INDUCTION OF MOUSE CYP2J BY PYRAZOLE IN THE EYE, KIDNEY, LIVER, LUNG, OLFATORY MUCOSA, AND SMALL INTESTINE, BUT NOT IN THE HEART

QIANG XIE, QING-YU ZHANG, YAN ZHANG, TING SU, JUN GU, LAURENCE S. KAMINSKY, AND XINXIN DING

Wadsworth Center, New York State Department of Health and School of Public Health, State University of New York at Albany, Albany, New York

(Received May 22, 2000; accepted July 11, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:

We have recently shown that rat CYP2J4 is inducible by pyrazole in liver, small intestine, and olfactory mucosa. The aim of the present study was to determine whether mouse CYP2Js are also inducible by pyrazole, which was known to induce CYP2A5 in mouse liver and kidney, but not in lung or olfactory mucosa. CYP2J proteins were detected in mouse liver, lung, kidney, heart, eye, olfactory mucosa, and small intestine by immunoblot analysis with an anti-CYP2J4 antibody. The microsomal level of the CYP2J4-related P450s in various mouse tissues ranked in the order of small intestine > olfactory mucosa > liver > kidney ≥ heart > lung > eye. Induction of the CYP2J proteins was observed in the eye, liver, lung, kidney, olfactory mucosa, and small intestine, but not in the heart, after daily i.p. injection of pyrazole at 120 or 200 mg/kg for 3 days. CYP2J proteins were induced similarly in C57BL/6 and DBA/2 mice. CYP2A5 was detected in the small intestine in addition to liver and olfactory mucosa; however, treatment with pyrazole induced CYP2A5 in the liver, but not in the olfactory mucosa or the small intestine. Induction of CYP2J mRNAs was also observed by RNA blot analysis with a CYP2J4 cDNA probe. RNA-polymerase chain reaction analysis showed that, in both untreated and pyrazole-treated mice, CYP2J5 was expressed in the kidney and liver, but not in the other tissues examined, whereas CYP2J6 was detected in all tissues examined. The different tissue selectivities in CYP2A5 and CYP2J induction by pyrazole suggest involvement of different regulatory mechanisms.

Ten CYP2J1 isoforms have been reported so far in the cytochrome P450 gene superfamily (Nelson et al., 1996), including rabbit CYP2J1 (Kikuta et al., 1991); human CYP2J2 (Wu et al., 1996); rat 2J3 and 2J4 (Wu et al., 1997; Zhang et al., 1997); mouse 2J5 (Ma et al., 1999), 2J6, 2J7, 2J8, and 2J9; and rat CYP2J10 (Scarborough et al., 1999). The metabolic activity of CYP2J2, 2J3, 2J4, and 2J5 has been characterized recently, and these isoforms were all active toward arachidonic acid (Wu et al., 1996, 1997; Zhang et al., 1997; Ma et al., 1999), forming metabolites believed to be important for cellular functions (Scarborough et al., 1999). In addition, CYP2J4 was active in the biosynthesis of retinoic acid from retinal (Zhang et al., 1998).

The potential biological importance of the CYP2Js prompted our recent study on their inducibility in rats by foreign compounds (Zhang et al., 1999). We found that pyrazole, a known inducer of mouse CYP2A5 and CYP2E1 (Su et al., 1998), significantly induced CYP2A5 expression in rat small intestine, olfactory mucosa, and liver. Interestingly, the induction of CYP2A5 by pyrazole occurred only in mice, and not in rats (Béréziat et al., 1995), and in mice, pyrazole induced CYP2A5 only in the liver and kidney, but not in the olfactory mucosa or lung (Su et al., 1998). Thus, it is important to determine whether pyrazole will induce CYP2J expression in mice and whether such induction is tissue selective.

The tissue distribution of mouse CYP2J5 and CYP2J6 has been examined recently. CYP2J5 is primarily expressed in the kidney and at lower levels in the liver, but not in the stomach, small intestine, muscle, lung, heart, colon, testis, or brain (Ma et al., 1999). CYP2J6 mRNA was detected mainly in the small intestine and at lower levels in the heart, lung, brain, kidney, and liver (Scarborough et al., 1999); but a full-length CYP2J6 protein was not detected in mouse tissue microsomes with an anti-peptide antibody, presumably because of instability of the protein. The tissue distribution of the other mouse CYP2J forms has not been characterized (Scarborough et al., 1999).

In this study, the expression and pyrazole inducibility of the mouse CYP2Js in the eye, heart, liver, lung, kidney, olfactory mucosa, and small intestine were examined. CYP2J proteins were detected on immunoblots with a polyclonal anti-CYP2J4 antibody. Evidence is provided that pyrazole induces CYP2J proteins in the eye, liver, lung, kidney, olfactory mucosa, and small intestine, but not in the heart. The induction of CYP2J proteins was accompanied by increases in CYP2J mRNA levels, as demonstrated by RNA-blot analysis with a CYP2J4 cDNA probe. Furthermore, isoform-specific RNA-PCR analysis and differential endonuclease digestion of CYP2J cDNA fragments showed that although CYP2J5 was expressed only in the kidney and liver, CYP2J6 was detected in the eye and olfactory mucosa, as well as the other tissues examined in both untreated and pyrazole-treated mice.

Materials and Methods

Animal Treatment and Immunoblot Analysis. Two-month-old male C57BL/6 and DBA/2 mice (about 20 g in body weight, obtained from Charles River Laboratories, Wilmington, MA) were used in this study. Animals were...
Hybridization with a 32P-labeled 0.9-kb CYP2J4 cDNA probe (nucleotides 172-1104 in the coding region; Zhang et al., 1997) was carried out at 58°C for 20 min. with a 32P-labeled ethidium bromide staining after agarose gel electrophoresis. Poly(A) was determined spectrally. The integrity of the RNA samples was assessed by TRIzol reagent (Life Technologies, Grand Island, NY). RNA concentration and protein concentrations of the prestained protein size markers from Bio-Rad (Hercules, CA). Protein weights of the proteins detected on immunoblots were estimated with use of the prestained protein size markers from Bio-Rad (Hercules, CA). Protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, IL) with bovine serum albumin as the standard.

Results

The tissue distribution of the mouse CYP2J proteins has been examined in a previous study by Ma et al. (1999) with an anti-peptide antibody to CYP2J5, which detected CYP2J5 in kidney and liver, but not in other tissues examined. The same group also indicated in a recent review (Scarborough et al., 1999) that an anti-peptide antibody to CYP2J6 failed to detect a full-length CYP2J protein in mouse tissue microsomes. In the present study, a polyclonal anti-rat CYP2J4 antibody was used to detect mouse CYP2J proteins on immunoblots. Of the five different mouse CYP2J isoforms reported (Scarborough et al., 1999), CYP2J6 has the highest amino acid sequence identity (94%) to rat CYP2J4 and would be recognized by the polyclonal anti-CYP2J4 antibody. The anti-CYP2J4 antibody may also recognize the other mouse CYP2J isoforms, which have 70 to 75% sequence identity with CYP2J6 (Scarborough et al., 1999).
As shown in Fig. 1, A–C, CYP2J-related proteins, which had similar migration positions as heterologously expressed CYP2J4 (data not shown), were detected in all tissues examined, including olfactory mucosa, eye, heart, liver, lung, kidney, and small intestine. Two bands, which were of about equal intensity but were not well resolved, were detected in the small intestine. In the liver, lung, heart, and kidney, the top band was much more prominent than the lower band, and only the top band was detected in the eye and olfactory mucosa. Quantitative analysis of the blots shown in Fig. 1, A–C, and additional ones from experiments not presented indicated that small intestinal microsomes had the highest level of CYP2J4-related proteins (both bands combined), more than 10-times higher than that of the liver. The microsomal level of CYP2J4-related proteins in the other tissues ranked in the order of olfactory mucosa > liver > kidney ≥ heart > lung > eye. It should be noted that S9 fractions, not microsomes, of the olfactory mucosa were analyzed in Fig. 1A. An additional band, with much lower migration position than the CYP2J proteins, was detected in heart microsomes (Fig. 1B); it remains to be determined whether it is a degradation product of a CYP2J protein.

Also shown in Fig. 1, treatment of C57BL/6 mice with pyrazole at 200 mg/kg (i.p.) for 3 days led to increases in the level of CYP2J proteins in all tissues examined, except the heart. Quantitative immunoblot analysis (Fig. 1D) of the relative levels of the CYP2J proteins in vehicle- or pyrazole-treated animals confirmed that there was no induction in the heart and indicated that the highest induction (about 18-fold) occurred in the olfactory mucosa. The extent of induction in the other tissues was about 4- to 10-fold. The two CYP2J-related bands were quantitated together. The intensity of both bands was increased in the small intestine after pyrazole treatment. In the liver, lung, and kidney, the top band was clearly induced, but it was difficult to judge whether the lower band was also induced because of its poor separation from the much more prominent top band. In other immunoblot experiments not shown, no difference was observed between vehicle- and pyrazole-treatment groups in the levels of NADPH-cytochrome P450 reductase for each tissue examined.

The dose-response of CYP2J protein induction by pyrazole was examined in liver, small intestine, and olfactory mucosa. As shown in Fig. 2, higher induction was observed in the olfactory mucosa than in the other tissues at all three doses tested. The induction in liver and small intestine was very small (≤2-fold) at 80 or 120 mg/kg. In all three tissues, a large increase in the extent of induction was seen between 120 and 200 mg/kg.

The induction of CYP2J proteins in liver, olfactory mucosa, and small intestine by pyrazole was also observed in DBA/2 mice, as shown in Fig. 3 for an experiment with 120 mg/kg pyrazole. Interestingly, in the same experiment, a large induction of CYP2A5 was seen in liver, with no induction in the olfactory mucosa or the small intestine. Similar results on CYP2A5 induction were also obtained with C57BL/6 mice (data not shown). The lack of induction of CYP2A5 by pyrazole in the olfactory mucosa, as well as in lung, of C57BL/6 mice was reported previously (Su et al., 1998), but the expression of CYP2A5 in mouse small intestine and its lack of induction by pyrazole have not been reported previously. The differential induction of CYP2A5 and the CYP2Js by pyrazole in these tissues suggests involvement of unique, isoform-dependent, and tissue-specific mechanisms.

To determine whether the induction of the CYP2Js involved pre-translational mechanisms, RNA-blot analysis was performed with use of a rat CYP2J4 cDNA probe. The probe is 93% identical with corresponding sequences in CYP2J6 cDNA and 79% identical with that of CYP2J5. As shown in Fig. 4, the probe detected a major band, about 2.0 kb, as well as a very faint band of about 2.4 kb in liver, lung, kidney, olfactory mucosa, and small intestine. Quantitative analysis using the level of β-actin mRNA to normalize the amounts of poly(A)+ RNA indicated that the CYP2J mRNAs corresponding to the major band were induced about 3-fold in liver, small intestine, and lung and about 2-fold in olfactory mucosa and kidney. The weak, upper band also appeared to be induced by pyrazole treatment although the densities were too low to be quantified accurately. Only trace amounts of CYP2J mRNAs were detected in the eye and the heart of either PBS- or pyrazole-treated mice, and the high background signals made it impracticable to determine whether there was any induction (data not shown).

The high sequence homology between CYP2J4 and CYP2J6 suggested that the signals detected on immunoblots and RNA-blot analysis with use of the anti-CYP2J4 antibodies and the CYP2J4 cDNA probe, respectively, most likely represent CYP2J6, instead of the other CYP2J isoforms. To confirm that CYP2J6 is expressed in these tissues, isoform-specific RNA-PCR was performed with total RNAs from liver, lung, kidney, olfactory mucosa, heart, eye, and small intestine. As shown in Fig. 5, CYP2J5 was only detected in liver and kidney, whereas CYP2J6 was detected in all tissues examined. The PCR primers were designed to distinguish CYP2J6 from CYP2J5, but may or may not detect the other CYP2J isoforms, for which the
Male C57BL/6 mice were treated with PBS (cont) or pyrazole (pyr) as described in the legend to Fig. 1. Poly(A)⁺ RNAs from the liver (Liv), lung (Lu), kidney (Kid), small intestine (Sl), and olfactory mucosa (OM) were prepared from pooled tissues of five or 20 (for OM) male mice in each group. Each lane contained about 1 μg of poly(A)⁺ RNA. The blots were hybridized first with a CYP2J4 cDNA probe and, after stripping, with a β-actin cDNA probe.

RNA-PCR was performed as described in Fig. 5, except that the primers used will amplify both CYP2J5 and CYP2J6. The 751-bp PCR products were either uncut (A), or treated with EcoRI (B) or PstI (C) as described under Materials and Methods. EcoRI cuts CYP2J5 to 185- and 566-bp fragments, whereas PstI cuts CYP2J6 to 343- and 408-bp fragments.

be determined whether both CYP2J5 and CYP2J6 were induced in liver and kidney and whether additional CYP2J forms were also induced.

**Discussion**

Several interesting findings were made in the present study on the tissue distribution of mouse CYP2Js and their induction by pyrazole. First, CYP2J-related proteins were detected, for the first time, in the eye, and, confirming previous studies in rats and mice, in other mouse tissues, including heart, liver, lung, kidney, olfactory mucosa, and small intestine. Second, CYP2J proteins, which may include CYP2J6 and possibly other CYP2J forms, were induced by treatment of mice with pyrazole in the liver, lung, kidney, olfactory mucosa, eye, and small intestine, but not in the heart. Moreover, not all tissues that responded to pyrazole treatment with induction of CYP2J had increased expression of CYP2A5, suggesting involvement of tissue-selective and isoform-dependent induction mechanisms. Third, consistent with previous findings on the induction of rat CYP2J4 by pyrazole (Zhang et al., 1999), induction of mouse CYP2J proteins was accompanied by increases in the CYP2J mRNA level. Finally, RNA-PCR results indicated that CYP2J6, but not CYP2J5, was expressed in the eye and olfactory mucosa and confirmed previous reports (Ma et al., 1999, Scarborough et al., 1999) that CYP2J5 is selectively expressed in the small intestine, heart, lung, kidney, and liver.

The regioselectivity of CYP2J-catalyzed arachidonic acid metabolism has been elucidated for CYP2J2, 2J3, 2J5, and 2J9, and partially for CYP2J4. CYP2J2 yields preferentially 14,15-EET, and, to a lesser extent, 5,6-, 8,9-, and 11,12-EET, as well as trace amounts of 19-HETE (Wu et al., 1996); CYP2J3 yields primarily 14,15-, 8,9-, and 11,12-EETs and 19-HETE, with a lesser extent of 5,6-EET (Wu et al., 1997); CYP2J4 yields primarily 19-HETE, with a lesser extent of undetermined EETs (Zhang et al., 1997); CYP2J5 yields primarily 14,15-, 11,12-, and 8,9-EETs and 11- and 15-HETE, with lesser quantities of 5,6-EET (Ma et al., 1999); and CYP2J9 yields primarily 19-HETE (Qu et al., 1999). These differences in arachidonic acid metabolite profiles of the various CYP2Js will likely produce differing functions of the CYP2Js in the organs selected for their expression.
The pleiotropic effects of pyrazole on P450 expression are intriguing. Pyrazole induces CYP2E1 by post-translational mechanisms (Song et al., 1989; Winters and Cederbaum, 1992), which appear not to be tissue-specific (Rosenberg and Mankowski, 1994; Wu and Cederbaum, 1994; Su et al., 1998). Pyrazole also induces CYP2A5 in the liver and kidney (Kojo et al., 1991; Emde et al., 1996; Su et al., 1998), apparently by stabilizing the mRNA (Aida and Negishi, 1991). It is likely that the induction of CYP2J by pyrazole may involve mechanisms similar to those found for the induction of CYP2A5 or CYP2E1 in the liver and the kidney. The induction of CYP2A5, however, does not occur in the lung, small intestine, or olfactory mucosa, where CYP2J is induced, which clearly indicates involvement of different mechanisms. Furthermore, the lack of induction of the CYP2J proteins in the heart suggests that nontissue-specific mechanisms such as stabilization of the CYP2J proteins by direct binding to pyrazole may not be involved. Of interest, heterologously expressed CYP2J6 was apparently unstable (Scarborough et al., 1999), which has stalled efforts to characterize its catalytic activity and led to the proposal that post-translational regulation may be important in controlling its steady-state level in mouse tissues. The induction of CYP2J in mouse liver, small intestine, and olfactory mucosa is consistent with previous findings on the induction of CYP2J4 in the same tissues in rats (Zhang et al., 1999). Thus, the mechanisms of CYP2J induction by pyrazole are conserved across species, which again differs from the species-dependent tissue-specificity of CYP2A5 induction in rats and mice.

P450 isoforms of the olfactory mucosa are generally refractory to induction by xenobiotics. Many classical P450 inducers failed to induce P450 in this tissue (Hadley et al., 1982; Bond, 1983; Longo et al., 1988; Su et al., 1998). The present finding that pyrazole induces CYP2J protein by as much as 18-fold in the olfactory mucosa represents the greatest induction of a P450 enzyme ever reported for this tissue. Previous studies have found modest induction of CYP2E1 by ethanol and acetone (Ding and Coon, 1990b; Longo and Ingelman-Sundberg, 1993) and CYP1A isoforms by Arochlor 1254 (Voigt et al., 1993) and cigarette smoke (Wardlaw et al., 1998). Members of the CYP2J subfamily are known to be active toward arachidonic acid, producing metabolites with biological activities in the regulation of vascular tone and ion transport (Scarborough et al., 1999). Although the specific metabolites of CYP2J6 have yet to be determined, the large induction of CYP2J proteins by pyrazole may present a good model for studying the potential roles of P450-mediated lipid metabolites in the regulation of mucus secretion and odorant clearance in response to prolonged exposure of the olfactory mucosa to foreign compounds.

This is the first demonstration of CYP2J expression in the eye. Although the ocular microsomal level of CYP2J proteins was very low, higher levels may be present in some parts of the eye because the P450 proteins would not be evenly distributed in such an organ with highly heterogeneous tissue and cell types. P450-Dependent monoxygenase activities were previously found in ocular tissues such as corneal epithelium, ciliary body, and retinal pigment epithelium, with the corneal epithelium having the highest P450-dependent activities in metabolizing arachidonic acid to biologically active compounds (Schwartzman et al., 1987). Of particular interest, the main P450-dependent metabolites of arachidonic acid in corneal epithelium are 12-hydroxy-5,8,10,14-eicosatetraenoic acid and 12-hydroxy-5,8,14-eicosatrienoic acid; the predominant (R) enantiomers of these metabolites are believed to be a modulator of Na+ - K+ -ATPase activity in the ocular tissue and a potent ocular proinflammatory compound, respectively (Stoltz et al., 1994). However, although multiple P450 isoforms have been detected in the eye, including CYP1A1/2, CYP2C, CYP2E1, CYP3A5, and CYP4A (Zhao and Shichi, 1995; Zhao et al., 1996; Offord et al., 1999), it is unclear which P450s are involved in the production of 12-hydroxy-5,8,10,14-eicosatetraenoic acid and 12-hydroxy-5,8,14-eicosatrienoic acid and their further metabolism in the cornea (Nishimura et al., 1991). The RT-PCR results showed that CYP2J6, but not CYP2J5, was expressed in the eye. The inducibility of the CYP2J proteins in the eye may help to identify whether CYP2J6 is active in these unique metabolic pathways.

The levels of the CYP2J proteins detected by the anti-CYP2J4 antibody were the highest in the smallest intestine. Two bands of about equal intensity were detected in intestinal microsomes. The lower band was not detected in the olfactory mucosa or the eye, therefore, it was probably not CYP2J6. It cannot be CYP2J5 either because CYP2J5 mRNA was not detected in the small intestine. Thus, the top band probably corresponds to CYP2J6, whereas the identity of the lower band remains to be determined. Interestingly, both bands were induced by pyrazole, suggesting that another pyrazole-inducible CYP2J-related isoform is expressed in the small intestine. Notably, it has been recently reported (Scarborough et al., 1999) that a specific anti-CYP2J6 peptide antibody failed to detect a full-length CYP2J protein in mouse tissue microsomes, whereas it did detect a much smaller (~25-kDa) band, presumably a degradation product. However, a 25-kDa band was not detected in the present study with the anti-CYP2J4 antibody. Therefore, it is questionable whether that band was derived from the CYP2J6 protein, and it remains to be seen whether the reported lack of detection of CYP2J6 protein was due to an inadequate sensitivity of the anti-peptide antibody.

The conserved inducibility of CYP2J in multiple rodent tissues implicates that human CYP2J6 may be similarly induced by pyrazole or other xenobiotic compounds. Only one CYP2J form, CYP2J2, has been identified in humans to date. It is expressed mainly in heart, and to a lesser extent in kidney, liver, and muscle (Wu et al., 1996). CYP2J2 expression was also detected in the liver and olfactory mucosa of human fetuses (Gu et al., 2000). CYP2J2 is active toward arachidonic acid, forming multiple EETs (Wu et al., 1996). Thus, potential induction of CYP2J2 by xenobiotic compounds such as pyrazole may lead to significant disturbances in the homeostasis of these regulatory molecules. Further studies on the mechanism of rodent CYP2J induction by pyrazole are warranted to establish a molecular basis for predicting the inducibility in humans.

Acknowledgments. We acknowledge the use of the Molecular Genetics Core of the Wadsworth Center.

References
Ding X and Coon MJ (1990a) Immunochemical characterization of multiple forms of cytochrome P450 in rabbit nasal microsomes and evidence for tissue-specific expression of P450s in NM and Nm. Mol Pharmacol 37:489–496.
Ding X and Coon MJ (1990b) Induction of cytochrome P450 isozyme 3a (I-P450IEI) in rabbit olfactory mucosa by ethanol and acetone. Drug Metab Dispos 18:742–745.