Genetic differences in oxygen toxicity are correlated with cytochrome P-450 inducibility
(hyperoxia/microsomal enzymes/pulmonary pathology)

JANET C. GONDER*,†, RICHARD A. PROCTOR‡, AND JAMES A. WILL*§

Departments of *Veterinary Science, College of Agriculture and Life Sciences, and †Anesthesiology, and ‡Medical Microbiology and Medicine, Medical School, University of Wisconsin–Madison, Madison, WI 53706

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ABSTRACT Susceptibility to oxygen toxicity was studied in three inbred and two hybrid strains of mice. Because in vitro studies have shown that the cytochrome P-450 enzymes can produce oxygen radicals and H₂O₂, we tested the hypothesis that inductibility of these enzymes might play a role in oxygen toxicity. Mice responsive to hepatic microsomal enzyme induction by aromatic hydrocarbons (C3H/HeJ, C3H/HeN, C3H/HeJ × DBA/2J designated C3D2F₁/J), C3H/HeN × DBA/2J, [NI]) were more sensitive to the toxic effects of 100% oxygen exposure than were genetically unresponsive mice (DBA/2J). DBA/2J mice survived significantly longer exposure periods with less lung damage. Lung and liver cytochrome P-450 levels increased 2- to 3-fold in C3H and F₁ mice during 100% oxygen exposure (maximum levels at 72–96 hr) and subsequently fell prior to death. No increases were seen in cytochrome P-450 levels in DBA/2J mice. Metabolic pathways involving cytochrome P-450 enzymes may initiate or modulate oxidative damage due to oxygen radicals. The differences in responsiveness of mice to microsomal enzyme induction may imply genetic differences in susceptibility to oxidative stress, may help to explain species differences in susceptibility, and may have long-term implications in therapeutics and patient care if similar inherited differences exist in humans.

Production of reactive oxygen species that damage membranes by lipid peroxidation is the primary cause of pulmonary injury following prolonged exposure to high concentrations of oxygen (1). The source of these reactive oxygen species is unknown. Research has concentrated on the mechanisms controlling increased activity of inherent protective pathways (superoxide dismutase, catalase, glutathione peroxidase), which are now relatively well understood (1–3).

Damage to plasma membranes, including pulmonary cells, has followed exposure to a number of substances, such as the herbicide parquat and anticancer antibiotics adriamycin and daunomycin, and has been related to the formation of superoxide radical anions, O₂⁻ (4–6). Potential sources of superoxide anions is the cytochrome P-450 enzyme system (7). Of note, O₂⁻ production by liver microsomes is enhanced following induction of these enzymes by phenobarbital (8). Longmuir et al. (9) and Rowe et al. (10) have reported in vivo and in vitro induction of murine hepatic cytochrome P-450 with exposure to high oxygen concentrations (1–4 ata). Because cytochrome P-450 induction is genetically controlled in mice (11), we hypothesized that the production of superoxide anion might also be genetically determined. The hypothesis that high oxygen concentrations would also induce lung cytochrome P-450 enzymes and cause enhanced susceptibility to oxygen toxicity was tested in inbred strains of mice. Strains of mice were chosen based on the ability to induce cytochrome P-450 enzymes with the aromatic hydrocarbon 3-methylcholanthrene. Responsiveness to aromatic hydrocarbon induction is genetically controlled by the aromatic hydrocarbon (Ah) gene locus (8). Oxygen sensitivity was assessed in aromatic hydrocarbon-responsive (C3H and C3D2F₁ hybrids) and aromatic hydrocarbon-unresponsive (DBA/2J) strains of mice.

MATERIALS AND METHODS

Animal Exposure. Male C3H/He/J and DBA/2J mice (20–25 g) were obtained from The Jackson Laboratory. C3H/HeN and F₁ hybrids (C3H/HeJ × DBA/2J, designated C3D2F₁/J, and C3H/HeN × DBA/2J, designated C3D2F₁/N) were from the Research Animal Resources Center, University of Wisconsin. For all experiments, mice 7–9 weeks of age were exposed to oxygen in a 2.14 m³ Plexiglas chamber. Oxygen concentration was maintained at >98% with a flow of 8–10 liters/min. Survival times in 100% oxygen were determined for each strain (12 mice per strain).

Cytochrome P-450 Assays. Following 0, 12, 24, 48, 72, and 96 hr of 100% oxygen exposure (12 mice per group per strain), lung and liver microsomes were prepared (12) and cytochrome P-450 content was determined by the carbon monoxide difference spectra of dithionite-reduced microsomal suspensions (13). A molar extinction coefficient of 91 cm⁻¹·mM⁻¹ was used for both liver and lung.

Histologic Procedures. Lung damage was assessed in 20 animals of each strain following 0, 24, 48, 72, and 96 hr of 100% oxygen exposure. Lungs were instilled via the trachea with Bouin’s fixative at 13 cm of water pressure, embedded in paraffin, sectioned 5 μm thick, and stained with hematoxylin/eosin or Miller’s elastic Van Geisen. Average vascular medial thickness was determined morphometrically by evaluation of 50- to 200-μm vessels in lung sections stained with Miller’s elastic Van Geisen. Medial thickness was determined based on measurements of the outside diameter and inside diameter of the vessels, using the outside and inside elastic lamina, respectively, as references to the limitation of the extent of muscular media. Compensation for differences due to vessel size was made by expressing average medial thickness as the ratio of the medial thickness of the vessel to its outside diameter.

Statistical Analysis. Responses between strains were compared by using one-way analysis of variance. The level of significance was set at P < 0.05.

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†Present address: Animal Resources Center, East Carolina University, School of Medicine, Greenville, NC 27834.
1. different of liver cytochrome that of (121.5 mice statistically oxygen decreased concentrations were less pronounced were F1 after even edema was severe by 72 hr. C3H/HeN; FN, C3H/HeJ; C3D2F1/N, and C3D2F1/J, were 98.5, 98.5, and 95.5 hr, respectively, and were not statistically different. The mean survival time for DBA/2J mice (121.5 ± 5.0 hr) was significantly longer (P < 0.05) than that of the other strains.

Cytochrome P-450. No changes were detected in lung or liver cytochrome P-450 content in DBA/2J mice during 100% oxygen exposure (Figs. 1 and 2). However, in the C3H and F1 hybrid mice, liver and lung microsomal cytochrome P-450 concentrations were significantly increased at 72 and 96 hr but decreased prior to death.

Based on microscopic examination of lung tissue, strain differences were also evident (Fig. 3). Pulmonary injury was less pronounced in DBA/2J mice than in C3H and F1 hybrids even after longer exposure times. Alveolar and perivascular edema was evident as early as 48 hr in C3H mice and was severe by 96 hr (Fig. 3A). Considerable perivascular infiltration of polymorphonuclear and mononuclear leukocytes was characteristic of C3H and F1 strains.

Pulmonary vascular medial thickness was significantly increased (P < 0.05) above control values in C3H and C3D2F1 hybrids following 72 and 96 hr of 100% oxygen exposure (Table 2). In DBA/2J mice, vascular medial thickness was unchanged through 72 hr and decreased significantly at 96 hr.

**DISCUSSION**

Sensitivity to high oxygen concentrations may be related to the induction of monoxygenase activity including cytochrome P-450 enzymes. Exposure to high oxygen concentrations also induces the antioxidant enzymes catalase and superoxide dismutase in several species (2, 14). Although these protective enzymes are genetically controlled in mice (15, 16), the genetic polymorphisms in different inbred strains are not well-defined, and enzyme activities do not appear to correlate with sensitivity to oxygen. C3H mice have high liver catalase activity (17) yet are sensitive to high oxygen concentrations. Strain differences in lung catalase activity have not been reported. We have shown that oxygen induction of cytochrome P-450 enzymes in mice parallels the genetic responsiveness to aromatic hydrocarbon induction. The DBA/2J mice survived significantly longer exposure periods in 100% oxygen with less histologic evidence of lung damage than C3H and F1 hybrid mice. Generation of superoxide anions by way of cytochrome P-450 enzyme systems may contribute significantly to the free radical-mediated damage in oxygen toxicity.

The decrease in cytochrome P-450 values prior to death may be related to a deterioration of cell function due to lipid

<table>
<thead>
<tr>
<th>Strain</th>
<th>Survival time, hr</th>
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<tbody>
<tr>
<td>C3H/HeJ</td>
<td>92.0 ± 2.8</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>98.5 ± 3.5</td>
</tr>
<tr>
<td>C3D2F1/J</td>
<td>98.5 ± 2.1</td>
</tr>
<tr>
<td>C3D2F1/N</td>
<td>95.5 ± 4.9</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>121.5 ± 5.0*</td>
</tr>
</tbody>
</table>

Each value is presented as the mean ± 1 SD for 12 animals.

*Significantly different (P < 0.05) when compared to other strains.

**Fig. 1.** Cytochrome P-450 concentrations in liver microsomes of five strains of mice following 100% oxygen exposure. CJ, C3H/HeJ; CN, C3H/HeN; FN, C3D2F1/J; FJ, C3D2F1/N; D2, DBA/2J. Bars indicate ± 1 SD for eight determinations. * Significantly different (P < 0.05) when compared to controls (0 hr).
peroxidation. NADPH-dependent lipid peroxidation causes degradation of cytochrome P-450 in adrenal cortex mitochondria (18). Peroxidative damage in oxygen-poisoned lung and liver microsomes may similarly affect cytochrome P-450 enzymes.

The severe perivascular and alveolar edema seen in oxygen-sensitive C3H and F1 hybrid mice may result from vasoconstriction, as indicated by increased pulmonary vascular medial thickness. Similar changes have been reported in hyperoxic rabbits (19). Cook et al. (20) presented data that superoxide anions or hydroxyl radicals cause vasoconstriction in isolated pulmonary artery segments, intrapulmonary arteries, and pulmonary veins. In the isolated, perfused rabbit lung, serotonin removal is significantly inhibited in a dose/time-related manner by superoxide and hydroxyl radicals (20). The increased concentrations of serotonin that alter hemodynamics and the endothelial damage caused by oxygen free radicals may then lead to increased vascular permeability and edema.

Metabolism of a large number of therapeutic agents that are substrates for the cytochrome P-450 system are highly oxygen dependent (21). The toxicity of these agents and drugs is mediated by O2•− production. Compounds such as paraquat (4, 22) and the anthracycline antibiotics (5) produce O2•− by way of interactions with the P-450 enzyme system. Moreover, induction of the P-450 system is required before paracetamol (23), nitrofurantoin (24), and several inhalant anesthetics (25) can cause lipid peroxidation. Agents that generate free radicals by means of induction of monooxygenases may lead to the peroxidative damage characteristic of oxygen toxicity. Indeed, one might conceptualize that these agents produce oxygen toxicity at ambient oxygen concentrations.

It appears that increased sensitivity to hyperoxia is, like aromatic hydrocarbon induction of cytochrome P-450, a dominant trait. The inbred strains (C3H and DBA/2J) have distinctly different responses (high and low sensitivity, respectively) and the F1 hybrids respond like the C3H parent strain.

We have shown that oxygen toxicity parallels the genetic control of cytochrome P-450 enzyme induction and that oxygen toxicity develops at a time when oxygen has induced

![Graph](image_url)

**Fig. 2.** Cytochrome P-450 concentrations in lung microsomes of five strains of mice following 100% oxygen exposure. CJ, C3H/HeJ; CN, C3H/HeN; FN, C3D2F1/N; F1, C3D2F1/J; D2, DBA/2J. Bars indicate ± 1 SD for four determinations. *, Significantly different (P < 0.05) when compared to controls (0 hr).

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**Table 2.** Pulmonary vascular medial thickness in five strains of mice following 100% oxygen exposure

<table>
<thead>
<tr>
<th>Strain</th>
<th>0 hr</th>
<th>12 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H/HeJ</td>
<td>0.570 ± 0.025</td>
<td>0.490 ± 0.042</td>
<td>0.540 ± 0.031</td>
<td>0.590 ± 0.033</td>
<td>0.720 ± 0.030*</td>
<td>0.820 ± 0.040*</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>0.465 ± 0.029</td>
<td>0.480 ± 0.040</td>
<td>0.560 ± 0.035</td>
<td>0.570 ± 0.040</td>
<td>0.750 ± 0.040*</td>
<td>0.790 ± 0.050*</td>
</tr>
<tr>
<td>C3D2F1/N</td>
<td>0.440 ± 0.020</td>
<td>0.500 ± 0.020</td>
<td>0.510 ± 0.030</td>
<td>0.530 ± 0.035</td>
<td>0.650 ± 0.030*</td>
<td>0.610 ± 0.030*</td>
</tr>
<tr>
<td>C3D2F1/J</td>
<td>0.430 ± 0.040</td>
<td>0.510 ± 0.038</td>
<td>0.530 ± 0.035</td>
<td>0.555 ± 0.045</td>
<td>0.630 ± 0.034*</td>
<td>0.660 ± 0.033*</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>0.515 ± 0.025</td>
<td>0.465 ± 0.030</td>
<td>0.490 ± 0.020</td>
<td>0.440 ± 0.042</td>
<td>0.490 ± 0.040</td>
<td>0.380 ± 0.025†</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ratio of vascular medial thickness to the outside diameter of the vessel ± 1 SD for four animals. Hours of 100% oxygen exposure are indicated.
*Significantly increased (P < 0.05) when compared to controls (0 hr).
†Significantly decreased (P < 0.05) when compared to controls (0 hr).
FIG. 3. Strain differences in pulmonary damage following 100% oxygen exposure for 96 hr. (A) Lung of C3H/HeJ mouse after breathing 100% oxygen for 96 hr. (B) Lung of DBA/2J mouse after breathing 100% oxygen for 96 hr. (Bars = 100 μm.)

the cytochrome P-450 enzyme system. These data suggest a direct role for the P-450 system in the development of oxygen toxicity, perhaps by increased production of superoxide anions. Oxygen not only induces the cytochrome P-450 system but also serves as a substrate. Thus, the toxicity of oxygen might be enhanced when given to patients exposed to a variety of drugs or agents that also induce the P-450 system, especially if those patients have a genetic predisposition toward cytochrome P-450 enzyme induction.