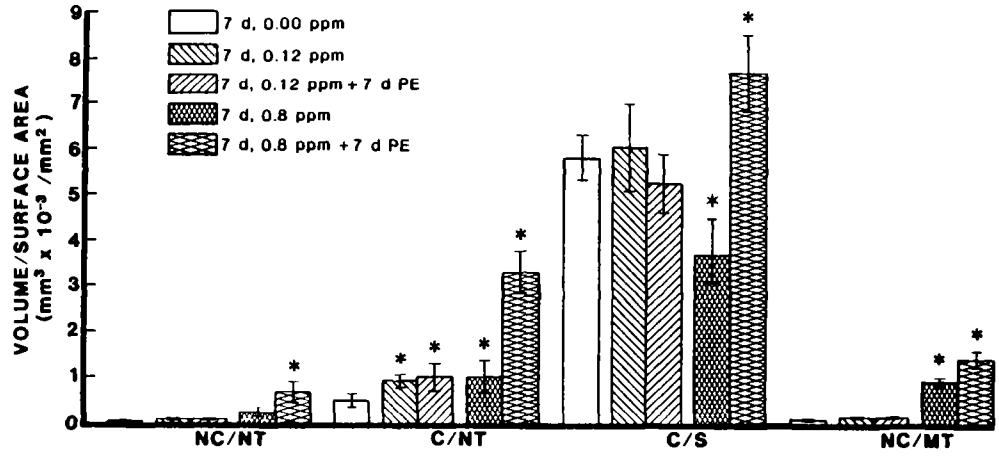

Figure 1. Amount of stored intraepithelial mucosubstances in anterior nasal cavity. Nonciliated surface epithelium lining nasoturbinate (NC/NF); ciliated surface epithelium lining nasoturbinates (C/NT); ciliated surface epithelium lining nasal septum (C/S); nonciliated surface epithelium lining maxilloturbinate; days (d); post exposure (PE); parts per million (ppm). * = Significantly different from the 7 day, 0.00 ppm group ($p < 0.05$).

ciliated epithelium lining the medial aspect of the nasal turbinate (C/NT) in the ozone-exposed animals. In contrast, there was an approximately 30-35% decrease in the amount of mucosubstance in the adjacent ciliated epithelium lining the nasal septum (C/S) immediately after the 7 day, 0.8 ppm ozone exposure.

Rats exposed to 0.12 ppm ozone for 7 days and then immediately killed had similar increases in the volume densities of mucosubstances within the C/NT compared to those in the 0.8 ppm ozone-exposed animals. Unlike the response in the nasal epithelium of 0.8 ppm ozone-exposed rats, the 0.12 ppm-exposed rats had no significant differences in the amount of epithelial mucosubstances within the NC/NT or the NC/MT, compared to air controls (Fig. 1).

Changes in the volume densities of mucosubstance were still present in some of the specific intranasal locations of ozone-exposed rats 7 days post-exposure (Fig. 1). In all the rats that were exposed to 0.8 ppm ozone for 7 days and then held in room air for 7 days before being killed, the amounts of epithelial mucosubstances in all four of the intranasal regions examined were significantly greater than in those animals exposed to filtered air (0.0 ppm ozone) or in those exposed to 0.8 ppm ozone and killed immediately after exposure. For example, the volume density of mucosubstance within the C/NT was approximately 7 times greater in animals exposed to 0.8 ppm ozone and killed 7 days post-exposure compared to air controls (3.21 + 0.49 nL/mm^2 compared to 0.44 + 0.09 nL/mm^2) and three times that in 0.8 ppm-exposed rats killed immediately after exposure (3.21 + 0.49 nL/mm^2 compared to 0.97 + 0.32 nL/mm^2). The amounts of total mucosubstances in the C/NT, NC/MT, and C/S of these ozone-exposed rats killed 7 days post-exposure were approximately 7, 28, and 0.3 times greater, respectively, than those in the same tissues of air controls. Although the volume densities of the mucosubstance in the C/S were significantly decreased in rats immediately killed after the 0.8 ppm ozone exposure, the volume densities were significantly increased above those in air controls 7 days post-exposure.

There were no significant differences in the volume densities of mucosubstances in the C/NT between rats that were exposed to 0.12 ppm ozone and killed immediately after the 7 day exposure and those killed 7 days after the exposure. Both groups of rats had approximately twice as much mucosubstance in this location compared to that in the air control group. The amount of mucosubstance in this nasal epithelium of the 0.12 ppm-exposed rats killed 7 days after exposure was approximately one-third the amount in rats exposed to 0.8 ppm and killed at the same post-exposure time point.

In addition, there were significant changes in the amounts of mucosubstances within the epithelium lining the nasopharyngeal airway (section 4) after ozone exposure. Figure 2 graphically illustrates the differences in the volume densities of mucosubstance in the three epithelial regions of the nasopharynx for the animals exposed to various ozone exposure regimes. Rats that were exposed to 0.8 ppm ozone and killed immediately after the 7 day exposure had similar amounts of intraepithelial mucosubstance, compared to controls, along the lateral, dorsal, and ventral walls of the nasopharynx immediately after the 7 day exposures. Approximately 2-3 times more mucosubstances were present in the lateral, dorsal, and ventral walls of 0.8 ppm ozone-exposed animals killed 7 days post-exposure compared to control rats.

Rats exposed to 0.12 ppm ozone and killed immediately after the 7 day exposure or 7 days after the end of exposure had quantities of nasopharyngeal mucosubstances in the surface epithelium that were not significantly different from those in air controls.

DISCUSSION

The results of this study indicate that exposure to low concentrations of ozone induces significant changes in the amount of stored secretory product in the surface epithelium of the

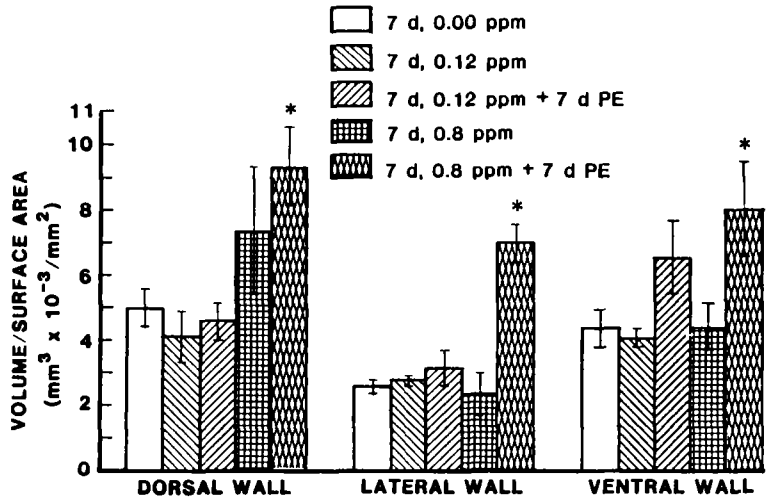

Figure 2. Amount of stored intraepithelial mucosubstances in nasopharynx. Days (d); parts per million (ppm). * = Significantly different from the 7 day, 0.00 ppm group ($p < 0.05$).

anterior nasal cavity and nasopharynx in the rat. Most of these quantitative changes in nasal intraepithelial mucosubstances were enhanced 7 days after the short-term exposure.

Rats exposed to ozone appeared to have two concomitant protective responses in the anterior nasal cavity after 7 days of exposure: (1) hypersection of mucus for the nasal septum and (2) increased amounts of glycoconjugates in adjacent transitional epithelium, which normally contains little intraepithelial mucosubstances.

Persistence and actual increases in nasal epithelial mucosubstances after a post-exposure period have not been reported previously. All or part of this increase in the transitional epithelium appeared to be due to an increase in the number of secretory cells, although a thorough morphometric analysis of total epithelial cells and differential cell counts must be performed before this can be substantiated. Morphometric analysis of the amount of secretory product per cell must also be done in future studies to determine if there are changes in intracellular storage.

Ozone-induced changes in the amount of stored mucosubstances in the nasal and nasopharyngeal airways may affect and reflect alterations in the surface epithelial layer, mucociliary clearance, and possibly intranasal airflow. Ozone has been shown to decrease tracheal mucociliary clearance, but its effect on nasal mucociliary clearance has not been studied. Such alterations could modify upper respiratory tract defense and leave the lower respiratory tract vulnerable to greater burdens of airborne pollutants or infectious particles that could induce pulmonary disease.

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