Long-Term Exposure to Ozone Increases Acute Pulmonary Centriacinar Injury by 1-Nitronaphthalene: I. Region-Specific Enzyme Activity

RENEE C. PAIGE, FRED H. ROYCE, CHARLES G. PLOPPER, and ALAN R. BUCKPITT

ABSTRACT
To test whether exposure to ozone alters pulmonary cytochrome P450 monoxygenase-mediated metabolism of xenobiotics, rates of 1-nitronaphthalene (1-NN) metabolism were measured in microsomes prepared from trachea, intrapulmonary airways, and distal lung of rats exposed to filtered air (FA) or ozone (O₃) (0.8 ppm 8 h/day for 90 days). Regioisomeric glutathione conjugates derived from intermediate epoxides were measured by HPLC. Compared with FA, rates of glutathione conjugate formation in distal lung (including the central acinus) were elevated 2-fold in O₃-exposed rats. Activity for one conjugate formation in distal lung (including the central acinus) were elevated 2-fold in O₃-exposed rats. There was a 2 ± 0.5-fold increase in immunodetectable CYP 2B protein in microsomes from the same lung subcompartment (P < .05). Immunodetectable protein was expressed in nonciliated epithelial (or “Clara”) cells and not associated with ciliated epithelial cells. No differences between O₃- and FA-exposed rats were noted in 1-NN metabolism or CYP 2B activity in trachea or intrapulmonary airways. This study emphasizes that cellular and biochemical alterations associated with long-term O₃ exposure vary considerably by location within the lung. Long-term exposure to O₃ elevates both CYP 2B activity and 1-NN metabolism in an airway-specific manner.

Oxidant environments, such as those found in urban areas, have long been known to alter the metabolic activity of the respiratory system. The morphological and biochemical changes associated with long-term exposure to ozone render the ciliated bronchiolar epithelial cell, a primary target of acute ozone toxicity, resistant to further oxidant injury. However, these changes may ultimately alter the susceptibility of this cell type to other toxicants found as copollutants in ambient air. The role of the cytochrome P450 monoxygenases in the activation of environmental contaminants is well documented and data suggest long-term oxidant exposure may alter the activity of cytochrome P450 isoforms found in the lung.

Information on the impact of ozone exposure on cytochrome P450 activity in the lung is complex and difficult to interpret. For example, P450 1A1 activity is reportedly decreased (Rietjens et al., 1988) or slightly elevated (Takahashi et al., 1985) after intermediate periods of ozone exposure (up to 14 days). Cytochrome P450 2B1 activity is either elevated by as much as 1.4-fold or not at all during this time period (Takahashi et al., 1985; Rietjens et al., 1988). However, CYP 2B1 protein increases 2-fold on Western blots of lung homogenates from ozone- compared with filtered air-exposed rats (Takahashi et al., 1994). At ozone concentrations of 0.5 ppm or below, exposures for up to 1 year show some dose-dependent elevation in activity. Concentrations of 0.2 and 0.5 ppm produce marked elevations in total microsomal cytochrome P450, NADPH-cytochrome P450 reductase, and enzyme activities for both CYP 1A1 and 2B1 (Filipowicz and McAuley, 1986; Takahashi and Miura, 1987), but exposure to 0.1 ppm does not produce an elevation in activity for CYP 1A1. Possibly adding to the confusion is that activity was measured in microsomes of whole-lung homogenates, despite the fact that ozone-induced injury in the lung is very focal. Averaging rates of xenobiotic metabolism in microsomes from whole lung could mask critical changes in metabolism in target airways. The proportion of the total number of lung cells most affected by ozone, and therefore most likely to undergo metabolic adaptation, is very small fraction of the whole (Barr et al., 1988). Conducting airway epithelium represents less than 4% of all lung cells (Hyde et al., 1991) and the terminal bronchiolode represents an even smaller fraction of that total. In a previous study (Watt et al., 1998) microsomal

ABBREVIATIONS: FA, filtered air; CYP, cytochrome P450; 1-NN, 1-nitronaphthalene; GSH, glutathione.
CYP 2E activity was found to be unaltered by long-term exposure to ozone in any lung subcompartment isolated by microdissection.

This and the companion study (Paige et al., 2000) address the impact of ozone tolerance on the toxicity of other compounds found as copollutants. 1-Nitronaphthalene (1-NN) is a nitroaromatic found as a copollutant with ozone in urban atmospheres whose toxicity appears to be related to metabolic activation catalyzed by CYP 2B (Verschoyle et al., 1993). In animal models, treatment with 1-NN results in a well characterized dose- and airway-selective toxicity involving necrosis of ciliated and nonciliated epithelial cells (Paige et al., 1997). The present study addresses whether oxidant exposure modulates P450 activity. First, we determined whether the rates of metabolism of this P450-activated toxicant (1-NN) were different in specific airway subcompartments of ozone- and filtered air-exposed rats. We then determined whether CYP 2B activity and protein expression differed in these same compartments. The companion study addresses whether changes in CYP 2B activity and 1-NN metabolism result in altered susceptibility to the acute toxicity of 1-NN in the whole animal.

Materials and Methods

Animals. Male Sprague-Dawley rats (275–300 g) were purchased from Charles River Laboratories (Hollister, CA). Animals were housed in stainless steel, open-mesh cages within 4.2-m³ exposure chambers (Hinners et al., 1988) ventilated with high efficiency particulate air (HEPA) and charcoal-filtered air at 2.1 m³/min. Animals were allowed free access to food and filtered, deionized water and were kept on 12-h light/dark cycle in American Association for the Accreditation of Laboratory Animal Care-approved facilities at the California Regional Primate Research Center for at least 5 days before use in experiments.

Ozone Exposure. Ozone was generated from medical-grade oxygen with an electric discharge ozonizer (Erwin Sanders Elektroapparatebau GmbH, Uetze-Eltze, Germany). The ozone concentration in the exposure chamber was continuously measured with an ultraviolet ozone monitor (Dasibi Corporation, Glendale, CA). Calibration of the monitor was performed according to the national reference method (United States Code of Federal Regulations, 1988) and was traceable to National Institute of Standards and Technology absolute ozone photometer serial 4 located at the California Air Resources Board Quality Assurance Laboratory (Sacramento, CA). A mean target concentration of 0.8 ppm was achieved with a standard deviation of less than 4%, and the maximum of the range did not exceed 10% of the mean. Animals were exposed from 12:00 AM to 8:00 AM for 90 days. A control group of age-matched, filtered air-exposed animals was sampled concurrently with ozone-exposed animals.

Chemicals and Reagents. 1-NN was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was recrystallized from ethanol before use (melting point 61.5°C). GSH was purchased from Fluka Chemical Corp. (Milwaukee, WI). Glutathione S-transferase was purified from mouse liver cytosol by affinity chromatography (Simons and Vander Jagt, 1981). Pentoxyresorufin, NADP, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and horse heart cytochrome c were purchased from Sigma-Aldrich (St. Louis, MO). Waymouth’s medium 752/1, containing 5 mM HEPES, was purchased from Life Technologies (Grand Island, NY). Low-melting-point agarose (Comatil) was purchased from FMC BioProducts (Rockland, MD). [14C]1-Nitronaphthalene was prepared synthetically from [14C]naphthalene as previously described for tritium-labeled product (Watt et al., 1999). The radiochemical purity of the final product was greater than 99% as assessed by HPLC. The final specific activity of [14C]1-NN was 2 mCi/mmol. All other chemicals were reagent grade or better.

Microsome Preparation. The procedure for obtaining defined specimens of the lung by blunt dissection has been described previously in detail (Plopper et al., 1991). Rats were anesthetized with sodium pentobarbital, and the trachea was exposed and cannulated. The lungs were inflated with 1% agarose in Waymouth’s medium and cooled in Waymouth’s medium at 4°C for 30 min. The airways were then stripped intact from the lobe by microdissection. Subcompartments isolated were trachea, intrapulmonary airways, and the distal lung (Fig. 1). The latter contains the central acinus, the transition between the conducting airways and the gas exchange region. For CYP 2B and NADPH-cytochrome P450 reductase activity measurements, liver and blood vessels consisting of arteries and veins throughout the lung also were sampled. The liver, trachea, and microdissected lung subcompartments were homogenized with a glass tissue homogenizer in 20 mM Tris, 150 mM KCl, 0.2 mM sodium EDTA, 0.5 mM dithiothreitol, and 15% glycerol, pH 7.4, at 4°C. Cell debris was removed by centrifugation at 18,000g at 4°C for 20 min. Microsomal pellets were recovered from the postmitochon-

![Fig. 1. Schematic of methods for preparation of microsomes from defined lung subcompartments. Using microdissection, trachea, intrapulmonary airways, and distal lung subcompartments are isolated. For P450 2B and reductase activity measurements, liver samples and blood vessels consisting of arteries and veins throughout the lung also were collected for microsome preparation.](image-url)
drial supernatant by ultracentrifugation at 100,000g for 75 min, and resuspended in 0.1 M NaH₂PO₄, pH 7.4. Protein concentration was determined by microassay (Bio-Rad, Hercules, CA) based on the Bradford method (Bradford, 1976) with standard curves generated using bovine serum albumin.

**1-Nitronaphthalene Metabolism.** Incubations were prepared on ice in a final volume of 300 μl of 0.1 M sodium phosphate buffer (pH 7.4). Incubations contained 300 μg of microsomal protein, glutathione transferases (three 1-chloro-2,4-dinitrobenzene units), 1 mM 1-NN, and an NADPH-generating system (consisting of 0.14 mM NADP, 3.8 mM glucose 6-phosphate, 0.1 unit of glucose 6-phosphate dehydrogenase, and 10 mM MgCl₂). Incubations were allowed to proceed for 20 min at 37°C. The reaction was terminated by adding 1 volume of ice-cold methanol and samples were stored at −20°C overnight for protein to precipitate. Microsomes were prepared on 3 separate days, each day consisting of two filtered air- and two ozone-exposed rats. Incubations were prepared in triplicate and included a control without the NADPH-generating system.

**Sample Preparation and HPLC Analysis of 1-NN Metabolites and Glutathione Conjugates.** The methods for the isolation and quantitative measurement of 1-nitronaphthalene glutathione conjugates (Fig. 2) have been described previously (Watt et al., 1999; Watt and Buckpitt, 2000). All glutathione adducts yielded similar spectra by tandem mass spectrometry analysis and the regiochemistry of each metabolite was definitively assigned by proton NMR (Watt et al., 1999). Briefly, the incubation mixture was centrifuged at 13,000g for 30 min at 4°C to remove the protein, and the remaining supernatant was evaporated under vacuum to dryness and stored at −20°C until analysis. Samples were reconstituted in water and chromatographed on a Phase Sep C18 reversed phase column (25 cm × 4.6 mm i.d.; 5-μm particle). The eluates were monitored by UV absorbance at 256 nm and peak area measurements were obtained with Millennium 32 software (Waters Corporation, Milford, MA). A mobile phase of 0.06% triethylamine in water (pH 3.1) (solvent A) and 1:1 acetonitrile:solvent A (solvent B) was run at a flow rate of 1.0 ml/min with a linear increase from 10 to 32% solvent B over the course of 60 min. Sufficient quantities of isolated, purified conjugates were not available to act as quantitative standards for measurement by UV detection. Accordingly, quantitative measurement of 1-NN glutathione conjugates generated by microsomes from pulmonary subcompartments relied on standards prepared from hepatic microsomal incubations containing [14C]-1-NN (at defined specific activity), glutathione, glutathione transferases, and NADPH-generating system. Complete radiochromatographic profiles were obtained (Fig. 3) and peaks, corresponding to glutathione conjugates, were summed and appropriate background counts were subtracted.

![Fig. 2. Possible pathways of 1-nitronaphthalene metabolism. 1-Nitronaphthalene can undergo oxidative (shown here) or reductive metabolism. Oxidative metabolism predominates in the lung generating epoxides via cytochrome P450 monooxygenases. Conjugates derived from the 7,8-epoxide predominate in lung microsomal incubations (solid arrows). The diastereomers of these glutathione conjugates were separated and quantitated by HPLC using methods described previously (Watt et al., 1999). Although formation of the 3,4-epoxide (dashed arrow) is possible, there is no experimental evidence for this in either lung or liver. Reprinted with permission (Watt et al., 1999).](image-url)
from these to obtain area unit/nanomolar ratios. The ratios were used as standards to measure rates of metabolism in incubations of airway subcompartments. Retention time standards, consisting of a mixture of 1-NN glutathione conjugates prepared from individual metabolites previously identified by both mass spectral and NMR techniques, were run before, during, and after each set of samples. Glutathione conjugates are referred to in numeric form (Fig. 2).

Microsomal Activity of Cytochrome P450 2B. Cytochrome P450 2B activities were measured by O-deethylation of pentoxyresorufin (Sigma-Aldrich) based on established methods (Rettie et al., 1986). Incubations were performed for 15 min at 37°C in a shaking incubator. The incubation consisted of 50 μg (lung) or 2.5 μg (liver) of microsomal protein, 1 mM pentoxyresorufin, 15 mM MgCl₂, 0.1 M sodium phosphate, pH 7.4, in a final volume of 200 μL. The reaction was initiated by the addition of 10 μL of an NADPH-generating system. The incubations were terminated by the addition of 400 μL of ice-cold methanol and protected from light at −20°C until time of analysis. Rates of formation of resorufin were measured by HPLC using a fluorescence detector with an excitation wavelength of 535 nm and an emission wavelength of 585 nm as described previously (Plopper et al., 1993). Standard curves were run with each HPLC run using a series of known concentrations of resorufin. Microsomes were prepared on 3 separate days, each day consisting of two filtered air- and two ozone-exposed rats. Incubations, prepared in triplicate, included a control without the NADPH-generating system.

Activity of NADPH-Cytochrome P450 Reductase. Microsomal NADPH-cytochrome P450 reductase was measured spectrophotometrically by determining the rate of reduction of cytochrome c by standard methods (Guengerich, 1994). Briefly, microsomes (50 μg of lung or 2.5 μg of liver) were added to 10 mM horse heart cytochrome c (Sigma-Aldrich), 0.1 mM NADPH, 0.3 M phosphate buffer, pH 7.4, in a final volume of 500 μL and absorbance was measured at 550 nm. Microsomes were prepared on 3 separate days, each day consisting of two filtered air- and two ozone-exposed rats. Incubations, prepared in triplicate, included a control without NADPH.

Western Blot Analysis for 2B Expression in the Distal Lung Subcompartment. The results of the activity assays indicated a possible increase in CYP 2B protein expression in the distal lung subcompartment. From the limited amount of microsomal protein remaining from activity determinations, four samples each were available from the distal lung subcompartment of ozone- and filtered air-exposed animals. Protein (30 μg) from each microsomal sample was mixed with loading buffer (Laemmli, 1970), heated at 100°C for 5 min, loaded into wells of 7.5% precast mini polyacrylamide gels (Bio-Rad), and electrophoresed at 150 V for 60 min. Proteins in the gel were electrophoretically transferred to polyvinylidene difluoride membrane. The membrane was blocked with 5% nonfat dry milk and rinsed with Tris-buffered saline containing 0.1% Tween 20 and then incubated with goat anti-CYP 2B antibodies generously supplied by Dr. Richard Philpot, National Institutes of Health, and characterized previously (Serabjit-Singh et al., 1979) at 1:20,000 for 1 h. The membrane was then incubated with a biotinylated donkey anti-goat secondary antibody at 1:150,000 (Vector Laboratories, Burlingame, CA) along with horseradish peroxidase-conjugated streptavidin (Bio-Rad) at 1:2000 for 1 h. After a rinse with Tris-buffered saline containing 0.1% Tween 20, the membrane was incubated with a chemiluminescence reagent (Renaisseance Reagent Plus; NEN Life Sciences, Boston, MA) per manufacturer's instruction and bound antibodies detected by exposure to radiographic film. A series of films were exposed to ensure that radiographs used were within the dynamic range of the radiographic film. The radiograph of the blot was scanned on a Powerlook (Umax, Hanchu, Taiwan) flatbed scanner. The resultant image was imported into Adobe Photoshop (Adobe Systems, San Jose, CA) to add labels, and final images were printed on an Epson 900 color printer. Relative abundance of CYP 2B protein was quantitated using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The density of the immunoreactive CYP 2B band for filtered air-exposed animals was averaged. From this, the relative abundance of CYP 2B in ozone-exposed rat airway microsomes was calculated and expressed as mean ± standard deviation.

Immunohistochemical Localization of Cytochrome P450 450 2B in the Distal Lung. Ozone- and filtered air-exposed rats were killed on the morning after the 90th exposure. The lungs were fixed for immunohistochemistry for 1 h in 1% paraformaldehyde instilled into the lungs by tracheal cannula under 30 cm of fluid pressure. The lungs were removed from the chest, the left lobe was grossly dissected, and embedded in paraffin. Sections (5–6 μm) were cut on a Reichert-Jung SuperCut Microtome and affixed to Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA). Paraffin sections were deparaffinized in xylene, rehydrated in a graded ethanol series, and incubated for 30 min in 3% H₂O₂ to eliminate endogenous peroxidase activity. Slides were then incubated for 30 min with 5% goat serum and incubated overnight with antibodies against CYP 2B raised in rabbit at 1:20,000. The bound antibody was detected using the Vectastain Immunoperoxidase kit per manufacturer's instruction (Vector Laboratories). Images were captured using a DAGE MTI VE100 video camera (Michigan City, IN) mounted on a Zeiss AxioScope MC80 with a 20× lens. The camera was interfaced with a Macintosh Centris 650 running NIH image software. Labels and magnification bars were added in Adobe Photoshop and final images were printed on an Epson 900 color printer.

Statistical Analysis. Rates of generation of 1-NN glutathione conjugates are expressed as picomoles per microgram per minute (mean ± 1 standard deviation). Cytochrome P450 2B activity is expressed as picomoles of resorufin per microgram per minute released by O-deethylation of pentoxyresorufin (mean ± 1 standard deviation). NADPH-cytochrome P450 reductase activity is expressed as nanomoles of reduced cytochrome c per microgram per minute (mean ± 1 standard deviation). Comparisons between filtered air- and ozone-exposed animals were made using a two-tailed Student's t test with significance assigned at P < .05 (Glantz, 1997).

Results

1-Nitronaphthalene Metabolism. The rate of 1-NN metabolism, measured as the rate of formation of glutathione conjugates, was markedly different between lung subcom-
partments. The rate of formation of 1-NN glutathione conjugates in the distal lung subcompartment was approximately 2-fold higher than in the intrapulmonary airways and nearly 100-fold higher than that observed in the trachea.

After long-term exposure to ozone, only the distal lung subcompartment exhibited altered 1-NN metabolism compared with filtered-air controls. The rates of formation of conjugates 2, 4, 5, and 6, which are derived from the 7,8- and 5,6-epoxides, were increased significantly (2-fold) in the distal lung subcompartment of ozone- compared with filtered air-exposed rats (Fig. 4A). The rates of formation of conjugates 3 and 7, which are relatively minor conjugates generated during the incubation, were not significantly different in microsomal incubations of ozone- and filtered air-exposed animals. Although conjugate 3 was often undetectable in ozone-exposed animals, there were no indications that the proportion of each conjugate formed was different in filtered air- and ozone-exposed animals. In both groups, the formation of conjugates 2 and 4 predominated.

The rates of microsomal 1-NN metabolism in intrapulmonary airways of filtered air- and ozone-exposed rats were not significantly different (Fig. 4B). In both groups, the formation of conjugates 2 and 4 predominated. As reported previously, rates of metabolism of 1-NN in the trachea were very low (Watt et al., 1999). Due to low rates of metabolism, peaks derived from extracts of tracheal microsomal metabolism could not be assigned with complete confidence, but there did not appear to be an induction of 1-NN metabolism in ozone-exposed animals (data not shown).

Cytochrome P450 2B and NADPH Reductase Activity. Long-term exposure to ozone increased CYP 2B activity 3-fold in microsomes isolated from distal airways, relative to that in filtered air-exposed animals ($P < .02$) (Fig. 5A). No changes were observed in CYP 2B activities in ozone-exposed versus filtered air-microsomes prepared from any of the other lung subcompartments examined (trachea, intrapulmonary airways, blood vessels) or from liver. Although NADPH-cytochrome P450 reductase activity varied significantly by subcompartment, activities were not altered by exposure to ozone in any of the subcompartments studied (Fig. 5B). Activities in the intrapulmonary airways were significantly greater than in blood vessels (7.0-fold, $P < .0001$) and distal lung (3.1-fold, $P < .001$). Long-term exposure to ozone did not affect metabolism in the liver; no changes were observed in cytochrome P450 2B (Fig. 5A) or NADPH-cytochrome P450 reductase activity (Fig. 5B).

Western Blot Analysis and Immunohistochemistry of 2B Expression. Western blot analysis of microsomal proteins isolated from the distal lung subcompartment of filtered air- and ozone-exposed animals revealed a significant $2 \pm 0.5$-fold increase in immunodetectable CYP 2B protein in ozone-exposed rats ($P < .05$) (Fig. 6). This is based on comparison of blots of microsomal proteins from distal lung of four filtered air- and four ozone-exposed animals. Although there did not appear to be a difference in the abundance of 2B protein detectable by Western blot in microsomes prepared from intrapulmonary airways of filtered air- and ozone-exposed animals, very limited amounts of material were available and thus, the response could not be measured quantitatively.

CYP 2B protein was localized to nonciliated (“Clara”) cells and was not expressed in ciliated cells (Fig. 7A). Although some nonciliated cells from ozone-exposed animals appeared to stain darker for immunodetectable CYP 2B (Fig. 7B), immunohistochemistry is shown only to emphasize that ciliated bronchiolar epithelial cells do not express immunodetectable CYP 2B in either filtered air- or ozone-exposed animals.

Discussion

Human populations are exposed to both oxidant gases and bioactivated toxicants found as constituents of polluted ambient air. Prolonged exposure to either class of pollutants generally results in cellular and metabolic adaptations that render the lungs tolerant to further acute injury and inflam-
duction of the present study demonstrates region-specific elevations in P450 activity using the CYP 2B-selective substrate pentoxyresorufin and in the rates of metabolic activation of 1-nitronaphthalene to epoxide intermediates. These elevations occurred only in sites of enhanced 1-nitronaphthalene-induced cytotoxicity after long-term ozone exposure. The central acinus is a region of the lung associated with high susceptibility to acute ozone injury and is the primary site of tolerance associated with long-term exposure. A wide range of cellular and metabolic adaptive changes occurs during prolonged ozone exposure that renders this region resistant to further oxidant stress. This study, however, clearly demonstrates that the potential of this region of the lung to metabolically activate toxic substrates is markedly increased after a long-term ozone exposure protocol that induces tolerance to oxidant injury.

Among the adaptive metabolic changes associated with long-term ozone exposure are alterations in cytochrome P450 monoxygenase activity. The responses of P450 isoforms 1A1, 2B1, and 2E1 to ozone exposure have been characterized previously, with variable results. The variability appears to depend on exposure concentration and duration, and methods of sampling and analysis. When data on P450 activity from similar exposure regimens are compared, the results remain difficult to interpret: both increases (Takahashi et al., 1985) and decreases (Rietjens et al., 1988) in pulmonary CYP 1A1 activity have been reported after a 7-day exposure to 0.8 ppm ozone with increases (Takahashi et al., 1985) or no change in CYP 2B activity (Rietjens et al., 1988). With the exception of recent work on CYP 2E1 (Watt et al., 1998) that found no change in microsomal activity in isolated lung subcompartments after a 90-day exposure to 0.8 ppm ozone, all measurements of P450 activity used microsomes prepared from homogenates of whole lung or isolated Clara cells. With the development of methods for the isolation of specific lung subcompartments by microdissection (Plopper et al., 1991) and a sensitive method for the isolation and identification of metabolites of 1-nitronaphthalene (Watt et al., 1999), we were able to more carefully characterize changes in metabolic potential after ozone exposure. Additionally, in contrast to the majority of prior studies, the present work used a well characterized exposure scenario known to result in oxidant tolerance. The pathologic response to this exposure regimen (Boorman et al., 1980), along with the response of many of the detoxification enzymes, has been characterized previously. The long-term ozone exposure used in this study is associated with elevations in several antioxidant enzymes (Plopper et al., 1994) and intracellular glutathione (Duan et al., 1996) in the distal subcompartment of the lung. Using airway microdissection to isolate specific subcompartments of the lung, including
those associated with oxidant tolerance and elevated susceptibility to a metabolically activated cytotoxicant, the present study found focal increases in CYP 2B activity and 1-nitronaphthalene metabolism in ozone-exposed rats. From this study, it appears that regional alterations in metabolic potential are responsible for the increased cytotoxicity in the central acinus of ozone-tolerant rats despite elevated phase II enzymes and intracellular glutathione.

The selective increase in CYP 2B activity in the distal lung of ozone-exposed rats is most likely not a generalized result of increased numbers of Clara cells in the central acinus. Although Clara cell hyperplasia is a well characterized response of the central acinus to long-term exposure to ozone (Dodge et al., 1994), elevations in P450 activity after ozone exposure appear to be isoform selective. Elevations in activity are also not likely due to differences in the subcellular fractions isolated by ultracentrifugation from filtered air-compared with ozone-exposed rats because earlier work showed no alterations in marker enzyme activities for endoplasmic reticulum (Watt et al., 1998). Although the mechanism for the observed region-selective induction in P450 activity is not evident, it is important to note that very different regulatory mechanisms are involved for each of the P450 isoforms evaluated so far: CYP 1A1 activity is regulated via the Ah receptor, CYP 2E1 activity is controlled by post-translational stabilization, and CYP 2B activity appears to be regulated through the barbie box (Ioannides, 1996). CYP 2B activity is also regulated through a constitutively activated receptor that transactivates a phenobarbital-responsive enhancer module upstream of CYP 2B genes (Kawamoto et al., 1999; Sueyoshi et al., 1999). Additionally, although the region-selective induction of CYP 2B appears to be responsible for the increase in 1-nitronaphthalene metabolism in rats, this may not be the case for other species. In mice, for example, CYP 2F2 rapidly metabolizes 1-nitronaphthalene with a relatively low $K_m$ (Schultz et al., 2001). Therefore, to fully appreciate the impact of oxidant exposure on the toxicity of bioactivated copollutants, the response of multiple genes needs to be addressed, including both phase I- and phase II-metabolizing enzymes. Genomic analysis using microarray technology provides a rapid method by which to screen a large number of genes for alterations in expression after ozone exposure. More global approaches, including DNA microarray and proteomics, are currently being used in an attempt to assess changes in gene levels/protein expression after ozone exposure.

The present study and its companion demonstrate that long-term exposure to the oxidant air pollutant ozone results in increased P450 activity that translates to enhanced rates of metabolic activation of a substrate and ultimately, enhanced cytotoxicity. 1-Nitronaphthalene, a model bioactivated toxicant, is found as a copollutant with ozone in polluted urban atmospheres. Nitronaphthalenes are the single largest genotoxic component found in ambient air and the genotoxicity of these nitroaromatics is dependent upon oxidative metabolism by cytochrome P450 monoxygenases (Grosovsky et al., 1999). Given that polycyclic aromatic hydrocarbons are slowly absorbed by airway epithelium and undergo extensive metabolism at the site of deposition (Gerde et al., 1997), the genotoxicity of these compounds may be greatly potentiated by the airway-specific alterations in metabolic activity after long-term exposure to ozone. The present study demonstrates that long-term exposure to an oxidant air pollutant enhances the potential for activation of a ubiquitous toxicant. The companion study (Paige et al., 2000) documents significantly increased cytotoxicity in the same region of the lung.

Acknowledgments

We acknowledge the technical assistance of the following people: Aimin Chang, Ning Sun, Cristi Lux-Miglacciao, Christina Strandgaard, Pratibha Daftari, and Alison Weir. We also acknowledge the expert technical assistance of Brian Tarkington and the staff at the California Regional Primate Research Center Exposure Facility.

References


Send reprint requests to: Dr. Renee Paige, Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616. E-mail: rpaige@ucdavis.edu

Ozone Exposure Increases 1-Nitronaphthalene Metabolism | 941