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RAPID COMMUNICATION

Inhibitory Monoclonal Antibodies to Human Cytochrome P450 2D6

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ABSTRACT. Two monoclonal antibodies (MAbs) have been isolated that bind to human P450 2D6 and inhibit 2D6 catalyzed bufuralol 1-hydroxylation by 90%. One but not both of the MAbs immunoblotted 2D6. The MAbs were highly specific to 2D6 and did not cross-react with other P450s. Inhibitory monoclonal antibodies will be useful for determining the contribution of 2D6 to the metabolism of a wide variety of 2D6 and other P450 substrates in human tissues containing multiple P450s. BIOCHEM PHARMACOL **54**;1:15–17, 1997.
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KEY WORDS. cytochrome P450 2D6; inhibitory monoclonal antibodies; human 2D6 P450; baculo-expressed P450; bufuralol

The large multiplicity of human P450 forms, their different overlapping substrate and product specificity, and their heterogeneous distribution require methods for determining tissue content and their contribution to the metabolism of specific drugs and other xenobiotics as well as endobiotics. The human P450 2D6 has an especially important position among the human P450s since it functions broadly in the metabolism of a large variety of drugs [1, 2], as well as being polymorphic in the human population. Individuals who are extensive metabolizers with normal P450 2D6 levels excrete 10–200 times more of the urinary metabolite of debrisoquine, 4-hydroxy-debrisoquine, than do poor metabolizers. Family studies indicate that the phenotype is due to an autosomal recessive trait with an incidence between 5 and 10%. This polymorphism can be relevant to a large number of commonly used drugs [1, 2]. Since many drugs are metabolized by 2D6, as well as other P450s, it is important to know the amount of the drug metabolism catalyzed by 2D6 relative to that metabolized by other P450s.

MAbs§ are precise, stable, and highly specific reagents that can quantitatively measure the amount of individual P450s. In addition, an MAb highly inhibitory to the enzyme activity of a specific P450 can measure the quantitative contribution of that individual P450 to the metabolism of diverse P450 substrates in the presence of multiple forms of P450 contained in a tissue [3]. Previously, we reported the isolation of inhibitory MAbs to human P450s 3A4 and 2E1. These have been used to measure the amount and contribution of human P450 2E1 [4] and 3A4 [5] mediated drug metabolism to a variety of substrates. Here we report the isolation of two MAbs, 512-1-8 and 50-1-3, that inhibit human P450 2D6 by 90%. These MAbs can identify the extent to which currently used drugs and those in development are metabolized by 2D6 and thus will permit the exclusion of drugs significantly metabolized by 2D6 from 2D6-deficient individuals. It will also elevate the degree of precision for the screening of drugs, drug choice, and drug dosage related to 2D6.

MATERIALS AND METHODS

Human P450 2D6 cDNA was inserted into and expressed from a baculovirus or vaccinia vector [6]. Cell pellets were pooled, diluted 1:2 with buffer A (0.1 M KPI pH 7.4, 20% glycerol, 1 mM EDTA, 0.35 mM DTT, 0.4 mM AEBSF, and 2 μM leupeptin), sonicated, and disrupted. Protein concentration was 10–15 mg/mL, and the cell suspension was ultracentrifuged at 100,000 *g*. The resulting pellet was resuspended to a protein concentration of 4 mg/mL. Sodium cholate was added to a concentration of 1%, and the suspension was stirred for 2.5 hr. The suspension was re-centrifuged for 60 min at 100,000 *g*, and the supernatant containing the P450 2D6 hemoprotein was dialyzed against 4 vol. of buffer A containing no sodium cholate. The extract with approximately 10 μg of 2D6 was used for the immunization followed by the isolation of hybridomas and MAbs as described [4, 5]. Control extracts and 2D6 containing extracts were compared to select 2D6 specific positive clones. The ELISA and immunoblot assay used are common and have been described previously [4, 5].

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§ *Abbreviations:* MAbs, monoclonal antibodies; DTT, dithiothreitol; AEBSF, 4-(2-Aminoethyl)-benzenesulfonyl-flouride; and IB, immunoblot.

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TABLE 1. MAbs to human P450 2D6: Lack of cross-reactivity to 12 other P450s

P450	MAb 512-1-8	MAb 50-1-3
bv*Wild	0.05–0.07	0.05–0.07
bv h2D6	1.38	0.95
2D6 mics	0.46	0.31
h1A2, h2B6, h2C8	<0.08	<0.05
h2C9, h2E1, h3A4	<0.08	<0.05
h3A5, m1A1, m1A2	<0.08	<0.05
r2A1, r2B1, rb4B1	<0.08	<0.05

* bv = baculovirus expressed; 2D6 mics = 2D6 microsomes from lymphoblastoid cells; m = mouse; r = rat; rb = rabbit; h = human. All P450s were vaccinia expressed except those noted as bv.

MAb Inhibition of Human P450 2D6 Catalyzed Bufuralol Metabolism

Ascites fluid containing MAbs at a protein concentration ranging from 10 to 500 μg/mL was preincubated with 10–50 pmol of human P450 2D6 expressed in microsomes of lymphoblast cells in 100 μL of 50 mM potassium phosphate buffer (pH 7.4) at 37° for 5 min, and the mixture was diluted with potassium phosphate buffer to 0.97 mL. Bufuralol or phenanthrene and NADPH (1 mM) were added to a final volume of 1.0 mL to initiate the reaction. A control MAb made against unrelated egg white lysozyme was used as a negative control. For cross-reactivity studies of MAb inhibition activity, phenanthrene was used as the substrate for all of the P450s tested [4, 5].

RESULTS

More than 700 hybridoma clones were formed from the fusion of myeloma cells with spleen cells from mice immunized with expression extracts containing P450 2D6. Twelve hybridomas produced MAbs that bound the P450 2D6. Two of the MAbs, 512-1-8 and 50-1-3, were found to be highly specific for human P450 2D6 as measured by their binding by ELISA (Table 1) to P450 2D6 and their lack of binding to twelve other cytochrome P450s expressed from vaccinia or baculovirus vectors. These were human P450s 1A2, 2B6, 2C8, 2C9, 2E1, 3A4, and 3A5, mouse 1A1 and 1A2, rat 2A1 and 2B1, and rabbit 4B1. Figure 1 shows an IB assay of MAb 512-1-8 that was positive with 2D6 and showed no cross-reactivity with human P450s. MAb 50-1-3, although inhibitory to 2D6, did not yield an immunoblot with 2D6 nor did any of the P450s tested in Fig. 1.

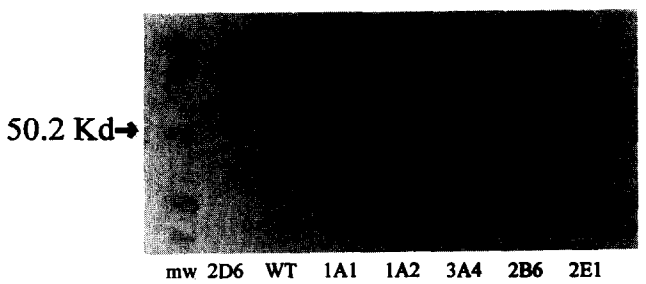

FIG. 1. Immunoblot of MAb 512-1-8 with human P450s. Each well was loaded with 2 pmol of baculovirus expressed P450. Abbreviations: MW = molecular weight standards, and WT = wild-type control (no P450).

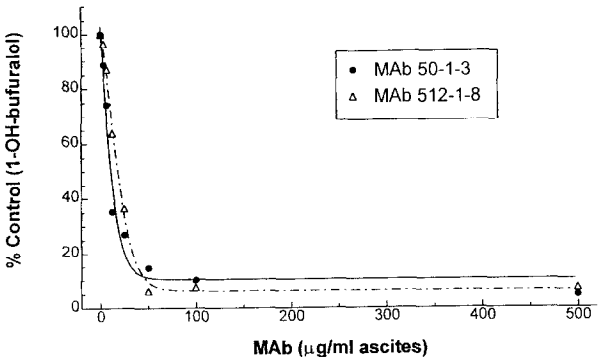

FIG. 2. Inhibition by MAbs of human 2D6 catalyzed bufuralol 1-hydroxylation. Bufuralol metabolism was performed as previously described [7]. Control activity = 1.46 nmol/min/nmol P450.

Highly Specific MAb 512-1-8 and 50-1-3 Inhibition of P450 2D6 Enzyme Activity

Bufuralol is commonly used as a substrate to measure 2D6 enzyme activity [7]. Figure 2 shows the strong inhibitory activity of MAb 512-1-8 and MAb 50-1-3 on P450 2D6 enzyme activity as measured by 1-OH bufuralol formation. Thus, P450 2D6 activity was inhibited by both MAb 512-1-8 and MAb 50-1-3 by more than 90%. MAb 512-1-8 exhibited both strong immunoblotting and 90% inhibitory activity of human P450 2D6, whereas MAb 50-1-3 was a strong inhibitor but did not immunoblot the 2D6.

Phenanthrene is a substrate metabolized by all the P450s examined. Figure 3 shows the metabolism of phenanthrene by 2D6 and six other human P450s. MAb 512-1-8 almost completely inhibited 2D6 catalyzed phenanthrene metabolism but had no effect on phenanthrene metabolism catalyzed by 1A2, 2B6, 2C8, 2C9, 2E1, and 3A4. MAb 50-1-3 exhibited almost identical inhibitory activity to 2D6 and a similar lack of cross-reactivity to other human P450s.

DISCUSSION

Cytochrome P450s are a ubiquitous class of enzymes that are the primary instruments for the metabolism of xenobiotics such as drugs, carcinogens, and environmental chemicals as well as several classes of endobiotics such as steroids and prostaglandins. There are multiple forms of the P450s, each with its own specificity. In many cases, a substrate xenobiotic drug or carcinogen is metabolized by several of the cytochrome P450s. Cytochrome P450 2D6 is a major and well characterized polymorphic P450 in the human population [1, 2]. This enzyme has also been called debrisoquine hydroxylase [1]. The polymorphism of 2D6 has wide clinical consequences, especially in the use of cardiovascu-

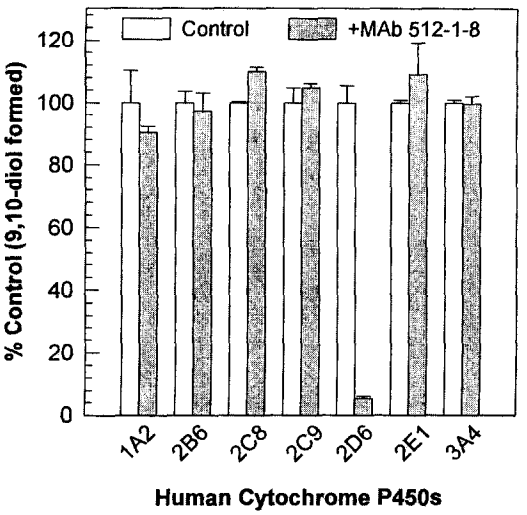

FIG. 3. MAb 512-1-8 inhibition of phenanthrene metabolism by 2D6 and lack of cross-reaction with other P450s. Phenanthrene metabolism was performed as previously described [8]. Control activities (in nmol/min/nmol P450) were as follows: 1A2 (4.30); 2B6 (6.58); 2C8 (1.31); 2C9 (4.16); 2D6 (0.70); 2E1 (1.33) and 3A4 (0.72).

lar drugs or drugs for psychiatric disorders [9] and for anti-arrhythmic drugs [10]. Many of these drugs have a large potential for adverse side-effects, and some of these effects in different individuals may result from the variability in 2D6 catalyzed disposition of the drug. Since P450s other than 2D6 may metabolize drugs that are also metabolized by 2D6, it is necessary to determine the contribution of 2D6 to the total metabolism of the drug. This is especially relevant to 2D6-defective individuals and their drug therapy. Drugs can also be screened for susceptibility to 2D6 metabolism with the specific inhibitory antibodies. The inhibitory based MAb analysis of the human P450 2D6 role in the metabolism of drugs will be extremely useful for determining appropriate drug selection as well as dosage level to individuals who vary in 2D6 levels. In general, the inhibitory monoclonal antibody [4, 5] can determine the percentage of a drug's metabolism catalyzed by a specific P450 relative to that catalyzed by other P450s. This information will be useful in drug development. MAbs to individual P450s can define the role of each P450 for a given drug. Inhibitory MAbs have been developed for P450 1A1 [3], 2E1 [4, 5], 3A4 [5], and 2D6 (this report). A comprehensive library of inhibitory monoclonal antibodies to the human P450s will present an opportunity to clearly define the function and interaction of individual P450s in the metabolism of specific drugs.

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