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Identification of a cytochrome P-450 in human fetal liver related to glucocorticoid-inducible cytochrome P-450HLp in the adult*

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The cytochromes P-450 are a multigene family of microsomal hemoproteins prominently found in the liver. These isozymes may be distinguished by differences in not only their primary structure, but also their substrate specificities and regulation of expression [1, 2]. Recent studies of human liver have confirmed that, in adult tissue, there are numerous forms of cytochrome P-450 structurally and functionly related to those found in experimental animals. These include: HLp, the glucocorticoid inducible, erythromycin *N*-demethylase homologous to P-450p in the rat and to LM3c in the rabbit [3]; HLj, the human ethanol-inducible *N*-nitrosodimethylamine demethylase orthologous to rat P-450j and rabbit LM3a [4]; HLc and HLd, the human orthologs of polycyclic aromatic hydrocarbon-inducible P-450c and P-450d, respectively, in the rat [5]; and HLx, a human isozyme related to the family of constitutive isozymes in untreated animals [6]. The human fetus has also been shown to actively metabolize many substrates of the cytochromes P-450 [7–10]. In addition, there are reports that microsomes isolated from human fetal livers contain proteins immunochemically-related to adult human liver cytochromes P-450 [9, 11]. In this report, we examined microsomes prepared from human fetal livers for the presence of proteins immunochemically-related to the five well characterized human cytochromes P-450 currently under study in our laboratory.

Methods

Liver specimens. Fetuses were obtained from therapeutic abortions performed prior to 12 weeks of gestation. The livers were removed, frozen in liquid nitrogen, and stored at −80°. Adult liver specimens were obtained from patients who had not received drugs known to induce HLp [3, 4] and were undergoing hepatic lobectomy or were brain dead renal transplant donors. Patient code numbers refer to individual specimens. Microsomes were prepared and stored as previously described [3]. Protein concentration was determined colorimetrically [12].

Antibody preparation and immunoblot analysis. Antibodies which specifically recognize human liver cytochrome P-450 HLj [4], HLc and HLd [5], or HLx [6] were prepared as described in the indicated references. Polyclonal antibodies against HLp were raised in goats as previously described [13]. Immunoblot analyses were performed and quantitated as previously described [3].

Results and discussion

Microsomes isolated from human fetal livers were subjected to electrophoresis in polyacrylamide gels, transferred to nitrocellulose filters, and then exposed to one of the anti-cytochrome P-450 antibodies. Analysis of these immunoblots (Fig. 1) demonstrated that the fetal liver microsomes did not contain detectable levels of proteins immunochemically-related to HLj [4], HLc and HLd [5], or HLx [6]. These results are in keeping with developmental studies in rats which indicate that P-450c and P-450d [14, 15] and P-450j (F. J. Gonzalez, personal communication, cited with permission) are absent in fetal liver, but then rise to adult levels during the neonatal period. To our knowledge, P-450g, a rat liver counterpart of HLx in humans, has not been studied in the rat fetus.

Cytochrome P-450p has been reported to be undetectable in rat fetal liver [15]. However, each of the human fetal microsomal samples we examined contained a protein that reacted with anti-HLp antibodies and displayed an electrophoretic mobility identical to that of HLp (Fig. 1). Scanning densitometry of these immunoblots demonstrated that the average amount of the HLp-related protein in five fetal preparations was 0.07 nmol HLp/mg protein (range, 0.03 to 0.09) (Table 1). By comparison, the average amount of immunoreactive HLp in hepatic microsomes prepared from adult specimens was 0.12 nmol/mg protein (range, 0.07 to 0.19) (Table 1). Thus, the concentration of immunoreactive HLp in adults is almost twice that of the HLp-related protein in fetal liver. However, in both adult and fetal microsomes, the amounts of the immunoreactive HLp

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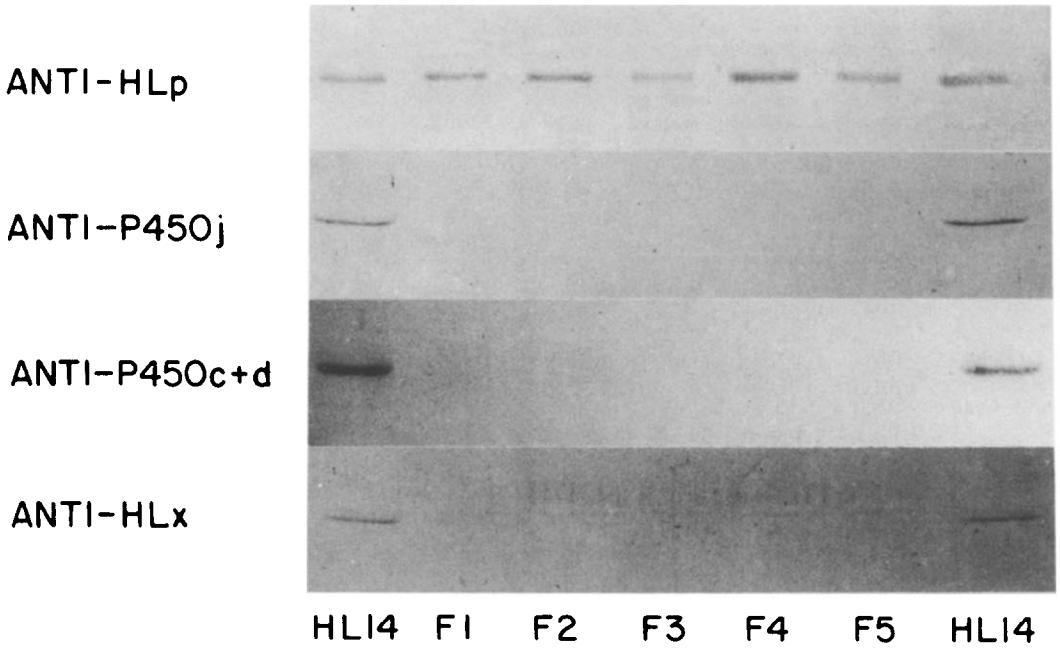

Fig. 1. Immunoblots of adult and fetal human liver microsomal proteins developed with antibodies that recognize HLp, HLj, HLc and HLd, or HLx. Liver microsomes isolated from the indicated human adult or fetal specimens were immunoblotted as described in Methods. In all cases, 50 μg of microsomal protein was applied to the polyacrylamide gels except for the anti-HLp immunoblot (15 μg).

proteins represented approximately one-third of total cytochrome P-450 measured spectrally as CO-binding hemoprotein (Table 1). Our human fetal liver microsomal samples 2, 3 and 5 had detectable rates of erythromycin *N*-demethylation (0.57, 0.71 and 0.89 nmol formaldehyde formed per min per mg microsomal protein respectively), an activity [3] specifically associated with P-450p and HLp. This enzyme activity was not reproducibily measurable (<0.1 nmol per min per mg protein) in the remaining two samples.

Our results are consistent with a previous report that human fetal liver microsomes contain proteins immunochemically-related to adult human cytochromes, P-450$_9$ and P-450$_5$ [9]. The latter isozyme may be immunochemically

Table 1. Quantitation of fetal and adult liver cytochromes P-450 reacting with antibody to HLp

Sample number*	Total cytochrome P-450† (nmol/mg protein)	Anti-HLp-reactive P-450‡ (nmol/mg protein)	% of total§
Fetus			
1	0.14	0.06	43
2	0.38	0.09	32
3	0.10	0.03	30
4	0.28	0.09	32
5	0.17	0.07	41
Adult			
2	0.46	0.19	41
3	0.28	0.11	39
6	0.33	0.14	42
7	0.25	0.12	48
10	0.44	0.16	36
12	0.44	0.07	18
14	0.42	0.07	17

* Microsomal samples were prepared as described in Methods.

† Total carbon monoxide binding cytochrome P-450 was determined by the method of Matsubara *et al.* [16].

‡ Immunoquantitation of the anti-HLp immunoreactive proteins was performed as described in Methods.

§ Immunoreactive protein ÷ total cytochrome P-450 × 100.

related to HLp [3, 17–19]. Human fetal liver also contains P-450 HFLa, a testosterone 6β-hydroxylase [10], which comprises a large fraction (36–50%) of the total fetal hepatic cytochrome P-450 [11]. However, unlike HLp, only a small amount (0–5%) of a protein immunochemically-related to HFLa is present in adult liver [11].

In the adult, HLp (also called P-450NF)* appears to be responsible for the metabolism of a large number of compounds including erythromycin [3], quinidine [20], nifedipine [18], aldrin [20], and the 6β-hydroxylation of testosterone and cortisol [20]. Therefore, the expression of a protein related to HLp in the fetal liver may influence the effects of xenobiotics in prenatal life. Moreover, our data suggest that, in humans, HLp, unlike HLj, HLc, or HLd, deviates radically from the developmental pattern observed for its orthologous isozyme in rats ([14, 15], and F. P. Gonzalez, personal communication, cited with permission]. In light of this qualitative interspecies difference in cytochrome P-450 gene expression, caution should be taken in extrapolating to humans the results of fetal toxicity testing in animals.

Note added in proof: After submission of this manuscript, a report appeared (M. Kitada, T. Kamataki, K. Itahashi, T. Rikihisa, and Y. Kanakubo, *J. biol. Chem.* **262**, 13534, 1987) which indicates that human fetal liver cytochrome P-450 HFLa may be structurally related to adult human liver cytochrome P-450 HLp.

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* Comparisons of the nucleotide and deduced amino acid sequences of HLp [17] and P-450NF [18] indicate that they are identical or highly related proteins.

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