Potentiation of Oxygen-Induced Lung Injury in Rats by the Mechanism-Based Cytochrome P-450 Inhibitor, 1-Aminobenzotriazole

BHAGAVATULA MOORTHY, KRISTEN M. PARKER, CHARLES V. SMITH, JOHN R. BEND, and STEPHEN E. WELTY

Department of Pediatrics, Baylor College of Medicine (B.M., K.M.P., C.V.S., S.E.W.), Houston, Texas; and Department of Pharmacology and Toxicology, University of Western Ontario (J.R.B.), London, Ontario

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ABSTRACT

In this investigation, we tested the hypothesis that the cytochrome P-450 (CYP) inhibitor 1-aminobenzotriazole (ABT) alters the susceptibility of rats to hyperoxic lung injury. Male Sprague-Dawley rats were treated i.p. with ABT (66 mg/kg), i.v. with N-benzyl-1-aminobenzotriazole (1 mmol/kg), or the respective vehicles, followed by exposure to 95% oxygen for 24, 48, or 60 h. Pleural effusion volumes were measured as estimates of hyperoxic lung injury, and lung microsomal ethoxyresorufin O-deethylation (EROD) (CYP1A1) activities and CYP1A1 apoprotein levels were determined by Western blotting. ABT-pretreated animals exposed to hyperoxia died between 48 and 60 h, whereas no deaths were observed with up to 60 h of hyperoxia in vehicle-treated animals. In addition, three of four ABT-treated rats exposed to hyperoxia for 48 h showed marked pleural effusions. Exposure of vehicle-treated rats to hyperoxia led to 6.3-fold greater lung EROD activities and greater CYP1A1 apoprotein levels than in air-breathing controls after 48 h, but both declined to control levels by 60 h. Liver CYP1A1/1A2 enzymes displayed responses to hyperoxia and ABT similar to the effects on lung CYP1A1. N-Benzyl-1-aminobenzotriazole markedly inhibited lung microsomal pentoxyresorufin O-depentylation (principally CYP2B1) activities in air-breathing and hyperoxic animals but did not affect lung EROD or liver CYP activities. In conclusion, the results suggest that induction of CYP1A enzymes may serve as an adaptive response to hyperoxia, and that CYP2B1, the major pulmonary CYP isoform, does not contribute significantly to hyperoxic lung injury.

Hyperoxia is used extensively for treatment of pulmonary insufficiency, as is encountered in prematurely born infants and in adult respiratory distress syndrome (Northway and Rosan, 1968; Fisher, 1980). However, hyperoxic therapy may contribute to tissue damage and the development of lung diseases, such as bronchopulmonary dysplasia (Northway and Rosan, 1968; Smith and Welty, 1999). Exposure of adult animals to hyperoxia alone causes lung damage (Clark and Lambertsen, 1971). The molecular mechanisms of hyperoxic lung injury are not completely understood, but it is logical that lung injury should be mediated by reactive oxygen species, e.g., superoxide, hydrogen peroxide, and hydroxyl radical, which may be generated in excessive amounts during hyperoxic exposures (Freeman and Crapo, 1981; Kehrer and Smith, 1994).

Cytochrome P-450 (CYP) enzymes have been implicated in the formation of reactive oxygen species (Tindberg and Ingelman-Sundberg, 1989; Gram, 1997; Khatsenko et al., 1997). Several studies have suggested CYP1A isoforms to contribute to oxygen toxicity (Gonder et al., 1985; Okamoto et al., 1993; Hazinski et al., 1995; Moorthy et al., 1997). Hyperoxia for 48 h induces pulmonary CYP1A1 in rats (Okamoto et al., 1993) and lambs (Hazinski et al., 1995). Furthermore, hyperoxia enhances CYP contents in aryl hydrocarbon-responsive mice, which are highly susceptible to hyperoxic lung injury, but not in aryl hydrocarbon-nonresponsive mice, which are relatively resistant to lung damage (Gonder et al., 1985).

We reported recently that exposure of adult rats to hyperoxia for up to 48 h resulted in induction of CYP1A1 and 1A2 in liver. However, CYP1A1/1A2 levels had declined dramatically between 48 and 60 h, coinciding with the onset of respiratory distress in these animals (Moorthy et al., 1997)...
suggesting that liver CYP1A enzymes also may contribute to resistance to lung injury. Although the lung is the primary target organ in which the damaging effects of hyperoxia have been characterized, the possible contributions of other organs such as the liver to hyperoxic lung injury need to be investigated.

Several reports are available on the effects of CYP inducers on modulation of hyperoxic lung injury (Mansour et al., 1988a,b; Thibeault et al., 1991). Hyperoxic injury in neonatal rats is potentiated by pretreatment with 3-methylcholanthrene (MC), which induces CYP1A1 (Thibeault et al., 1991). However, Mansour et al. (1988a,b) observed protection against hyperoxic lung injury with pretreatment of adult rats (Mansour et al., 1988a) and mice (Mansour et al., 1988b) with the CYP1A inducers MC and β-naphthoflavone (BNF). In contrast to studies with CYP inducers, little is known regarding the effects of CYP inhibitors on hyperoxic lung damage. Cimetidine does not protect rats from oxygen-induced lung injury (Todd et al., 1990), but attenuates oxygen toxicity in lambs (Hazinski et al., 1989). Because cimetidine is also a H₂ receptor antagonist and an interferon inducer (Levine et al., 1998), it is difficult to draw conclusions regarding the role of CYP enzymes in lung injury. Furthermore, cimetidine inhibits CYPs in an isozyme-selective manner, inactivating CYP2C6 and CYP2C11 but not CYP1A1, CYP2A1, CYP2B1/2B2, or CYP3A1/1A2 (Levine et al., 1998).

In the present investigations, we used 1-aminobenzotriazole (ABT), a mechanism-based inhibitor of CYP with relatively low isozyme selectivity (Mugford et al., 1992), to test the hypothesis that pretreatment of adult rats with ABT would attenuate hyperoxic lung injury in vivo. ABT inhibits major hepatic and pulmonary CYP isoforms, i.e., CYP1A1/1A2 and CYP2B1, but exhibits no measurable effects on cytochrome b₅, serum transaminases, flavin monooxygenase, or phase II enzymes activities (Mugford et al., 1992). Extensive dose-responsive studies in guinea pigs showed that 24 h after administration of 30 to 100 mg/kg ABT, pulmonary ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-depentylyase (PROD) activities were diminished by 40 to 60% and 60 to 80%, respectively (Knickle and Bend, 1992). Thus, we selected an ABT dose of 66 mg/kg for our studies in rats, in an attempt to achieve in the range of 50 to 70% inhibition of EROD and PROD activities in livers and lungs of ABT-treated rats. In addition, we investigated the effects on hyperoxic lung injury of BBT, which is an isozyme- and lung-selective inhibitor of CYP2B1 in vitro (Mathews and Bend, 1986) and in vivo (Knickle et al., 1994), to test the hypothesis that lung CYP2B1 contributes to hyperoxic lung injury.

**Materials and Methods**

**Animal Treatment**

**Effects of ABT.** Adult male Sprague-Dawley rats (2 months old) were obtained from Harlan Sprague-Dawley (Houston, TX). The animals were acclimatized for 7 days before the experiment and randomized into 12 groups of 3 to 5 animals each. ABT was administered i.p. in saline (2 ml/kg) at 0 (saline controls) or 66 mg/kg. Two hours later, half the animals in each treatment group were exposed to >95% O₂, with pure O₂ at 5 l/min, as we have described previously (Ramsay et al., 1998). The other animals were maintained in room air. Animals from all groups (saline/ABT; air/hyperoxia) were studied at 24, 48, and 60 h. Animals to be studied at 48 h were administered an additional dose of ABT (66 mg/kg) or saline (2 ml/kg), as appropriate, at 24 h, and animals to be studied at 60 h were dosed at 24 and 48 h. ABT + hyperoxia animals did not survive to 60 h and therefore were not studied further. Purified tap water and food (Purina Rodent Lab Chow 5001) were available ad libitum. A 12-h light/dark cycle was maintained. At the termination of these exposures, rats were anesthetized with sodium pentobarbital (200 mg/kg i.p.) and euthanized by exsanguination while under deep pentobarbital anesthesia.

**Effect of BBT.** Adult male Sprague-Dawley rats (2 months old) were obtained from Harlan Sprague-Dawley. Sixteen rats were randomized into four groups of four animals each. Two groups of animals were administered (i.v. by tail vein) BBT (1 μmol/kg) suspended by sonication in 1.2 ml/kg of 5% BSA/dimethyl sulfoxide, 6:0.15, as described by Orton et al. (1973). The remaining animals were given equivalent volumes of the vehicle alone. Two hours later, one group each of BBT- and vehicle-treated animals was exposed to >95% O₂, as described above, and the other animals were maintained in room air. After 24 h, one additional dose of BBT or vehicle was administered. All animals were sacrificed after 60 h.

**Chemicals**

Calcium chloride, Tris, sucrose, NADPH, ethoxyresorufin, glutathione reductase, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO). Methoxyresorufin was obtained from Molecular Probes (Eugene, OR). ABT was from Aldrich Chemical Co. (Milwaukee, WI). BBT was synthesized and purified as described previously (Mathews and Bend, 1986). Buffer components for electrophoresis and Western blotting were obtained from Bio-Rad (Richmond, CA). The primary monoclonal antibody to CYP1A1 was a generous gift from Dr. P. E. Thomas. Goat anti-mouse IgG conjugated with horseradish peroxidase was from Bio-Rad.

**Pleural Effusions and Preparation of Microsomes**

Pleural effusions were measured by carefully collecting the fluid in the thorax with preweighed gauze pads and measuring the weight gain. For preparation of microsomes, livers were excised, weighed, and homogenized in 10 mM Tris, pH 7.4, containing 0.25 M sucrose. Lungs were perfused with 10 mM phosphate-buffered saline, pH 7.4. Liver microsomes were prepared by the calcium chloride precipitation method (Cinti et al., 1972), whereas lung microsomes were prepared by differential centrifugation (Matsubara et al., 1974). Protein concentrations were estimated by the Bradford dye-binding method (Bradford, 1976).

**Enzyme Assays**

Total CYP contents were estimated by the method of Omura and Sato (1964). EROD (CYP1A1), PROD (CYP2B1), and methoxyresorufin O-demethylyase (MROD) (CYP1A2) activities in microsomes were assayed essentially according to the method of Pohl and Fouts (1980), as we have described previously (Moorthy et al., 1997).

**Electrophoresis and Western Blotting**

Liver microsomes (30 μg of protein) prepared from individual animals were subjected to SDS-polyacrylamide gel electrophoresis in 7.5% acrylamide gels. The separated proteins either were stained with Coomassie blue dye or were transferred to polyvinylidene difluoride membranes, followed by Western blotting, as described previously (Moorthy et al., 1997). For the Western analysis, a monoclonal antibody to CYP1A1, which cross-reacts with CYP1A2, and goat anti-mouse IgG conjugated with horseradish peroxidase were used as primary and secondary antibodies, respectively (Moorthy et al., 1997). Liver CYP1A1/A2 apoproteins were detected on the blots with hydrogen peroxide and 4-chloro-1-naphthol. Lung CYP1A1 protein was detected with 4-chloro-1-naphthol and diaminobenzidine in
the presence of hydrogen peroxide. Apoprotein levels were estimated by scanning the photographic negatives of the Western blots with laser densitometry, as described (Thomas et al., 1984). The Western blots were assessed quantitatively in the linear range of the detection system.

Statistical Analyses

Data are expressed as means ± S.E. Three-way ANOVA, followed by modified t tests, as appropriate, was used to assess significant differences arising from dosing of CYP inhibitors, inspired oxygen tension, and time. P values < .05 were considered significant.

Results

Effect of ABT/BBT on Animal Survival and Lung Injury in Oxygen. Animals treated with saline and exposed to hyperoxia showed no mortality through 60 h, but four of four animals treated with ABT and exposed to hyperoxia died between 48 and 60 h (Table 1). However, all animals treated with BBT, which selectively inhibits CYP2B1 in lung, survived exposure to 60 h of hyperoxia (Table 1). Air-breathing animals given ABT, BBT, or the corresponding vehicles showed no mortality through 60 h. Pleural effusions are abnormal accumulations of fluid in the pleural spaces and are indicative of damage to both the endothelial and epithelial fluid barriers in the lung. Saline-treated animals exposed to hyperoxia for 48 h showed no measurable pleural effusion volumes (Fig. 1). Three of four ABT-treated rats and none of four saline-treated rats exposed to hyperoxia for 48 h had measurable pleural effusions, whereas no effusions were observed in saline-treated Sprague-Dawley rats exposed to >95% O2 for 48 h, either in the present study (Fig. 1) or in our numerous previous studies with this animal model (Stenzel et al., 1993; Moorthy et al., 1997; Awasthi et al., 1998; Knight et al., 1998). All the saline-treated animals displayed enhanced pleural effusion volumes after 60 h of hyperoxia. Pleural effusions in the BBT-treated animals were not different from the effusions in the vehicle-treated animals exposed to hyperoxia for the same length of time (data not shown).

Three-way ANOVA indicated a combined effect of treatment with ABT, exposure to hyperoxia, and the time of exposure to ABT/hyperoxia on pleural effusion volumes. When the effects of each of these factors were examined individually, effects of hyperoxia, time of exposure to ABT/hyperoxia, and two-way interactions between each of these factors were observed, but three-way interactions were not indicated. ABT-treated animals exposed to hyperoxia for 48 h showed pleural effusion volumes that were greater than those of saline-treated animals exposed to hyperoxia for the same length of time, or in animals treated with ABT and exposed to hyperoxia for 24 h (Fig. 1). The pleural effusion volumes were greater in animals given saline and exposed to hyperoxia for 60 h than those in animals given saline or ABT and maintained in room air for 60 h, or in animals given saline and exposed to hyperoxia for 48 h (Fig. 1).

Effect of Hyperoxia and ABT on Lung CYP1A1 Activities and Contents. Exposure of animals to hyperoxia for 24 h did not change lung microsomal EROD (CYP1A1) activities, but 48 h of hyperoxia induced EROD activities by 6.3-fold (Fig. 2). By 60 h, lung EROD activities in the hyperoxic rats declined to control levels. Lung microsomal EROD activities did not change in ABT-treated air-breathing animals.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Room air</td>
<td>100%</td>
</tr>
<tr>
<td>ABT</td>
<td>100%</td>
</tr>
<tr>
<td>BBT</td>
<td>100%</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>100%</td>
</tr>
<tr>
<td>ABT + hyperoxia</td>
<td>100%</td>
</tr>
<tr>
<td>BBT + hyperoxia</td>
<td>100%</td>
</tr>
</tbody>
</table>

* n = 4 animals per group.
Moreover, treatment of animals with ABT followed by exposure to hyperoxia for 24 h did not alter EROD activities (Fig. 2). However, treatment with ABT prevented the increase in pulmonary EROD activities that were observed in saline-treated animals exposed to hyperoxia for 48 h (Fig. 2).

Pulmonary CYP1A1 apoprotein contents were enhanced by administration of ABT to the room air- as well as the hyperoxia-exposed animals at each time point (Fig. 3). Lung CYP1A1 contents of saline-treated animals were not changed after 24 h of hyperoxia, but were almost doubled after 48 h (Table 2). By 60 h, the pulmonary CYP1A1 levels in animals exposed to hyperoxia declined to 50% of the levels observed in air-breathing animals. In ABT-treated animals exposed to hyperoxia for 48 h, CYP1A1 apoprotein contents were ~1.5-fold greater than in saline-treated air-breathing animals, but were not different than the levels in saline-treated hyperoxic rats (Table 2). Densitometric analyses of lung microsomes revealed that ABT-treated air-breathing animals had 0.63-, 0.52-, and 0.62-fold higher concentrations of CYP1A1 after 24, 48, and 60 h, respectively, than did the corresponding saline-treated room air controls (Table 2).

Three-way ANOVA indicated a combined effect of these factors on lung CYP1A1 protein levels, but only ABT showed an individual effect. Significant two-way interactions were observed between hyperoxia and time, as well as hyperoxia and ABT. Three-way interactions also were noticed between ABT, hyperoxia, and time of exposure to ABT/hyperoxia. Modified t tests displayed several differences in CYP1A1 levels between individual treatment groups, as detailed in Table 2.

**Effects of Hyperoxia and ABT on Total Hepatic CYP Contents and Liver CYP1A1/1A2 Activities and Contents.** Hyperoxic exposure did not change total liver microsomal CYP contents at any time studied (Fig. 4), but ABT treatment decreased total CYP contents by ~50% at each time point.

In animals not treated with ABT, liver microsomal EROD activities (CYP1A1) were induced by 6.6-fold in animals exposed to hyperoxia for 48 h, although activities were not different after 24 h (Fig. 5A). Similar to our observations in lung, the hepatic EROD activities returned to control levels between 48 and 60 h of hyperoxia. Treatment of animals with ABT and exposure to hyperoxia for 48 h produced hepatic EROD activities that were higher than in saline- or ABT-treated air-breathing rats, but markedly lower than in the saline-treated animals exposed to hyperoxia for 48 h (Fig. 5A). CYP1A1 apoprotein was not detectable in livers of air-breathing animals or in animals exposed to hyperoxia for 24 h, but administration of ABT or 48 h of hyperoxia led to readily detectable levels of CYP1A1 (Fig. 6). Densitometric analyses of the Western transfers showed enhancements in the intensities of hepatic CYP1A1 after exposure to hyperoxia (Table 2). After 60 h of hyperoxic exposure, CYP1A1 was no longer detectable (Fig. 6; Table 2). ABT by itself led to greater levels of CYP1A1 apoproteins as early as 24 h after treatment, and the effect was observed through 60 h (Table 2).

Hepatic MROD activities, reflecting primarily CYP1A2, were greater in both saline- and ABT-treated animals after 48 h of hyperoxia than in air-breathing animals or in animals exposed to hyperoxia for 24 or 60 h (Fig. 5B). However, the increases in hepatic MROD activities in animals exposed to hyperoxia for 48 h were attenuated in the animals treated with ABT (Fig. 5B). In contrast to CYP1A1, hepatic CYP1A2 apoprotein levels were detectable in air-breathing rats, but the levels were not increased by hyperoxia through 48 h (Fig. 6; Table 2). CYP1A2 levels were enhanced by ABT in air-breathing animals at 24 h, and ABT-treated animals exposed to hyperoxia displayed CYP1A2 levels that were greater than in either group of saline-treated animals (Table 2). At 48 h, ABT-treated animals showed higher levels of hepatic CYP1A2 than did saline-treated rats (Table 2). At 60 h, ABT-treated air-breathing animals showed continued elevation of hepatic CYP1A2 levels, whereas the levels in saline-treated animals exposed to hyperoxia had declined markedly from the levels observed at 48 h to the levels observed in saline-treated air-breathing control rats (Table 2).

**Effects of BBT and Hyperoxia on Pulmonary and Hepatic CYP2B1 and CYP1A1/1A2 Activities.** In animals maintained in room air, BBT inhibited by 50% the activities of lung PROD, which is highly selective for CYP2B1 (Knickle et al., 1994), but did not measurably alter EROD activities (Table 3). BBT also inhibited lung PROD activities in the hyperoxic animals by ~50% and had no inhibitory effect on EROD (Table 3). BBT exposure did not change hepatic contents of total CYP, nor were PROD, EROD, or MROD activities altered (Table 4).

**Discussion**

The present studies were designed to test the hypothesis that the mechanism-based CYP inhibitor ABT would alter the susceptibility of rats to oxygen-induced toxicity in vivo. The accelerated death (Table 1) and enhanced lung injury (Fig. 1) in animals given ABT and exposed to hyperoxia, indicated marked potentiation of hyperoxic lung injury by ABT. We have shown previously (Stenzel et al. 1993; Knight et al., 1998) that pleural effusions were better indicators of hyperoxic lung injury in rats than were measurements of extravascular lung water and were comparable in power to determining bronchoalveolar lavage protein concentrations (Welty et al., 1995). The lungs of the specific animals used in
The sudden decline between 48 and 60 h of hyperoxia (Ah et al., 1997) is not consistent with differences between two groups are designated by only one superscript. The mechanisms of induction by hyperoxia are not understood.

The marked increases in lung EROD activities (Fig. 2) and CYP1A1 apoprotein levels (Fig. 3; Table 2) caused by exposure to hyperoxia for 48 h indicate induction of CYP1A1. CYP1A1 induction in lungs of male Sprague-Dawley rats after 48 h of hyperoxia also has been reported by Okamoto et al. (1993), but the mechanisms of induction by hyperoxia are not understood. Ah receptor-dependent mechanisms have been proposed in the induction of CYP1A1 by hyperoxia (Okamoto et al., 1993), but the absence of parallel effects of hyperoxia on glutathione S-transferase-α activities (Moorthy et al., 1997) is not consistent with Ah receptor-dependent mechanisms. The sudden decline between 48 and 60 h of hyperoxia of lung EROD activities (Fig. 2), expressed as per milligram of microsomal protein, and CYP1A1 apoprotein contents (Table 2) suggest mechanisms and responses relevant to the onset of lung injury. The decreases in activities and protein contents could be due either to the oxidative alterations or degradation of the respective enzymes, or could be secondary to increases in the levels of total protein concentrations in the fractions assayed. The quantities of protein isolated in the respective microsomal fractions were not different in the animals studied (data not shown), which suggests that the decreases in CYP1A1 activities and protein levels observed in animals after 60 h of hyperoxia cannot be attributed to dilution of lung proteins by proteins from filtered plasma or to recruitment of inflammatory cells into lungs of hyperoxic animals.

The lower pulmonary EROD activities in ABT-treated animals exposed to hyperoxia for 48 h than in saline-treated animals similarly exposed to hyperoxia (Fig. 2) are best attributed to inactivation of CYP1A1. ABT is a mechanism-based inhibitor of CYP1A enzymes in vivo and in vitro (Mathews and Bend, 1986; Ortiz de Montellano and Reich, 1986; Mugford et al., 1992). The absence of decreases in the EROD activities of lungs (Fig. 2) of air-breathing animals given ABT suggests insufficient production of the ABT metabolite (benzyne) that is responsible for inhibition of EROD by the constitutive CYP1A1 and possibly other enzymes responsible for basal EROD activities. Alternatively, CYP1A1 recovery may be sufficiently rapid to overcome effects on unstimulated levels (Ortiz de Montellano and Costa, 1986). In addition to CYP1A1, other CYP isoforms (e.g., CYP2B1) are affected by ABT (Knickle and Bend, 1992; Mugford et al., 1992). Pretreatment of animals with BBT (Table 3), which is selective for inhibition of CYP2B1 in lung (Knickle et al., 1994), did not alter the susceptibility of rats to hyperoxic lung injury. These data are not consistent with the hypothesis that inhibition of the catalytic activity of CYP2B1, the major pulmonary CYP isoform, contributes measurably to the potentiation of oxygen-induced lung injury by ABT. The lack of inhibition of hepatic EROD, MROD, or PROD activities by BBT was consistent with the findings of Knickle et al. (1994) that BBT at low doses is a liver-selective inhibitor of CYP2B1 in vivo.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent of Oxygen</th>
<th>Exposure Time</th>
<th>Pixel Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>h</td>
<td>Lung CYP1A1</td>
</tr>
<tr>
<td>Saline</td>
<td>21</td>
<td>24</td>
<td>840 ± 60</td>
</tr>
<tr>
<td>ABT</td>
<td>21</td>
<td>24</td>
<td>1387 ± 33</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;95</td>
<td>24</td>
<td>1035 ± 65</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;95</td>
<td>48</td>
<td>639 ± 91</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;95</td>
<td>48</td>
<td>974 ± 76</td>
</tr>
<tr>
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<td>48</td>
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<td>Saline</td>
<td>&gt;95</td>
<td>48</td>
<td>1604 ± 36</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;95</td>
<td>60</td>
<td>1135 ± 135</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;95</td>
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<td>1841 ± 59</td>
</tr>
<tr>
<td>Saline</td>
<td>&gt;95</td>
<td>60</td>
<td>565 ± 66</td>
</tr>
</tbody>
</table>

N.D., not detectable (the limit of detection was 80).

* * Significant differences at $P < .05$ from $a$ saline (air), $b$ ABT (air), $c$ saline (hyperoxia), $d$ ABT (hyperoxia), and $e$ the same group at the preceding time point. The differences between two groups are designated by only one superscript.
Increases in CYP1A1 apoprotein concentrations following administration of ABT have not been reported previously and the mechanisms responsible for this response are not known at this time. Ortiz de Montellano and Costa (1986) did not observe increases in liver CYP1A1 mRNA levels for up to 12 h after administration of a single dose of ABT (50 mg/kg) to male Sprague-Dawley rats, which suggests post-translational mechanisms (e.g., protein stabilization) of apoenzyme induction.

The increases in hepatic EROD (Fig. 5A) and MROD (Fig. 5B) activities after 48 h of hyperoxia that were paralleled by elevations in CYP1A1 and 1A2 apoprotein concentrations (Fig. 6; Table 2) indicate induction of the hepatic enzymes by hyperoxia (Moorthy et al., 1997). The decreases in total hepatic CYP contents (Fig. 4) and the attenuation of hyperoxia-induced increases in hepatic EROD (Fig. 5A) and MROD (Fig. 5B) activities by ABT are consistent with mechanistic inactivation of CYP isozymes by ABT, presumably through the alkylation of the heme moieties by benzyne, formed by oxidative metabolism of ABT (Ortiz de Montellano and Reich, 1986; Mugford et al., 1992). Although hepatic EROD activities were lower in the ABT-treated rats than in the saline-treated rats, the higher CYP1A1/1A2 apoprotein levels in the ABT-treated rats than in the saline-treated rats suggest that ABT and hyperoxia induce CYP1A1/1A2 independently (Fig. 6), although CYP1A1 activity is irreversibly inhibited by an oxidative metabolite of ABT. The mechanisms responsible for increased hepatic apoprotein contents of CYP1A1/1A2 following ABT treatment (Fig. 6) are not known at this time.

The exacerbation of hyperoxic lung injury and animal mortality by administration of ABT, in conjunction with attenuation of the increases in lung EROD and liver EROD and MROD activities observed in animals exposed to hyperoxia, suggests that increases of CYP1A1/1A2 monooxygenase activities may serve as adaptive responses to hyperoxia. The potentiation of hyperoxia-induced lung injury by ABT may result from inhibition of the putative adaptive responses, or may involve iron released from heme proteins secondary to oxidative stress.

**Table 3**

Effect of BBT and hyperoxia on lung CYP enzymes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PROD</th>
<th>EROD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + air</td>
<td>96.8 ± 4.5c</td>
<td>53.8 ± 9.3</td>
</tr>
<tr>
<td>BBT + air</td>
<td>45.4 ± 7.1a</td>
<td>38.6 ± 5.5</td>
</tr>
<tr>
<td>Vehicle + hyperoxia</td>
<td>25.0 ± 2.8a</td>
<td>20.6 ± 4.2b,c</td>
</tr>
<tr>
<td>BBT + hyperoxia</td>
<td>13.8 ± 1.6b,c</td>
<td>21.0 ± 6.3b,c</td>
</tr>
</tbody>
</table>

^a–d Statistically significant differences at \( P < .05 \) from\(^a\) vehicle (air), \(^b\) BBT (air), \(^c\) vehicle (hyperoxia), and \(^d\) BBT (hyperoxia). The differences between two groups are designated by only one superscript.
ABT-mediated CYP alkylation. In fact, the ABT-heme adduct undergoes further metabolism, leading to release of one mole of iron per mole of CYP enzyme inactivated (Ortiz de Montellano and Reich, 1986). Given the concentrations of CYP in rat lung of ~0.1 nmol/mg protein, the increases in iron would be small, but still could be significant in exacerbating pulmonary toxicity. In intracellular concentrations of reducto-active iron might augment tissue injury in hyperoxia-exposed animals through increased formation of hydroxyl radicals via Fenton-like reactions, or by propagating lipid peroxidation (Kehler and Smith, 1994; Knight et al., 1998). The reports of Bysani et al. (1990) and Paller and Jacob (1994) provide evidence for CYP enzymes as intracellular sources of reducto-active iron and lend credence to the idea that ABT may potentiate oxygen-induced lung injury at least in part through mechanisms involving iron-mediated oxidation of tissue macromolecules. That ABT, which inhibits CYP2B1 by reacting covalently with the apoprotein rather than the heme moiety (Woodcroft et al., 1997), did not potentiate lung injury by oxygen is consistent with the idea that CYP heme inactivation by ABT plays a role in the potentiation of hyperoxic lung injury.

Clinical trials of the administration of cementidine to premature infants undergoing oxygen therapy are currently in progress with the goal to alleviate the toxic effects of oxygen, presumably through inhibition of CYP enzymes (T. A. Hazinski, personal communication). The augmentation of oxygen-mediated lung injury in rats by administration of ABT and the protection against hyperoxic lung injury in lambs treated with cementidine (Hazinski et al., 1989) suggest different mechanisms of inhibitory action on different CYP enzymes. Nonetheless, the present results with ABT indicate that more detailed investigations into the relationships between specific CYP functions and hyperoxic lung injury are needed to help develop rational strategies for the optimal use of CYP inhibitors or inducers to the treatment of lung disease in premature infants or adults undergoing oxygen therapy.

Acknowledgements

We thank Dr. P. E. Thomas for providing us with monoclonal antibody against CYP1A1 used in these studies.

References


TABLE 4

Effect of BBT and hyperoxia on liver CYP enzymes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total CYP Content</th>
<th>PROD</th>
<th>EROD</th>
<th>MROD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.52 ± 0.037</td>
<td>0.25 ± 0.064</td>
<td>0.72 ± 0.11</td>
<td>1.11 ± 0.08</td>
</tr>
<tr>
<td>BBT</td>
<td>0.44 ± 0.019</td>
<td>0.29 ± 0.044</td>
<td>0.64 ± 0.05</td>
<td>1.13 ± 0.17</td>
</tr>
<tr>
<td>Vehicle + hyperoxia</td>
<td>0.49 ± 0.052</td>
<td>0.22 ± 0.057</td>
<td>0.77 ± 0.15</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>BBT + hyperoxia</td>
<td>0.38 ± 0.070</td>
<td>0.20 ± 0.024</td>
<td>0.60 ± 0.07</td>
<td>0.83 ± 0.11</td>
</tr>
</tbody>
</table>


Send reprint requests to: Bhagavatula Moorthy, Ph.D., Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
E-mail: bmoorthy@neo.bcm.tmc.edu