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Altered Regulation of Cytochrome P-450 Enzymes in Choline-Deficient Cirrhotic Male Rat Liver: Impaired Regulation and Activity of the Male-Specific Androst-4-ene-3,17-dione 16α-Hydroxylase, Cytochrome P-450$_{UT-A}$, in Hepatic Cirrhosis

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SUMMARY

Total microsomal cytochrome P-450 levels were decreased, to about 50% of control, in liver of male rats made cirrhotic by the prolonged intake of a choline-deficient diet. We have suggested previously that this decrease in cytochrome P-450 levels is not a generalized one, but is selective for certain forms of the enzyme. In the present study, levels of six cytochrome P-450 forms including the sex-specific cytochrome P-450 forms, P-450$_{UT-A}$, P-450$_{PCN-E}$, and P-450$_{UT-I}$, were quantitated immunochemically in hepatic microsomes prepared from control and cirrhotic male rats and were related to changes in the regioselectivity of cytochrome P-450-mediated androst-4-ene-3,17-dione hydroxylation in these fractions. The principal finding of this study was that the male-specific androst-4-ene-3,17-dione 16α-hydroxylase was decreased in cirrhotic microsomes to about 20% of control. The content of P-450$_{UT-A}$ decreased concurrently from about 0.40 to less than 0.01 nmol/mg of microsomal protein. Other pathways of androst-4-ene-3,17-dione hydroxylation were also affected, but to different extents than the 16α-hydroxylase. 6β-Hydroxylation decreased in cirrhotic microsomes to about 45% of control, despite a marked decrease in P-450$_{PCN-E}$ from 0.27 to less than 0.002 nmol/mg of microsomal protein. The rate of androst-4-ene-3,17-dione 7α-hydroxylation underwent a less pronounced reduction in cirrhosis to about two-thirds of control microsomal activity, and levels of the cytochrome P-450 associated with this activity, P-450$_{UT-F}$, were decreased in proportion with the decrease in total microsomal cytochrome P-450. 16β-Hydroxylase activity was unaffected by the cirrhotogenic process. From spectral binding studies it was apparent that androst-4-ene-3,17-dione elicited a high affinity type I interaction in control microsomal fractions (K_s = 4.5 μM), whereas no interaction was apparent in cirrhotic liver microsomes. Levels of three other forms of cytochrome P-450—P-450$_{PB-C}$ (a constitutive form inducible by phenobarbital), P-450$_{ISF-G}$ (a major isosafrole-inducible form), and P-450$_{UT-I}$ (the major female sexually-differentiated isozyme)—were apparently unaltered in cirrhosis. These findings are consistent with the assertion that specific forms of cytochrome P-450 are subject to altered regulation in hepatic cirrhosis.

Cytochrome P-450 is the principal catalytic component of the hepatic microsomal mixed function oxidase system that is active in the biotransformation of lipophilic drugs and endogenous compounds to more hydrophilic metabolites. It is now recognized that the unusually broad substrate specificity of this system is due to the existence of a number of forms of P-450 (1–3). Although the absolute number of forms is open to speculation, at least 12 distinct proteins have been described by several laboratories (2–5).

Levels of microsomal P-450 are reduced in humans with chronic liver disease and, as a consequence, the drug oxidation capacity of these individuals is impaired (6). Following *in vivo* studies of hepatic drug clearance (7) and *in vitro* estimation of microsomal oxidase activity (6), it was suggested that selective changes in different mixed function oxidase activities may occur in chronic liver disease that relate to changes in individual forms of P-450. As yet, no direct studies of P-450 composition have been undertaken in humans with chronic hepatocellular disease. Studies of experimental liver disease in animals, such as the carbon tetrachloride inhalation model of hepatic cirrhosis (8), have demonstrated that P-450 in hepatic microsomes from cirrhotic animals has a reduced capacity to bind (9) and catalyze the oxidation (10, 11) of drug substrates. Hepatic heme turnover was normal in this model (12) and the capacity for microsomal P-450 induction was retained (11). In light of these findings, altered regulation of basal P-450 levels was proposed

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ABBREVIATIONS: P-450, cytochrome P-450; EDTA, ethylenediaminetetraacetic acid; IgG, immunoglobulin fraction of sera.

as a feasible underlying cause of impaired hepatic drug oxidation. Recent studies, in the choline-deficient rat model of hepatic cirrhosis, suggested that chronic liver disease is associated with the selective suppression of some P-450s (13).

It is now clear that certain P-450s catalyze the regiospecific hydroxylation of androst-4-ene-3,17-dione (14–18). Accordingly, this endogenous steroid substrate was used to probe the absolute and relative changes in hydroxylase activities in microsomal fractions from choline-deficient cirrhotic and choline-supplemented control rat liver. In addition, levels of specific P-450s were quantitated by immunoblotting to examine the relationship between the development of hepatic cirrhosis and the expression of individual forms of P-450.

Materials and Methods

Chemicals. [4-^{14}C]Androst-4-ene-3,17-dione (specific activity 59 mCi/mmol) was obtained from Amersham Australia, Sydney, New South Wales. Unlabeled androst-4-ene-3,17-dione (androstenedione), 6β-hydroxy- and 16α-hydroxyandrost-4-ene-3,17-dione, as well as all biochemicals, were purchased from Sigma Chemical Co., St. Louis, MO. 7α-Hydroxyandrost-4-ene-3,17-dione was obtained from Professor D. N. Kirk and the Medical Research Council Steroid Reference Collection, Queen Mary's College, London, United Kingdom. 16β-Hydroxyandrost-4-ene-3,17-dione was prepared enzymatically by the action of 3β-hydroxysteroid dehydrogenase (Sigma Chemical Co.) on 3β,16β-dihydroxyandrost-5-ene-17-one (Medical Research Council collection). Other hydroxylated testosterone and androst-4-ene-3,17-dione standards were obtained from either Sigma, the Medical Research Council Steroid Collection, or Steraloids Inc., Wilton, NH.

Components of the choline-deficient (and choline-supplemented) diet were obtained through normal retail outlets. Salts and vitamins, for inclusion into synthetic diets, were purchased either from Sigma or Roche Pty. Ltd., Dee Why, New South Wales, Australia. Solvents and miscellaneous chemicals were from Ajax Chemicals, Sydney, New South Wales, Australia and were at least analytical reagent grade.

Animals. Male Wistar rats (starting weight 90–130 g) were obtained from the animal facility at the Westmead Hospital Institute of Clinical Pathology and Medical Research. Animals were held in wire cages under conditions of constant temperature (22°), humidity, and lighting (12-hr dark/light cycle).

Animal treatment. The choline-deficient diet used in the production of experimental hepatic cirrhosis was prepared as described elsewhere (13). Choline-supplemented diet was prepared by the addition of 0.4% choline chloride to the choline-deficient synthetic diet. Animals received water and either the choline-deficient or the choline supplemented diet *ad libitum* over a 30-week period, until the time of sacrifice. After an overnight fast, microsomal fractions were prepared from individual rat livers as before (13). The final microsomal fractions were stored at −70° as suspensions in 50 mM potassium phosphate, containing 20% glycerol (pH 7.4), until required for use.

Androstenedione hydroxylase activity. Microsomal androstenedione hydroxylase activity was assayed essentially by the procedure of Gustafsson and Ingelman-Sundberg (19). Briefly, microsomal fractions from control or cirrhotic rat liver (2.5 mg of protein/ml in 100 mM potassium phosphate buffer containing 1 mM EDTA, pH 7.4) were incubated with [^{14}C]androstenedione (final concentration 0.19 mM; 4 × 10^5 dpm; added in 50 μl of acetone) for 10 min at 37° in the presence of 1 mM NADPH. Incubations (final volume 4.0 ml) were terminated by the addition of 5.5% zinc sulfate and centrifuged, and the supernatant was extracted with chloroform. The organic phase was evaporated to dryness under N_2, reconstituted in a small volume of chloroform, and applied to thin layer chromatography plates (silica gel 60 F_{254} type, 20 × 20 cm × 0.25 mm thickness, and activated 15 min at 100° before use; E. Merck, Darmstadt, West Germany). Plates were developed twice in the solvent system $CHCl_3$:ethyl acetate (1:2, v/v) as described by Waxman *et al.* (16). Zones corresponding to hydroxylated androstenedione standards were visualized under UV light and scraped into vials for scintillation spectrometry (Aquasol scintillant, New England Nuclear Corp., Boston, MA).

Spectral binding assays. Studies of the spectral binding interactions of androstenedione in liver microsomes from choline-supplemented control and choline-deficient cirrhotic rats were conducted at 37° as described elsewhere (9). Substrate-elicited spectral changes were recorded after μl additions of the steroid (stock solution in dimethylformamide). Spectral dissociation constants (K_s) and maximal absorbance change values (ΔA_{max}) were derived from x axis and y axis intercepts, respectively, of double reciprocal plots of androstenedione concentration versus spectral change magnitude (20).

Other assays. Protein was determined by the method of Lowry *et al.* (21) with bovine serum albumin as standard, and P-450 levels were estimated by the procedure of Omura and Sato (22).

Purified cytochrome P-450 isozymes[1] and antibodies. Cytochromes P-450$_{UT\text{-}A}$, P-450$_{PCN\text{-}E}$, P-450$_{PB\text{-}C}$, P-450$_{UT\text{-}F}$, P-450$_{UT\text{-}I}$, and P-450$_{ISF\text{-}G}$ were isolated as described by Guengerich *et al.* (2). Antisera were raised in rabbits as detailed elsewhere (2) and IgG fractions were subsequently obtained (23).

Quantitation of the six individual P-450 isozymes in control and cirrhotic microsomal fractions was performed by immunoblotting, as described elsewhere (2, 18, 24); when appropriate, cross-adsorptions were done to improve antigen-antibody selectivity (18).

Androstenedione hydroxylase activities of purified P-450 isozymes are as follows (turnover numbers): P-450 2c/UT-A, 7.1 nmol of 16α-hydroxy metabolite/min/nmol of P-450 (17); P-450 PB-4/PB-B, 10.7 nmol of 16β-hydroxy metabolite/min/nmol of P-450 (17); P-450 3/UT-F, 8.9 nmol of 7α-hydroxy metabolite/min/nmol of P-450 (17); P-450 PB-2a/PCN-E, <0.2 nmol of 6β-hydroxy metabolite/min/nmol of P-450 (18).

Analysis of data. In these experiments, mean values of data obtained from choline-deficient cirrhotic and control hepatic microsomes were compared using the unpaired Student's t test (two-tailed).

Results

Alterations in androstenedione hydroxylation in hepatic cirrhosis. The data in Table 1 clearly indicate that formation of three of the four principal hydroxylated metabolites of androstenedione is greatly decreased in hepatic microsomal fractions from the choline-deficient cirrhotic rat. Perhaps the most interesting point to emerge from these data is that the four hydroxylation pathways are affected to different extents in cirrhotic liver. Thus, 6β-hydroxyandrostenedione formation was decreased to 45% of control activity (0.48 ± 0.18 nmol of metabolite formed/min/mg of protein in cirrhotic liver microsomes compared with 1.06 ± 0.18 in control fractions). 7α-Hydroxylation was decreased to a lesser extent (to 68% of control activity), but the 16α-hydroxylation pathway was dramatically decreased to 22% of the control level. In contrast, 16β-hydroxylation in cirrhotic hepatic microsomes was not significantly different from that in control microsomes. The mean total hydroxylation of androstenedione in control liver was 2.79 nmol of products formed/min/mg of protein, whereas this figure was only 1.22 nmol of products formed/min/mg of protein in cirrhotic hepatic microsomes.

Spectral analysis of androstenedione binding in mi-

[1] Equivalent forms of P-450 include: i) P-450$_{UT\text{-}A}$:P-450 2c (17), P-450h (3), P-450 RLM$_5$ (15), and P-450$_{16\alpha}$ (31); ii) P-450$_{UT\text{-}F}$:P-450 3 (17) and P-450a (25); iii) P-450$_{PB\text{-}B}$:P-450 PB-4 (16) and P-450b (25); iv) P-450$_{PCN\text{-}E}$:P-450 2a (18) and PCN-P-450 (26); v) P-450$_{UT\text{-}I}$:P-450 2d (17), P-450i (3), and P-450$_{16\beta}$ (27); vi) P-450$_{ISF\text{-}G}$:P-450d (28) and isosafrole P-450 (29); and vii) P-450$_{PB\text{-}C}$:P-450 PB-1 (30).

TABLE 1

Cytochrome P-450 and androst-4-ene-3,17-dione hydroxylation in hepatic microsomes from choline-deficient cirrhotic and choline-supplemented control rats[a]

Microsomes	Cytochrome P-450[b]	Androst-4-ene-3,17-dione hydroxylation			
		6β	7α	16α	16β
	nmol/mg protein	nmol product formed/min/mg protein			
Control	1.42 ± 0.12	1.06 ± 0.18	0.37 ± 0.06	1.18 ± 0.12	0.18 ± 0.05
Cirrhotic	0.68 ± 0.20	0.48 ± 0.18	0.25 ± 0.08	0.26 ± 0.09	0.23 ± 0.05
Percentage of control	48	45	68	22	128
p[c]	<0.001	<0.001	<0.01	<0.001	NS[d]

[a] Values are presented as means ± standard deviations of six (control) or nine (cirrhotic) individual microsomal fractions.
[b] Spectral determination of total holo-P-450.
[c] p values are for comparison of cirrhotic to control animal values.
[d] NS, not significant.

crosomal fractions. Androstenedione elicited a characteristic type I optical difference spectrum in hepatic microsomes from choline-supplemented control rats with λ_{max} = 386 nm and λ_{min} = 422 nm (Fig. 1A). From double reciprocal plots of the binding data (as shown, for example, in Fig. 1B), a value of 4.5 ± (0.4) μM was determined for the spectral dissociation constant (K_s) of the androstenedione binding reaction with P-450 in control microsomal fractions; the maximal absorbance change (ΔA_{max}) was determined to be 1.10 (± 0.12) × 10^{-2} absorbance units/nmol of P-450. In contrast to these findings, no type I spectral interaction was observed when androstenedione was added to microsomal fractions from cirrhotic rat liver (Fig. 1A).

Immunoquantitation of P-450 forms in hepatic cirrhosis. Levels of two male-specific P-450s, namely, P-$450_{UT\text{-}A}$ and P-$450_{PCN\text{-}E}$, were markedly decreased in microsomes from cirrhotic rat liver (Table 2). P-$450_{UT\text{-}A}$ levels were only about 2% of those in control microsomes, whereas P-$450_{PCN\text{-}E}$ levels were decreased to less than 1% of control levels. In contrast, P-$450_{UT\text{-}F}$, an isozyme not subject to sex differentiation, was decreased in proportion to the overall decrease in microsomal P-450, that is, only by about one-half. P-$450_{PB\text{-}C}$ was present at identical levels in both microsomal preparations, a finding that suggests that this form is regulated by factors completely different from those of other isozymes that were evaluated. The female-specific P-$450_{UT\text{-}I}$ was not present at measurable levels in either control or cirrhotic liver microsomes. Similarly, the major isosafrole-inducible form, P-$450_{ISF\text{-}G}$, was present only at very low levels in both types of microsomes.

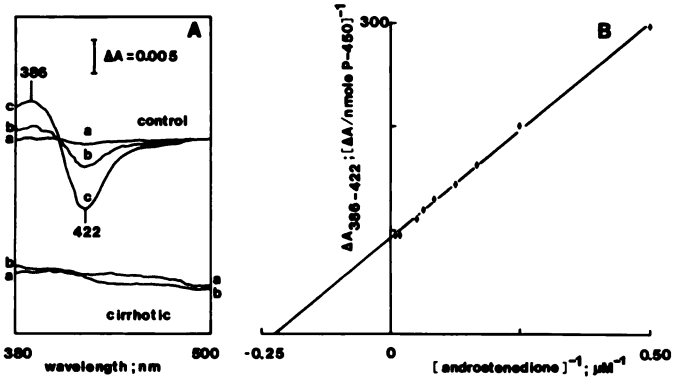

Fig. 1. A. Androst-4-ene-3,17-dione-induced binding spectra in control and cirrhotic rat hepatic microsomes. In the control microsomal fraction, tracing *a* indicates the baseline of equal light absorbance, tracing *b* indicates the difference spectrum elicited by 8 μM androstenedione, and tracing *c* indicates the difference spectrum elicited by 220 μM androstenedione. In the cirrhotic microsomal fraction, tracing *a* indicates baseline and tracing *b* indicates the difference spectrum elicited by 200 μM androstenedione. B. Double reciprocal plot of androstenedione binding in control rat hepatic microsomes.

Discussion

The results of the present investigation provide compelling evidence that levels of individual P-450 isozymes are altered to different extents in the choline-deficient cirrhotic rat liver. Such an occurrence had been postulated from earlier studies, but it now seems clear that the pathophysiological state of cirrhosis is associated with the altered regulation of specific forms of P-450.

In recent years the regulation of P-450s has been the subject of increasing interest. Thomas *et al.* (28) have shown that two polycyclic hydrocarbon-inducible isozymes of P-450 are under similar regulatory control. Similarly, it is now apparent that the levels of four phenobarbital-inducible P-450s are correlated with each other (24). The successful isolation of constitutive forms of P-450 has permitted studies of their regulation to be undertaken.

Most recently, the physiological factors controlling the regulation of sex-specific constitutive P-450 isozymes have been investigated. Two forms, P-$450_{UT\text{-}A}$ and P-$450_{PCN\text{-}E}$, are now known to be male specific and are virtually absent from untreated female rat liver microsomes (17, 18, 31–33). The studies of Gustafsson and co-workers (31) have shown that the expression of at least one of these isozymes, P-$450_{16\alpha}$ (which appears to be similar to or identical to P-$450_{UT\text{-}A}$) is under hypothalamic-pituitary-gonadal control. Both castration and hypophysectomy of male rats decrease levels of P-$450_{UT\text{-}A}$, and it is now apparent that the synthesis of this form of P-450 is regulated by a "male pattern" of growth hormone secretion by the pituitary (18, 31, 34–36). A "female-type" pattern of growth hormone secretion regulates the synthesis of the female-specific steroid sulfate 15β-hydroxylase known as P-$450_{15\beta}$ (27, 37); this isozyme is termed P-$450_{UT\text{-}I}$ in one of our laboratories (18). The results of the present study, in which P-$450_{UT\text{-}A}$ levels fell to about 2% of those in control male hepatic microsomes and androstenedione 16α-hydroxylation decreased to 22% of control activity, therefore demonstrate that the demasculinizing effect of either castration or hypophysectomy is also produced in male rats by hepatic cirrhosis.

Less information is available concerning the factors involved in the regulation of other constitutive forms of P-450. The second male-specific isozyme, P-$450_{PCN\text{-}E}$, seems also to be subject to endocrine control (18). This cytochrome appears to

TABLE 2

Immunochemical quantitation of individual P-450s in hepatic microsomes from choline-deficient cirrhotic and choline-supplemented control rats

Microsomes	Cytochrome P-450[a]					
	$P\text{-}450_{UT\text{-}A}$	$P\text{-}450_{PCN\text{-}E}$	$P\text{-}450_{PB\text{-}C}$	$P\text{-}450_{UT\text{-}F}$	$P\text{-}450_{UT\text{-}I}$	$P\text{-}450_{ISF\text{-}G}$
	nmol/mg of protein					
Control	0.40 ± 0.04	0.27 ± 0.07	0.24 ± 0.08	0.11 ± 0.01	<0.06	<0.03
Cirrhotic	0.009 ± 0.001	<0.002	0.23 ± 0.06	0.053 ± 0.023	<0.06	<0.03
Percentage of control level	2	<1	96	48	ND[b]	ND

[a] Values are presented as means ± standard deviations of individual determinations in three separate microsomal preparations.
[b] ND, not different from control.

be active in the 6β-hydroxylation of C_{19} steroids such as androstenedione. However, in the present study, levels of $P\text{-}450_{PCN\text{-}E}$ were decreased in cirrhosis to less than 1% of control levels, even though androstenedione 6β-hydroxylation was only decreased to 45% of control activity (Tables 1 and 2). Waxman *et al.* (18) noted that the rabbit antibody to rat $P\text{-}450_{PCN\text{-}E}$ effectively inhibited 6β-hydroxylase activity in control microsomes but that 20% of control activity remained. Thus, it is possible that some other P-450s, present in control microsomes, are active in androstenedione 6β-hydroxylation and that these forms are unaffected by cirrhosis. Two other proteins with 6β-hydroxylase activity have been described, namely, P-450g of Ryan *et al.* (3) and $P\text{-}450RLM_3$ of Cheng and Schenkman (15). The interesting observation that androstenedione binding was not apparent in cirrhotic rat hepatic microsomes suggests the absence from those fractions of an isozyme, or isozymes, with high affinity for the steroid. However, the precise relationship between spectral binding of the steroid to microsomal P-450 and hydroxylase activities is unclear.

$P\text{-}450_{UT\text{-}F}$ is not subject to endocrine control via the hypothalamic-pituitary-gonadal axis and it is not sex differentiated (18). Slightly higher levels of $P\text{-}450_{UT\text{-}F}$-associated 7α-hydroxylase activity are observed in female rat hepatic microsomes (18). Thus, the finding that this activity was relatively refractory to the pathophysiological effects of cirrhosis on hepatic drug metabolism, whereas the male-specific steroid hydroxylase activities were decreased, raises the interesting possibility that hypothalamic-pituitary-gonadal impairment may occur in cirrhosis.

Levels of the other constitutive form of P-450 included in the present study, $P\text{-}450_{PB\text{-}C}$, were unchanged in cirrhotic liver compared with controls. This is convincing evidence that the effects of hepatic cirrhosis on P-450s are quite selective. Regulation of the major isosafrole-inducible isozyme, $P\text{-}450_{ISF\text{-}G}$, also did not appear to be altered in cirrhosis; levels were found to be less than 0.03 nmol/mg of microsomal protein in both control and cirrhotic microsomal fractions (Table 2).

The prolonged administration to rats of a lipid-rich, choline-deficient diet results in fatty infiltration of liver cells with focal necrosis followed by fibrosis and regeneration, and ultimately leads on to cirrhosis (13, 38). The model resembles that of human alcoholic cirrhosis in its histological features. It is therefore of interest that human males with alcoholic cirrhosis commonly complain of impotence and exhibit testicular atrophy, loss of male hair distribution and gynecomastia. Although the pathophysiology of these demasculinizing and feminizing effects in humans with cirrhosis is undoubtedly complex (39), the possibility that some changes could be related to altered hepatic steroid metabolism has not been previously considered. Recent studies in this laboratory using a different model of cirrhosis, produced by chronic carbon tetrachloride inhalation in rats, have revealed alterations in male-specific androstenedione and testosterone hydroxylase activities that are very similar to those described here.[2] Thus, it would appear that these effects are attributable to cirrhosis and not to nutritional or toxicological factors during treatment of animals. Further studies are now required to establish the mechanistic basis of reduced levels of male-specific P-450 isozymes and associated pathways of drug oxidation that occur in hepatic microsomes from cirrhotic male rats and to determine whether these changes are important in regard to the human situation.

References

1. Guengerich, F. P. Isolation and purification of cytochrome P-450, and the existence of multiple forms. *Pharmacol. Ther.* **6**:99–121 (1979).
2. Guengerich, F. P., G. A. Dannan, S. T. Wright, M. V. Martin, and L. S. Kaminsky. Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic and immunochemical properties, and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. *Biochemistry* **21**:6019–6030 (1982).
3. Ryan, D. E., S. Iida, A. W. Wood, P. E. Thomas, C. S. Lieber, and W. Levin. Characterization of three highly purified cytochromes P-450 from hepatic microsomes of adult male rats. *J. Biol. Chem.* **259**:1239–1250 (1984).
4. Cheng, K. C., and J. B. Schenkman. Purification and characterization of two constitutive forms of rat liver microsomal cytochrome P-450. *J. Biol. Chem.* **257**:2378–2385 (1982).
5. Waxman, D. J., and C. Walsh. Phenobarbital-induced rat liver cytochrome P-450: purification and characterization of two closely related isozymic forms. *J. Biol. Chem.* **257**:10446–10457 (1982).
6. Farrell, G. C., W. G. E. Cooksley, and L. W. Powell. Drug metabolism in liver disease: activity of hepatic microsomal drug metabolizing enzymes. *Clin. Pharmacol. Ther.* **26**:483–492 (1979).
7. Klotz, U., C. Fischer, P. Muller-Seydlitz, J. Schulz, and W. A. Miller. Alterations in the disposition of differently cleared drugs in patients with cirrhosis. *Clin. Pharmacol. Ther.* **26**:221–227 (1979).
8. Marshall, W. J., and A. E. M. McLean. The effect of cirrhosis of the liver on microsomal detoxications and cytochrome P-450. *Br. J. Exp. Pathol.* **50**:578–583 (1969).
9. Murray, M., and G. C. Farrell. Different effects of carbon tetrachloride toxicity and cirrhosis on substrate binding to rat hepatic microsomal cytochrome P-450. *Biochem. Pharmacol.* **33**:687–689 (1984).
10. Villeneuve, J.-P., A. J. J. Wood, D. G. Shand, L. Rogers, and R. A. Branch. Impaired drug metabolism in experimental cirrhosis in the rat. *Biochem. Pharmacol.* **27**:2577–2581 (1978).
11. Farrell, G. C., and L. Zaluzny. Microsomal protein synthesis and induction of cytochrome P-450 in cirrhotic rat liver. *Aust. J. Exp. Biol. Med. Sci.* **62**:291–301 (1984).
12. Farrell, G. C., and L. Zaluzny. Hepatic heme metabolism and cytochrome P-450 in cirrhotic rat liver. *Gastroenterology* **89**:172–179 (1985).
13. Murray, M., L. Zaluzny, and G. C. Farrell. Drug metabolism in cirrhosis: selective changes in cytochrome P-450 isozymes in the choline-deficient rat model. *Biochem. Pharmacol.* **35**:1817–1824 (1986).
14. Wood, A. W., D. E. Ryan, P. E. Thomas, and W. Levin. Regio- and stereoselective metabolism of two C_{19} steroids by five highly purified and reconstituted rat hepatic cytochrome P-450 isozymes. *J. Biol. Chem.* **258**:8839–8847 (1983).
15. Cheng, K.-C., and J. B. Schenkman. Testosterone metabolism by cytochrome P-450 isozymes RLM_2 and RLM_5 and by microsomes. *J. Biol. Chem.* **258**:11738–11744 (1983).

[2] M. Murray, L. Zaluzny, and G. C. Farrell, unpublished observations.

16. Waxman, D. J., A. Ko, and C. Walsh. Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J. Biol. Chem.* **258:**11937–11947 (1983).
17. Waxman, D. J. Rat hepatic cytochrome P-450 isoenzyme 2c: identification as a male-specific, developmentally induced steroid 16α-hydroxylase and comparison to a female-specific cytochrome P-450 isoenzyme. *J. Biol. Chem.* **259:**15481–15490 (1984).
18. Waxman, D. J., G. A. Dannan, and F. P. Guengerich. Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24:**4409–4417 (1985).
19. Gustafsson, J.-A., and M. Ingelman-Sundberg. Regulation and substrate specificity of a steroid sulfate-specific hydroxylase system in female rat liver microsomes. *J. Biol. Chem.* **250:**3451–3458 (1975).
20. Schenkman, J. B., H. Remmer, and R. W. Estabrook. Spectral studies of drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.* **3:**113–123 (1967).
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275 (1951).
22. Omura, T., and R. Sato. The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239:**2379–2385 (1964).
23. Kaminsky, L. S., M. J. Fasco, and F. P. Guengerich. Production and application of antibodies to rat liver microsomal cytochrome P-450. *Methods Enzymol.* **74C:**262–272 (1981).
24. Dannan, G. A., F. P. Guengerich, L. S. Kaminsky, and S. D. Aust. Regulation of cytochrome P-450: immunochemical quantitation of eight isozymes in liver microsomes of rats treated with polybrominated biphenyl congeners. *J. Biol. Chem.* **258:**1282–1288 (1983).
25. Thomas, P. E., L. M. Reik, D. E. Ryan, and W. Levin. Regulation of three forms of cytochrome P-450 and epoxide hydrolase in rat liver microsomes. Effects of age, sex, and induction. *J. Biol. Chem.* **256:**1044–1052 (1981).
26. Elshourbagy, N. A., and P. S. Guzelian. Separation, purification, and characterization of a novel form of cytochrome P-450 from rats treated with pregnenolone 16α-carbonitrile. *J. Biol. Chem.* **255:**1279–1285 (1980).
27. MacGeoch, C., E. T. Morgan, J. Halpert, and J.-A. Gustafsson. Purification, characterization and pituitary regulation of the sex-specific cytochrome P-450$_{15\alpha}$-hydroxylase from liver microsomes of untreated female rats. *J. Biol. Chem.* **259:**15433–15439 (1984).
28. Thomas, P. E., L. M. Reik, D. E. Ryan, and W. Levin. Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochromes P-450c and P-450d, by structurally diverse xenobiotics. *J. Biol. Chem.* **258:**4590–4598 (1983).
29. Fisher, G. J., H. Fukushima, and J. L. Gaylor. Isolation, purification, and properties of a unique form of cytochrome P-450 in microsomes of isosafrole-treated rats. *J. Biol. Chem.* **256:**4388–4394 (1981).
30. Waxman, D. J., and C. Walsh. Cytochrome P-450 isozyme 1 from phenobarbital-induced rat liver: purification, characterization, and interactions with metyrapone and cytochrome b_5. *Biochemistry* **22:**4846–4855 (1983).
31. Morgan, E. T., C. MacGeoch, and J.-A. Gustafsson. Hormonal and developmental regulation and expression of the hepatic microsomal 16α-hydroxylase cytochrome P-450 apoprotein in the rat. *J. Biol. Chem.* **260:**11895–11898 (1985).
32. Kamataki, T., K. Maeda, M. Shimada, K. Kitani, T. Nagai, and R. Kato. Age-related alteration in the activities of drug-metabolizing enzymes and contents of sex-specific forms of cytochrome P-450 in liver microsomes from male and female rats. *J. Pharmacol. Exp. Ther.* **233:**222–228 (1985).
33. Morgan, E. T., C. MacGeoch, and J.-A. Gustafsson. Sexual differentiation of cytochrome P-450 in rat liver: evidence for a constitutive isozyme as the male-specific 16α-hydroxylase. *Mol. Pharmacol.* **27:**471–479 (1985).
34. Einarsson, K., J.-A. Gustafsson, and A. Stenberg. Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. *J. Biol. Chem.* **248:**4987–4997 (1973).
35. Gustafsson, J.-A., A. Mode, G. Norstedt, and P. Skett. Sex steroid induced changes in hepatic enzymes. *Annu. Rev. Physiol.* **45:**51–60 (1983).
36. Mode, A., J.-A. Gustafsson, J.-O. Jansson, S. Eden, and O. Isaksson. Association between plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. *Endocrinology* **111:**1692–1697 (1982).
37. MacGeoch, C., E. T. Morgan, and J.-A. Gustafsson. Hypothalamopituitary regulation of cytochrome P-450$_{15\alpha}$ apoprotein levels in rat liver. *Endocrinology* **117:**2085–2092 (1985).
38. Oler, A., and B. Lombardi. Further studies on a defect in the intracellular transport and secretion of proteins by the liver of choline-deficient rats. *J. Biol. Chem.* **245:**1282–1288 (1970).
39. Van Thiel, D. H., J. S. Gavaler, and R. R. Schade. Liver disease and the hypothalamic pituitary gonadal axis. *Semin. Liver Dis.* **5:**35–45 (1985).

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