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Identification of an inducible form of cytochrome P-450 in human liver

(NH_2-terminal sequence/dexamethasone/macrolide antibiotics/triacetyloleandomycin)

P. B. WATKINS*†, S. A. WRIGHTON*, P. MAUREL*‡, E. G. SCHUETZ*, G. MENDEZ-PICON§, G. A. PARKER§, AND P. S. GUZELIAN*¶

*Division of Clinical Toxicology and Environmental Medicine, Department of Medicine and §Department of Surgery, Medical College of Virginia, Richmond, VA 23298; and †Department of Medicine, McGuire Veterans Administration Hospital, Richmond, VA 23249

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ABSTRACT **It has not yet been determined whether human liver contains inducible cytochromes P-450 similar to those that catalyze the oxidative metabolism of foreign substances in animals. We carried out immunoblot analyses of liver microsomes isolated from eight patients and found that each contained a cytochrome P-450, termed HLp, that reacted with antibodies directed against P-450p, a rat liver cytochrome that is inducible by the anti-glucocorticoid pregnenolone-16α-carbonitrile, by glucocorticoids, by anti-seizure drugs, and by such macrolide antibiotics as triacetyloleandomycin. In the two patients who received dexamethasone and anti-seizure medications and in the one patient who was given triacetyloleandomycin, the concentrations of immunoreactive HLp and the ability to demethylate erythromycin and/or to convert triacetyloleandomycin to a metabolite that forms a spectral complex with cytochrome P-450 heme (catalytic properties unique to P-450p in rat liver) were significantly higher as compared to the values for patients who received no inducing drugs. We purified HLp to homogeneity and found that it was immunochemically related to P-450p and to its homologue in the rabbit (LM3c), actively demethylated erythromycin in a reconstituted system, exhibited electrophoretic mobility identical to that of P-450p, and shared 57% homology in its NH_2-terminal amino acid sequence with that of a pregnenolone-16α-carbonitrile-inducible rat cytochrome P-450. We conclude that HLp is a human representative of the multigene family of the glucocorticoid-inducible cytochromes P-450.**

The cytochromes P-450 are a family of hemoproteins, abundant in the endoplasmic reticulum of the hepatocyte, that catalyze the oxidative metabolism of many drugs, environmental chemicals, and endogenous compounds (1). An important characteristic of some of the forms of cytochrome P-450 is that they are inducible. For example, different forms of liver cytochrome P-450 accumulate in rats treated by a member of one of three "classes" of inducers (as reviewed in ref. 2) typified, respectively, by phenobarbital (P-450b, P-450e), 3-methylcholanthrene (P-450c, P-450d), and pregnenolone-16α-carbonitrile (PCN) (P-450p). Since the amounts and types of cytochromes P-450 in the liver may be rate-limiting for metabolism of foreign chemicals, enzyme induction may play an important role in such clinically relevant phenomena as interactions among therapeutic drugs (3), metabolic "idiosyncrasy" in hepatotoxic drug reactions (4), and interindividual differences in susceptibility to toxic effects of environmental chemicals (5).

There is abundant, albeit indirect, evidence that human liver also contains cytochromes P-450 that are inducible. For example, exposure of humans to inducers of animal cytochromes P-450 including such drugs as phenobarbital (6), macrolide antibiotics (7), or diphenylhydantoin (8), or environmental chemicals such as organochlorine pesticides (9) or polychlorinated biphenyls (10), accelerates the disappearance of administered substrates for the cytochromes P-450 from the blood or the appearance of metabolites of such model drugs in the breath (11). Such patients may also exhibit increased urinary excretion of metabolites of endogenous substrates such as 6β-hydroxy derivatives of cortisol (12–15). Additional evidence for liver enzyme induction in humans are proliferation of the smooth endoplasmic reticulum in hepatocytes, as judged by electron microscopic examination of liver biopsies (16), increased urinary excretion of glucaric acid (a breakdown product of a constituent of the endoplasmic reticulum) (17), and increased concentration of CO-binding hemoprotein or drug oxidizing activities in liver microsomes prepared from such patients (18, 19). However, although it has been possible to purify at least six individual polypeptide forms of human liver cytochrome P-450 (20), there has to date been no clear evidence which, if any, of these cytochromes are inducible.

We were prompted to look for an inducible cytochrome P-450 in humans when we discovered that P-450p, a form of cytochrome P-450 in rat liver originally purified from animals treated with the anti-glucocorticoid PCN (21), is inducible not only by PCN, but also by commonly used therapeutic agents such as the glucocorticoid dexamethasone (Dex) (22), the macrolide antibiotics [especially triacetyloleandomycin (TAO) (23)], and phenobarbital (22). P-450p is unique in being inducible by three different categories of agents and also in being able to catalyze the *N*-demethylation of erythromycin and TAO (23). These macrolide antibiotics are converted into metabolites that bind tightly to the heme moiety of P-450p, forming a stable spectrally detectable complex (23). Since we have previously shown (23) that in rat liver microsomes P-450p is exclusively responsible for the *N*-demethylation of erythromycin and the formation of the TAO–metabolite complex, these two measurements provide specific and convenient means to quantitate holocytochrome P-450p. Indeed, we found that liver microsomes prepared from mice, gerbils, hamsters, and rabbits contain cytochromes P-450 that exhibit immunoreactivity with anti-P-450p antibodies and also catalyze erythromycin demethylation and formation of the TAO–metabolite complex (24). Moreover, these cytochromes are inducible by most of the same agents active in the rat (24). We also isolated and purified from TAO-

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Abbreviations: Dex, dexamethasone; TAO, triacetyloleandomycin; PCN, pregnenolone-16α-carbonitrile.

‡Present address: Institut National de la Santé et de la Recherche Médicale, U 128, Centre National de la Recherche Scientifique, B.P. 5051, 34033 Montpellier Cedex, France.
¶To whom reprint requests should be addressed at: Box 267, MCV Station, Richmond, VA 23298-0001.

treated rabbits a liver cytochrome P-450 (LM3c) that is immunochemically related to P-450p and found that LM3c shares 73% homology with P-450p in its NH_2-terminal amino acid sequence (23). In the present report, we examined eight surgical liver specimens obtained from patients with well-documented medication histories and have found that each contained a cytochrome P-450 immunochemically and functionally similar to cytochrome P-450p. This cytochrome was induced in the two patients who received large doses of Dex and in the one patient who electively received TAO.

MATERIALS AND METHODS

Human Liver Specimens. Specimens were obtained at surgery from eight patients under protocols approved by the Committee for the Conduct of Human Research at our institution. All patients had normal serum transaminase and bilirubin levels and each received atropine prior to general anesthesia. Patient 1 was a male renal transplant donor. Documentation of medical history and medications received was unavailable. Patient 2 was a 69-yr-old white male who underwent a hepatic lobectomy for primary hepatocellular carcinoma. The liver specimen provided from the operating room was histologically normal without evidence of tumor or cirrhosis. This patient had a history of hypertension treated with hydrochlorothiazide. However, the only medications he received during the 3 days before surgery were four oral doses each of erythromycin base (1 g every 6 hr) and neomycin (1 g every 6 hr). Patient 3 was a 27-yr-old white male who became a renal transplant donor after a motor vehicle accident. He had no prior medical illnesses and was not taking medications. He received only vitamin K and potassium chloride prior to surgery. Patient 4 was a 33-yr-old black female who became a renal transplant donor after rupture of a basilar artery aneurysm. She was otherwise healthy and had been taking no medications. She received a total of 80 mg of Dex, 840 mg of phenobarbital, and 300 mg of diphenylhydantoin in divided doses during the 2 days before surgery. Patient 5 was a 70-yr-old white male who underwent a hepatic lobectomy for metastases of colon cancer. He had no other medical illnesses and was taking no medications. He volunteered to take TAO prior to the operation. While in the hospital, he received a total of 4.25 g of TAO over 4 days [250 mg orally every 6 hr (five doses) followed by 500 mg orally every 6 hr (six doses)]. Patient 6 was a 26-yr-old white male who became a renal transplant donor after a motor vehicle accident that caused severe brain injury. He was previously healthy and did not take any medications. He was given cimetidine (1 g in four divided doses intravenously) on the day before surgery. Six hours before surgery, the patient's blood pressure fell and he was given 300 mg of hydrocortisone and dopamine by intravenous infusion. Patient 7 was a 42-yr-old white female undergoing hepatic lobectomy for metastatic colon cancer. She was otherwise healthy and was not taking any medications. Patient 8 was a 52-yr-old white male who became a renal donor after sustaining a massive stroke. He was known to have hypertension but had not been taking medications prior to his admission. During the 3 days of his hospitalization prior to surgery, he received 72 mg of Dex, 600 mg of diphenylhydantoin, and 1200 mg of phenobarbital in divided doses by intravenous infusion.

All liver specimens were transported from the operating room on ice. The samples were minced, homogenized (five passes in a Potter–Elvehjem tissue grinder), and microsomes were prepared immediately by differential centrifugation according to the method of vanderHoeven and Coon (25) and stored at −70°C. Each of the assays was performed on all eight specimens simultaneously. Protein concentration was measured colorimetrically (26).

Immunoquantitation. Appropriate quantities of microsomes from each patient previously determined to give an immunoreactive response in a linear range were subjected to electrophoresis (27) on polyacrylamide gels (10%) for a total of 3 hr (30 mA per gel). After 1 hr, the current was temporarily interrupted and a second loading was made by adding to each well a different amount of microsomes prepared from patient 4. At completion of electrophoresis, the separated proteins were electrophoretically transferred to nitrocellulose paper, which was then immunoblotted (28) with specific goat antibodies directed against P-450p or LM3c (23, 24). The intensity and size of each developed band was quantitated by a Colorscan C-4100 densitometer (Optronics International, Chelmsford, MA). Coomassie staining of the gels was used to establish that there had been complete transfer of protein.

CO-Binding Hemoprotein. Dithionite-reduced microsomes were assayed by the method of Omura and Sato (29).

Erythromycin Demethylation. The 1-ml reaction mixture contained microsomal samples diluted to 1.0 mg/ml in 0.1 M potassium phosphate buffer (pH 7.4) containing erythromycin (Laboratoires Roussel, Paris) (0.4 mM). NADPH (Sigma) (1.0 mM) was added to initiate the reaction and after a 15-min incubation at 37°C, the formaldehyde formed was assayed by the method of Nash (30). For the reconstituted assay, 1 nmol of purified cytochrome P-450, 2 nmol of rat NADPH cytochrome P-450 reductase, and 30 μg of sonicated dilaurylphosphatidylcholine replaced the microsomes in the assay mixture.

TAO Complex Formation. The amount of TAO complex formed *in vivo* was measured by diluting microsomes from patient 5 to 1.0 mg/ml in 0.1 M potassium phosphate buffer (pH 7.4) and placing equal volumes of this suspension into the reference and sample cuvettes of an Aminco DW-IIa spectrophotometer. K_3FeCN_6 (10 μl, 2.0 mM) (Sigma) was added to the reference cuvette and an equal volume of water was added to the sample cuvette. The absorbance difference between 456 nm and 510 nm was determined after 10 min (25°C). The concentration of complex was calculated by using the extinction coefficient of 68 $mM^{-1} \cdot cm^{-1}$, which was previously calculated in rat microsomes (23). Liver microsomes from the other patients were assayed for the ability to form TAO complex *in vitro* by placing solutions of diluted microsomes (1.0 mg of protein per ml in 0.1 M potassium phosphate buffer, pH 7.4) in reference and sample cuvettes (37°C) and adding 4 μl of 5.0 mM TAO (Pfizer) (in dimethyl sulfoxide) to the sample cuvette. NADPH was then added (1 mM final concentration) to both sample and reference cuvettes and the formation of complex was monitored by measuring absorption at 456 nm. After 30 min, maximal absorbance was attained, and the concentration of complex was calculated.

Purification of the Human Cytochrome P-450. Liver microsomes prepared from patient 8 were solubilized with sodium cholate and the cytochrome P-450 immunochemically related to rat P-450p (hereafter referred to as HLp) was purified by the method of Wang *et al.* (20). The solubilized material (675 nmol) was applied to an *n*-octylamine-Sepharose 4B column (2.6 × 37 cm), the column was washed (20), and the HLp was eluted in buffer containing 0.06% Renex 690 (20). Those fractions containing HLp, as determined by immunoblots developed with anti-P-450p IgG, were combined and concentrated to 50 ml by ultrafiltration. The sample was dialyzed against 20 vol of buffer A (20) for 48 hr with four changes. Then the sample was applied to a series of Whatman DE51 (Bodman, Doraville, GA) (1.6 × 7 cm), DE52 (1.6 × 15 cm), and DE53 (1.6 × 25 cm) columns connected in series (20). HLp was the second cytochrome P-450 peak eluted by buffer B from the DEAE series of columns as described (20). The peak fractions containing HLp were combined and dialyzed for 24 hr with two changes of CM52 equilibration

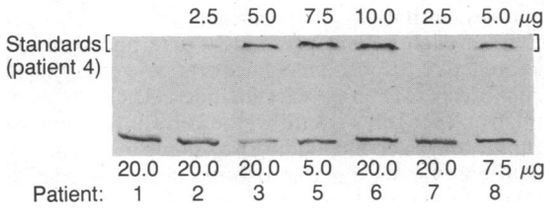

FIG. 1. Immunoblot analyses of human liver microsomes. Liver microsomes were prepared from eight patients as described, and the indicated amounts (5.0–20 μg, except for patient 4) were subjected to polyacrylamide gel electrophoresis. After 1 hr, electrophoresis was temporarily stopped and the indicated amounts of microsomes prepared from patient 4 (2.5–10 μg) were added to the wells of the gel. After completion of electrophoresis, the proteins were transferred to nitrocellulose sheets and blots were developed with anti-LM3c IgG. The intensity of the stained bands produced was measured by densitometry.

buffer (20). The sample was then applied to a Whatman CM52 column (1.6 × 30 cm), which was washed with equilibration buffer, and the cytochrome P-450 was eluted from the column by a NaCl gradient (20). HLp eluted as the first and major 417 nm absorbing peak and was found to be homogeneous as visualized by silver-stained 10% polyacrylamide gels. HLp was concentrated and the detergent was removed by chromatography on a small hydroxylapatite column (Bio-Rad) (20).

RESULTS

When the human liver microsomes were analyzed by immunoblotting with anti-P-450p or anti-LM3c IgG, each of the eight specimens reacted with both antibodies and produced a single band corresponding to a 51-kDa protein (Fig. 1). Microsomes from patient 4 yielded the highest densitometric value when expressed per mg of microsomal protein. The densitometric value was constant on repetitive analysis and was proportional to the amount of applied microsomal protein in the range of 2.5 to 10.0 μg (data not shown). Serial dilutions of microsomes prepared from patient 4 were used as standards on the blots to ensure that the staining density of the immunoreactive bands for all microsomal samples were within the established linear range and to establish that there had been uniform transfer of proteins from the entire gel (Fig. 1). There was a wide variation in relative amounts of the human protein immunochemically related to P-450p among the eight microsomal samples (Table 1). Nonetheless, the results for a given patient were in excellent agreement when the values calculated from blots developed with anti-P-450p IgG (Table 1) were compared to those from blots developed with anti-LM3c IgG (Fig. 1). The amounts of immunoreactive protein in microsomes from patients 4, 5, and 8 were 2- to 5-fold higher than the values for other patients (Table 1). Patients 4 and 8 had received high doses of Dex. Patient 5 received only TAO. The amounts of immunoreactive protein in microsomes prepared from patient 2 (who had received erythromycin base) and from patient 6 (who had received hydrocortisone) are not significantly different from the values for patients 3 and 7, who received no medications.

Next, the microsomal samples were incubated with TAO and NADPH to measure their ability to form the TAO–metabolite complex. Only the microsomes isolated from the patient who had received TAO preoperatively contained TAO–metabolite complex, which had been formed *in vivo* (patient 5). No additional TAO–metabolite complex could be formed *in vitro* with these microsomes. When the amount of TAO–metabolite complex formed in each sample was calculated per mg of microsomal protein and ranked as a percentage of the highest value (patient 4), the results correlated well with the amounts of immunoreactive protein (Table 1). The highest values for TAO–metabolite complex formation were in patients 4, 5, and 8, who had received drugs that are potent inducers of P-450p. However, the correlation was not perfect in that TAO–metabolite complex formation was undetected in patient 7 and was only about one-half the relative value for immunoreactive protein in patient 5 (Table 1).

The final characteristic of rat P-450p examined in the microsomes isolated from the human livers was the ability to demethylate erythromycin. The highest activity was found in the microsomes from patient 4 (Table 1). The activities in other samples ranked relative to the highest value were in excellent agreement with the relative amounts of immunoreactive protein. The only discrepancy was patient 5, who had received TAO and demonstrated high values for immunoreactive protein and TAO–metabolite complex formation but low values for erythromycin demethylase activity. However, this is exactly the pattern that would be expected if the induced cytochrome P-450 in this patient were inhibited by a TAO–metabolite complex formed *in vivo*, just as P-450p is inhibited when induced by TAO treatment in rats (31). Implicit in this argument is the assumption that erythromycin demethylation in human liver is primarily catalyzed by the inducible immunoreactive protein in each sample. We verified this assumption in human liver, as we have previously for rat liver (23), by demonstrating that the presence of either anti-P-450p IgG or anti-LM3c IgG in the reaction mixture blocked >75% of the erythromycin demethylase activity in the microsomes of patient 4 (Fig. 2). In contrast, antibodies to the major phenobarbital-inducible form of

Table 1. Properties of human liver microsomes

Parameter	Patient number							
	1	2	3	4	5	6	7	8
Anti-LM3c immunoblot	36	36	17	100	88	30	26	81
Anti-P-450p immunoblot	36	37	20	100	96	38	32	93
TAO complex	25	30	<15	100	45	20	<15	90
Erythromycin demethylation	32	40	17	100	20	27	30	96
CO-binding hemoprotein	58	58	35	100	40	41	31	79
Medications	Unknown	Erythromycin base, neomycin	None	Dex, phenobarbital diphenylhydantoin	TAO	Cimetidine, hydrocortisone	None	Dex, phenobarbital diphenylhydantoin

The indicated parameters were measured for each of the eight microsomal samples and were ranked as percentage of the respective values for patient 4, which were as follows: immunoblots, arbitrary units; TAO complex, 0.22 nmol·mg^{-1}; erythromycin demethylation, 5.9 nmol of formaldehyde formed per min·mg^{-1}; CO-binding protein, 0.80 nmol/mg. The indicated medications were administered preoperatively.

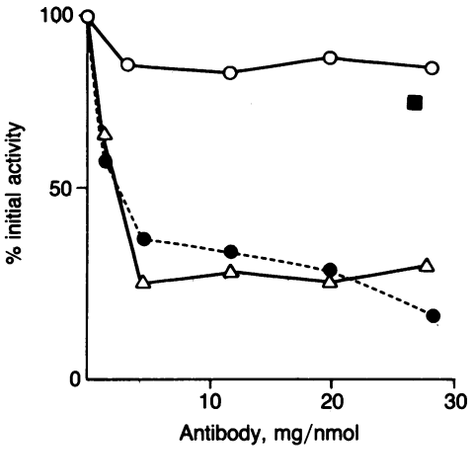

FIG. 2. Effect of anti-cytochrome P-450 antibodies on erythromycin demethylase activity in microsomes from patient 4. Antibodies were added in the indicated amounts to a standard erythromycin demethylation reaction mixture and, after 30 min at 25°C, the reaction was initiated by addition of NADPH. Results are given as percentage of a control incubation containing no antibody, which produced 5.9 nmol of formaldehyde per min per mg of microsomal protein. Anti-P-450p IgG (△); anti-LM3c (●); anti-P-450b (■); unimmunized goat IgG (○).

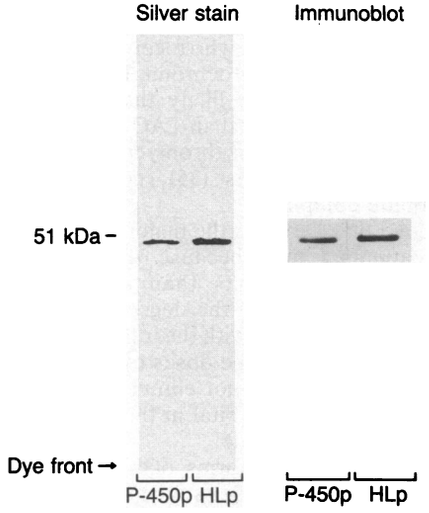

FIG. 3. Comparisons of purified HLp and P-450p. The purified proteins were subjected to electrophoresis on 10% polyacrylamide gels and silver-stained (*Left*) or electrophoretically transferred to nitrocellulose paper and immunoblotted with anti-P-450p IgG (*Right*) as described.

cytochrome P-450 in rat liver (anti-P-450b) or nonimmune goat IgG produced little inhibition of this activity.

Total CO-binding hemoprotein, measured by difference spectroscopy, was also determined in each sample, and the results are expressed relative to the highest value (patient 4) (Table 1). The patients who did not receive Dex (for example, patient 1) had values for total cytochrome P-450 that were a higher percentage of the value for patient 4 (58%) than were the relative amounts of immunoreactive protein (30%). This suggests that a higher percentage of total cytochrome P-450 in patients 4 and 8 consists of the cytochrome immunochemically related to P-450p. In contrast, patient 5 had relatively higher amounts of immunoreactive protein (88%) as compared to either total cytochrome P-450 (40%) or to the amount of TAO–metabolite complex formed (45%). Hence, this patient may have accumulated excess immunoreactive apocytochrome, as has been proposed to occur in rat liver (2).

By using published methods (20), we isolated and purified the cytochrome P-450 immunochemically related to rat P-450p from patient 8 by monitoring column eluate fractions for protein reactive with anti-P-450p IgG on immunoblots. This human liver cytochrome, which we term HLp, contained 12.5 nmol of CO-binding hemoprotein per mg of protein, appeared homogeneous as judged by electrophoresis on polyacrylamide gels stained with silver, and exhibited a mobility identical to that of P-450p corresponding to a size of 51 kDa (Fig. 3). Purified HLp reacted with anti-P-450p (Fig. 3) and anti-LM3c IgG (not shown) on immunoblots. Two other cytochromes P-450 purified from the same liver exhibited different electrophoretic mobilities and failed to react with either antibody (not shown). In a reconstituted system, HLp demethylated erythromycin at a rate (1.9 nmol·min^{-1}/nmol of HLp) comparable to that exhibited by P-450p (2.0 nmol·min^{-1}/nmol of P-450p) and LM3c (3.2 nmol·min^{-1}/nmol of LM3c). NH_2-terminal amino acid sequence analysis was performed on HLp (Fig. 4) and revealed little homology with P-450p (33%) or rabbit LM3c (27%).

DISCUSSION

In this study, we have isolated and characterized a specific molecular form of human liver cytochrome P-450, termed HLp, which is immunochemically related to the glucocorticoid-inducible P-450p in the rat and LM3c in the rabbit. HLp also resembles P-450p and LM3c in catalyzing erythromycin demethylation and in converting TAO to a metabolite complex. The concentration of HLp was significantly increased in the two patients who had received Dex, phenobarbital, and diphenylhydantoin, and in one patient who had received TAO. Since these were also the only patients who were given drugs that strongly induce P-450p in rat liver, we conclude that the increased concentrations of HLp represents induction and not genetic differences.

Proof of HLp inducibility would require serial liver biopsies in a drug-treated human. Nevertheless, our conclusion is supported by a previous study in which the concentrations of total CO-binding hemoprotein in liver biopsies from eight patients treated with TAO were increased over that measured in untreated patients (33), and this increase was accounted for by a cytochrome present as a TAO–metabolite complex. Our

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
HLp	-	ALA	LEU	ILE	PRO	ASP	LEU	ALA	MET	GLU	THR	TRP	LEU	LEU	LEU	ALA	VAL	SER	LEU	VAL	LEU
LM3c	MET	ASP	LEU	ILE	PHE	SER	LEU	GLU	THR	TRP	VAL	LEU	LEU	ALA	ALA	SER	LEU	VAL	LEU	LEU	TYR
P450p	MET	ASP	LEU	ILE	PHE	MET	LEU	GLN	THR	SER	SER	LEU	LEU	ALA	ALA						
P450/PCN	MET	ASP	LEU	LEU	SER	ALA	LEU	THR	LEU	GLU	THR	TRP	VAL	LEU	LEU	ALA	VAL	VAL	LEU	VAL	LEU

FIG. 4. The NH_2-terminal amino acid sequence of HLp was determined as described (23) by using a 470A gas phase sequencer. The yields of the amino acids (pmol) per cycle for HLp were as follows: 2, 74; 3, 58; 4, 59; 5, 42; 6, 23; 7, 42; 8, 43; 9, 46; 10, 12; 11, 5; 12, 23; 13, 23; 14, 23; 15, 23; 16, 23; 17, 21; 18, identified as Ser-DTT adduct; 19, 17; 20, 17; and 21, 10. Corresponding published sequences of LM3c, P-450p (23) and P-450/PCN (40) are provided for comparison.

finding of high amounts of immunoreactive protein in the microsomes from patient 5, who received TAO, indicates that HLp is a major form of cytochrome P-450 induced by TAO in human liver. It is also likely that HLp is induced by macrolide antibiotics other than TAO, because liver biopsies of patients treated with erythromycin proprionate (34), an inducer of P-450p in rats (35), contain an erythromycin–metabolite complex.

We believe that Dex was the major inducer of HLp in the livers of patients 4 and 8 because (*i*) Dex is a more potent inducer of P-450p in rats than are phenobarbital or diphenylhydantoin and (*ii*) the degree of induction of HLp corresponds more closely with the total dose of Dex received preoperatively than with the doses of the antiseizure drugs. However, our studies do not eliminate the possibility that phenobarbital or phenobarbital in the presence of Dex may also induce this cytochrome.

Most of the inducible forms of liver cytochrome P-450 (including P-450p and LM3c) studied to date in animals are either absent or represent a small fraction of the total cytochrome P-450 in the basal steady state. Therefore, we were surprised to find readily measurable amounts of HLp in the livers of patients receiving no drugs. Indeed, a recent survey of drug-oxidizing activities in 33 human liver microsomal samples (36) revealed, without exception, the presence of warfarin-hydroxylating activity at the R-10 position, a reaction that is stereoselectively catalyzed by rat P-450p (2). If, as preliminary data suggest (L. Kaminsky, personal communication), HLp supports R-10 warfarin hydroxylation, then HLp is a prominent form expressed widely in humans. Since P-450p is inducible in cultured rat hepatocytes exposed to corticosterone (37), it is possible that the level of circulating glucocorticoids sustains the expression of HLp. Another intriguing possibility is that, because macrolide antibiotics are widely used in commercial feed for poultry and other farm animals (38), people may be continuously exposed to small amounts of these inducers of HLp.

The NH_2-terminal amino acid sequence of HLp showed little homology to that of P-450p or LM3c. However, a shift in the alignment of the sequence of HLp by two amino acids results in greater homology between HLp as compared to LM3c or P-450p (Fig. 4). Recently, others (39) have isolated a rat liver cytochrome P-450 (termed P-450/PCN) that is inducible by PCN and phenobarbital, is identical to P-450p in its carboxyl-terminal amino acid sequence based on nucleic acid sequencing of cloned cDNAs to the mRNAs for these proteins (24) and, yet, differs from P-450p in its NH_2-terminal amino acid sequence (Fig. 4). However, P-450/PCN shares 57% homology (83% for positions 10–21) in NH_2-terminal amino acid sequence with HLp (Fig. 4). Since genomic hybridization studies using cloned cDNA coding for P-450/PCN (32) have indicated that there are multiple genes related to P-450/PCN in all species examined (including humans), it is likely that HLp belongs to a genetically related family of PCN-inducible forms of cytochrome P-450 that are catalytically similar and are conserved in mammalian species.

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