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Inhibitory Monoclonal Antibody to Human Cytochrome P450 2B6

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ABSTRACT. The human cytochrome P450 2B6 metabolizes, among numerous other substrates, diazepam, 7-ethoxycoumarin, testosterone, and phenanthrene. A recombinant baculovirus containing the human 2B6 cDNA was constructed and used to express 2B6 in Sf9 insect cells. The 2B6 was present at 1.8 ± 0.4% of the total cellular protein and was purified to a specific content of 13.3 nmol/mg protein. Mice were immunized with the purified 2B6, and a total of 811 hybridomas were obtained from the fusion of NS-1 myeloma cells and spleen cells of the immunized mice. Monoclonal antibodies (MAbs) from 24 of the hybrids exhibited immunobinding to 2B6 as determined by ELISA. One of the MAbs, 49-10-20, showed a strong immunoblotting activity and was highly inhibitory to 2B6 enzyme activity. MAb 49-10-20 inhibited cDNA-expressed 2B6-catalyzed metabolism of diazepam, phenanthrene, 7-ethoxycoumarin, and testosterone by 90–91%. MAb 49-10-20 showed extremely high specificity for 2B6 and did not bind to 17 other human and rodent P450s or inhibit the metabolism of phenanthrene catalyzed by human 1A2, 2A6, 2C8, 2C9, 2D6, 2E1, 3A4, and 3A5. MAb 49-10-20 was used to determine the contribution of 2B6 to the metabolism of phenanthrene and diazepam in human liver. In ten liver samples, MAb 49-10-20 inhibited phenanthrene metabolism variably by a wide range of 8–42% and diazepam demethylation by 1–23%. The degree of inhibition by the 2B6 specific MAb 49-10-20 defines the contribution of 2B6 to phenanthrene and diazepam metabolism in each human liver. This technique using inhibitory MAb 49-10-20 determines the contribution of 2B6 to the metabolism of its substrates in a human tissue containing multiple P450s. This study is a prototype for the use of specific and highly inhibitory MAbs to determine individual P450 function. BIOCHEM PHARMACOL **55**;10:1633–1640, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. monoclonal antibody; human cytochrome P450 2B6; baculovirus; vaccinia virus; cDNA expression; phenanthrene; diazepam; testosterone; 7-ethoxycoumarin

Cytochrome P450s are the principal enzymes for the metabolism of xenobiotics such as drugs, carcinogens, and environmental chemicals as well as endobiotics such as steroids and fatty acids. P450‖-dependent activation of carcinogenic compounds may yield highly reactive intermediates that can damage DNA and initiate tumorigenesis [1]. Certain inherited P450-mediated drug oxidation polymorphisms include the debrisoquine/sparteine polymorphism and the mephenytoin polymorphism [2]. Generally, individual differences in relevant P450 levels may determine responsiveness to the variable therapeutic or toxic effects of drugs or the activation of carcinogens.

A number of studies have detected cytochrome P450 2B enzymes in the livers of animal species, including rats, rabbits, and mice [3]. In rodents, the basal hepatic P450 2B protein content is low, and is expressed very poorly (usually <5% of the total P450 content) but is highly inducible by diverse chemicals, of which the prototype is phenobarbital [4, 5]. P450 2B enzymes in rodents and 2B6 in humans metabolize a variety of xenobiotics and endobiotics [3–5]. Yamano *et al.* [6] reported the isolation of a cDNA clone of 2B6 from a human liver library, Czerwinski *et al.* [7] detected 2B6 mRNA in human liver, and Mimura *et al.* [8] partially purified 2B6 from human liver microsomes.

In this study, we constructed a recombinant baculovirus containing human 2B6 cDNA for expression of 2B6 in Sf9 insect cells. The expressed 2B6 was purified and used as an immunogen for MAb production. MAb 49-10-20 was found to exhibit strong binding by IB to 2B6. We then used vaccinia virus-expressed human P450 2B6 to examine the effect of MAb 49-10-20 on 2B6-catalyzed metabolism of phenanthrene [9], DZ [10], testosterone [11], and 7-EC [12], each of which was inhibited by 90%. Immunobinding MAbs are precise tools for determining the content of individual P450s in tissues. MAbs inhibitory to 2E1 and 3A4 have determined the contribution of these human

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‖ *Abbreviations:* P450, cytochrome P450; MAb, monoclonal antibody; 2B6, human cytochrome P450 2B6; DZ, diazepam; NDZ, *N*-demethyldiazepam; PA 9,10-diol, phenanthrene 9,10-dihydrodiol; B[a]P t-9,10-diol, benzo[*a*]pyrene *trans*-9,10-dihydrodiol; 7-EC, 7-ethoxycoumarin; MOI, multiplicity of infection; and IB, immunoblot.

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P450s to specific drug and carcinogen metabolism in the presence of multiple forms of P450 in human liver [13–15]. MAb 49-10-20 is highly specific to 2B6 and does not bind or inhibit other P450s examined. It was used to determine both the amount of P450 2B6 in human liver and the contribution of 2B6 to the metabolism of two substrates, DZ and phenanthrene, in a collection of human liver samples.

MATERIALS AND METHODS

Chemicals

DZ was purchased from USP Convention, Inc. The DZ metabolite NDZ was provided by Hoffmann–La Roche. 2-Oxoquanzepam (OQZ), used as internal standard, was provided by the Schering–Plough Corp. Testosterone and the hydroxytestosterone metabolite were obtained from Steraloids (Wilton, NH). Phenanthrene and B[a]P t-9,10-diol were purchased from the NCI Chemical Carcinogen Repository. 7-EC was purchased from the Sigma Chemical Co., and NADPH was obtained from Boehringer Mannheim.

Construction and Expression of Recombinant Baculovirus

Previously cloned [6] human 2B6 cDNA was used to obtain the coding region of 2B6 by partial digestion with *Eco*RI from pUC9. The fragment was ligated into pGEM-7Z, from which the 2B6 open reading frame was isolated by using *Xba*I and *Kpn*I, followed by blunt-ended repair with Klenow DNA polymerase and insertion into the *Bam*HI digested, blunt-ended site of pBlubac2 (Invitrogen). Recombinant virus was constructed according to the manufacturer's protocol (Invitrogen) and isolated using X-gal for color selection of recombinant virus in conjunction with Southern blot hybridization for the presence of the cDNA in the virus. After two rounds of plaque purification, the virus was amplified, and the presence of cDNA and the absence of contaminating virus in the high titer stock (used for expression) were checked simultaneously by polymerase chain reaction (PCR) using standard primers (Invitrogen). The 5′-end of the PCR product was sequenced to confirm the presence of the start codon of the coding sequence of P450 2B6 (*Bcl*I cuts three base pairs upstream of the start codon). The expression of P450 2B6 from baculovirus in Sf9 insect cells was performed as previously described [16].

Purification of 2B6

Purification of 2B6 was performed with a modification of the protocol of Gillam *et al.* [17]. Sf9 cell membrane fractions were diluted to a protein concentration of 2 mg/mL of buffer A (25 mM of phosphate buffer, pH 7.4, containing 20% glycerol, 1.0 mM of EDTA, and 1.0 mM of dithiothreitol). Sodium cholate (10% stock solution) was added dropwise up to 0.8%, and the solution was stirred for 2 hr at 4°. The 100,000 *g* supernatant was loaded onto a DEAE-Sephacel column (Pharmacia LBK). After washing with 200 mL of buffer A, 2B6 was eluted with buffer A containing 100 mM of potassium phosphate (pH 7.4). The 2B6 fraction was dialyzed against buffer A (pH 6.5) containing 0.5% Triton N101. The sample was applied to a 1.5 × 6 cm CM-Sepharose column (Pharmacia) that had been equilibrated with 25 mM of buffer A (pH 6.5). The column was washed sequentially with 200 mL of the equilibrate buffer. 2B6 was eluted with 100 mM of potassium phosphate buffer (pH 7.4) in buffer A. 2B6 was detected at 405 nm and collected with an autosampler collector. The detergent was reduced and protein was concentrated to 3 mg/mL using a 30 K membrane cutoff in an Amicon concentrator. The spectral properties [18] and protein content [19] were as described.

Immunization of Mice and Production of Hybridomas

Six female BALB/c mice were immunized by i.p. injection weekly for 3 weeks with 10 or 30 μg of purified baculovirus-expressed human 2B6 protein emulsified in 0.2 mL of complete Freund's adjuvant for the first injection, and then with incomplete Freund's adjuvant for subsequent injections [20]. Three days after the third injection, the mouse serum was examined by ELISA. The mice were killed and spleens were removed. The hybridization of spleen cells with the myeloma cells and hybridoma growth were described previously [14, 15].

Media, Hybridoma Growth, and Screening for Antibody Production

RPMI 1640 (Life Technologies, Inc.) supplemented with 20% fetal bovine serum (Bio Whittaker), 2 mM of L-glutamine, and 1 mM of sodium pyruvate was used as complete medium. The myeloma cell line NS-1, supplied by the Frederick Cancer Research and Development Center (FCRDC), resistant to 8-azaguanine, was used as a myeloma fusion partner. After fusion, newly formed hybrids were grown in complete medium containing 100 μM of hypoxanthine (H), 0.4 μM of aminopterin (A), and 16 μM of thymidine (T) (HAT medium) supplemented with 10% hybridoma cloning factor (IGEN, Inc.). RPMI 1640 (not supplemented) was used as the washing medium [14, 15, 21].

Screening for the Production of Antibodies

Indirect ELISA was performed by the generally accepted method [22] using alkaline phosphatase-conjugated goat F(ab′)2 fragment to mouse IgG (Fc specific), and mouse IgM (μ chain specific). Beckman immunoassay plates were coated with baculovirus-expressed 2B6 in 1× coating solution (KPI Laboratories) at 1.5 pmoL/well (100 μL). Due to the low yield of highly purified expressed protein, we used partially purified baculovirus-expressed 2B6 for the purposes of screening hybrids. We also used lysate from Sf9 cells infected with wild-type baculovirus, which went

through the same partial purification procedure to check for the specificity of the antibodies. As the hybrids began to grow, the spent medium from each individual well with hybrid growth was screened for the presence of antibody to 2B6 as described above. Each individual well on the plates that had cell growth was assigned a number. Each positive well was rescreened and then cloned using complete medium with 10% hybridoma cloning factor. Hybrids of interest were cloned at least three times.

Antibody Characterization

Isotyping was carried out using the Ouchterlony immunodiffusion technique as described [21] and provided by the mouse monoclonal antibody typing kits (The Binding Site).

Immunoblot-Quantitative Analysis of 2B6 in Human Liver Microsomes with MAb 49-10-20

An MAb-based IB method with enhanced chemiluminescence, SuperSignal™ CL-HRP Substrate System from Pierce, was used to determine the amount of 2B6 proteins in human liver microsomes. A standard curve of 2B6 IB was made with purified 2B6, which was loaded on a SDS–PAGE gel (180 V for 1 hr). 2B6 Protein was transferred onto nitrocellulose filters, and incubated with MAb 49-10-20 for 2 hr. MAb binding was detected using peroxidase-conjugated goat F(ab′)2 anti-mouse IgG (Fcγ fragment specific) and SuperSignal™ CL-HRP Substrate System. The 2B6 band probed by MAb 49-10-20 was measured by a densitometer. The amount of human liver 2B6 was determined by references to the standard 2B6.

MAb 49-10-20 Inhibition of P450 2B6

MAb 49-10-20 was preincubated with 25 or 50 pmol of vaccinia virus vector expressed 2B6, 3A4 as control, or human liver microsomes (100 pmol P450) in 100 μL of 50 mM potassium phosphate buffer (pH 7.4) at 37° for 5 min. The mixture was diluted with buffer to a final volume of 1 mL. The substrate was dissolved in 10 μL of methanol to which was added either 200 μM of DZ, 200 μM of phenanthrene, 200 μM of 7-EC, or 250 μM of testosterone to a final concentration as indicated. The reaction was initiated by the addition of NADPH (1 mM) at 37°. An amount of anti-lysozyme MAb HyHel (IgG) equivalent to MAb 49-10-20 was used as a control for nonspecific binding. Reactions were followed for 30 min and terminated with 5 vol. of dichloromethane (DCM). OQZ was used as internal standard for the metabolism of DZ, B[a]P t-9,10-diol as internal standard for the metabolism of phenanthrene, B[a]P *cis*-4,5-diol as internal standard for the metabolism of 7-EC, and 6-hydroxyprogesterone as internal standard for the metabolism of testosterone. Extracts of products were dissolved in mobile phase and analyzed immediately by reversed-phase HPLC. The metabolites formed were identified by comparing their retention times with authentic standards.

HPLC

HPLC was performed on a Hewlett–Packard model HP1050 liquid chromatograph equipped with an HP model 1050 autosampler, a ternary solvent delivery system, and a multiple-wavelength or dioarray detector, which are controlled by Hewlett–Packard HPLC 2D or 3D ChemStations software installed in a Compaq Deskpro 5133 personal computer. The metabolites of phenanthrene [9], DZ, testosterone [14], and 7-EC [23] were separated as previously described and identified with authentic standards.

RESULTS

Construction and Expression of 2B6 from a Recombinant Baculovirus Vector

Three separate viruses resulting from one transfection were plaque-purified, and the most actively expressed was amplified to a high-titer stock. Expression in insect Sf9 cells was obtained at an MOI between 0.5 and 5 pfu/cell. The highest 2B6 expression was observed with an MOI of 1.0 at 4–5 days postinfection. The expression of 2B6 decreased 5 days after infection with the virus (Fig. 1A). With an MOI of 1, the highest expression of 2B6 was obtained by adding 1 μg/mL of hemin (Fig. 1B). The infected Sf9 cells were harvested 4 days later. Expression of 2B6 was 354 pmol P450/mg of protein, or 85 nmol P450/L culture suspension ($\sim 1.6 \times 10^6$ cells/mL). The maximum absorbance peak bound to CO was at 452 nm. 2B6 represented 1.8% of total cellular protein.

Monoclonal Antibody Production, Screening, and Cross-reactivity

2B6 was found to be stable in Sf9 cells, which facilitated purification. The purification results are summarized in Table 1 and Fig. 2A. 2B6 was purified by a two-column procedure, as described in Materials and Methods. The procedure resulted in a 67% purification of 2B6 as determined with a computing densitometer (Molecular Dynamics). The recovery of 2B6 was 13.3%. A total of 811 hybridoma cells were obtained after immunization of mice with purified baculovirus-expressed human 2B6 followed by hybridization of the mouse spleen cells with myeloma cells. ELISA analysis found MAbs from 24 hybridomas positive to 2B6. Of the 24 hybridomas, eight grew well and were stable, and of the eight hybridomas, one, MAb 49-10-20, exhibited excellent immunoblotting activity and strongly inhibited the 2B6-catalyzed metabolism of phenanthrene and DZ by 91%. MAb 49-20-10, exhibiting the optimal desired properties, was selected for further study and development.

Table 2 shows an ELISA analysis of the binding specificity of MAb 49-10-20. The MAb 49-10-20 showed strong binding to both baculovirus and vaccinia virus expressed 2B6 and no significant cross-reactivity to expressed human 1A1, 1A2, 2A6, 2C8, 2C9, 2D6, 2E1, 3A4, and 3A5; rat 2A1, 3A1, 4A1, and 2B1; mouse 1A1 and 1A2; and rabbit

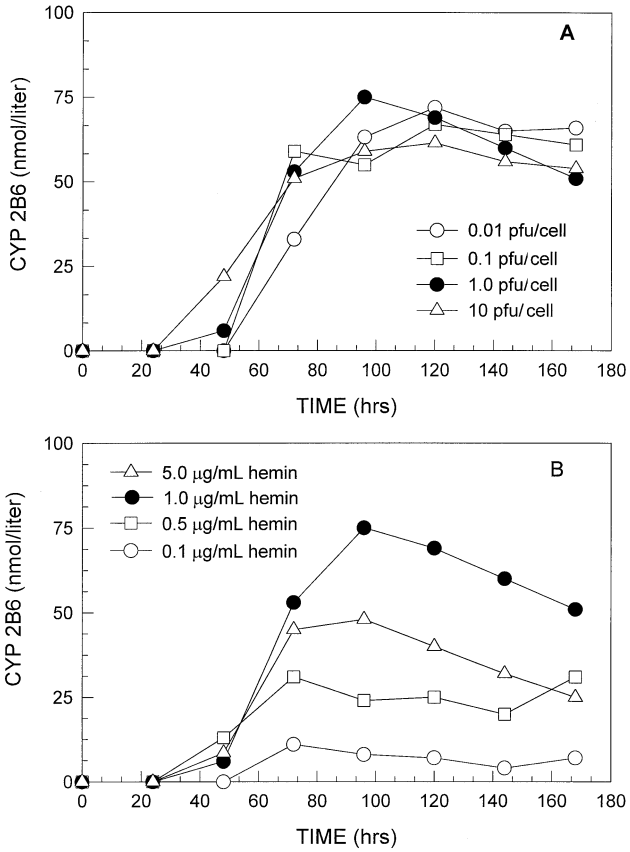

FIG. 1. Optimum expression of P450 2B6 in Sf9 insect cells. (A) Sf9 cells (1.6 × 10^6/mL) were infected with the indicated amounts of baculovirus. After 24 hr, hemin (1.0 μg of hemin/mL) was added as a hemin–albumin complex. (B) P450 2B6 expression in Sf9 cells in IPL-41 medium containing 0.1 to 5 μg/mL of hemin. Sf9 cells (1.6 × 10^6/mL) were infected with an MOI of 1 (1 pfu/cell). After 24 hr, hemin–albumin complex was added at the final concentration indicated. P450 2B6 expression was determined by CO difference spectra. Each value is the mean of six experiments.

4B1. Thus, MAb 49-10-20 shows extraordinarily high specificity for 2B6.

Figure 2B shows an IB analysis of MAb 49-10-20 specificity to baculovirus and vaccinia virus expressed 2B6 extract, purified human 2B6, and, as controls, human 3A4 and 2E1. MAb 49-10-20 showed a strong IB positive to 2B6 expressed from either baculovirus or vaccinia virus, and a

TABLE 1. Purification of baculovirus-expressed P450 2B6 in Sf9 insect cells

	P450 2B6 (nmol)	Specific content (nmol P450/mg protein)	Recovery (%)
Cell lysate	120	0.35	100
Solubilized membrane protein	85	1.8	70
DEAE column fraction	35	7.2	29
CM column fraction	16	13.4	13.3

TABLE 2. Cross-reactivity of MAb 49-10-20 to human and animal cytochrome P450s by ELISA

Human P450	O.D.	Animal P450	O.D.
vv Wild-type	0.08	vv Wild-type	0.08
bv Wild-type	0.06	bv Wild-type	0.06
vv h2B6	1.46	vv r2B1	0.02
bv h2B6	1.29	vv r3A1	0.06
bv h1A1	0.04	vv m1A1	0.08
vv h1A2	0.04	vv m1A2	0.08
vv h2A6	0.06	vv r2A1	0.07
vv h2C8	0.04	vv r4A1	0.06
vv h2C9	0.02		
vv h2D6	0.06		
vv h2E1	0.07	vv r4B1	0.09
vv h3A4	0.07		
vv h3A5	0.02		

ELISA was performed using expressed P450s from vaccinia virus (vv) and/or baculovirus (bv), 2 pmol/well of each P450. The MAbs (10 μg/mL) used were from culture fluid. The O.D. values are means of triplicate samples. Letters preceding P450s indicate: h, human; m, mouse; and r, rat.

negative IB to baculovirus-expressed human 2E1 and 3A4 and vaccinia virus expressed rat 2B1. MAb 49-10-20 did not show IB to expressed human 1A1, 1A2, 2A6, 2C8, 2C9, 2D6, and 3A5. The MAb showed a strong IB with human liver microsomes, making it a useful probe for the quantification of 2B6 in human liver. Although MAb

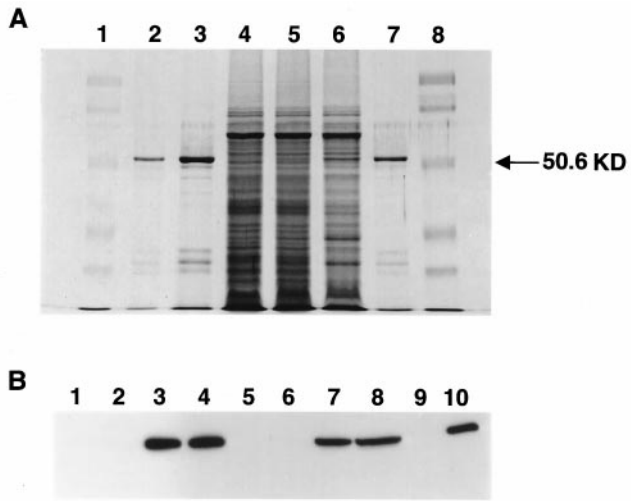

FIG. 2. (A) SDS–PAGE of baculovirus expressed, extracted, and purified P450 2B6 (Coomassie blue stained). Lane 1, MW standards; 2, purified P450 2B6 (10 μg); 3, purified P450 2B6 (30 μg); 4, Sf9 cells infected with wild-type baculovirus (30 μg); 5, Sf9 cell lysate containing expressed P450 2B6 (30 μg); 6, 0.8% cholate extract (30 μg); 7, purified P450 2B6 (20 μg); and 8, MW standards. (B) IB analysis of rat and human liver microsomes (HLM), and expressed human P450s using MAb 49-10-20. Lane 1, MW standards; 2, vaccinia virus expressed wild-type control (30 μg); 3, baculovirus expressed P450 2B6 (0.1 pmol); 4, vaccinia virus expressed P450 2B6 (0.1 pmol); 5, baculovirus expressed P450 3A4 (0.5 pmol); 6, baculovirus expressed P450 2E1 (0.5 pmol); 7, HLM 40 (50 μg); 8, HLM 41 (50 μg); 9, vaccinia virus expressed rat 2B1 (0.5 pmol); and 10, rat liver microsomes (50 μg).

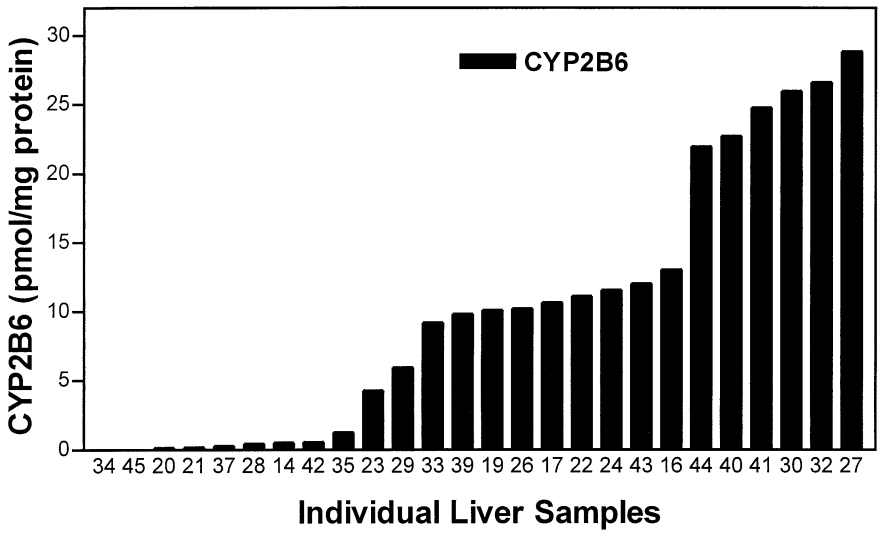

FIG. 3. Content of P450 2B6 in individual human liver microsomes probed with MAb 49-10-20. Purified P450 2B6 as standard was loaded on each gel. Human liver microsomes were loaded at 60 and 30 μg of protein. IB was performed as described in Materials and Methods.

49-10-20 did not react with expressed rat 2B1, it gave a positive band with rat liver microsomes. We do not know the nature of the antibody receptor in these rat microsomes.

Figure 3 shows the IB analysis of 2B6 protein in 26 samples of human liver microsomes as measured with MAb 49-10-20. The variability of 2B6 content in human liver microsomes was very large, ranging from nondetectable to 28 pmol/mg of human liver protein in the highest 2B6 content liver. The average 2B6 content was 10.9 pmol/mg protein with a standard deviation of 9.5. In comparison, an analysis of 3A4 content showed 3A4 at a much higher level ranging from 5 to 906 pmol/mg protein in human liver [14]. Figure 3 shows three populations of 2B6 levels among the 26 human liver microsome samples. We do not know the basis for this distribution, which may be related to different sample sources or genetic determinants.

Inhibitory Activity of MAb 49-10-20

Table 3 shows the strong inhibitory effects of MAb 49-10-20 on the 2B6-catalyzed metabolism of four 2B6 substrates: phenanthrene, DZ, 7-EC, and testosterone. In each case, the magnitude of this inhibition was large and consistent at 90–91%. In order to use the MAbs as inhibitory probes, their inhibitory activity should be high (in the range of 90%).

Figure 4 shows that the inhibitory effect of the MAb 49-10-20 was highly specific to 2B6, and that the MAb did not inhibit phenanthrene metabolism by eight other human P450s.

Analysis of 2B6 Contribution to Metabolism with MAb 49-10-20

Table 4 shows an analysis of the contribution of 2B6 to the metabolism of phenanthrene and DZ in human liver microsomes using MAb 49-10-20 as the inhibitory probe. The percent inhibition of 2B6-catalyzed metabolism by the 2B6-specific MAb 49-10-20 determines the contribution of 2B6 to the total metabolism of the substrate. The addition of MAb 49-10-20 caused an inhibition of phenanthrene metabolism by 3–28%, indicating considerable variability in the 2B6 catalyzed phenanthrene metabolism in ten liver samples. The relatively low level of the 2B6 role in phenanthrene metabolism is consistent with the low content of 2B6 in human liver and the phenanthrene metabolism catalyzed by numerous other competitive liver P450s [9].

DZ (Valium) is one of the most common clinically used drugs in the world. Human P450s catalyze DZ conversion to temazepam by C3-hydroxylation and to NDZ by N1-demethylation [10]. The 2B6, however, specifically cata-

TABLE 3. MAb 49-10-20 inhibition of DZ, phenanthrene, 7-EC, and testosterone metabolism by expressed cytochrome P450 2B6

Substrate	Metabolite measured	Activity* (nmol/min/nmol P450)		% Inhibition
		Control	MAb 49-10-20	
DZ	NDZ	3.25	0.33	90.2
Phenanthrene	PA 9,10-diol	21.7	1.95	91.0
7-EC	7-OH coumarin	3.22	0.32	90.1
Testosterone	16β-OH testosterone	1.98	0.18	90.8

Mab 49-10-20 was preincubated with vaccinia virus expressed 2B6 for 5 min at 37° and diluted to 1 mL with phosphate buffer. Substrates (200 or 250 μM) and NADPH (1 mM) were used. The reaction was carried out for 30 min. The separation and analysis of the metabolites were as described as Materials and Methods.

*Values are the means of duplicate determinations.

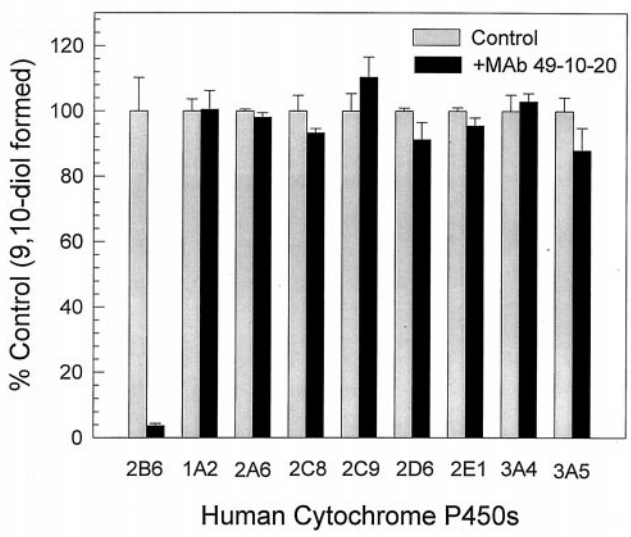

FIG. 4. Specificity of MAb 49-10-20 inhibition of phenanthrene metabolism by nine expressed human P450s. The incubation and separation were done as described in Materials and Methods. Data are the means ± SD of three incubations. Control activities were as follows (nmol PA 9,10-diol formed/min/nmol P450): 2B6 (6.58), 1A2 (4.30), 2A6 (3.24, PA 9-phenol), 2C8 (1.31), 2C9 (4.16), 2D6 (0.70, PA 9-phenol), 2E1 (1.33), 3A4 (0.72), and 3A5 (1.05).

lyzes DZ conversion to NDZ at a high activity level but does not catalyze temazepam formation. The 2B6-catalyzed formation of N-demethylation of DZ by expressed 2B6 was inhibited 90% by MAb 49-10-20 (Table 3). The inhibition by MAb 40-10-20 of DZ demethylation in ten human liver microsome samples ranged from 8 to 15%, with one sample inhibited by 23%. The low level of 2B6 contribution is expected because of the low level of 2B6 in human liver [24] and the very high level of 3A4, which is highly active in DZ demethylation [10]. A major metabolite of testosterone formed by expressed 2B6 is 16β-hydroxytestosterone [11]. MAb 49-10-20 does not inhibit its formation in human liver microsomes. Thus, although MAb 49-10-20 inhibits the conversion of testosterone to 16β-OH testosterone by expressed 2B6, the lack of inhibition of this reaction in human liver microsomes by MAb 49-10-20 indicates that 2B6 has no role in 16β-OH testosterone formation in human liver.

DISCUSSION

Cytochrome P450 2B6 is a human P450 isoform that metabolizes a variety of substrates that include DZ [10], 7-EC, ethylmorphine [12], testosterone [11], pentoxyresorufin, benzphetamine, aniline, and cyclophosphamide [3], phenanthrene [9], benzo[*a*]pyrene [25], and nicotine [26]. However, the contribution of 2B6 to the metabolism of these substrates in human liver is not known. In this study, we report the isolation of an MAb to 2B6, which is highly specific for both the binding and inhibition of human 2B6. MAb 49-10-20 can be used to determine the amount of 2B6 present in human liver and to measure the contribution of 2B6 to the metabolism of any 2B6 substrate in the presence of the multiplicity of P450s in human liver.

To obtain human P450 2B6 for use as an immunogen, we constructed a baculovirus expression system containing 2B6 cDNA to produce abundant amounts of 2B6, which was purified and used successfully as an immunogen for MAb preparation. Monoclonal antibodies from 24 hybrids exhibited immunobinding to 2B6 by ELISA and IB. We selected for study MAb 49-10-20, which yields a strong IB with 2B6 with very high specificity. This MAb was used to determine the amount of 2B6 protein in human liver. The immunobinding MAb can determine the distribution of 2B6 in individuals and be used to phenotype possible polymorphisms of the 2B6 protein in humans. We found a wide distribution of 2B6 in 26 human liver samples. The amount of 2B6 in liver was 1/30 or less than the 3A4 level. The level of 2B6 was also reported to be considerably less than 1A2, 2A6, 2C, 2D6, and 2E1 [24]. MAb 49-10-20 was also a potent and specific inhibitor of 2B6 catalysis. Thus, MAb 49-10-20 inhibited by 90% the metabolism of phenanthrene, DZ, 7-EC, and testosterone.

In previous studies, we reported the isolation and inhibitory activity of MAbs to human 3A4 [14], 2E1 [15], and

TABLE 4. MAb 49-10-20 inhibition of phenanthrene and DZ metabolism by human liver microsomes

HLM	PA 9,10-diol formed*		% of Inhibition	NDZ formation*		% of Inhibition
	Control	MAb 49-10-20		Control	MAb 49-10-20	
HL39	4.87	2.80	42.5	2.39	1.83	23.5
HL40	3.44	2.81	18.1	1.24	1.12	9.7
HL41	3.47	2.85	17.9	2.33	2.18	6.4
HL42	2.80	2.01	28.2	1.79	1.58	11.4
HL43	2.97	2.19	26.1	1.61	1.37	15.4
HL44	1.82	1.57	14.0	0.50	0.47	6.1
HL45	0.63	0.61	2.9	0.123	0.11	8.0
HL59	4.26	3.92	7.9	0.18	0.16	10.3
HL60	0.62	0.56	9.3	0.078	0.071	9.5
HL72	0.90	0.82	8.6	0.13	0.12	8.1

MAb 49-10-20 was preincubated with HLM for 5 min at 37° and diluted to 1 mL with phosphate buffer. Phanenthrene (200 μM) or DZ (200 μM) and NADPH (1 mM) were used. The reaction was carried out for 30 min. The separation and analysis of the metabolites were as described as Materials and Methods.

*Values, expressed in nmol/min/nmol P450, are the means of duplicate determinations.

2D6 [27]. The 3A4 enzyme is considered the major contributor to the metabolism of a very large variety of clinically used drugs. The 2D6 is polymorphic and, like 3A4, metabolizes a large number of substrates [2, 24]. 2E1 metabolizes small molecules in addition to some larger molecular size drugs. In contrast, the relatively few studies related to 2B6 have indicated that the known substrates of 2B6 are considerably less than those for 3A4, 2E1, and 2D6.

The use of baculovirus [16] or vaccinia [25] expression systems for the production of single cytochrome P450s permits the determination of the substrate and product specificity of the individual P450. This important information, however, does not yield information on the quantitative contribution of the individual P450 to the metabolism of a substrate in a tissue containing multiple P450s. This is especially true when more than one or many P450s engage in the metabolism of a particular substrate. Other factors determining the role of an individual P450 in a tissue are the amount of the P450 present in the liver or other tissues and the amount of other P450s present that compete for the same substrate. MAbs can determine the amount of P450 protein present in the tissue, and the amount of inhibition by an MAb specific for a single P450 defines the contribution of that P450 in the presence of multiple P450s. The IB, by measuring the amount of a specific P450 protein, can elucidate polymorphisms that are not under the control of structural genes but rather can arise from changes in regulatory mechanisms, or in regulatory genes that result in unique P450 phenotypes. Thus, MAbs to P450 represent a powerful tool for mapping P450 structure and function.

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