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DISTRIBUTION OF CYTOCHROMES P-450, CYTOCHROME b_5, AND NADPH–CYTOCHROME P-450 REDUCTASE IN AN ENTIRE HUMAN LIVER

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Abstract—In rat liver there appear to be significant differences between lobes in the concentration of individual cytochrome P-450 isozymes (Sumner and Lodola, *Biochem Pharmacol* **36**: 391–393, 1987). Because studies in patients often rely on small pieces of liver obtained from diverse anatomical locations, it seemed important to determine if the cytochromes P-450 were also heterogeneously distributed in human liver. Accordingly, tissue was obtained from ten different locations in a single human liver including those most commonly biopsied by percutaneous needles, and by surgeons during laparotomy. The differences observed between locations in the microsomal concentrations of carbon monoxide-binding protein (total cytochrome P-450), cytochrome b_5, and NADPH–cytochrome P-450 reductase appeared to be small and were not statistically significant. Likewise, no significant differences were observed between locations in the specific content of HLp, HLp3, HLj, HLx or P450MP. However, the specific concentrations of HLd varied almost 2-fold between the microsomes and this was statistically significant in some cases ($P < 0.05$). Our results suggest that, in human livers, regional differences in the content of cytochromes P-450 are generally small but may be significant for some isozymes. With the exception of HLd, tissue obtained by percutaneous or surgical liver biopsies is probably representative of the entire organ with regard to the enzymes assayed.

The hepatic cytochromes P-450 are a multigene family of enzymes that play a critical role in the metabolism of many drugs and xenobiotics. There are marked differences between patients in the liver content of some of the cytochromes P-450, and this may result in variable responses to many drugs and other xenobiotics [1]. These "oxidative polymorphisms" appear to largely reflect genetic factors [1]; however, nongenetic factors may also be involved. In rodents, for example, it is well established that liver content and catalytic activity of some of the cytochromes P-450 are greatly influenced by animal gender and by treatment with a wide variety of xenobiotics, including many medications. Although many of the genes coding for rodent cytochromes P-450 have been shown to be highly conserved in other mammals including humans [2], it is generally unknown whether the mechanisms involved in the regulation of these enzymes are also highly conserved. If their regulation is conserved, the liver content of the cytochromes P-450 in patients could be influenced significantly by a variety of factors including many commonly used medications, cigarette smoking, and gender.

One approach to defining factors underlying cytochrome P-450 polymorphisms has been to examine individual P-450 proteins or their catalytic activities in liver tissue obtained from patients, and to correlate these results with patient characteristics such as medication and smoking histories [3–9]. In many instances, only a small portion of the patient's liver is available for analysis, such as a small core of tissue obtained by percutaneous needle biopsy or a small wedge of tissue obtained during abdominal surgery, and it has been assumed that the information obtained from the samples on the expression of the cytochromes P-450 is representative of the entire liver. This assumption has been called into question by a recent report that the concentrations of total cytochrome P-450, and of the specific P-450 proteins inducible by phenobarbital and 3-methylcholanthrene, differ significantly in microsomes prepared from tissue obtained from different lobes, and from different locations within the same lobe of rat liver [10, 11]. If the distributions of the various forms of cytochrome P-450 in the human liver also vary depending on location within the liver, the results of analysis of tissue obtained from a single biopsy site may not be representative of the entire liver.

We recently received an entire human liver from an adult male patient. Microsomes were prepared from tissue obtained at ten anatomical locations, including those most frequently biopsied. Each sample of microsomes was analyzed with regard to the concentration of five different human P-450 proteins, total cytochrome P-450, cytochrome b_5, and NADPH–cytochrome P-450 reductase activity. In addition, we assayed each sample of microsomes for its ability to catalyze the N-demethylation of erythromycin and *N*-nitroso-dimethylamine (NDMA), catalytic activities characteristic of HLp and of HLj respectively [3, 5]. The results presented

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here indicate that, within this liver, regional differences in content and activity of these enzymes generally appeared to be small. More importantly, tissue obtained from the most common locations for percutaneous and surgical (wedge) liver biopsies appeared to be representative of the entire organ with regard to all enzymes assayed, with the possible exception of HLd.

METHODS

Patient data. The entire liver was obtained from a 30-year-old male patient who became an organ donor after he developed a subarachnoid hemorrhage. He had previously been healthy and he was not taking medications. Donor organ surgery was performed within 24 hr of his admission to the hospital and 20 hr after he was placed on a respirator. The patient's serum aspartate aminotransferase, alanine aminotransferase, bilirubin, alkaline phosphatase, albumin and prothrombin time were all within normal limits. In the operating room the patient received 30,000 units of intravenous heparin and dopamine (4 μg/kg/min) for 3 hr in addition to general anesthesia. The liver was rapidly cooled by *in situ* perfusion with a buffered saline and glucose solution (Eurocollins solution, 4°). The liver was then removed from the donor and transported on ice to a potential recipient at The University of Michigan Medical Center. The entire liver became available for our studies when the transplant recipient died intraoperatively and no alternate recipient could be found. The ten biopsies of liver tissue were obtained within 6 hr after removal of the liver from the donor.

Tissue processing and preparation of microsomes. Wedges of hepatic tissue (~10 g) were excised from the ten locations shown in Fig. 1. Location 6 is the most common site biopsied by percutaneous needle, and sites 2 and 4 are the most common locations for an intraoperative biopsy. All specimens were flash frozen in liquid nitrogen. Hepatic microsomes were prepared simultaneously from all tissue specimens as previously described [3].

Antibodies and immunoblot analyses. Antibodies that specifically recognize human cytochromes HLp [3], HLx [4], and HLd [12] were prepared as described in the indicated references. Murine monoclonal antibodies were prepared as previously described [4] against HLj [13] and P450MP purified as indicated below. Immunoblot analyses were performed and quantified as previously described [4].

Purification and characterization of human liver cytochrome P450MP. Human liver microsomes (2000 mg) obtained from a patient previously termed patient 11 [4] were solubilized and the amino-octylamino-Sepharose 4B and diethylaminoethyl (DEAE)-cellulose columns were run as described in detail elsewhere [3]. The fractions in the second heme-containing peak eluting off the DEAE columns were combined and dialyzed against 30 vol. of buffer C (5 mM potassium phosphate, pH 6.5, 20% glycerol, 0.1 mM EDTA, and 0.2% Tergitol NP-10) with three changes over 48 hr. The dialyzed sample was then applied at 1 mL/min to a CM-52 column (1.6 × 28 cm) equilibrated with 250 mL of buffer C. The column was washed with 250 mL of buffer C, and the hemoprotein was eluted by the application of 500 mL of 0 to 150 mM NaCl gradient in buffer C. Those fractions eluting in the major heme-containing peak were combined.

The combined fractions were then dialyzed overnight against 50 vol. of 5 mM potassium phosphate (pH 7.25) buffer containing 20% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol (DTT) and 0.2% Tergitol NP-10. The dialyzed sample was then applied at 1 mL/min to an hydroxylapatite column (1.6 × 5 cm) equilibrated with 50 mL of the dialysis buffer. The column was then washed with 50 mL each of 40 and 90 mM potassium phosphate (pH 7.25) buffers containing the same additives as the dialysis buffer.

The combined fraction was then concentrated 3-fold and dialyzed against 125 vol. of 5 mM Tris–HCl buffer containing 20% glycerol, 1.0 mM DTT, and 1.0 mM EDTA with two changes over 2 days. The dialyzed fraction was then applied at 1 mL/min to a DEAE-Sepharose CL-6B column (1.6 × 7.5 cm) equilibrated with 100 mL of dialysis buffer. The hemoprotein did not bind tightly and was eluted with dialysis buffer. The detergent was removed from this fraction by application to a small hydroxylapatite column.

The final fraction was found to contain only one protein as visualized in silver-stained sodium dodecyl sulfate (SDS)–polyacrylamide gels and had a CO-binding heme content of 14 nmol/mg protein. Amino-terminal amino acid analysis of P450MP by automated Edman degradation [5] yielded a 19 amino acid sequence identical to that previously reported for the form of human liver P-450 responsible for the metabolism of mephenytoin and is therefore presumed to be P450MP [14].

Statistical analysis. The determination of each enzyme activity or concentration was performed in triplicate on each of the ten samples of microsomes. For each enzyme, the results were subjected to a one-way analysis of variance followed by the Tukey multiple comparison procedure [15]. This analysis compared the three values obtained in microsomes from each location with the three values obtained in microsomes from each of the other nine locations. This conservative analysis was used to determine whether inconsistency in the assay procedures alone could account for the observed differences between liver locations. For each enzyme, the mean of values obtained at all ten locations was also calculated, and 95% confidence intervals were calculated as the mean ± two standard deviations of the mean.

Other assays. Total cytochrome P-450 was measured as CO-binding protein [16]. Cytochrome b_5 concentration [17], erythromycin *N*-demethylase activity [3], *N*-nitroso-dimethylamine *N*-demethylase activity [5], and NADPH–cytochrome P-450 reductase activity [18] were measured as previously described at 37°.

RESULTS

Microsomes were prepared from tissue obtained at ten locations in a single human liver (Fig. 1). The levels of total CO-binding protein (total cytochrome P-450) and of cytochrome b_5, and the activities of

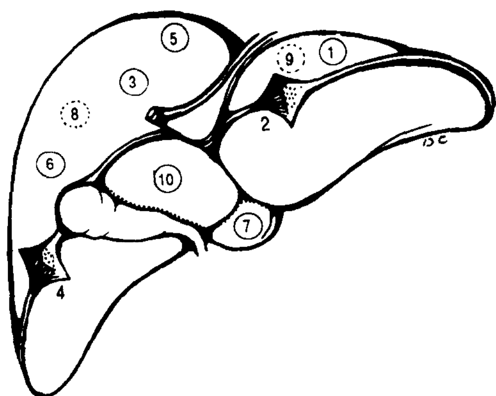

Fig. 1. Biopsy locations in the liver. Approximately 10 g of tissue was removed from each of the ten locations shown. The anterior of the liver is shown in the view obtained during laparotomy. Biopsies 8 and 9 were obtained from the opposite side of the liver.

NADPH–cytochrome P-450 reductase, NDMA *N*-demethylase and erythromycin *N*-demethylase were determined in each sample of microsomes. The values obtained at each location are indicated in Table 1 as the means of triplicate measurements (except for cytochrome b_5) with the calculated standard deviations. Since each location was biopsied only once, the standard deviations shown for each location reflect the reproducibility of the assay results and not distribution of the enzyme within a specific region of the liver.

To determine if the differences observed between locations could not have been due to inconsistencies in our assay methods alone, for each enzyme, the mean value obtained at each location was compared with that obtained at the other nine locations using the Tukey multiple comparison test [15]. According to this conservative analysis, the difference between the lowest erythromycin *N*-demethylase activity (0.35 nmol/min/mg protein at location 7) and the highest catalytic activity towards this substrate (0.66 nmol/min/mg protein in location 2) was significant ($t = 5.11$, $P < 0.05$). No other pairs of results from any of the other assays differed significantly, including the relatively large difference between the highest and lowest values for NADPH cytochrome P-450 reductase activity (141 at location 1 and 212 at location 6; $t = 4.96$). The content of cytochrome b_5 varied from 0.441 nmol/mg (location 9) to 0.493 nmol/mg (location 3); however, only two measurements were obtained in each specimen and the differences appeared to be within the error of the assay. Therefore, with one possible exception, the intraliver variations in the concentrations of the enzymes tested may not be significant.

If the distribution of these enzymes is indeed homogeneous, the true liver specific concentration of each enzyme could be best estimated as the mean of the measurements obtained in biopsies from each of the ten locations. As shown in Table 1, the overall mean concentrations of cytochrome P-450 (0.415 nmol/mg) and of cytochrome b_5 (0.471 nmol/mg), and the overall mean NADPH reductase

Table 1. Distribution of various enzyme activities in human liver microsomes*

Location	P-450 (nmol/mg protein)	ERMD (nmol formaldehyde formed/min/mg protein)	NDMAD	b_5 (nmol/mg protein)	NADPH reductase (nmol/min/mg protein)
1	0.356 ± 0.022	0.44 ± 0.09	1.19 ± 0.11	0.478	141 ± 1
2	0.422 ± 0.032	0.66 ± 0.04	1.45 ± 0.15	0.484	185 ± 30
3	0.416 ± 0.021	0.51 ± 0.02	1.34 ± 0.07	0.493	182 ± 18
4	0.390 ± 0.005	0.52 ± 0.09	1.10 ± 0.25	0.444	182 ± 18
5	0.457 ± 0.034	0.48 ± 0.06	1.35 ± 0.25	0.458	179 ± 8
6	0.420 ± 0.013	0.56 ± 0.09	1.22 ± 0.12	0.478	212 ± 16
7	0.406 ± 0.075	0.35 ± 0.12	1.22 ± 0.12	0.485	183 ± 28
8	0.460 ± 0.030	0.50 ± 0.03	1.38 ± 0.11	0.476	201 ± 8
9	0.409 ± 0.051	0.54 ± 0.08	1.30 ± 0.15	0.441	202 ± 10
10	0.408 ± 0.052	0.50 ± 0.06	1.31 ± 0.15	0.469	201 ± 16
Mean	0.415	0.51	1.29	0.471	187
95% Conf. intervals	0.355–0.475	0.35–0.67	1.09–1.49	0.436–0.506	148–226

* Activities of the various enzymes were determined as described in Methods. Values are the means ± SD of triplicate determinators except for cytochrome b_5 where the average of two measurements obtained in each specimen is shown. Abbreviations: P-450, cytochrome P-450; ERMD, erythromycin *N*-demethylase activity; NDMAD, NDMA *N*-demethylase activity; b_5, cytochrome b_5, and NADPH reductase, NADPH–cytochrome P-450 reductase.

Table 2. Distribution of specific isozymes of cytochrome P-450 in the human liver microsomes*

Location number	Isozyme†				
	HLp/HLp3	P450MP	HLx	HLd	HLj
1	95 ± 4	76 ± 1	69 ± 6	40 ± 12	105 ± 16
2	94 ± 4	84 ± 9	81 ± 11	46 ± 2	120 ± 10
3	93 ± 13	72 ± 14	94 ± 7	72 ± 5	100 ± 9
4	114 ± 15	93 ± 14	88 ± 18	42 ± 1	84 ± 8
5	103 ± 13	87 ± 8	71 ± 7	62 ± 6	105 ± 10
6	104 ± 9	97 ± 14	94 ± 9	56 ± 3	109 ± 1
7	109 ± 4	83 ± 3	71 ± 4	78 ± 2	100 ± 10
8	102 ± 13	100 ± 15	95 ± 6	64 ± 4	106 ± 9
9	95 ± 2	102 ± 9	77 ± 4	77 ± 3	92 ± 3
10	86 ± 12	105 ± 15	106 ± 12	64 ± 7	94 ± 1
Mean	99.5 ± 8.4	89.9 ± 11.2	84.6 ± 12.7	60.1 ± 13.9	101.5 ± 10.0
95% Conf. intervals	(82.7–116.3)	(67.4–112)	(109–59.3)	(32.4–87.8)	(121–81.5)

* The levels of the various isozymes were determined by immunoblot analyses performed as described in Methods. The values are the means ± SD of triplicate determinations.

† All values are expressed as percent of immunoreactive protein present in microsomes from a unrelated patient (patient No. 23 in Ref. 4).

(187 nmol/min/mg), erythromycin *N*-demethylase and NDMA *N*-demethylase (0.51 and 1.29 nmol formaldehyde formed/min/mg respectively) activities are comparable to those previously reported in human liver microsomes [3, 5, 9]. The 95% confidence intervals of these means did not appear to be wide and, with the exception of the NADPH–cytochrome P-450 reductase activity determined at location 1, the result determined in the tissue from each location fell within the 95% confidence intervals of the mean for all locations. In particular, each assay value determined for tissue obtained from the locations of either a percutaneous biopsy (location 6) or a surgical wedge biospy (location 2 and 4) fell within 20% of the respective mean determination for all locations.

Next, we determined the distribution of five specific P-450 proteins in the liver by analyzing each of the ten samples of microsomes for content of immunoreactive HLp, P450MP, HLx, HLd, and HLj. This was determined by densitometric analysis of immunoblots developed with form specific antibodies. Each assay was performed in triplicate on each sample of microsomes, and the mean values ± the standard deviations of the means are shown in Table 2. Our monoclonal antibody to HLp identified two closely spaced protein bands (a "doublet") on the immunoblots, indicating that this patient expressed HLp3 [19]. HLp3 is a cytochrome P-450 closely related to HLp in structure and catalytic activity, and it appears to be expressed in only 20% of the population [19]. The specific content listed in Table 2, therefore, reflects the combined total of these proteins. However, by visual inspection of the blots, there was no obvious difference between locations in the relative intensities of the HLp and HLp3 bands. The intraliver variations observed in HLp/HLp3, HLj, HLx and P450MP were not significant according to the Tukey multiple comparison test. However, significant differences in the concentrations of HLd were observed between location 1 and locations 3, 7, 8, 9 and 10 ($t = 7.19$, 8.85, 5.39, 8.32 and 5.39 respectively, $P < 0.05$), between locations 2 and 9 ($t = 6.97$, $P < 0.05$), between locations 3 and 4 ($t = 6.74$, $P < 0.05$), and between location 4 and locations 7 and 9 ($t = 8.09$ and 7.87 respectively, $P < 0.05$). For each form of P-450 assayed, the specific content determined in microsomes prepared from each location fell within two standard deviations of the mean of measurements at all locations (Table 2). With the exception of HLd, the concentrations of each of the proteins determined in microsomes prepared from locations 2, 4 and 6 fell within 20% of the mean values for all determinations.

Finally, we reasoned that if the differences between locations in the concentrations of HLp/HLp3 and HLj were in fact significant, we might see a correlation between the content of each enzyme protein and its characteristic catalytic activity in each sample of microsomes. However, no correlation was observed between the specific concentrations of HLp/HLp3 and the ability to demethylate erythromycin ($r = 0.08$) or between the specific concentration of HLj and the ability to catalyze NDMA demethylation ($r = 0.03$). This suggests that the distribution of these forms and their catalytic activities were uniform across the ten sites examined.

DISCUSSION

It is now well established that within the functional unit of mammalian liver, the hepatic acinus, hepatocyte content of drug-metabolizing enzymes is heterogenous [20]. It has been reported recently that, in addition to acinar heterogeneity, there are significant interlobe and intralobe variations in the microsomal concentration of total cytochrome P-450 and of P-450 proteins in rat liver [10, 11]. Similar regional differences in human liver may have important implications since it is rarely possible to obtain a whole human liver for analysis, and studies often rely on small amounts of tissue obtained from diverse anatomical locations.

We here report the results of assays of various enzyme activities and of five P-450 proteins in microsomes prepared from ten different locations of an entire adult male liver. In general, intraliver differences in the content and catalytic activity of the enzymes measured appeared to be small. Indeed, the conservative multiple analysis statistics we employed suggested that the majority of these differences could have been due to inconsistency in our assay methods alone. Additional evidence suggesting that the specific concentration of some of the drug metabolizing enzymes may be homogeneous in human liver is that there was no correlation between either the content of HLp/HLp3 and erythromycin *N*-demethylase activity or between the content of HLj and NDMA *N*-demethylase activity in the microsomes. We have shown previously that in human liver microsomes anti-HLp IgG can inhibit >95% of the erythromycin *N*-demethylase activity present and anti-HLj IgG can inhibit up to 78% of the NDMA *N*-demethylase activity [5, 21]. Furthermore, we have demonstrated that, in microsomes isolated from a large number of different human liver specimens, an excellent correlation exists between the content of HLp and HLj and their respective catalytic activities [3, 21, and unpublished results]. Our failure to find these correlations in the liver microsomes of our patient further suggests that the regional differences in the concentrations of these enzymes may not be significant.

Differences between microsomes in content of HLd appeared to be significant, however. Thus, a study of this patient which relied exclusively on measurements of HLd determined in tissue obtained by surgical biopsy in location 2 or 4 would have underestimated the mean value for all locations of this liver by almost 30% (Table 2). This suggests that the distribution of HLd in this liver may be independent of that of other cytochromes P-450, which appeared to be homogeneous in the liver. It has been reported that treatment of rats with phenobarbital or 3-methylcholanthrene increases interlobe differences in the distribution of the induced P-450 proteins [11]. The selective variation in concentration of HLd therefore may have resulted from exposure of this patient to inducers of this enzyme. However, our patient had received no known enzyme inducers prior to surgery, he was not a smoker, and he had no history of unusual environmental exposures. Furthermore, the specific content of HLd in the liver microsomes of our patient was similar to that observed in the majority of the other livers we have assayed [4]. It was not possible to confirm the differences in HLd protein by correlating them with differences in catalytic activity since an HLd-specific substrate has not yet been identified. It should be pointed out, however, that the regional differences observed in the specific content of HLd are modest compared to the greater than 10-fold variation in liver HLd concentration observed between patients [4]. It would obviously be desirable to repeat our studies in additional whole livers obtained from patients with a variety of medication and environmental exposure histories, but this will be difficult with the current demand for transplant organs.

In summary, intraliver differences in the content of the measured enzyme proteins and catalytic activities were generally small in this patient. With the exception of HLd concentration, the results of each assay performed on tissue obtained from the most common sites for percutaneous liver biopsy or for surgical wedge liver biopsy were within 20% of the mean value for all locations.

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