Abstract  
Semiquantitative measurements of chronic inflammation of the centriacinar region (proximal acinus of lung) were compared between 20 Miami and 18 Los Angeles residents (ages 11–30 years) for whom smoking histories were available. Mean extent and severity scores of four lung sites were higher for Los Angeles than Miami residents, with effect of city statistically significant for extent ($P=0.02$). Also, maximum scores for extent and severity by city were significantly greater for Los Angeles residents ($P=0.02$, each), but not by smoking history. Smokers did have higher scores for mean extent and severity (by lung site and smoking history), but neither this nor inclusion of smoking and city in the model reached significance. With respect to maximum extent and maximum severity scores, a stratified comparison of cities by smoking history showed a trend (not significant) toward higher scores for Los Angeles residents. Mean extent and severity scores for the lower lobe were higher for basilar sections than for apical sections (each $P<0.001$). Cumulative data indicate that expanded pathologic studies are essential for efforts to complete a convergence of epidemiological and experimental data implicating exceedences of the Federal ozone standard as a contributor to human lung injury.

Keywords  
Lung · Ozone · Centriacinar · Human · Autopsy

Introduction

Our previous study had shown that severe centriacinar region (CAR) injury was frequently present in the lungs of young Los Angeles residents who died suddenly from vehicular accidents, homicide, or other violence [21]. The pathologic data obtained at that time converged with a large body of epidemiological and experimental data [1, 2, 3, 16] to suggest strongly that ambient levels of ozone caused human lung injury. An opportunity to verify that relationship became available through the cooperation of Medical Examiner Offices in Miami (Dade County) and Los Angeles (Los Angeles County). Those two metropolitan areas were selected for differing levels of ozone in their ambient community atmospheres. Los Angeles was the central consideration in view of frequent exceedences of the National Ambient Air Quality Standard (NAAQS) for ozone, i.e., 0.12 ppm 1-h average (parts per million), as opposed to infrequent exceedences in Miami. It should be noted that the 0.12-ppm NAAQS ozone standard is less stringent than the World Health Organization and California State 1-h mean standards for ozone of 0.056 ppm and 0.09 ppm, respectively. Of further pertinence, the newly introduced 8-h annual mean standard for ozone has been reported to be greater for Los Angeles than for Miami, namely 0.099 ppm and 0.034 ppm, respectively [14]. Complications in the implementation of this project reduced the number of cases available for demographic–pathologic correlations and this obviated definitive conclusions regarding adverse effects of ambient ozone exposure. However, the finding of a greater degree of lung injury in the Los Angeles res-

Disclaimer. Although the research described in this article has been supported by the United States Environmental Protection Agency through Cooperative Agreement CR 821576-01-0 with the University of Southern California, it does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
Fig. 1 Lung tissue sample site (sagittal sections). Site 1: a most lateral lung slice, with the section away from the pleura (“central”) and in the posterior half of the lower lobe. Site 2: lateral portion of lobe, with section abutting pleura at the base of the lobe. Site 3: medial slice of lobe, with section from anterior aspect and at a level that is below hilum (“central”). Site 4: most medial slice of the lung, with section of lower lobe taken about midway between base and apex (most superoanterior section), and at the level of the hilum (note presence of main stem bronchus). This lung slice is oriented with its posterior margin facing the posterior margin of the adjacent lung slice, and includes a portion of the upper lobe and lingula.

Methods

Processing of lungs

Lungs were obtained from autopsies of young residents of Miami (Dade County) and Los Angeles (Los Angeles County) who died suddenly from homicide, vehicular accident, or other violence between 1995 and 1997. Eligibility criteria for lung accessioning were sudden death (at the scene or dead on arrival at the emergency room), residency in Los Angeles or Miami for at least 5 years, age from 12–30 years, no historical record or autopsy evidence of drug use, absence of disease on gross examination at autopsy, and autopsy examination less than 3 days postmortem. The main stem bronchus of a left lung (or right lung when left lung was not suitable due to trauma) was cannulated with a loose-fitting plastic tube and secured at the bronchial margin with an 18-gauge hypodermic needle inserted into a cork. The lung was immersed in a 90-l tank of 10% phosphate-buffered formalin and fixed by perfusion inflation at 25 cm water pressure for 72 h or more. After fixation, lungs at the Miami site were shipped to the principal investigator inflation at 25 cm water pressure for 72 h or more. After fixation, lungs at the Miami site were shipped to the principal investigator and accessioned along with lungs from Los Angeles’ cases. Accessioning of lungs, gross examination, tissue processing, microscopic study, and semiquantitative measurements were carried out without the principal investigator being aware of the source of the lungs. The gross appearance of each lung was described before and after serial sectioning with the use of an electrical rotary slicer. A peer review of the research protocol [26] agreed with the following: use of the lower lobe of the left lung for the semiquantitative measurements; examination of four tissue sections from each lower lobe in accordance with specific topographical sites as shown in Fig. 1 [specifically, posterolateral central (site 1), lateral basal (site 2), anteromedial central (site 3), and superoanterior (site 4)]; and retention of the upper lobe and lingula for pending quantitative measurements of emphysema by means of diazo print methodology [28]. The sections were processed for paraffin embedding, sectioned at 4 µM, and stained with hematoxylin and eosin (H and E).

Semi-quantitative measurements

Semi-quantitative measurements of CAR alterations were made in accord with the definition of CAR as a microscopic topographical unit involving the transition zone between the terminal membranous bronchioles and ductal-alveolar respiratory units [10]. A search for CAR alterations in tissue sections at each topographical site (Fig. 1) under 80× magnification was done by sequential field examination with the use of a stage micrometer. Severity of CAR alteration was based on one or more of a complex of findings, with at least a mild respiratory bronchiolitis (some degree of macrophage desquamation) as an invariable component. Additional alterations of the proximal acinus that may or may not have been present included lymphocytic-plasmacellular infiltration of bronchioalveolar walls, interstitial bronchiolar and/or alveolar infiltration by macrophages, bronchiolar and/or airspace dilation (with or without acinar wall derangement), peribronchiolar fibrosis, and/or epithelial hyperplasia. Every instance of a CAR alteration was scored for severity and extent on a scale of 1–10. The degree of macrophage desquamation and other alterations, if present, determined an overall scoring. A score of 5 or more was considered to be a severe CAR alteration and this usually was a complex that included macrophage desquamation filling at least half of the lumen of a respiratory bronchiole, and/or one or more of the following alterations that alone was at least a score of 5: interstitial tissues infiltrated by macrophages, peribronchiolar fibrosis, epithelial hypertrophy and/or hyperplasia, and/or bronchioalveolar dilatation with derangement of air spaces. Every CAR alteration in each lung tissue section (lung sites as shown in Fig. 1) was recorded as to severity (0–10; no CAR alteration at some sites), and a single score was assigned per section as a subjective integration (“best judgment mode”) of all scores per section. The extent of CAR alteration was estimated as the relative proportion (0–10) of lung parenchyma per section containing altered CAR structures, with a score of 5 reflecting CAR alterations that involved approximately 50% of lung parenchymal tissue in that one section.