Use of Human Lung Tissue for Studies of Structural Changes Associated with Chronic Ozone Exposure: Opportunities and Critical Issues

Morton Lippmann

Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY 10987

Definitive information on the chronic effects of exposure to ozone (O₃) in humans is not available. There is a strong concern that ozone could produce chronic lung damage in humans on the basis that exposures are ubiquitous at levels that produce transient symptoms, function deficits, and lung inflammation in humans and chronic lung damage in laboratory animals. Both prospective and national population surveys suggest an association between chronic O₃ exposure and reduced lung function, and a pilot investigation of autopsied lungs of accident victims in Los Angeles reported an unexpectedly high incidence of disease in the centriacinar region, the lung region known to receive the highest dose of inhaled O₃. This paper discusses the advantages and limitations of further studies of structural changes in human lung tissue in relation to chronic O₃ exposure. The major advantages of such studies are that a) measurable effects may be related to realistic chronic exposures, b) the effects may be described quantitatively and compared directly to those obtained in chronic animal inhalation exposures, and c) evidence for chronic effects may be obtained much more rapidly than in prospective studies. The major limitations are the difficulties in obtaining sufficient reliable information on residential history, physical activity out-of-doors, and smoking and other confounding exposures to lung irritants from next of kin, and limited availability of adequate air quality data for determining ambient concentrations at places of residence and/or outdoor exercise. The paper also discusses approaches to minimizing these limitations in the design of specific studies. — Environ Health Perspect 101(Suppl 4):209–212 (1993).

Key Words: Chronic ozone exposure, respiratory bronchiolitis, centriacinar region disease, human lung tissue, post-mortem analyses, retrospective exposure assessment

Introduction

While it is well established that short-term exposures of humans to ozone (O₃) produce a plethora of transient responses such as reduced ventilatory function; increased symptoms, permeability, and reactivity (1); and an influx of inflammatory cells and mediators (2), there is relatively little known about the roles of repetitive transient exposures and the responses they induce in the development of cumulative lung damage and/or disease. Many of the transient responses produced by exposures to O₃ are similar to those produced by cigarette smoke, a known causal factor for chronic lung disease. Since about half of the U.S. population lives in communities having O₃ concentrations that exceed the current National Ambient Air Quality Standard, there is an ample basis for research on the effects of chronic O₃ exposure.

While past research studies on the chronic effects of O₃ have not been definitive, there are some provocative indications that there may be substantial adverse effects. The indications include: greater rate of loss of lung function in nonsmoking men and women in both Glendora, California, (high oxidant) and Long Beach, California (moderate oxidant and moderate SO₂), than in Lancaster, California (moderate oxidant and low SO₂) (3,4); reduced baseline lung function when annual average O₃ concentration is greater than 40 ppb, based on a national population sample (5); and an unexpectedly high incidence of centriacinar region disease in the lungs of adolescents and young adults examined post-mortem in Los Angeles County (6).

There are a variety of ways in which epidemiologic research can provide evidence of adverse chronic health effects in humans resulting from long-term exposure to O₃ and/or the other ambient air pollutants that coexist with it. Prospective cohort studies in well-defined populations of interest could be performed with suitable and careful measurements of exposure, activity patterns, symptoms, lung function, etc. However, it may be hard to justify such a study at this time for several reasons, including lack of firmer evidence that chronic effects are occurring, the very high costs of properly performed prospective studies, and the long time frame for results (i.e., at least 7–10 years).

For the above reasons, retrospective human lung studies may be most appropriate at this stage of inquiry, despite the great difficulties in adequate characterization of past exposure to O₃ and copollutants and adequate evaluation of confounding and modifying factors. Such studies have their own inherent advantages (i.e., the existence and extent of early chronic lesions in the peripheral lung tissues can be quantified), and no other kind of human studies can provide such information. Thus, quantitative comparisons of the extent of lesions in the lungs of well-matched individual cases that have lived in areas with different pollutant exposures can indicate whether there is an association between pollution and chronic lung damage. Furthermore, if there are differences in lung structure associated with chronic exposure, the nature and extent of such differences would provide an extremely valuable resource for designing follow-up studies of function and symptoms in living populations and chronic inhalation exposure studies in laboratory animals. In other words, knowledge of the structural changes that occur in humans should guide the selection of end points and measurement methods that are

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likely to produce significant results in more conventional studies.

This brief paper outlines the rationale for a study of chronic effects of O<sub>3</sub> exposure based on postmortem lung tissue as well as the opportunities and problems that face an investigator in conducting such a study.

**Rationale**

There are a series of specific factors that help establish the appropriateness of a study of postmortem lung tissue of individuals having definable variations in chronic exposure to oxidant air pollutants. First, predictive lung uptake models (7,8) indicate that delivered ozone (O<sub>3</sub>) dose is greatest in respiratory acinus of humans, rats, and other species. This region of the lung is inaccessible for studies based on direct in vivo examination, except that cells from this region can be recovered by bronchoalveolar lavage along with cells from adjacent regions. Second, chronic and sub-chronic exposures of rats (9) and monkeys (10) at near ambient levels of exposure produce changes in epithelial cell size and distribution in terminal bronchioles and immediately distal airways. These exposures also produce evidence of lung inflammation. All of these results are consistent with predicted uptake sites for O<sub>3</sub>. Third, intermittent exposures of monkeys (4 weeks on, 4 weeks off) produce changes that are similar to or greater than those seen in monkeys exposed continuously (and, therefore, having twice the total exposure) (10). These results have implications for both seasonal and daily patterns of human exposure. Fourth, the structural changes seen in the chronic and subchronic exposures in rats and monkeys are associated with the functional changes consistent with emphysema and a stiffening of the lungs, both of which correspond to premature aging of the lungs (11). Fifth, an autopsy study of 107 lungs from 14- to 25-year-old fatal injury victims in Los Angeles County by Sherwin and Richters (6) showed that 27% had what the authors judged to be severe degrees of structural abnormalities and bronchiolitis not expected for such young subjects, and another 48% of them had similar, but less severe, abnormalities. In the absence of corresponding analyses of lungs of comparable subjects from communities having much lower levels of air pollution, the possible association of the observed abnormalities with chronic O<sub>3</sub> exposure remains speculative. Some of the abnormalities observed could have been due to smoking and/or drug abuse, although the authors noted that published work on the association between smoking and small airway effects showed lesser degrees of abnormality (12).

**Hypothesis**

The lung abnormalities produced by sub-chronic and chronic O<sub>3</sub> exposures in rats and monkeys at near peak ambient levels are sufficiently similar to those seen in 14- to 25-year-old residents of Los Angeles to suggest that long-term ambient exposures to O<sub>3</sub> contributed to these effects. Furthermore, the data suggest that such exposures, if continued over a greater proportion of normal life span, could lead to reduced ventilatory capacity later in life and perhaps to chronic lung diseases such as chronic obstructive lung disease and emphysema.

**Discussion**

The kinds and degrees of abnormalities seen in the studies involving analyses of animal and human lung tissues discussed above would be largely subclinical and poorly related to conventional lung function indices. Measurement of spirometry and pulmonary flow resistance are generally controlled by airway calibre in the large and mizeductive airways, whereas the locus of damage associated with O<sub>3</sub> is in the small airways, which normally contribute little to overall flow restriction at early stages of disease progression.

The lungs of rats, monkeys, and humans were all examined at relatively young ages. Thus, there is concern that continued chronic O<sub>3</sub> exposure could lead to further progression of the structural and functional changes and thereby accelerate the normal rate of loss of lung function with age in a manner analogous to the accelerated loss of function associated with chronic cigarette smoke exposure.

**Research Opportunities**

To test the hypothesis that O<sub>3</sub> exposure can cause or facilitate an accelerated loss of lung function with age in human adults, it is necessary to show that there are significant differences in age-adjusted lung abnormalities in appropriately matched populations living in areas of relatively high and relatively low ambient O<sub>3</sub> concentrations.

Additional requirements, aside from appropriate matching or adjustment for smoking, age, gender, ethnicity, etc., would include climate and lifestyle. High oxidant, low acidic aerosol California communities would best be matched by other Pacific Coast communities that have relatively low levels of both types of secondary pollution, such as Santa Barbara, California; Portland, Oregon; Seattle, Washington; Victoria and Vancouver, British Columbia; etc. Cities in the midwest with moderately high oxidant and acid aerosol concentrations, such as Chicago, Illinois; Cleveland, Ohio; Detroit, Michigan; Buffalo, New York; and Toronto, Canada, might be matched with more westerly cities that have lower concentrations of such secondary pollutants such as Minneapolis, Minnesota; Milwaukee, Wisconsin; Kansas City, Missouri; and St. Louis, Missouri. For hot, humid cities, Houston, Texas, with high oxidant concentrations, could be matched with lower oxidant communities in Florida such as Tampa Bay, Orlando, Miami, and Fort Lauderdale to minimize possible confounding by differences in ambient temperature and humidity.

**Specific Exposure-Related Research Needs**

Of all the criteria pollutants, O<sub>3</sub> probably has the most extensive data base for ambient community levels. Quality-assured federal and state network data are readily available, and exposure modeling for locations within a monitored area is relatively straightforward. Temporal variations on a daily and seasonal level are largely predictable, and as a secondary pollutant, O<sub>3</sub> concentration variations within local regions are less extreme than those for primary pollutants such as carbon monoxide and lead. Some of the same considerations apply to acidic sulfate particles, another class of secondary pollutant that also deposits preferentially in small conducting airways.

The health effects associated with sulfates are most likely due to the associated hydrogen ion rather than the ammonium ion or sulfate itself (13). The H<sup>+</sup>/SO<sub>4</sub><sup>2-</sup> ratio is highly variable, and SO<sub>4</sub><sup>2-</sup> concentration data are usually available only on the basis of 24-hr averages every sixth day. For chronic effects studies, the available data on SO<sub>4</sub><sup>2-</sup>, SO<sub>2</sub>, O<sub>3</sub>, temperature, and humidity are thought to be sufficient to permit good estimates of long-term average exposure to SO<sub>4</sub><sup>2-</sup> and H<sup>+</sup>, at least for an examination of potential interaction of acidic sulfates and ozone in the production of accelerated aging of the human lung.

The development of protocols for obtaining residential and personal risk factors information on fatal injury victims whose lungs are analyzed is a specific research need. For those for whom such information can be obtained reliably and who have no complications of smoking or occupational exposures to lung irritants, cumulative O<sub>3</sub> exposure based on ambient concentrations at pollution...
monitoring sites adjacent to or surrounding the residence or work sites can be calculated.
To assess the cumulative exposures of individuals whose lungs are studied, these data should be obtained: a) residential histories—inclusive years at each address; b) distances from nearest continuous quality-assured monitoring sites for O3 and other pollutants at each residential address; c) participation in outdoor activities, sports, and regular exercise (including intensity, duration, location, and time of day); d) history of acute or chronic lung diseases; e) cigarette smoking as well as occupational and hobby exposures; f) residential exposures to confounding factors environmental tobacco smoke (ETS), unvented gas and kerosene cookers or space heaters, wood smoke, mulls, mildew, etc.; and g) commuting patterns resulting in different levels and types of air pollution exposure.

The development of alternate indices of cumulative pollutant exposure for correlation with activity patterns to yield individual exposure metrics is another research need. The exposure indices would then be correlated with the extent of observed lung abnormalities. Pollutant data resources of reasonably reliable quality include a) EPA and local monitoring data for O3, NOx, SO2, SO42-, and PM10; b) weather bureau data such as temperature, humidity, wind speed and direction; and c) airport visibility data (which can serve as surrogates for fine particle concentrations).

With regard to research needed to develop methodologies for retrospective exposure assessment, some preliminary research of this type has been performed at New York University (14) and Harvard (15); further research in this area is continuing. It involves improving and validating predictive models by using available pollutant concentrations and meteorological data bases. While the preliminary work is encouraging, much more needs to be done.

Retrospective exposure assessment research needs for chronic O3 epidemiology studies include delineation, investigation, and development. Delineation of the influence of various factors on local outdoor O3 concentrations and indoor/outdoor (I/O) O3 ratios is necessary for this type of research. Outdoor factors include sources of O3 scavengers such as NO from motor vehicles, elevation, and local micrometeorology. Factors affecting the I/O ratio include air exchange ratios and the nature of indoor surface sinks for O3. Investigation of the reliability of models for estimating ambient concentrations of H+ from intermittent (every sixth day) measurements of SO42- or continuous measurements of fine particle mass or light-scatter coefficient is another research need. Also necessary is the delineation of the influence of various factors on local outdoor aerosol H{eq}^{+}\text{ concentrations and I/O ratios. Major factors here are the strengths of the indoor and outdoor sources of ammonia (NH}_3\text{) and the rates of neutralization of H}_2\text{O by NH}_3\text{ from outdoor and indoor sources. A final retrospective exposure assessment research need is development and validation of exposure models that combine air concentration data and activity data to yield personal estimates of total hourly or daily inhalation rates.}

The design and evaluation of a personal history questionnaire about residential and occupational histories and personal risk factors of the accident victims whose lungs are to be analyzed is also needed in exposure-related research. Among the data that should be acquired, as possible, for each individual are: a) analysis of blood for COHb and/or cotinine as well as for evidence of substance abuse that might produce lung abnormalities; b) residential history from next of kin, to be verified to the extent possible by information from driver's licenses, school records, etc.; c) occupational history, if any, from next of kin, to be supplemented by employers' records; d) patterns of outdoor activity from next of kin, supplemented by records of team sports, running clubs, etc.; and e) records on location of nearest air pollution monitoring site(s) for residences lived in for the past 10 years or longer, if appropriate, along with a listing of the pollutants monitored at each site.

A final research need is the identification of suitable cities and collaborating pathologists for maximizing the range of chronic ozone exposures and access to suitable lungs in those communities. Consider possibilities for matching for climate, socioeconomic levels, and other potentially confounding factors. In terms of the selection of the communities that may provide the best opportunities for the collection of lungs having the greatest range of air pollutant exposures of interest, it would be ideal to have a) high ozone with low H+; b) high H+ with low ozone, c) high ozone with high H+ and d) low ozone with low H+.

In reality, there are no large communities that meet any of these criteria, except that large portions of the greater Los Angeles area fall into the first category. Areas with complex terrain and variations in altitude, such as Los Angeles, can include people with highly variable pollutant mixtures. On the other hand, many large metropolitan areas in the midwest and along the Atlantic coast have relatively uniform concentrations of secondary pollutants such as O3 and H+.

The rate at which numbers of lungs that can be obtained from young accident victims and processed in a uniform manner sufficient to permit sensitive quantification of pathological abnormalities, localized morphometry, and cell type distributions will be quite limited, especially for the smaller cities or regions, it may not be feasible or desirable to look for differences in means of responses by city or region. An alternative is to treat the results on each individual case as an independent observation related to that individual's cumulative exposure scores for O3. There should be enough O3 monitoring data in the higher O3 areas to devise a numerical value for each case with long residence in a high or moderate O3 area. It may or may not be useful or desirable to semiquantitatively rank them by H+ exposure based on measured sulfate levels and background knowledge of atmospheric chemistry (14).

**Specific Health Effects Assay Needs**

The Sherwin and Richters (6) study, while a pioneering effort of great interest, raised more critical issues than it settled. The distinctly abnormal centriacinar lesions they found could have been due less to air pollution than to cigarette smoking, drug abuse, or other stresses of the lower socioeconomic status (SES) that affected many of the individuals studied. Furthermore, the prospects of obtaining adequate background data on residence, occupation, and other critical variables on these and similar individuals are relatively poor. Another problem is that tissues were sampled for up to 48 hr postmortem. Information from animal exposure studies suggests that samples should be collected within one hour or less to obtain satisfactory results. The animal studies also indicate that some additional analytic protocols should be performed on
human lungs in future studies, to improve the prospects of seeing changes of interest in the tissues receiving the highest doses of oxidant, and to provide complementary analyses for interspecies comparisons.

It should be possible to overcome many of the limitations of the Sherwin and Richters protocols in designing future studies of human lung tissue from individuals chronically exposed to different levels of air pollution. For example, it may be possible to obtain fresher tissues from transplant donors. If such a source is used, the prospects of gathering adequate personal history data from next of kin should be better than for medical examiner cases. Selection of more optimal protocols for tissue analyses should be based on the recommendations of an expert panel convened to design such protocols.

The major developmental need for measuring the health impact of chronic ozone exposure is to refine and standardize the pathological protocols used in selecting target populations by age, location, background, etc. and used for selecting, storing, processing, inflating, fixing, preparing, devising analytical protocols for, managing data, etc., for human lungs. Consideration must be given to matching end points to those that have been or can be used in the chronic animal exposure studies. In terms of standardization of pathological analyses, there is a need to convene one or more expert panels of pulmonary pathologists with research backgrounds in irritant responses and let them establish suitable analytic criteria that would be used for all specimens.

**Summary and Conclusions**

Parallel studies on quantitative methods for retrospective exposure assessment need to be undertaken along with methods for quantitatively characterizing lung pathology, morphometry, and cell distributions. Neither aspect is trivial or easy, but the opportunities to do both are quite real and feasible.

Arrangements need to be made for coordination, standardization of protocols and procedures, and quality assurance for each collaborating investigator and group of investigators. Workshops should be conducted at the beginning and intermediate stages, and the results and experiences among the different groups should be used to develop and refine more optimal protocols. Furthermore, consideration should be given to the need for and benefits from continuing interchange of information with toxicologists performing chronic animal inhalation studies.

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