Short Communication

CLONING CYP2D21 AND CYP3A22 CDNAS FROM LIVER OF MINIATURE PIGS

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ABSTRACT:

To compare the identity of the primary structure of drug-metabolizing cytochrome P450 between miniature pigs and humans, two cDNA clones, coding for miniature pig CYP2D21 and CYP3A22, were isolated. The deduced amino acid sequences of CYP2D21 and CYP3A22 were 78.3 and 75.0% identical to human CYP2D6 and CYP3A4, respectively. These values were nearly the same as those of bovine, dog, and some rodent isoforms, and 12.2 to 18.4% lower than those of nonhuman primates such as cynomolgus monkeys, Japanese monkey, and marmosets. These data indicate that miniature pig P450s are genetically not so close as monkey P450s to human P450s as previously expected. The recombinant CYP2D21 enzyme, however, showed bufuralol 1'-hydroxylase activity, suggesting that miniature pig CYP2D21 is capable of metabolizing some of the same substrates associated with human CYP2D6 despite its low identity to human counterparts.

The miniature pig is small in size (~50 kg body weight in adult animals) and has been developed through selective breeding so as to overcome the shortcomings arising from the large body size of domestic pigs (Khan, 1984). The miniature pig is becoming a popular alternative to traditional nonrodent species in pharmacological and toxicological studies. Porcine hepatocytes continue to find increasing application in bioartificial liver devices (Watanabe et al., 1997). These uses are, in part, based on findings that many anatomical, physiological, and biochemical properties of domestic and miniature pigs are close to those of humans (Swindle and Smith, 1998). However, it has been well recognized that qualitative and quantitative species differences in drug-metabolizing systems are present among animal species including man (Caldwell, 1981). Thus, information on the primary structure of drug-metabolizing enzymes of experimental animal species and information on the identity of those enzymes between human and experimental animal species is important to explain species differences in drug-metabolizing systems and to predict drug metabolism in the human body. Genetic distance of some porcine genes to human orthologs has been clarified. Regarding porcine CYP genes, some forms of P4501 are concerned with endogenous metabolism such as CYP5A (accession no. L131128), CYP11B1 (D38590), CYP19 (U37311), CYP21 (M83939), and CYP51 (AB042975) have been isolated from domestic pigs. These forms shared amino acid sequence identities ranging from 73 to 95% to human orthologous genes. cDNA cloning of the entire coding sequence of two drug-metabolizing P450s, namely CYP2D25 and CYP3A29, from domestic pigs has been reported (Postlind et al., 1997; Nissen et al., 1998). Additionally, the partial sequences of cDNAs for five drug-metabolizing CYP2Cs (2C32, 2C33, 2C34, 2C35 and 2C42) from domestic pigs have been reported (Zaphiropoulos et al., 1995; Nissen et al., 1998). However, study of cDNA cloning from miniature pig has not yet been reported. The aims of the present study were to demonstrate in miniature pigs the primary structure of drug-metabolizing P450s, especially CYP3A and CYP2D, which are responsible for the metabolism of many prescribed drugs, and to compare these with those of human orthologs.

Materials and Methods

Animals. Two 24-week-old male Göttingen miniature pigs of about 15 kg in body weight were used in this study. Livers were stored at −80°C until use for the preparation of RNA and microsomes. All experiments were performed in accordance with the Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985).

Construction and Screening of the Miniature Pig Liver cDNA Library. Poly(A)+ RNA was prepared from the liver of an untreated male miniature pig by oligo(dT)-cellulose column chromatography. A cDNA library was constructed as described previously (Sakuma et al., 1994). Approximately 5 × 107 plaques were screened by plaque hybridization using the cDNA fragments of human CYP2D6 and cynomolgus monkey CYP3A8 as probes.

Expression of CYP2D21 in Yeast Cells and Analysis of Bufuralol Hydroxylation Activity. An expression plasmid for CYP2D21 was constructed with the pAAH5 yeast expression vector. The resulting expression plasmid, pAAMS2D, contains native coding, six bases of 5'-noncoding sequences, and 83 bases of 3'-noncoding sequences. Transformation of the yeast, Saccharomyces cerevisiae AH22, cultivation of the recombinant yeasts, preparation of microsomal fractions, and determination of P450 contents were carried out as described previously (Sakuma et al., 1994). Measurement of the bufuralol 1'-hydroxylation activity was carried out as described previously (Yokoi et al., 1996). The kinetic parameters of bufuralol 1'-hydroxylation of microsomes from the recombinant yeasts or miniature pig liver were calculated from Lineweaver-Burk plots.

Results and Discussion

To clarify the identity of the primary structure of P450 between miniature pigs and humans, two cDNA clones containing the entire coding sequence of CYP2D or CYP3A were isolated from a cDNA...
library prepared from the liver of a male miniature pig: MS2D\(^2\) (accession no. D89502) and MS3A (AB006010). MS2D consisted of 1613 bp including 1509 bp of an open reading frame. MS3A consisted of 1653 bp, including 1509 bp of an open reading frame. Both P450s were designated as CYP2D21 and CYP3A22 by the cytochrome P450 nomenclature committee. The nucleotide sequences of CYP2D21 and CYP3A22 appear in the Genome Sequence Database, DNA DataBank of Japan, European Molecular Biology Laboratory, and National Center for Biotechnology Information databases with the accession numbers D89502 and AB006010, respectively.

CYP3A46 (AB052266). Those isoforms share 81.5, 83.9, and 76.5% amino acid sequence identities with CYP3A22, respectively, indicating that three domestic pig CYP3A isoforms are not an ortholog of CYP3A22.

Regarding the substrate specificity of CYP2D isoforms in domestic and miniature pigs, Skaanild and Friis (1999) reported that livers of miniature pig and domestic pig have no CYP2D6 activity (debrisoquine 4-hydroxylase activity). They concluded in a later report (Skaanild and Friis, 2002) that dextromethorphan O-demethylase and bufuralol 1'-hydroxylase activities, other marker activities, may be catalyzed by CYP2B isoforms in domestic and miniature pigs. Low debrisoquine 4-hydroxylase activity of domestic pig CYP2D25 was confirmed by an assay using recombinant enzymes (Hosseinpour and Wikvall, 2000). By contrast, Jurima-Romet et al. (2000) demonstrated the evidence for the catalysis of dextromethorphan O-demethylhylation by a CYP2D6-like enzyme in domestic pig liver. These discrepancies raised the question of whether CYP2D enzymes of domestic or miniature pigs retain the functional similarity to human CYP2D6. Therefore, CYP2D21 was expressed in yeast cells. The recombinant CYP2D21 showed bufuralol 1'-hydroxylase activity. The apparent \( K_m \) and \( V_{max} \) values calculated from Lineweaver-Burk plots were 0.98 \( \mu M \) and 1.8 pmol/min/nmol P450, respectively. Those values of the human CYP2D6 expressed in the same expression system are 4.2 \( \mu M \) and 2.4 pmol/min/nmol P450, respectively (Yokoi et al., 1996). These results indicate that the miniature pig possesses a CYP2D enzyme in its liver, and it retains the capacity to metabolize a substrate of human CYP2D6. This, together with the finding of Skaanild and Friis (2002), implies that the contribution of CYP2D21 to the metabolism of bufuralol is smaller than those of other P450s, such as CYP2B.

The amino acid sequence of domestic pig CYP2D25 showed 97.8% identity to that of miniature pig CYP2D21. There are 10 amino acid differences between CYP2D21 and CYP2D25, and one located in the putative substrate recognition site (SRS) for CYP2 enzyme (SRS-1 through SRS-6) described by Gotoh (1992): 204th, Q or L for 2D21 and M and 1.8 pmol/min/nmol P450, respectively. Those values of the human CYP2D6 expressed in the same expression system are 4.2 \( \mu M \) and 2.4 pmol/min/nmol P450, respectively (Yokoi et al., 1996). These results indicate that the miniature pig possesses a CYP2D enzyme in its liver, and it retains the capacity to metabolize a substrate of human CYP2D6. This, together with the finding of Skaanild and Friis (2002), implies that the contribution of CYP2D21 to the metabolism of bufuralol is smaller than those of other P450s, such as CYP2B.

\(^2\) The P450s coded by MS2D and MS3A were designated as CYP2D21 and CYP3A22, respectively, by the cytochrome P450 nomenclature committee. The nucleotide sequences of CYP2D21 and CYP3A22 appear in the Genome Sequence Database, DNA DataBank of Japan, European Molecular Biology Laboratory, and National Center for Biotechnology Information databases with the accession numbers D89502 and AB006010, respectively.
the possibility that CYP2D21 and CYP2D25 have distinct substrate specificity.

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Laboratory of Drug Metabolism, Division of Pharmacobio-dynamics, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan (T.Sa., T.Sh., K.M., T.K.) and Department of Toxicology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, Japan (T.Sa.)

References


