Short Communication

ROLE OF MOUSE CYP2E1 IN THE O-HYDROXYLATION OF P-NITROPHENOL: COMPARISON OF ACTIVITIES IN HEPATIC MICROSONES FROM CYP2E1(−/−) AND WILD-TYPE MICE

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ABSTRACT:
Enzymatic activities are routinely used to identify the contribution of individual forms of cytochrome P450 in a particular biotransformation. P-nitrophenol O-hydroxylation (PNPH) has been widely used as a measure of CYP2E1 catalytic activity. However, rat and human forms of CYP3A have also been shown to catalyze this activity. In mice, the contributions of CYP3A and CYP2E1 to PNPH activity are not known. Here we used hepatic microsomes from Cyp2e1(−/−) and wild-type mice to investigate the contributions of constitutively expressed and alcohol-induced murine CYP2E1 and CYP3A to PNPH activity. In liver microsomes from untreated mice, PNPH activity was much greater in wild-type mice compared with Cyp2e1(−/−) mice, suggesting a major role for CYP2E1 in catalyzing PNPH activity. Hepatic PNPH activities were not significantly different in microsomes from male and female mice, although the microsomes from females have dramatically higher levels of CYP3A. Treatment with a combination of ethanol and isopentanol resulted in induction of CYP3A proteins in wild-type and Cyp2e1(−/−) mice, as well as CYP2E1 protein in wild-type mice. The alcohol treatment increased PNPH activities in hepatic microsomes from wild-type mice but not from Cyp2e1(−/−) mice. Our findings suggest that in untreated and alcohol-treated mice, PNPH activity may be used as a specific probe for CYP2E1 and that constitutively expressed and alcohol-induced forms of mouse CYP3A have little to no role in catalyzing PNPH activity.

The O-hydroxylation of p-nitrophenol (PNPH) is an activity often used as a measure of CYP2E1 catalytic activity in hepatic microsomes (Louis et al., 1994; Morimoto et al., 1995; Kessova et al., 1998; Longo et al., 2000; Allis et al., 2001; Sapone et al., 2003). However, this activity is also catalyzed by expressed forms of human and rat CYP3A (Zerilli et al., 1998; Kobayashi et al., 2002) and rat CYP1A2 (Kobayashi et al., 2002). A role for CYP3A in this reaction was confirmed in intact rats and rat hepatocytes by studies showing that dexamethasone treatment, which induces CYP3A but not CYP2E1 (Sinclair et al., 2000), similar to the findings of Sakuma et al. (2002). This work was supported in part by the Department of Veterans Affairs and National Institutes of Health (grant AA12898).

ABBREVIATIONS: PNPH, p-nitrophenol O-hydroxylation.

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from tails. Three hundred ninety-four of 398 markers tested were identical in wild-type and Cyp2e1(−/−) mice. All animal protocols were approved by the internal animal review boards at the Department of Veterans Affairs Medical and Regional Office Center and at Dartmouth College. At the commencement of the experiment, mice weighed 18 to 26 g and were 3 months old. All mice were fed control liquid diet (Lieber-DeCarli) containing maltose-dextrin for an initial 2-day period. Animals to be treated with the alcohols were then administered 2.8% (w/v) ethanol and 0.4% (w/v) isopentanol for 7 days in a liquid diet. This diet was made isocaloric with the control diet by adjusting the maltose-dextrin content in the alcohol-containing diet, as directed by the manufacturer. Carbon dioxide was used to anesthetize the mice before they were sacrificed by cervical dislocation. Microsomes were prepared from liver homogenates as described (Sinclair et al., 2000a).

**Immunochemical Analyses of Cytochromes P450.** CYP2E1 was measured immunochemically in hepatic microsomes, as described (Sinclair et al., 2000a), using a commercially available polyclonal antibody against human CYP2E1 (Oxford Biomedical Research). Hepatic microsomes from each Cyp2e1(−/−) mouse used in this study were analyzed for CYP2E1 to confirm the genotype. CYP3A was measured immunochemically, as described (Sinclair et al., 2000a). The immunoblots were scanned into Adobe Photoshop with an HP Precision Scanner (Hewlett Packard, Palo Alto, CA) and quantified using OneDScan (Scanalytics Inc., Fairfax, VA). When samples were analyzed on separate gels, an identical sample was included in each gel, and the values were normalized to this sample. Each band was scanned in triplicate. The error bars in the densitometric graphs represent the range of mean values from two animals.

**Additional Assays.** PNPH activity was measured in liver microsomes, as described (Louis et al., 1994). Proteins were determined by the procedure of Lowry et al. (1951), using bovine serum albumin as a standard.

**Statistical Analyses.** Values represent the means and S.E.M. or range, as indicated in the legends. PNPH data from four or more mice per treatment were first analyzed by analysis of variance, followed by the Student Newman-Keuls multiple comparisons test. *p* Values <0.05 indicated significance.

### Results and Discussion

As found previously (Sinclair et al., 2000a), hepatic levels of CYP3A protein were very low in adult male Cyp2e1(−/−) mice (Fig. 1). Similar low hepatic levels were also observed in adult male wild-type mice (Fig. 1). Hepatic levels of CYP3A were over 4-fold greater in females than in males, in both Cyp2e1(−/−) and wild-type mice (Fig. 1). Yet, in untreated mice, PNPH activity was similar in hepatic microsomes from male and female mice (Fig. 2). These findings suggest that constitutive, female-specific forms of CYP3A, CYP3A41 and CYP3A44 (Sakuma et al., 2000, 2002), do not contribute to PNPH activity. The PNPH activities observed for untreated male mice in this study (Fig. 2) are similar to those reported by Carlson (2003), in studies comparing hepatic PNPH activities in untreated wild-type and Cyp2e1(−/−) male mice.

Treatment with a combination of ethanol and isopentanol for 7 days increased PNPH activity in hepatic microsomes from wild-type mice of both genders (Fig. 2, *p* < 0.001). In contrast, treatment of Cyp2e1(−/−) mice with the alcohols resulted in no increase in PNPH activity. As found previously in Cyp2e1(−/−) mice (Sinclair et al., 2000a), the alcohol treatment increased a higher-molecular-weight CYP3A protein (Fig. 3A), which is most likely CYP3A11 (Sakuma et al., 2000). Our findings in adult male Cyp2e1(−/−) mice, that the alcohol treatment did not increase PNPH activity (Fig. 2) despite a 5-fold increase in CYP3A protein (Fig. 3A, A and B) suggest that CYP3A induced by the alcohols has no appreciable PNPH activity. In male wild-type mice, as in the knockout mice, the alcohol treatment increased only the higher molecular weight CYP3A protein, whereas in female wild-type mice, the alcohol treatment increased both the higher- and lower-molecular-weight forms of CYP3A (Fig. 3A). In mice of both genders, the alcohol treatment appeared to cause a greater increase in CYP3A in wild-type mice compared with Cyp2e1(−/−) mice (Fig. 3, A and B).

We confirmed by immunoblots that there was no CYP2E1 protein in any of the microsomal samples from Cyp2e1(−/−) mice used in these studies (Fig. 3, C and D for two representative mice of eight total). In both male and female wild-type mice, pretreatment with ethanol plus isopentanol for 7 days resulted in 3- to 4-fold increases...
in hepatic microsomal CYP2E1 protein (Fig. 3, C and D). Our findings that the fold increases in CYP2E1 protein (Fig. 3D) are similar to the fold increases in PNPH activity (Fig. 2) and that the alcohol treatment did not increase PNPH activity in *Cyp2e1*(/H11002/)/H11002/) mice suggest that CYP2E1 is the major catalyst of PNPH activity in hepatic microsomes from alcohol-treated mice.

In contrast to the findings in rats (Zerilli et al., 1998; Kobayashi et al., 2002; DiPetrillo et al., 2002) and humans (Zerilli et al., 1998), our findings that female-specific and alcohol-induced forms of mouse CYP3A do not contribute appreciably to PNPH activity suggest that minor differences between species in the amino acid sequences of CYP3A are responsible. Differences in substrate specificities of CYP3A have been detected for different forms of CYP3A within the same species. Although 6β-hydroxylation of testosterone is widely used as a measure of CYP3A in several species, a newly identified human form of CYP3A, CYP3A43, when expressed in baculovirus, has very low activity (Domanski et al., 2001). Even single amino acid changes within cytochromes P450 have been shown to dramatically alter their activities. For example, in CYP2E1, a Leu209Ile mutation caused a major decrease in its intrinsic activity for PNPH (Spatzenegger et al., 2003).

In summary, we have found that hepatic PNPH activities were similar in untreated male and female mice, despite dramatically greater levels of CYP3A in females. Alcohol treatment caused an increase in PNPH activity in wild-type mice, but not in *Cyp2e1(−/−)* mice, despite induction of CYP3A in both lines. The results suggest that constitutively expressed and alcohol-induced forms of mouse CYP3A have little to no role in hepatic PNPH activity. Therefore, in microsomes isolated from the livers of untreated and alcohol-treated mice, PNPH activity can be used as a specific probe for CYP2E1.

### References


