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MORPHOMETRIC ANALYSIS OF FOCAL LUNG INJURY CAUSED BY LOW LEVELS OF NITROGEN DIOXIDE

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ABSTRACT

Changes in cells of the terminal bronchiolar epithelium in response to subchronic exposure to 0.5 and 2.0 ppm NO_2 were evaluated using morphometric techniques. Oriented sections through the distal portion of randomly selected terminal bronchioles were used to determine the relative number of ciliated and nonciliated (Clara) cells, as well as their mean volume and surface areas. Exposure to 0.5 ppm NO_2 caused no detectable changes in either of these types of cells. Exposure to 2.0 ppm caused a 19% reduction in the relative number of ciliated cells. The mean volume of the remaining ciliated cells was slightly increased and there was an 18% reduction in the ciliated surface area/cell. Clara cells were unchanged in number but showed evidence of spreading on the basement membrane surface and a loss of their protruberant dome giving a 23% decrease in the mean luminal surface area/cell.

Keywords: Airway epithelium, morphometry, oxidants, stereology.

INTRODUCTION

Exposures to low levels of oxidants such as O_3 and NO_2 causes structural changes in the terminal bronchioles and their adjacent alveoli (Stephens et al., 1972; Stokinger et al., 1957). Quantitative studies of these changes in cell structure have been limited because of the oriented nature of the bronchioalveolar junction. In addition, the effect of exposure to low levels of these oxidants on lung structure has not been rigorously studied. Techniques have recently been devised to reproduce isolated cross-sections of terminal bronchioles. Morphometric methods have also been developed to analyse the cells in this oriented structure (Barry and Crapo, 1985). In the current study, the extent of injury caused by nitrogen dioxide at sufficiently low concentrations relevant to current problems in ambient air pollution is defined.

METHODS

Six-week old Fisher 344 rats were exposed to two doses of NO_2 for 6 weeks while age and weight matched rats that breathed filtered room

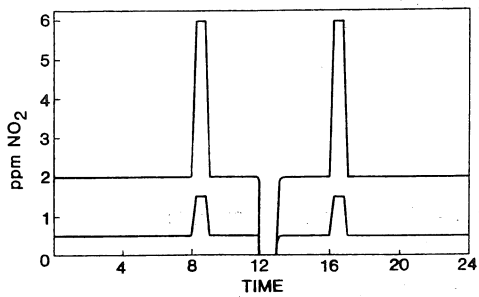

Figure 1. Rats were exposed to NO_2 7 days a week and 23 hours a day according to the gas profiles depicted above. Two one-hour peaks to 3 times the background levels were included in the exposure profiles.

air served as controls. All animals were exposed in stainless steel Rochester type chambers having a volume of 323 m^3. The diurnal exposure profiles of the NO_2 gases are shown in Figure 1. The twice daily peaks were adapted to simulate likely gas profiles in the urban environment. The total gas flow was maintained at 323 1/min and the NO_2 concentration was monitored using continuous chemiluminescent analyzers. For ease of discussion, the two doses of NO_2 will be referred to by the baseline levels, e.g. 0.5 and 2.0 ppm NO_2. After the exposures, rats were sacrificed and their lungs fixed with 2% glutaraldehyde in 0.084 M cacodylate buffer by intratracheal instillation under a constant 20 cm pressure.

Sample isolation: The left lobe of the lung was cut transversely. Four 2 mm thick slices were obtained, 2 each from the upper and lower one-third of the left lobe. These tissue slices were cut into chunks approximately 2 x 4 x 4 mm in size and embedded in large blocks of epoxy resin. Terminal bronchioles, the portion of the conducting airway immediately before the gas exchange area of the lung, were dissected out from randomly selected tissue blocks. The plastic block was first softened by mild heat and then cut with a razor blade to slices < 0.5 mm thick. Airways and alveolar ducts can be easily identified in these thin slices. Small blocks, each containing a terminal bronchiole, were isolated. The blocks were trimmed so that the block face showed a full cross-section of the terminal bronchiole. These terminal bronchiole blocks were then glued on regular blank EM blocks for ultramicrotomy. Thin sections of 5 terminal bronchioles from each of 8 controls and 8 exposed animals of both age groups were picked up on 1 mm hole parlodion-coated grids. The entire terminal bronchiolar epithelium were photographed on 20-25 sequential electron micrographs at 2000x and printed at 8500x on 11" x 14" photographic paper. Two lines perpendicular to the basement membrane were drawn on each micrograph to define the portion of the epithelium to be analyzed on each micrograph.

Morphometric techniques: The epithelial tissue area (A_{EP}), basement membrane length (L_{BM}), and luminal surface lengths (L_{LM}) were measured on each micrograph with a digitizing tablet interfaced to an IBM

personal computer. The results were summed to yield the total values for the terminal bronchiole. Point and intercept counting were done using a Merz curvilinear overlay to compensate for any non-random distribution of terminal bronchiolar cells. Point hits were catagorized into major bronchiolar cell types (e.g. ciliated cells and Clara cells). Intercepts with the epithelial basement membrane and luminal surface of each cell type as well as intercepts with the surface of cilia were also recorded. The number of nuclear profiles of each cell type were first counted on each micrograph and then totaled for the entire terminal bronchiole.

Morphometric formulas: To evaluate the effects of the NO_2 exposures, the thickness of the epithelium and characteristics of the epithelial cells were calculated.

The cross-section of the terminal bronchiole is assumed to be a perfect circle whose radius (R_1) and area (A_1) can be calculated from the measured circumference of the basement membrane. The bronchiolar lumen was also assumed to be a circle. Its area (A_2) was derived by deducting the epithelial area from A_1. R_2, the radius for A_2, can then be derived and the average thickness of the epithelium was then equal to $r_1 - r_2$.

The tissue volumes, surface areas, and cell numbers of bronchiolar cells in a unit volume of terminal bronchiolar epithelium can be derived by the following formulas (Weibel, 1979):

$$V_V = P_i / P_{EP} \quad (1)$$

$$S_V = 2I_i / L \quad \text{or} \quad 2I_{BM} / L \quad (2)$$

$$N_V = N_A / H \quad (3)$$

P_i = Point hits on ciliated or Clara cells.

P_{EP} = Point hits on terminal bronchiolar epithelium.

I_i = Number of intercepts with the luminal or basement membrane surfaces of ciliated or Clara cells.

I_{BM} = Number of intercepts with the entire epithelial basement membrane surface.

L = Length of the test line in the reference volume.

N_A = Number of nuclear profiles of ciliated or Clara cells on a cross-section of the terminal bronchiole with a known tissue area (A_{EP}).

H = The average length of each cell type's nuclei parallel to the direction of sectioning (the mean caliper length). H was used instead of the mean caliper diameter (D) because the terminal bronchioles were cut in a selected orientation.

Tissue volumes, however, are frequently changed by exposure to oxidants. The surface area of the terminal bronchiolar basement membrane on the other hand was not altered by the oxidant treatment which is demonstrated by the fact that the circumference of the terminal bronchioles in control and treated animals remained the same. We expressed our data, consequently, per unit surface area of epithelial basement membrane.

$$V_{BM} = V_V \times A_{EP} / L_{BM} \quad (4)$$

$$S_{BM} = I_i / I_{BM} \quad (5)$$

$$N_{BM} = N_V \times A_{EP} / L_{BM} \quad (6)$$

The cell characteristics, such as mean cell volume, mean cell luminal surface area, and mean cell basement membrane surface area, are calculated by the following formulas:

$$V = V_{BM} / N_{BM} \quad (7)$$

$$SA = S_{BM} / N_{BM} \quad (8)$$

RESULTS

NO_2 exposures affects mainly the ciliated cells and the Clara cells of the terminal bronchioles. Figure 2 illustrates the characteristics of these two cell types in control and exposed rats. Morphometric analysis revealed that the terminal bronchiolar epithelium was not injured by prolonged exposure to 0.5 ppm NO_2. Ciliated cells in the terminal bronchioles of rats exposed to 2.0 ppm NO_2 were reduced in number and the mean cell ciliated surface, but were increased in mean cell volume when compared with controls. Clara cells from these same terminal bronchioles exhibited a smaller luminal surface area and a broader mean cell basement membrane surface area. The Clara cell was shown to be the progenitor of ciliated cells (Evans et al., 1972; Evans et al., 1976). The structural changes observed with the Clara cells probably represent the presence of increased intermediate cells as a result of Clara cell division in response to loss of ciliated cells. We conclude that injuries to bronchiolar epithelium caused by low levels of exposure to NO_2 were dose dependent and that both ciliated and Clara cells in the terminal bronchioles were susceptible to 2.0 ppm NO_2 exposure.

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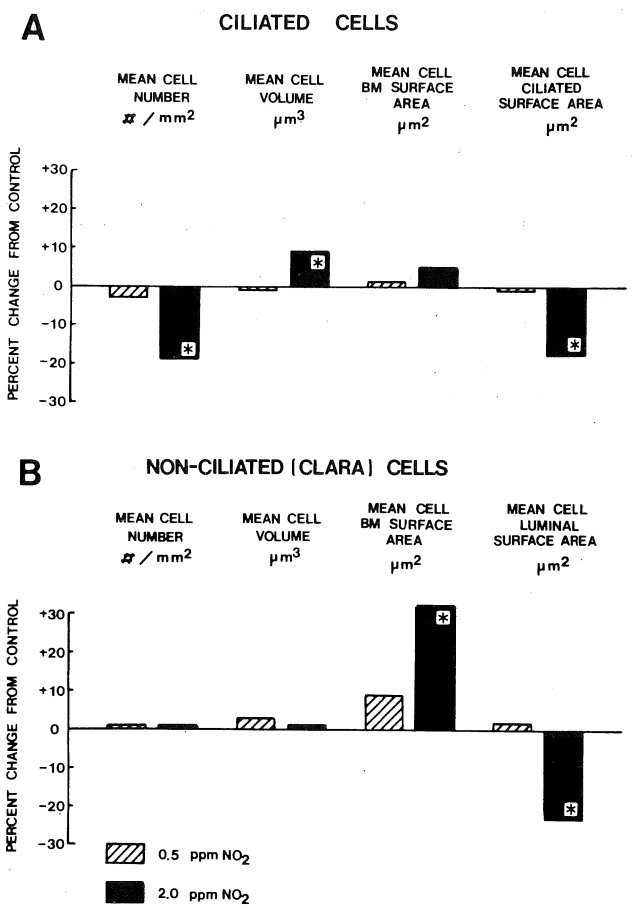

Figure 2. Changes of the characteristics of terminal bronchiolar cells occurring in rats exposed to either 0.5 or 2.0 ppm NO_2 for 6 weeks. A. Changes in the ciliated cells. B. Changes in the Clara cells. BM=basement membrane, *$p < 0.05$ when compared with control values.

REFERENCES

Barry BE, Crapo JD. Application of morphometric methods to study diffuse and focal injury in the lung caused by toxic agents. CRC Crit Rev Tox 1985; 14: 1-32.

Evans MJ, Stephens RJ, Cabral LJ, Freeman G. Cell renewal in the lungs of rats exposed to low levels of NO_2. Arch Environ Health 1972; 24: 180-188.

Evans MJ, Johnson EV, Stephens RJ, Freeman G. Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO_2 or O_3. Lab Invest 1976; 35: 246-257.

Stephens RJ, Freeman G, Evans MJ. Early response of lungs to low levels of nitrogen dioxide. Arch Environ Health 1972; 24: 160-179.

Stokinger HE, Wagner WD, Dobrogorski OJ. Ozone toxicity studies. III. Chronic injury to lungs of animals following exposure to a low level. Arch Int Health 1957; 16: 514-522.

Weibel ER. Stereological Methods. I. Practical Methods for Biological Morphometry. London: Academic Press, 1979.