Site-Specific Metabolism of Naphthalene and 1-Nitronaphthalene in Dissected Airways of Rhesus Macaques

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ABSTRACT

Studies in rodents have demonstrated the importance of cytochrome P450 monooxygenases in generating reactive metabolites that produce Clara cell injury. Pulmonary P450 activities in rodents are much higher than those in primates, raising the issue of relevance of rodent data to primates. Few studies on P450-catalyzed activation of cytotoxicants in subcompartments of primate lung have been reported. Accordingly, infant monkey airway subcompartments, including trachea, proximal, midlevel, distal airways, and parenchyma, were incubated with naphthalene or 1-nitronaphthalene to define metabolism at both high (500 μM) and low (50 μM) substrate concentrations. There was a relatively even distribution of metabolizing activities for naphthalene across subcompartments, but at high concentrations of 1-nitronaphthalene, lower airways (midlevel airway through parenchyma) showed higher bioactivation than upper airways. Dihydrodiol was the predominant water-soluble metabolite of naphthalene generated by all subcompartments, whereas covalently bound metabolites accounted for the greatest percentage of 1-nitronaphthalene metabolites, especially in lower airways. As anticipated, the amounts of metabolite covalently bound as a percentage of total metabolite formed increased dramatically with the 10-fold increase in substrate concentration. With both substrates, the formation of water-soluble metabolites was approximately 100 times less than observed previously in rodents. We conclude that 1) there are significant quantitative differences between rhesus and rodents in substrate bioactivation; 2) the distribution of metabolizing activities for naphthalene but not 1-nitronaphthalene is significantly different for rodents and primates; and 3) a very high percentage of the metabolites generated, particularly for 1-nitronaphthalene, is bound covalently to cellular proteins.

Naphthalene is a polycyclic aromatic hydrocarbon generated as a by-product of various combustion processes, including gasoline use and cigarette smoking (IARC, 2002). Nitration of naphthalene and its methylated congeners in the gas phase forms 1-nitronaphthalene and corresponding methylnitro derivatives. A significant fraction of the total mutagenicity associated with ambient particulates in southern California has been attributed to the presence of these compounds (Gupta et al., 1996).

Naphthalene and 1-nitronaphthalene undergo metabolic activation catalyzed by the cytochrome P450 monooxygenases to form electrophilic intermediates that seem to be linked to the cytotoxic injury observed in the respiratory airway epithelial cells of rodents treated with these compounds (Verschoyle et al., 1993; for review, see Buckpitt et al., 2002). Previous studies correlated the species- and site-selective susceptibility to naphthalene injury with the rate of naphthalene metabolism and total protein-bound metabolites in different airway subcompartments from rodent lungs (Buckpitt et al., 1995; Cho et al., 1995). 1-Nitronaphthalene cytotoxicity also showed airway selectivity (Paige et al., 1997). In addition, the rate of formation of regioisomeric epoxides from 1-nitronaphthalene differed substantially in target and nontarget tissues (Watt and Buckpitt, 2000). This suggests that site-selective toxicity associated with naphthalene and 1-nitronaphthalene exposure is, in part, related to P450 activity.

A number of cytochrome P450 monooxygenases have been identified in the lung (Willey et al., 1996), and catalytic...
activities of a few of these enzymes have been reported using either purified proteins or recombinant enzymes (Lanza et al., 1999; Shultz et al., 2001). In general, the amount of P450 protein present and the P450 activity of both microsomal and intact airway preparations from lungs of nonhuman primates or humans are 10- to 100-fold or more lower than those observed in rodents (for review, see Hukkanen et al., 2002). For example, recent comparative metabolic studies of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol showed that the rate of α-hydroxylation was 160 pmol/min/mg in mouse lung microsomal incubations (Jalas et al., 2003), whereas the highest rate in human lung was 0.057 pmol/min/mg (Smith et al., 2003). An exception to the striking difference in metabolic rates between rodents and humans has been reported with dichloroethylene; biotransformation rates in lung microsomes from humans were only 2- to 3-fold lower than those from mice (Dowsley et al., 1995). The high degree of localization of cytochrome P450 monooxygenases in airway epithelial cells in rodents suggests that rodent-primate differences in xenobiotic metabolism might be due to the fact that comparisons of P450-dependent metabolism were done using microsomes from whole lung rather than airway subcompartments. When comparing P450 activities in microsomal incubations prepared from specific subcompartments of the lungs of rat and rhesus macaque, the rhesus macaque preparations yielded lower activities in all cases, but most of the differences were 2- to 3-fold, not 10- to 100-fold (Lee et al., 1998). Moreover, the rat and the rhesus monkey had a different distribution of P450 isofrom activities. The distribution of CYP1A1 and 2B1 activities was more homogenous in rhesus macaques, whereas CYP2E1 activity was highest in the distal bronchiole and parenchyma than in the proximal airways (Lee et al., 1998). In rats, CYP1A1 and CYP2B2 activities were highest in the parenchyma, whereas CYP2E1 activity was highest in the airways. Whether the distribution of P450 activities for isofrom-selective substrates correlates with the distribution of metabolic activity with pulmonary cytotoxicants is unknown.

The studies described in this report provide a detailed assessment of the formation and nature of metabolites from two P450-activated pulmonary cytotoxicants, naphthalene and 1-nitronaphthalene, in lung subcompartments of infant rhesus macaques, a species anatomically and physiologically similar to the human. The studies show a much lower substrate bioactivation than in rodents yet a similar formation of reactive metabolites.

Materials and Methods

Animals and General Experimental Protocol. Male rhesus macaques (Macaca mulatta) monkeys used for this study were colony-born at the California National Primate Research Center located on the University of California, Davis, campus. Care of these animals complied with the provisions of the Institute of Laboratory Animal Resources (NRC, 1996) and conformed to practices established by the American Association for Accreditation of Laboratory Animal Care. Six male infant rhesus monkeys were housed from birth until necropsy at 6 months of age in social groups in large 4.2-m² stainless steel and glass inhalation exposure chambers updated from those described previously by Hinners et al. (1986). The chambers were prefilled with high-efficiency particulate-filtered air and activated charcoal adsorber to eliminate most air pollutants from the chamber. A central air handling system supplied chemical, bacteriological, and radiological filtered air at a 2.1 m³/min flow rate for a complete air change every 2 min. Before necropsy, monkeys were euthanized with an overdose of pentobarbital after being dosed with Telazol (8 mg/kg i.m.) and anesthetized with Diprivan (0.1–0.2 mg/kg/min i.v.). The animals were then necropsied after exsanguination via the systemic aorta.

Chemicals and Reagents. Deficient Waymouth’s MB 752/1 medium (without glutathione, I-cysteine, L-cystine, L-glutamine, and L-methionine) was purchased from Invitrogen (Carlsbad, CA). Waymouth’s MB 752/1 medium with L-glutamine was purchased from Sigma-Aldrich (St. Louis, MO). Safety-Solve liquid scintillation fluid was purchased from Research Products International Corp. (Mt. Prospect, IL). Thioether conjugate standards of naphthalene (glutathione, cysteine and cysteinyl-glycine) were prepared by reacting naphthalene oxide with the appropriate thiol as described previously (Buonarati et al., 1990). 1,2-Dihydroxy-1,2-dihydronaphthalene was purchased from Aldrich Chemical Co. (Milwaukee, WI). Glutathione conjugate standards for 1-nitronaphthalene were generated in liver microsomal incubations, and their identities were established by coelution with previously characterized metabolites (Watt et al., 1999). All other chemicals purchased from a commercial vendor were reagent grade or better.

Radioactive Chemicals. [1-14C]Naphthalene (specific activity 52 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). [14C]-1-Nitronaphthalene was prepared in the laboratory by the nitration of [1-14C]naphthalene with a stoichiometric amount of ammonium nitrate and excess trifluoroacetic anhydride in chloroform. The product was purified using normal-phase chromatography. The radiochemical purity of the final product was tested by reverse-phase HPLC and shown to be ≥99% pure. Unlabeled naphthalene or 1-nitronaphthalene was added to achieve final specific activities of 5 and 10 dpm/pmol at 500 and 50 μM substrate concentrations, respectively.

Tissue Preparation, Incubation, and Preparation of Samples for Analysis. Airway subcompartments (trachea, proximal, midlevel, distal airways, and parenchyma) were obtained as described previously (Plopper et al., 1991) and were placed in deficient Waymouth’s medium at 4°C. A small piece of each airway subcompartment was placed in 200 mM methane sulfonic acid, 5 mM diethylenepentaacetic acid for glutathione determination (Lakritz et al., 1997). The remaining dissected airway subcompartments were placed into separate silanized vials containing 1 ml of oxygenated Waymouth’s medium (Sigma-Aldrich) with 27 mM sodium bicarbonate, 15 mM HEPES, and the pH was adjusted between 7.4 and 7.5 by dropwise addition of 1 N NaOH for incubation with either naphthalene or 1-nitronaphthalene. The individual airways were then incubated with either 500 μM 14C-labeled or 50 μM 14C-labeled naphthalene or 1-nitronaphthalene, added in 10 μl of methanol, for 4 h at 37°C in a shaking water bath. After incubations, samples were stored in a −20°C freezer until sample preparation. The contents of the incubation vial were extracted twice with an equal volume of hexane to remove nonmetabolized, volatile substrate. Methanol (2 ml) was added to each sample and mixed on a vortex mixer before storing overnight at −20°C. Samples were centrifuged at 9000g for 15 min at 0°C to remove the protein. The protein pellet was saved for protein covariant binding analysis, and the supernatant was evaporated to dryness and saved for HPLC analysis of water-soluble metabolites.

HPLC Analysis of Water-Soluble Metabolites of Naphthalene and 1-Nitronaphthalene. Metabolites generated in incubations of airway subcompartments were separated by methods described previously in Buckpitt et al. 1995 (naphthalene) and Watt et al. (1999) (1-nitronaphthalene). Briefly, the final dried residue of the methanol/medium extract from the incubation was reconstituted in water, and aliquots were injected onto a Spherisorb ODS-2 column (25 cm × 4.6 mm i.d.; 5-μm particle) (Waters, Milford, MA). The mobile phase consisted of 0.06% triethylamine adjusted to pH 3.1.
(with phosphoric acid) and acetonitrile. Solvent composition was adjusted from 5 to 7% acetonitrile at time 0 to 7.5 to 13.2% acetonitrile over the first 75 min of a 100-min run at a flow rate of 1 mL/min to separate naphthalene and 1-nitronaphthalene metabolites. Naphthalene metabolite standards (1,2-dihydroxy-1,2-dihydronaphthalene, glutathione conjugates, and cysteinyln-glycine conjugates) and 1-nitronaphthalene metabolite standards (glutathione conjugates derived from the C₆,C₆- and C₇,C₈-epoxides) were run on the same gradient and spiked into various samples for comparison. The column eluate was monitored with a Hewlett Packard series 1100 UV detector at 256 nm, and 1-min fractions were collected and counted for 5 or 10 min each in a liquid scintillation counter. Quantitative measurements of metabolite formation were calculated based on ¹⁴C elution from the column.

Protein Covalent Binding. Protein pellets saved after centrifugation of the sample were washed multiple times with 5 volumes of methanol until an aliquot contained less than 100 dpm/ml. Proteins were dissolved in 1 N NaOH, and protein content was measured using a modification of the Lowry method (Lowry et al., 1951) with bovine serum albumin as a standard.

HPLC Analysis of Reduced Glutathione. Measurements of reduced glutathione content were conducted using HPLC with electrochemical detection as described by Lakritz et al. (1997). Individual airway tissues were homogenized in 200 μL of 200 mM methanolic sulfonic acid/5 mM diethylenetriaminepentaacetic acid, samples were centrifuged (12,000 g, 20 min), and aliquots of the supernatant were used for analysis. The pellets were resuspended in 1 N NaOH, and an aliquot was taken for protein determination as described above.

Statistical Analysis. When water-soluble metabolites of naphthalene and 1-nitronaphthalene were below our limit of detection, 0.001 pmol/mg protein was used for those samples because this number is below the detection limits of both naphthalene (limit of detection 0.008 pmol/mg protein) and 1-nitronaphthalene (limit of detection 0.016 pmol/mg protein) metabolites. Data were analyzed using SigmaStat (SPSS Inc., Chicago, IL) for one-way analysis of variance (ANOVA). Once a statistically significant difference was identified among groups (p < 0.05), all pairwise multiple comparison procedures (Bonferroni t test) were done to determine statistical differences between groups (p < 0.05).

Results

Formation of Water-Soluble and Covalently Bound Metabolites of Naphthalene in Airway Incubations. Earlier in vitro and in vivo work in rodents has shown that naphthalene is metabolized to a 1,2-epoxide that is subsequently converted to 1,2-dihydroxy-1,2-dihydronaphthalene (diol), 1-naphthol, and a number of thioether conjugates (Fig. 1A).

Figure 2, A and B, summarizes the findings of naphthalene metabolism at a 50 μM substrate concentration by microdissected airway explants of monkey lung. In addition to those metabolites specifically identified in the radiochromatographic profiles, a number of less abundant, unidentified metabolites were measured and summed to provide an assessment of the total metabolism. In the parenchyma, the dihydrodiol content was 0.115 nmol/mg protein, significantly higher (p < 0.05) than that in the proximal and distal airways (Fig. 2A). Total naphthalene metabolism was highest in the midlevel airway (0.88 nmol/mg protein) and lowest in the distal airway (0.13 nmol/mg protein) (Fig. 2B). Naphthalene metabolites that covalently bound to proteins accounted for 26% of the total metabolites in the distal airway and 11 to 15% of the total metabolites produced in incubations of other airway subcompartments. Cysteinyln-glycine conjugates accounted for between 0 and 5% of the total metabolites, depending upon the airway subcompartment. The total formation of thioether conjugates (glutathione plus cysteinyln-glycine) accounted for about 9% of the total metabolites produced. The amounts of diol generated were variable and represented 41% of the total metabolites in the parenchyma but only 5.7% in the midlevel airways.

Increasing the substrate concentrations in the incubation to 500 μM resulted in a corresponding increase in product formation but also in a striking alteration in the profile of metabolic products generated (Fig. 2, C and D). There was an n of 3 for each airway subcompartment; however, no trachea samples were obtained. Total naphthalene metabolism was highest in the midlevel airway (3.27 nmol/mg protein) and lowest in both proximal and distal airways (0.98 nmol/mg protein) (Fig. 2D). Total reactive naphthalene metabolites bound covalently to proteins varied from 25 to 65% of total metabolites in midlevel and distal airways, respectively. As a percentage of the total metabolites, cysteinyln-glycine conjugates were highest in the proximal (7.9%) and lowest in the distal (1.6%) airway incubations. However, the amounts of glutathione conjugates generated were lower than the detection limit in the proximal airway and below 3.0% of the total metabolism in all airway levels. The relatively small amounts of glutathione conjugates did not seem to result from lack of intracellular glutathione in subcompartments from juvenile monkeys (Table 1). Diol varied from 14% of the total metabolites in the midlevel airway to 34% in parenchyma.

A comparison of the formation of protein-bound reactive and total metabolites at two different naphthalene concentrations (50 or 500 μM) in dissected airways is shown in Fig. 3A. The quantities of total naphthalene metabolites (water-soluble plus covalently bound) generated at 500 μM compared with 50 μM varied from 2.9-fold higher in the proximal airway to 7.7-fold higher in both distal airway and parenchyma. Similarly, the formation of reactive naphthalene metabolites bound covalently at 500 μM varied from 6.9 to 18.6 to 37.4-fold that observed at 50 μM substrate concentration in proximal, distal airway, and parenchyma, respectively. The ratio of reactive to total metabolite formation at both substrate concentrations is lowest in the midlevel airway and highest in the distal airway (Fig. 3A).

Formation of Water-Soluble and Covalently Bound Metabolites of 1-Nitronaphthalene in Airway Incubations. A number of water-soluble metabolites of 1-nitronaphthalene generated by lung tissue have been identified and characterized in previous studies conducted in rats (Watt et al., 1999). These metabolites include glutathione conjugates derived from the C₅,C₆- and C₇,C₈-epoxides (Fig. 1B). In addition to the metabolites specifically identified by chromatography with the reference standards, several unidentified metabolites were measured and summed to assess the total metabolites in incubations of monkey lung subcompartments with 1-nitronaphthalene (50 or 500 μM). The data showing the formation of water-soluble metabolites, covalently bound reactive metabolites, and the percentage of each class of metabolites to total formed in airway subcompartments incubated with a 50 μM concentration of 1-nitronaphthalene is presented in Fig. 4, A and B. Total 1-nitronaphthalene metabolism varied from 0.11 nmol/mg protein in the proximal airway to 0.35 nmol/mg protein in the...
Fig. 1. Pathways of naphthalene (A) and 1-nitronaphthalene (B) metabolism.
parenchyma (Fig. 4B). Reactive metabolites covalently bound to proteins accounted for more than 30% of the total metabolites at all airway levels, with the distal airway having the highest percentage at 55%. No C₅,C₆- or C₇,C₈-epoxide-derived glutathione adducts were detected in either proximal or midlevel airways. However, in other airway subcompartments, glutathione adducts from C₅,C₆- and C₇,C₈-epoxides accounted for 5 to 12% of the total metabolites formed.

At 500 μM 1-nitronaphthalene, no differences in the amounts of water-soluble metabolites were observed in different airway levels. In contrast, reactive metabolite formation was significantly higher (p < 0.05) in the parenchyma (1.35 nmol/mg protein) in comparison with the trachea, proximal, and midlevel airways with respective values of 0.25, 0.31, and 0.74 nmol/mg protein (Fig. 4C). In addition, the formation of reactive, covalently bound metabolites in the distal airway (1.16 nmol/mg protein) was significantly higher than that in the trachea and proximal airways (p < 0.05). Total substrate metabolism was lowest (0.48 nmol/mg protein) in the proximal airway and highest (1.67 nmol/mg protein) in the parenchyma at the 500 μM substrate concentration (Fig. 4D). In the parenchyma and distal airway, substrate metabolism was higher than that in the trachea, proximal, and midlevel airways (p < 0.05) (Fig. 3B). In midlevel airway, substrate metabolism was higher than that in the trachea (p < 0.05). Reactive metabolite-protein adducts accounted for 48% of the total metabolites in the trachea and 82% of the total metabolites in the distal airway.

TABLE 1
Reduced glutathione levels in airway subcompartments of rhesus monkeys

<table>
<thead>
<tr>
<th>Airway Subcompartment</th>
<th>GSH Level nmol/mg protein</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>1.6 ± 0.9</td>
<td>4</td>
</tr>
<tr>
<td>Proximal airway</td>
<td>1.8 ± 1.4</td>
<td>5</td>
</tr>
<tr>
<td>Midlevel airway</td>
<td>1.5 ± 1.4</td>
<td>6</td>
</tr>
<tr>
<td>Distal airway</td>
<td>4.6 ± 2.2</td>
<td>6</td>
</tr>
<tr>
<td>Parenchyma</td>
<td>10.9 ± 11.4</td>
<td>6</td>
</tr>
</tbody>
</table>

GSH, glutathione.
C₅,C₆- or C₇,C₈-epoxide glutathione conjugates detected were below 2% of the total metabolites in all airway levels (Fig. 4D).

Increasing substrate concentration from 50 to 500 µM increased total metabolism from 2.4-fold in the trachea to 11.3-fold in the distal airway (Fig. 3B). Similarly, the 10-fold higher substrate concentration led to a 3.8-fold increase in reactive metabolite covalent binding in the trachea and to a 17-fold increase in the distal airway. The percentage of bound 1-nitronaphthalene metabolites to total metabolites was lowest in the trachea and highest in the distal airway at both substrate concentrations.

**Discussion**

A number of cytochrome P450 monooxygenases have been detected either at the transcript or protein level in lungs of rodents and primates (for review, see Ding and Kaminsky, 2003). In rodents, the importance of these P450s in the metabolic activation of a number of inert chemicals to cytotoxic or carcinogenic intermediates is well accepted (for review, see Yost, 2001). In contrast, the significance of these P450s in catalyzing metabolic activation reactions in humans is not well established primarily because of the lack of quantitative data on cellular protein expression and, in some instances, differences in catalytic activities of the P450 orthologs in rodents versus humans. Moreover, when P450 distribution within the airway trees was measured using isoform-selective substrates, there was a significant difference in pulmonary distribution between rats and monkeys (Lee et al., 1998).

In the current study, the distribution of naphthalene metabolism in the lung was relatively homogenous with slightly higher activities noted in the parenchyma and potentially midlevel airways at both substrate concentrations. In comparison, at the 500 µM substrate concentration, 1-nitronaphallene was metabolized primarily in the parenchyma and distal airway.
thalene metabolites generated in the monkey parenchyma and distal airways were higher than those observed in other airway levels, which is consistent with the distribution of CYP2E1 activity in the monkey (Lee et al., 1998). In monkeys, CYP1A1 and CYP2B activities (which were 20–50% of those in rodents) were more uniformly distributed throughout the airway tree than in rats, whereas CYP2E1 activity was mainly found toward the end of the airway. In rats, CYP1A1 and CYP2B activities were highest in the parenchyma, whereas CYP2E1 activity was highest in the airways.

The overall amounts of naphthalene dihydrodiol and glutathione conjugates formed in rhesus lung explants were approximately 70 times less than in comparable incubations with mouse airways (Buckpitt et al., 1995), a finding that is consistent with the results of comparative mouse, monkey, and human lung microsomal incubations (Buckpitt and Bahnson, 1986; Buckpitt et al., 1992). In contrast to the dramatic differences observed in the amount of water-soluble naphthalene metabolites formed between rodents and nonhuman primates, the differences in the levels of reactive metabolites bound were not as substantial (Cho et al., 1994). Reactive naphthalene metabolite binding varied from slightly less than 1 nmol/mg protein in mouse upper airways to 3 to 4 nmol/mg protein in more distal airways at 500 μM substrate (Cho et al., 1994). In the current studies at the same substrate concentrations, the amounts of bound metabolite were slightly less than 1 nmol/mg protein in airways and slightly higher than this in the parenchyma. Thus, although there are differences in the amount of metabolite bound in mouse compared with monkey, these differences were not 10- to 100-fold. In contrast to the small amounts of reactive naphthalene metabolites generated in mouse parenchyma (Cho et al., 1994), monkey parenchyma generated the largest amounts of reactive metabolites at the high substrate concentration.

Dihydrodiol was the predominant water-soluble naphthalene metabolite generated in incubations with both concentrations of naphthalene. This is consistent with previous data in rhesus monkey (Buckpitt et al., 1992) and human (Buckpitt and Bahnson, 1986) lung microsomal incubations. It is
not clear whether metabolism to the dihydriodiol represents a true detoxication reaction because the diol can be further metabolized to the 1,2-quinone by dihydriodiol dehydrogenase (Smithgall et al., 1988), and the redox activity and electrophilic nature of this metabolite make it a potential suspect in toxicity (Penning et al., 1999). Moreover, evidence has been presented for a trihydroxymercapturic acid metabolite in the urine of mice treated with high doses of naphthalene, a finding that supports the in vivo formation of a diol epoxide intermediate (Pakenham et al., 2002).

Both C5,C6- and C7,C8-epoxides of 1-nitronaphthalene are generated in rodent microsomal incubations and C7,C8-epoxides predominate in the lung (Watt et al., 1999). Moreover, CYP2F2 shows regioselectivity for epoxide formation at C7,C8 (Shultz et al., 2001). The regioselectivity noted in rodent lungs was not observed in monkeys, and this is consistent with the fact that CYP2F is undetectable in rhesus macaque airways (Baldwin et al., 2004). Although it is not possible to directly compare the metabolism of 1-nitronaphthalene to water-soluble metabolites in rhesus and rodent because different experimental approaches were used (rodent metabolism studies were conducted using microsomal preparations; Watt et al., 1999), it seems that the amounts of glutathione conjugates generated were considerably lower in rhesus compared with rat lung, with similar levels of GSH or glutathione S-transferase in airways of both species (Duan et al., 1993, 1996). Metabolites generated by reductive processes were not detected in previous rodent microsomal incubations or in the current study. This is consistent with in vivo studies showing only small amounts of naphthylamine in the urine of 1-nitronaphthalene-treated rats (Halladay et al., 1999).

Interesting differences were observed in the percentages of metabolite bound covalently from naphthalene and 1-nitronaphthalene. At low naphthalene concentrations, less than 27% of the total metabolites were bound covalently, whereas 30 to 55% of the total metabolites generated from 1-nitronaphthalene were bound covalently. As expected, at higher substrate concentrations, larger percentages of the total metabolites became bound covalently to proteins. With both substrates, the amount of metabolite bound covalently accounted for 25 to 65% of the total naphthalene metabolites and 48 to 82% of the total 1-nitronaphthalene metabolites.

Earlier work has provided evidence for the importance of CYP2F2 in the metabolic activation and subsequent cytotoxicity of naphthalene in rodent models. The kinetics of naphthalene metabolism by heterologously expressed murine CYP2F2 (Kₘ and kₐₗ values of 3 µM and 104 s⁻¹, respectively; Shultz et al., 1999) and excellent correlations between metabolic activities in various lung subcompartments and across species with the amounts of immunoreactive protein present (Baldwin et al., 2004) suggests that this isozyme is quantitatively important in the initial step of naphthalene metabolism. The small amounts of naphthalene metabolites generated in dissected airway subcompartments in the present study is fully consistent with recent results showing that although CYP2F2 isozyme is detected at the mRNA level in rhesus (R. M. Baldwin, unpublished data); the protein is not detected in quantitative immunoblotting experiments (Baldwin et al., 2004). Although CYP2F2 also metabolizes 1-nitronaphthalene (Kₘ and kₐₗ of 21 µM and 17.1 s⁻¹), the quantitative contribution of this isozyme to the initial epoxidation and the toxicity of 1-nitronaphthalene is unclear. Previous work with isozyme-selective P450 inhibitors supported the involvement of CYP2B in the metabolic activation of 1-nitronaphthalene (Verschoyle et al., 1993), and this is consistent with the nearly equal susceptibility of the rat and mouse to 1-nitronaphthalene toxicity.

Overall, the data from the current work have demonstrated dramatic rodent-primate differences in the metabolism of two pulmonary toxicants that require P450 activation to elicit toxicity. Moreover, the distribution of metabolic activation and the profiles of metabolites are very different in primates and rodents. These differences raise important questions about the use of data obtained in rodent bioassays for chemicals such as naphthalene, which seem to be very weak respiratory tract carcinogens. The finding of detectable levels of naphthalene in both fat and breast milk in a significant percentage of humans tested raise legitimate concerns about the potential toxicity of this agent (IARC, 2002). With a single exception of the data set available on 4-ipomeanol metabolism and toxicity, we lack sufficient information to indicate whether the studies conducted in rodents translate well to the human. 4-Ipomeanol is a bioactivated furan that results in lung-selective toxicity in almost every species tested (rats, mice, rabbits, dogs, and cattle (for review, see Gram, 1997; Dutcher and Boyd, 1979). Good correlations between the rates of metabolic activation of 4-ipomeanol and target tissue toxicity have been observed. Human lung microsomes and cell lines showed relatively low rates of metabolic activation (McLemore et al., 1990). Subsequent phase I and II clinical trials with 4-ipomeanol produced no evidence for lung toxicity; indeed, doses were limited by evidence of hepatic toxicity (Rowinsky et al., 1993; Lakhanpal et al., 2001).

At face value, our work would suggest that naphthalene poses a very limited hazard to human populations. However, this is based on several assumptions: 1) that rhesus macaques are a good model for the human; 2) that the activities measured here are a result of small amounts of P450 protein spread over many of the cells in the specimens obtained; 3) that the results obtained in juvenile animals in this study would be similar to results obtained with adult animals; and 4) that overall metabolism is more important than the formation of metabolites that bind covalently to protein. On these latter points, previous work has shown that the levels of P450 activity are lower in early postnatal animals, which are more susceptible to naphthalene toxicity (Fanucchi et al., 1997). As shown in these studies, a high percentage of the overall metabolites generated bind covalently to proteins, and although there are differences in the amounts bound in rodents compared with primates, these are generally less than 2- to 3-fold. Ongoing studies are focused on determining the importance of specific adducts in the mechanisms of cytotoxicity of naphthalene and 1-nitronaphthalene. If such relationships can be established, adduct formation might be used to determine whether exposed human populations are capable of generating sufficient quantities of precursor metabolites to be harmful.

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References


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