Benzene Metabolism in Human Lung Cell Lines BEAS-2B and A549 and Cells Overexpressing CYP2F1

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ABSTRACT: Benzene is an occupational and environmental toxicant. The main human health concern associated with benzene exposure is leukemia. The toxic effects of benzene are dependent on its metabolism by the cytochrome P450 enzyme system. The cytochrome P450 enzymes CYP2E1 and CYP2F2 are the major contributors to the bioactivation of benzene in rats and mice. Although benzene metabolism has been shown to occur with mouse and human lung microsomal preparations, little is known about the ability of human CYP2F to metabolize benzene or the lung cell types that might activate this toxicant. Our studies compared bronchiolar derived (BEAS-2B) and alveolar derived (A549) human cell lines for benzene metabolizing ability by evaluating the roles of CYP2E1 and CYP2F1. BEAS-2B cells that overexpressed CYP2F1 and recombinant CYP2F1 were also evaluated. BEAS-2B cells overexpressing the enzyme CYP2F1 produced 47.4 ± 14.7 pmols hydroxylated metabolite/10^6 cells/45 min. The use of the CYP2E1-selective inhibitor diethyldithiocarbamate and the CYP2F2-selective inhibitor 5-phenyl-1-pentyne demonstrated that both CYP2E1 and CYP2F1 are important in benzene metabolism in the BEAS-2B and A549 human lung cell lines. The recombinant expressed human CYP2F1 enzyme had a K_m value of 3.83 μM and a V_{max} value of 0.01 pmol/pmol P450 enzyme/min demonstrating a reasonably efficient catalysis of benzene metabolism ($V_{max}/K_m = 2.6$). Thus, these studies have demonstrated in human lung cell lines that benzene is bioactivated by two lung-expressed P450 enzymes. © 2004 Wiley Periodicals, Inc. J Biochem Mol Toxicol 18:92–99, 2004; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jbt.20010

KEYWORDS: **Benzene; Lung; Liver; Cytochromes P450**

INTRODUCTION

Although benzene can cause acute drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, and unconsciousness or even death [1], the main human health concern associated with chronic benzene exposure is acute myelogenous leukemia [2–5]. Benzene causes tumors of the Zymbal gland, oral cavity, and skin in the rat, and Zymbal gland tumors, malignant lymphomas, lung carcinomas, and other types of tumors in the mouse [6]. In the mouse, but not in the rat, hematopoietic hyperplasia has been observed, suggesting that the mouse may represent a better model for benzene-induced leukemia than the rat [7].

Outside of its effects on the central nervous system, the toxic effects of benzene are dependent on its metabolism by the cytochrome P450 enzyme system. The highest concentration of the cytochrome P450 enzyme system can be found in the liver, but expression has been reported in almost all tissues including lung, small intestine, kidney, colon, and brain [8]. Benzene is first metabolized to benzene oxide, and this then nonenzymatically rearranges to form phenol [9]. Phenol is further metabolized to hydroquinone, catechol, and 1,2,4-trihydroxybenzene [10]. Hydroquinone and catechol can be further converted by bone marrow myeloperoxidase to reactive metabolites [11].

The cytochrome P450 enzymes CYP2E1 and CYP2F2 have been found to play significant roles in the metabolism of benzene. Oxidation of benzene by CYP2E1 to reactive intermediates is a prerequisite of cellular toxicity, and it has been found that mice deficient in CYP2E1 activity developed no benzene-induced myelotoxicity or hematotoxicity [12]. Using rabbit hepatic microsomes and six purified isozymes of hepatic P450, Koop et al. [13] identified CYP2E1 as a primary isozyme involved in benzene oxidation. By using the relatively specific CYP2E1 inhibitor, diethyldithiocarbamate (DDTC), Masuda et al. [14] found that the metabolism of benzene in both mouse liver and

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mouse lung was inhibited. Gut et al. [15] showed that DDTC almost completely suppressed benzene oxidation to both soluble and protein-bound metabolites in rat liver, further indicating that 2E1 was involved in benzene oxidation at this level. Chaney and Carlson [16] showed that 300 μM DDTC decreased benzene metabolism in rat liver microsomes by 96% while a 54% decrease was seen in rat lung microsomes. Using chemical inhibitors, Powley and Carlson [17] showed that CYP2F2 was much more important in the mouse lung than in the liver and that benzene was metabolized to nearly the same extent in mouse pulmonary microsomes by CYP2E1 and CYP2F2. Using wild-type and CYP2E1 knockout mice, Sheets and Carlson [18] found that CYP2E1 has a higher rate of metabolism but lower affinity for benzene than does CYP2F2 and confirmed that CYP2F2 metabolizes benzene primarily in lung compared to liver.

Cell types are important when considering benzene-metabolizing capability within the lung, but little is known about the specific cell types associated with the metabolism of benzene by the lung or the cytochrome P450 isozymes involved in those cells. A cDNA library screen from human lung tissue identified the CYP2F1 gene that encodes a cytochrome P450 enzyme capable of bioactivating a number a pulmonary-selective toxicants [19]. However, little is known about the ability of this human form of CYP2F to metabolize benzene. Using microsomes prepared from human lymphoblastoid cells containing human CYP2F1, Lanza et al. [20] showed that CYP2F1 metabolizes 3-methylindole (3MI) to its putative reactive intermediates and catalyzes the epoxidation of naphthalene to a reactive epoxide. Nichols et al. [21] showed that BEAS-2B cells transfected with CYP2F1 were susceptible to 3MI-mediated cytotoxicity. Using RT-PCR, human bronchial mucosal cells have been shown to express mRNA for several cytochrome P450 enzymes, including 1A1, 2C8, 2C18, 2A6, 2B6, 2E1, 2F1, and 3A5 [22–24]. The immortalized human cell line BEAS-2B is a subclone of transformed adult human bronchial epithelial cells that express phenotypic characteristics of nonciliated mucus-secreting epithelial cells (Clara cells) and was obtained by infection of bronchial cells with an adenovirus 12-simian virus 40-hybrid preparation [25].

Nichols et al. [25] showed that the BEAS-2B cell line is a reasonable model cell for toxicological studies because it expresses differentiation characteristics of human lung explants grown on collagen with serum-free medium supplemented with grow factors. They suggested that these cells represent a model that can be used to test the carcinogenic potential of chemicals that can be bioactivated to mutagenic metabolites. Van Agen et al. [26] also stated that the immortalized human bronchial epithelial cell culture BEAS-2B may provide a good in vitro model which simulates the effects on bronchial epithelial tissue of inhalation exposure to environmental carcinogens and that BEAS-2B cells have a high metabolic capacity.

The human adenocarcinoma cell line A549 is derived from alveolar epithelial type II cells, a cell type that expresses several CYP forms and therefore possesses metabolic activities toward xenobiotics [27]. Type II cells are the progenitor cells for Type I epithelial cells and are one of the few lung cells in which P450 isoenzymes are localized suggesting a possible role in the oxidative metabolism of drugs in the lung [28]. They express most of the major constitutive and inducible CYP forms found in lung epithelial cells including 1A1, 1B1, 2B6, 2C8-19, 2E1, 3A5, and 3A7. CYP2E1 expression has been established in several studies, while CYP2F1 and CYP4B1 mRNAs have not been detected in A549 cells but have been found in whole lung tissue [27].

To further understand the importance of both lung cell types and cytochrome P450 isozymes in benzene metabolism, the objectives of this research were to compare bronchiolar-derived and alveolar-derived human cell lines for benzene metabolizing ability and to further evaluate the roles of CYP2E1 and CYP2F in the metabolism of benzene by using cell lines overexpressing CYP2F and recombinant microsomal CYP2F1.

MATERIALS AND METHODS

Cells

Normal BEAS-2B cells, BEAS-2B cells overexpressing CYP2F1, and recombinant CYP2F1 were obtained as previously described [20,21]. A549 cells were obtained from American Type Culture Collection (ATCC # CCL-185).

Chemicals

[^{14}C]Benzene (60 mCi/mmol or 50 mCi/mmol) was obtained from Moravek Biochemicals (Brea, CA). The radiochemical purity was 99.9%. Chemicals used in the microsomal and cell metabolism experiments included L-ascorbic acid, NADH, and NADPH obtained from Sigma Chemical Co. (St. Louis, MO); magnesium chloride and potassium phosphate (monobasic) from Mallinckrodt Specialty Chemicals (Paris, KY); and potassium chloride and potassium phosphate (dibasic) from J. T. Baker (Phillipsburg, NJ). Diethyldithiocarbamic acid (DDTC) was obtained from Sigma Chemical Co. (St. Louis, MO), and 5-phenyl-1-pentyne (5PIP) was obtained from Lancaster Synthesis (Windham, NH).

BEGM (bronchial epithelial growth media) was ordered as a Bullet Kit from Clonetics (BioWhittaker) Corp., catalog # CC-3170. Bovine serum albumin (BSA) and fibronectin were obtained from Sigma Chemical Co. (St. Louis, MO), and Vitrogen 100 was obtained from Cohesion Corp. (Palo Alto, CA). Ham's F12 media and EDTA were obtained from Gibco/Invitrogen Corp. (Carlsbad, CA). Fetal bovine serum was obtained from Gemini Bio-Products (Woodland, CA), and penicillin, streptomyocin, and amphotericin B were obtained from Fischer Corp. (Grand Rapids, MI).

Cell Culture

BEAS-2B Cells

BEAS-2B cells were grown in T-75 flasks coated with a solution containing fibronectin (0.01 mg/mL), Vitrogen 100 (0.03 mg/mL), bovine serum albumin (0.01 mg/mL), and BEGM media (0.88 mL/mL solution). Once the cells grew to near confluence, they were detached with 0.25% trypsin/0.5% polyvinylpyrrolidone (PVP) solution. The cells were then centrifuged for 10 min at 125 × g. The media was poured off, and the cells were resuspended in 1–2 mL of potassium phosphate buffer (0.1 M, pH 7.4) for the benzene metabolism assay.

A549 Cells

The cell culture media for the A549 cells was a Ham's F12 media + PSF (penicillin/streptomyocin/fungicin). Added to this media was 10% fetal bovine serum. When the cells covered the bottom of the flask (3–4 days), they were divided. The media was aspirated and 2 mL of 1% trypsin were added to the flask. The trypsin solution contained 0.25% trypsin and 0.1% EDTA in Hank's balanced salt solution with 0.l M Hepes, pH 7.4, buffer (HBSS-Hepes buffer). When sufficient numbers of cells had been grown, they were trypsinized, combined in a 50 mL conical tube, and centrifuged at 1000 rpm and the cell pellet was resuspended in 2 mL of potassium phosphate buffer (0.1 M, pH 7.4) for the benzene metabolism assay.

Cell Incubation

The method used for determining the cellular metabolism of benzene in this study was developed by Powley and Carlson [29–31] and was a modification of the method developed by Chaney and Carlson [16]. An incubation mixture containing 0.2 mL of the BEAS 2B or A549 cell suspension, 0.7 mL potassium phosphate buffer (0.1 M, pH 7.4) with ascorbate (0.1 M), 0.080 mL magnesium chloride (25 mM), and 20 μl [^{14}C]benzene was placed in 3 mL reacti-vials fitted with resealable Teflon septa and incubated at 37°C for 10 min. Nonlabeled benzene was added with the ^{14}C benzene to obtain the desired concentrations. Therefore, the specific activity of the ^{14}C benzene used in the incubations varied due to dilution with the nonlabeled benzene. The reaction was initiated by injecting 0.1 mL of a solution containing NADPH (10 mM) and NADH (10 mM), and the resulting mixture was incubated at 37°C in a metabolic shaker for 45 min. When inhibitors were used, diethyldithiocarbamic acid (DDTC; 300 μM final concentration) or 5-phenyl-1-pentyne (5P1P; 5μM final concentration) was incubated with [^{14}C]benzene.

The reaction was stopped by adding 1 mL of cold ethyl acetate containing benzene (114 mM), phenol (7 mM), catechol (14 mM), hydroquinone (26 mM), 1,2,4 trihydroxybenzene (10 mM), and butylated hydroxytoluene (91 mM). Extraction of the radiolabeled metabolites was maximized by vortexing the mixture, placing it on ice for 10 min, and repeating. The mixture was centrifuged at 833 × g at 4°C for 20 min. A portion of the organic layer was placed in a screw top vial and stored at −80°C until analysis.

The components of the organic layer were analyzed using a Shimadzu HPLC system with a Beckman octadecyl silane (ODS) column (5 μM; 4.6 mm × 25 cm) and a Beckman Ultrasphere ODS guard column (5 μM; 4.6 mm × 4.5 cm). The mobile phases were deionized water purified by a Milli-Q Reagent Water System (A) and HPLC-grade methanol (B) connected to a Packard 505TR Flow Scintillation Analyzer. The flow rate throughout each run was constant at 1 mL/min for 55 min. Flow composition was as follows; 0–5 min: 0% B; 5–10 min: increase to 20% B; 10–15 min: 20% B; 15–30 min: increase to 35% B; 30–36 min: increase to 100% B; 36–45 min: 100% B; 45–50 min: decrease to 0% B; 50–55 min: 0% B. Sample injections were 80 or 200 μl. Each mL of HPLC eluent was mixed with 3 mL of Ultima Flo AP scintillation cocktail (Packard Instrument Co., Inc., Meriden, CT) prior to analysis by the Flow Analyzer. The radioactive metabolites were identified by the time of their elution compared to the UV peaks of the unlabeled standards. The metabolites were quantified as pmol metabolite/mg microsomal protein (or per 10^6 cells)/45 min. This time period was in keeping with our previous studies on tissues [16–18,29–32]. Since the primary metabolite was phenol with only traces of hydroquinone and catechol, derived from further metabolism of this metabolite, being identified, the results are expressed as total hydroxylated metabolite. No measurable 1,2,4-trihydroxybenzene was measured in the incubations (<1–20 pmols depending upon specific activity in individual assay).

Kinetic and Statistical Analysis

Kinetic factors were determined using the Lineweaver–Burk extrapolation method using Enzyme Kinetics Pro™ (ChemSW Inc., Version 2.34). Cell line comparisons were analyzed by using Student's two-tailed t test employing the SAS statistical program (SAS Institute Inc., Windows Version 5.0.2195). To determine the effectiveness of the inhibitors on the cell lines, control groups were compared with treated groups using one-tailed paired Student's t tests. The acceptable level of significance was set at $p < 0.05$.

RESULTS

Cells ($1–7 \times 10^6$) were harvested for use in each benzene incubation. The concentration of benzene used was 200 μM. As determined by trypan blue exclusion, this concentration of benzene caused very little or no cell death during the incubation. However, with the P450 inhibitor DDTC (300 μM) and 5P1P (5 μM) included in the incubation, 15–30% and 7–15% decreases were seen in cell viability, respectively. The number of viable cells determined after the incubation was used when calculating the total amount of metabolite produced by the cells during the incubation.

Normal BEAS-2B cells metabolized benzene, producing 60.4 ± 10.2 pmols hydroxylated metabolite/10^6 cells/45 min (Figure 1). BEAS-2B cells overexpressing the enzyme CYP2F1 produced 47.4 ± 14.7 pmols hydroxylated metabolite/10^6 cells/45 min (Figure 1). The greater metabolism in the normal BEAS-2B cells, compared with the BEAS-2B cells overexpressing CYP2F1 was not statistically significant. In the normal BEAS-2B cells, the cytochrome CYP2E1 inhibitor DDTC (300 μM) significantly reduced the amount of total benzene metabolite formed by 39% (Figure 2) while the CYP2F1 inhibitor 5P1P (5 μM) had no effect on total benzene metabolite produced (Figure 3). DDTC reduced the amount of total benzene metabolite produced by BEAS-2B cells overexpressing CYP2F1 by 70%, and 5P1P reduced it by 56% (Figure 4). Both of these reductions were not statistically significant but had low p-values of 0.06.

A549 cells metabolized benzene producing 11.7 ± 2.3 pmols hydroxylated metabolite/10^6 cells/45 min (Figure 5). DDTC (300 μM) had no effect on the rate of benzene metabolism by A549 cells (Figure 5). A 51% decrease was seen in total benzene metabolite produced in the A549 cells inhibited with 5P1P (5 μM) (Figure 5), which was statistically significant ($p < 0.05$).

Microsomes prepared from human lymphoblastoid cells containing recombinant human CYP2F1 have the ability to metabolize benzene. When incubated with 200 μM [^{14}C]benzene in two separate experiments, the microsomes containing human CYP2F1 produced 56.8 and 37.3 pmols total metabolite/mg protein/45 min. There were negligible amounts of other cytochromes P450 present with perhaps a trace of CYP1A1 according to the producer. Based on a P450 enzyme concentration of 22.6 pmols/mg microsomal protein, the turnover rates for these microsomes were 2.51 and 1.65 pmols total metabolite/pmol P450 enzyme/45 min. Both DDTC (300 μM) and 5P1P (5 μM) inhibited benzene metabolism in these microsomes containing human CYP2F1. 5P1P (5 μM) inhibited to a greater extent than DDTC (300 μM). When incubated

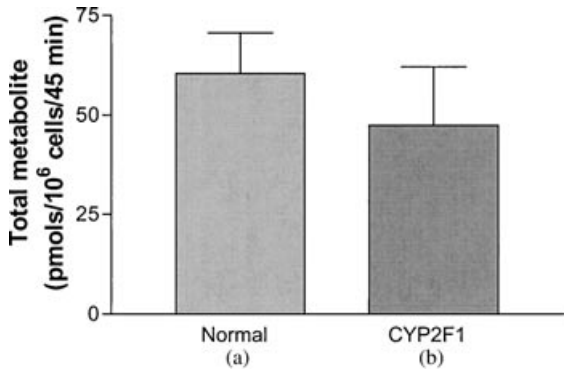

FIGURE 1. Comparison of total hydroxylated metabolite production by normal BEAS-2B cells and BEAS-2B cells overexpressing CYP2F1 incubated with 200 μM benzene. Cells were incubated with 200 μM benzene for 45 min at 37°C. Benzene metabolites were extracted immediately following the 45 min incubation and are presented as pmol of total hydroxylated metabolite/10^6 cells/45 min. Values are mean ± S.E. for (a) $n = 7$; and (b) $n = 4$.

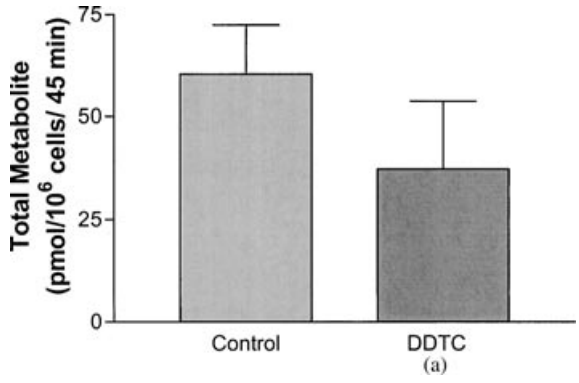

FIGURE 2. Effects of diethyldithiocarbamate (DDTC; 300 μM) on total hydroxylated metabolite production by the normal BEAS-2B cell line incubated with 200 μM benzene. Cells were incubated with 200 μM benzene for 45 min at 37°C. DDTC (300 μM) was added prior to incubation. Benzene metabolites were extracted immediately following the 45 min incubation and are presented as pmol of total hydroxylated metabolite/10^6 cells/45 min. Values are mean ± S.E., $n = 6$, (a) significant decrease ($p < 0.05$) from control values.

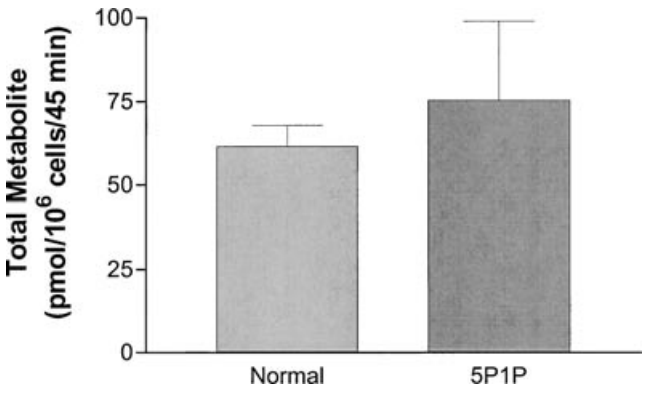

FIGURE 3. Effects of 5-phenyl-1-pentyne (5P1P; 5 μM) on total hydroxylated metabolite production by the normal BEAS-2B cell line incubated with 200 μM benzene. Cells were incubated with 200 μM benzene for 45 min at 37°C. 5PIP (5 μM) was added prior to incubation. Benzene metabolites were extracted immediately following the 45 min incubation and are presented as pmol of total hydroxylated metabolite/10^6 cells/45 min. Values are mean ± S.E. $n = 3$.

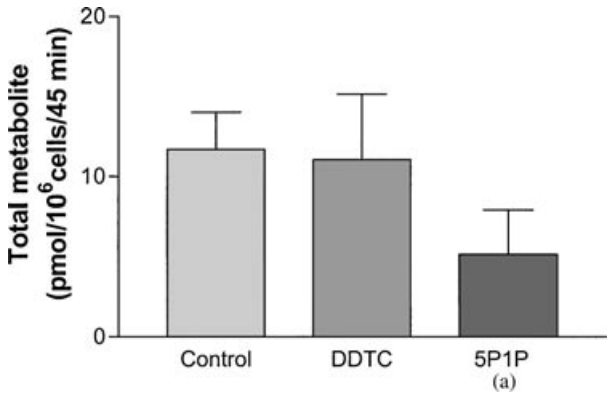

FIGURE 5. Effects of diethyldithiocarbamate (DDTC; 300 μM) and 5-phenyl-1-pentyne (5P1P; 5 μM) on total hydroxylated metabolite production by the A549 cell line incubated with 200 μM benzene. Cells were incubated with 200 μM benzene for 45 min at 37°C. The inhibitors DDTC (300 μM) or 5P1P (5 μM) were added prior to incubation. Benzene metabolites were extracted immediately following the 45 min incubation and are presented as pmol of total hydroxylated metabolite/10^6 cells/45 min. Values are mean ± S.E., $n = 4$, (a) significant decrease ($p < 0.05$) from control values.

with DDTC, the microsomes produced 12.5 pmols total metabolite/mg protein/45 min, while when incubated with 5P1P, they produced only 4.4 pmols total/mg protein/45min. The turnover rates were 0.52 pmols total metabolite/pmol P450 enzyme/45 min when inhibited with DDTC and 0.19 pmols total metabolite/pmol P450 enzyme/45 min when inhibited with 5P1P.

Recombinant CYP2F1 microsomes were studied by incubation with six concentrations of [^{14}C]benzene ranging from 1 to 50 μM. Using the Lineweaver–Burk method, a K_m value of 3.75 μM and a V_{max} value of 0.15 pmol/mg protein/min were determined for the single set of incubations (Figure 6). Based again on a P450 enzyme concentration of 22.6 pmols/mg microsomal protein and using the Lineweaver–Burk method, a K_m value of 3.83 μM and a V_{max} value of 0.01 pmol/pmol P450 enzyme/min ($V_{max}/K_m = 2.6$) were established for the same set of incubations.

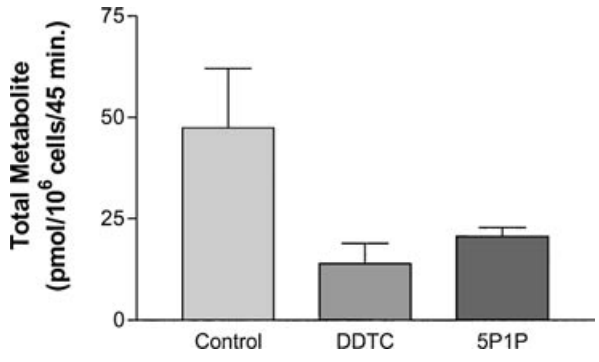

FIGURE 4. Effects of diethyldithiocarbamate (DDTC; 300 μM) and 5-phenyl-1-pentyne (5P1P) on total hydroxylated metabolite production by BEAS-2B cell line overexpressing CYP2F1 incubated with 200 μM benzene. Cells were incubated with 200 μM benzene for 45 min at 37°C. Inhibitors DDTC (300 μM) or 5P1P (5 μM) were added prior to incubation. Benzene metabolites were extracted immediately following the 45 min incubation and are presented as pmol of total hydroxylated metabolite/10^6 cells/45 min. Values are mean ± S.E., $n = 4$.

DISCUSSION

Cell types are important when considering the metabolizing capability within the lung as is their complement of cytochromes P450 capable of bioactivating or detoxifying xenobiotics. Human lung microsomes have been shown to metabolize benzene [32], but the activity was less than that in mouse lung microsomes [29,30]. Freshly isolated, mouse Clara cells metabolize benzene, and this activity is inhibited by both DDTC and 5P1P (Powley, personal communication). Studies have shown the importance of CYP2F2 in mouse lung as compared to mouse liver [17,18,29,30]. Surprisingly, when comparing the normal BEAS-2B cells with the BEAS-2B cells overexpressing CYP2F1, the normal BEAS-2B cells and the overexpressing cells metabolized benzene to a similar extent. These data suggest that the overexpression of CYP2F1 in BEAS-2B cells decreased the ability of the cells to metabolize benzene. This observation may be due to CYP2F1 having a high affinity for benzene but not being able to metabolize it as efficiently as CYP2E1. Our studies comparing mouse liver and lung microsomes from wild-type and CYP2E1 knockout mice suggest that CYP2E1 has a higher activity but lower affinity for benzene than does

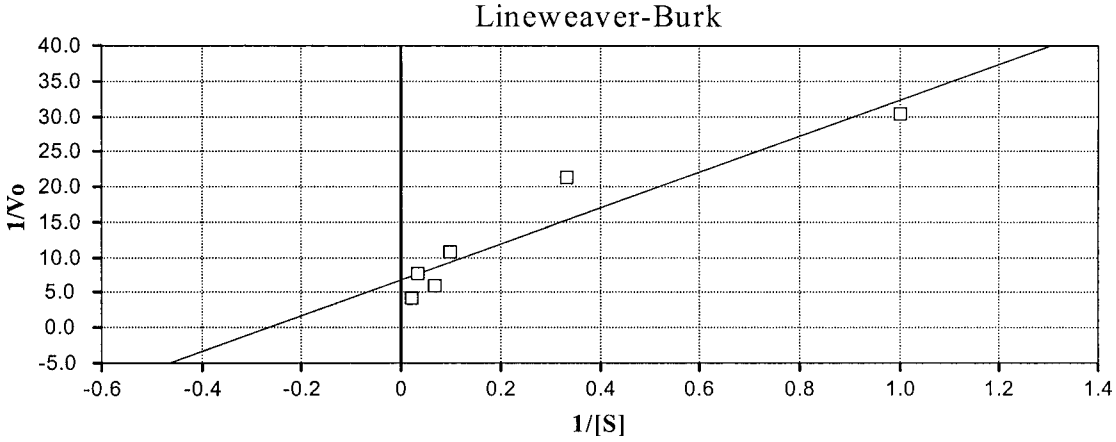

FIGURE 6. Lineweaver–Burk plot for benzene oxidation by lymphoblast microsomes containing recombinant CYP-2F1. Data are displayed as the inverse of pmol total hydroxylated metabolite/mg protein/min.

CYP2F2 [18]. The overexpression of CYP2F1 is most likely increasing the cells' ability to bind benzene, but because CYP2F1 has a low V_{max} value, the benzene may not be converted to metabolites as quickly as with normal BEAS-2B cells that contain variable low levels of CYP2F1 [21]. This would be in agreement with our previous studies on benzene metabolism in the livers and lungs of CYP2E1 wild-type and knockout mice. In those studies the apparent kinetic factors were different between the wild type mouse (K_m: 30.4 μM, V_{max}: 25.3 pmol/mg protein/min) and knockout mouse with no CYP2E1 (K_m: 1.9 μM, V_{max}: 0.5 pmol/mg protein/min) livers [18].

Inhibition of metabolism by DDTC in the normal BEAS-2B cell line indicates that CYP2E1 is present in these human bronchiolar derived cells. Benzene metabolism in the normal BEAS-2B cell line was not inhibited by 5P1P indicating that CYP2F1 is not present or not active. However, in the BEAS-2B cell line that overexpressed CYP2F1, there were decreases in metabolism when the cells were incubated with DDTC (300 μM) or 5PIP (5 μM). This suggests that CYP2F1 metabolized benzene when it is overexpressed and that CYP2E1 is active in both normal and BEAS-2B cells overexpressing CYP2F1. As expected, 5P1P decreased benzene metabolism in BEAS-2B cells overexpressing CYP2F1.

The human adenocarcinoma cell line A549 is derived from alveolar epithelial type II cells, a cell type that expresses several CYP forms and therefore possesses metabolic activities toward xenobiotics [27]. Type II cells are the progenitor cells for Type I epithelial cells and are one of the few lung cells in which P450 isoenzymes are normally expressed, suggesting a possible role in the oxidative metabolism of drugs in the lung [28]. The A549 cells metabolized benzene. The 51% decrease in the amount of total hydroxylated metabolite produced in the A549 cell line incubated with 5PIP implies that this inhibitor may affect some other P450 enzyme in these cells. Less likely is the possibility that, although CYP2F1 mRNA is not normally detected in this cell line, enough active enzyme was expressed to be inhibited by 5PIP. DDTC had no effect on total hydroxylated metabolite production indicating CYP2E1 may not be active or present within these cells.

Microsomes prepared from human lymphoblastoid cells containing recombinant human CYP2F1 metabolized benzene. 5P1P inhibited benzene metabolism, reinforcing the conclusion that CYP2F1 catalyzed benzene metabolism. DDTC inhibited benzene metabolism as well. These results may mean that CYP2F1 can be inhibited by DDTC, but experiments to confirm this hypothesis could not be pursued because the amount of expressed CYP2F1 was quite limited. When comparing the kinetic factors determined for the lymphoblast microsomes to the kinetic factors determined for the knockout mouse lung by Sheets and Carlson [18], the K_m values are very similar, but the V_{max} value for the lymphoblastoid cell microsomes is approximately 87.5% lower than the value determined for the knockout mouse lung. This could indicate a species difference with the regards to the CYP2F subfamily in the human lung and its ability to metabolize benzene or contribution by additional cytochromes P450 in the lung microsomes of mice.

In conclusion, CYP2F1 is active in benzene metabolism when it is overexpressed and CYP2E1 is active in both normal and BEAS-2B cells overexpressing CYP2F1. CYP2F1 is involved in benzene metabolism within alveolar epithelial type II cells and recombinant human CYP2F1 metabolizes benzene. Based on

these findings, human CYP2F1 may be an important contributor to benzene-induced toxicity in the human lung.

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