Down-Regulation of Rat Hepatic Microsomal Cytochromes P-450 in Microvesicular Steatosis Induced by Orotic Acid

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ABSTRACT

Microvesicular steatosis is an important component of the overall pathogenesis of drug-mediated liver injury. Although mitochondrial damage has a role in the development of microvesicular steatosis, the consequences of fatty change for hepatic gene function are unclear. The present study was undertaken to evaluate hepatic cytochrome P-450 (CYP) function in a rat model of microvesicular steatosis produced by the intake of diets containing 1% orotic acid (OA) that were administered for 5, 10, or 21 days. Hepatic triglyceride levels were increased to 3-fold of control after 5 days and were elevated further at 10 and 21 days. Cholesterol and phospholipid contents were increased after 10 and 21 days but not by 5 days of feeding. Microsomal androst-4-ene-3,17-dione hydroxylation activities mediated by CYP2C11 (16α-hydroxylation) and CYP3A2 (6β-hydroxylation) were decreased in liver from OA-fed rats for only 5 days, whereas CYP2A1/2-mediated steroid 7α-hydroxylation was decreased after 10 days; these observations were complemented by immunoblot analysis that demonstrated the impaired expression of the corresponding CYP proteins. CYP2C11 mRNA, the major CYP in male rat liver, was down-regulated in steatotic liver to 52% of control after 10 days; these dysregulations were due directly to OA-containing diets that histologically similar to that produced by hepatotoxic drugs and produces the rapid down-regulation of constitutive CYPs in rat liver. Analogous processes of lipid deposition in human liver after drug- or disease-related injury could precipitate adverse effects during subsequent drug therapy.

Accumulation of lipid within hepatocytes, or steatosis, often occurs after the ingestion of alcohol and certain drugs, including corticosteroids, tetracycline, and some nonsteroidal anti-inflammatory agents, as well as in inherited metabolic disorders and diseases with hepatic involvement (Farrell, 1994; Burt et al., 1998). Microvesicular steatosis is identified by the presence of multiple small lipid droplets in hepatic sections and appears to be secondary to mitochondrial injury (which results in impaired β-oxidation of fatty acids and impaired respiration leading to ATP depletion) and/or to decreased synthesis of lipoproteins by the liver (Breen et al., 1975; Fromenty et al., 1990a,b; Berson et al., 1998). Although early steatosis may be reversible, continued hepatotoxic injury may lead to fibrosis and eventual cirrhosis.

Experimental steatosis can be produced in rodents by a variety of approaches involving dietary manipulation. Thus, intake of diets deficient in lipotropes such as choline and methionine leads to the rapid deposition of lipid in liver, which significantly affects hepatic gene regulation (Rogers and Newberne, 1973; Lombardi and Smith, 1994). In previous studies, we described the down-regulation of the male-specific cytochrome P-450 (CYP) 2C11 in rat liver after 10 weeks of intake of a choline-deficient diet (Murray et al., 1992a). Continued intake of this diet for an additional 20 weeks produced hepatic cirrhosis, but the expression of CYP2C11 did not decline further over this period. Thus, CYP dysregulation was not due directly to chronic liver disease and was instead a relatively early event in the disease process. Because a number of therapeutically important agents are known to elicit microvesicular steatosis, the effects of lipid-mediated injury on hepatic drug metabolizing enzymes are of considerable clinical significance.

In initial experiments, we found that the short-term intake of diets containing orotic acid (OA) produces rapid and extensive steatosis that follows a microvesicular distribution. The present study evaluated CYP expression and function in this model of steatotic injury. The principal findings to emerge were that significant alterations in major constitutive CYPs occurred after only 5 days of dietary modulation and were optimal after only 10 days of dietary conditioning. The principal CYP in male rat liver, CYP2C11, was down-regulated at a pretranslational level in OA-induced steatosis. Analogous processes of microvesicular steatosis in human liver may significantly impair drug clearance and complicate drug therapy.

ABBREVIATIONS: CYP, cytochrome P-450; OA, orotic acid; RC, rat chow; SP, semipurified.
Experimental Procedures

Materials. [4,14C]Testosterone (specific activity, ,55 mCi/mmol); [4,14C]androst-4-ene-3,17-dione (androstenedione; specific activity, ,55 mCi/mmol); [α-32P]dCTP (specific activity, 3000 Ci/mmole), Hyperfilm-MP, ACS II, Hybond-N+ filters, and reagents for enhanced chemiluminescence were obtained from Amersham Australia (North Ryde, NSW, Australia). Retinyl acetate, retinol, α-tocopherol acetate, unlabeled testosterone, and androstenedione were purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxy steroid standards for thin-layer chromatography were obtained from Sigma Chemical Co. or the MRC Steroid Reference Collection (Queen Mary’s College, London, UK). Biochemicals were purchased from Sigma Chemical Co. or Boehringer-Mannheim (Castle Hill, NSW, Australia). Reagents for SDS-polyacrylamide gel electrophoresis were obtained from Bio-Rad laboratories (Richmond, CA). HPLC-grade solvents were obtained from Rhone-Poulenc Chemicals (Baulkham Hills, NSW, Australia). Analytical-grade reagents were purchased from Ajax Chemicals (Sydney, NSW, Australia). Components for experimental diets were obtained from ICN Biochemicals (Seven Hills, NSW, Australia).

Animal Treatments. Studies were performed in accordance with the guidelines of the Australian National Health and Medical Research Council and were approved by the University of New South Wales and Central Area Health Service Animal Care and Ethics Committees. Basal diets consisted of either powderd laboratory rat chow (RC; Young Stock Feeds, Young, NSW, Australia) or a high-sucrose-containing semipurified (SP) diet. Each kilogram of SP diet contained sucrose (600 g), casein (200 g), cellulose (110 g), corn oil (40 g), IIN salt mixture 4179 (40 g), ICN vitamin diet fortification mixture (10 g), α-tocopherol (20 mg), and retinyl acetate (8.7 mg). OA was added to some diets at a level of 1%.

Male Wistar rats (180–220 g; five animals per group) were placed on an experimental diet for 21 days and had free access to water over the same period of dietary manipulation; control animals were pair-fed (they received an amount of diet equal to that consumed on the previous day by rats in the SP/OA or RC/OA groups). In a subsequent experiment, rats (four per group) received either the SP/OA or SP/ control diets for 5 or 10 days. Rats were sacrificed under anesthesia, blood was obtained from the abdominal aorta, and the serum and plasma were stored at ,20°C for serum biochemical assays. Livers were harvested, perfused with cold saline, snap frozen in liquid nitrogen, and stored at 70°C for later RNA and lipid analysis. Washed hepatic microsomes were prepared according to standard procedures (Murray et al., 1983). The final microsomal pellets were resuspended in 50 mM potassium phosphate buffer (pH 7.4) that contained 1 mM EDTA and 20% glycerol and were stored at 70°C.

Female New Zealand White rabbits were immunized with CYP2C11, CYP3A2, CYP2A1, and NADPH-CYP reductase that had been isolated from rat liver according to standard protocols (Murray et al., 1992). IgG fractions were isolated from rabbit serum after immunization (Rad, Richmond, CA). Immunoblotting for CYP Apoproteins and NADPH-CYP Reductase in Rat Hepatic Microsomes. Rat hepatic microsomes (5 μg/lane unless otherwise specified) were incubated at 100°C for 5 min with 2% SDS and 5% 2-mercaptoethanol and subjected to electrophoresis on 7.5% polyacrylamide gels (Laemmli, 1970) with minor modifications (Murray et al., 1986). Proteins were then transferred to nitrocellulose sheets (Towbin et al., 1979) and incubated with one of the IgGs (3.7 μg/ml). Immunoreactive proteins were detected by enhanced chemiluminescence and autoradiography on Hyperfilm-MP, and the resultant signals were analyzed by densitometry (Bio-Rad, Richmond, CA).

Oligonucleotide Probes and CYP mRNA Analysis. A synthetic 50-mer oligonucleotide for CYP2C11, reverse complement of nucleotides 925 to 954 of the reported cDNA sequence (Yoshioka et al., 1987), was obtained from Rachel Forster Hospital (Raphael, NSW, Australia). The oligonucleotide for 18S RNA (Chan et al., 1984) was purchased from Bresapect (Adelaide, South Australia).

Total RNA was extracted from male rat liver by the guanidinium thiocyanate/CsCl method (Sambrook et al., 1989). Oligonucleotides were labeled using [α-32P]dCTP and deoxynucleotidyl transferase. RNA (10 μg) was electrophoresed on 1% agarose in the presence of 2.2 M formaldehyde and then transferred to Hybond-N+ nylon filters (0.45 μm; Amersham). Hybridization and washing conditions were as described previously (Jiang et al., 1994), and signals corresponding to CYP mRNAs were quantified using a Fuji PhosphorImager. To demonstrate equivalence of RNA loading between samples, filters were stripped and rehybridized to the α-32P-labeled 18S RNA probe.

Light Microscopy and Serum Biochemistry. After individual rat livers were removed, a small section of tissue was fixed in Millenol’s buffered formalin and histologic sections were prepared in the Histology Laboratory of the Institute of Clinical Pathology and Medical Research at Westmead Hospital. Blood was also taken from the abdominal aorta at the time of sacrifice, serum was prepared, and biochemical analyses were performed in the Clinical Chemistry Laboratory of the Institute of Clinical Pathology and Medical Research at Westmead Hospital.

Hepatic lipids were extracted using chloroform and methanol in the presence of 1% Triton X-100 (Janssen and Meijer, 1995). Total hepatic lipids were measured gravimetrically after the evaporation of chloroform under nitrogen and drying over silica gel in a vacuum desiccator to constant weight for several days. This extract was also used for the quantification of lipids using commercial kits (Boehringer-Mannheim GmbH, Mannheim, Germany): cholesterol (Chol MPR1 kit and the Presiset standard), triglycerides (Peridochrom triglycerides GPO-PAP kit and the Precimat standard), and phospholipids (PL MPR2 phospholipid kit).

Statistical Analysis. Data are expressed as mean ± S.E. throughout. All measurements were made in samples from individual rats. Comparisons between two groups were made using the Student’s t test (unpaired) or the Mann-Whitney U test (nonparametric data). Data from multiple treatment groups were subjected to single-factor ANOVA and Student-Newman-Keuls q test for comparisons between multiple treatments.

Results

Effect of Intake of OA-Containing Diets on Body Weight, Serum Biochemistry, and Hepatic Lipids in the Rat. Groups of rats were administered diets that were based on either standard rat chow (RC diet) or a semipurified diet that was high in sucrose (SP diet). OA (1%) was incorporated into the RC or SP diet that was administered to some animals to produce steatosis; pair-fed controls received either the RC or SP diet from which OA was excluded. Rats that received OA-containing diets gained weight over the 21-day period at rates that were not significantly different from those in corresponding controls. Thus, after 21 days,
rats that received the RC/control and RC/OA diets \((n = 5\) in each group) were 164 ± 7 and 151 ± 5\% of their respective initial weights, and rats that received the SP/control and SP/OA diets were 149 ± 3 and 138 ± 6\% of their respective initial weights. These effects of OA on weight gain were not statistically significant (Student’s \(t\) test).

Intake of the SP/OA diet, but not the RC/OA diet, produced an increase in relative liver to body weight (7.5 ± 0.3 versus 4.4 ± 0.1\% in SP/control; \(P < .001\)). Serum bilirubin was increased by the SP/OA diet (2.4 ± 0.2 versus 0.6 ± 0.2 \(\mu\)M in SP/control; \(P < .001\)), and there was a trend toward an increase in alanine aminotransferase activity (97 ± 26 versus 42 ± 6 U/liter) that did not attain significance \((P = .08)\). γ-Glutamyltranspeptidase was detected in serum from rats that received the SP/OA but not the SP/control diet \((P < .05,\) Mann-Whitney \(U\) test). Total hepatic lipid was increased to 22-fold of control in rats that received the SP/OA diet \((4200 ± 300\) versus 190 ± 20 mg/liver; \(P < .001\)). The apparent increase in total hepatic lipids after intake of the RC/OA diet was not significant because of extensive individual variation in the RC/OA livers \((420 ± 190\) versus 130 ± 30 mg/liver).

Light microscopy indicated the presence of multiple lipid droplets in the cytoplasm of rat hepatocytes after SP/OA feeding for 21 days (microvesicular change; Fig. 1). By comparison, the amount of lipid in SP/control livers was normal, as reflected by the smaller size of hepatocytes and the uniform distribution of nuclei in cells (Fig. 1). Liver sections from RC/OA and RC/control diet-fed rats exhibited a normal appearance (not shown).

Hepatic contents of the major lipid types were quantified after intake of the experimental diets. It is apparent from the data in Fig. 2 that hepatic cholesterol, total phospholipid, and, especially, total triglyceride contents were increased after intake of the SP/OA diet for 21 days to 4.7-, 2.1-, and 16-fold of those levels in SP/control livers; intake of the RC/OA diet did not significantly affect the hepatic lipid content.

**Microsomal CYP Function in OA-Induced Hepatic Steatosis.** Total CYP levels were decreased to 64\% of control in hepatic microsomes from rats that received the SP/OA diet \((0.75 ± 0.06\) versus 1.18 ± 0.06 nmol/mg protein; \(P < .001\)). By comparison, the RC/OA diet did not significantly affect total CYP in rat hepatic microsomal fractions from those levels in control liver. There was also a decline in NADPH-CYP-reductase activity produced by the SP/OA diet to 63\% of the activity in SP control liver \((730 ± 50\) versus 1160 ± 70 nmol/mg protein/min).

Because steroid hydroxylation activities provide information on the catalytic function of quantitatively important constitutive CYPs, these activities were measured in the present study. Pronounced decreases in the activities of microsomal CYPs 2C11, 3A2, and 2A1 were noted in livers of rats that received the SP/OA diet for 21 days (Fig. 3). Thus, in microsomes from SP/OA rat liver, androstenedione 6\(\beta\)-, 7\(\alpha\)-, and 16\(\alpha\)-hydroxylase activities were decreased to 31, 55, and 53\% of those in rat liver.
SP/control liver (Fig. 3A). Analogous measurements of testosterone hydroxylation activities corroborated these findings. Thus, the CYP2C11-mediated 2α-/16α-hydroxylations of testosterone were decreased to 48 and 49% of respective control (P < .01) and the CYP3A- and CYP2A1-dependent 6β- and 7α-hydroxylations of testosterone were decreased to 20% (P < .001) and 34% (P < .01) of control activities (Fig. 3B).

Consistent with the relatively low impact of the RC/OA diet on hepatic lipid composition after 21 days of intake, activities of androstenedione and testosterone hydroxylation pathways were essentially unchanged from RC/control. Exceptions, however, were the small but significant decreases in androstenedione 7α-hydroxylation to 75% of RC control (0.24 ± 0.03 versus 0.32 ± 0.03 nmol/mg protein/min; P < .05; Fig. 3) and testosterone 6β-hydroxylation to 73% of RC control (1.35 ± 0.03 versus 1.84 ± 0.15 nmol/mg protein/min; P < .05; Fig. 3). The trend toward decreased androstenedione 6β-hydroxylation activity (to 81% of RC/control) did not reach significance (P = .10).

The effect of OA-mediated steatosis on CYP expression was also evaluated at the protein level by immunoblotting (Fig. 4). Microsomal CYP2C11 apoprotein expression was decreased significantly after 21 days of intake of the SP/OA diet to 63 ± 17% of control levels (n = 3; P < .05). After 21 days of dietary intake, CYP2A and CYP3A immunoreactive proteins were also decreased to 52 ± 16 (P < .01) and 42 ± 6% (P < .001) of SP/control. In contrast with these findings, but consistent with steroid hydroxylation measurements, the microsomal expression of CYPs 2C11 and 2A was not decreased by intake of the RC/OA diet (results not shown). There was, however, a trend toward a decrease in CYP3A immunoreactive protein content (to 85 ± 4% of RC/control; P = .08).

The regulatory impairment in steatosis leading to altered hepatic CYP2C11 expression was further investigated. From Northern analysis, it emerged that expression of CYP2C11 mRNA was decreased after 21 days of intake of the SP/OA diet (Fig. 5). Densitometric measurements indicated that CYP2C11 mRNA was decreased in SP/OA rat liver to 52 ± 4% (n = 3) of SP/control (relative to 18S RNA). Thus, the down-regulation of CYP2C11 produced by dietary intake of OA in the SP diet occurs at a pretranslational level.

Early Alterations of Hepatic Lipid Content and Microsomal CYP Activities after Intake of SP/OA Diet. To attempt to clarify the relationship between hepatic lipid accumulation and the decrease in the microsomal content and activity of constitutive CYPs, additional groups of rats were administered the SP/OA diet for shorter periods. After 5 days of the SP/OA diet, the triglyceride content of rat liver was increased to about 3-fold of control but cholesterol and total phospholipid contents were unchanged. Continued intake of the diet for an additional 5 days increased the hepatic contents of cholesterol, triglyceride, and phospholipid, respectively, to 1.8-, 5.0-, and 1.8-fold of SP/OA control (Table 1). As indicated in Fig. 2, the corresponding increases produced by 21 days of intake of the SP/OA diet were to 4.7-, 16-, and 2.1-fold of SP/control.

From Table 1, it is apparent that androstenedione 6β-hydroxylation was decreased significantly after 5 and 10 days of dietary intake to 56 and 33% of the corresponding activities in SP/control microsomes. Similarly, CYP2C11-dependent 16α-hydroxylation of the steroid was decreased to 76 and 34% of the control activities at days 5 and 10. The activity of the 7α-hydroxylation pathway was decreased to 54% of control after 10 days but was unchanged from SP/control at day 5. CYP2C11 and CYP3A apoproteins were
days). In the latter case, however, because the number of
SP/OA after 5, 10, and 21 days; SP/control after 5, 10, and 21
ers or mean values from groups of animals were analyzed
cients were similar whether measurements in individual liv-
representing a wide range of lipid values. Correlation coeffi-
ships between individual steroid hydroxylation pathways

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of short-term intake of OA-containing diets on microsomal androstenedione hydroxylation activities and lipid contents in rat liver</td>
</tr>
<tr>
<td>Dietary Treatment</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SP/control diet, 5 days</td>
</tr>
<tr>
<td>SP/OA, 5 days</td>
</tr>
<tr>
<td>SP/control diet, 10 days</td>
</tr>
<tr>
<td>SP/OA, 10 days</td>
</tr>
</tbody>
</table>

aP < .05, bP < .01, cP < .001. Different from corresponding control at same time point.
dP < .05. Different from activity in SP/control group after 5 days of dietary intake.

decreased after 5 days of intake of the SP/OA diet (n = 4 per
group) to 65 ± 3 (P < .01) and 72 ± 10% (P < .05) of
corresponding control. By comparison, 10 days of intake de-
creased the expression of these proteins to 54 ± 4 (P < .001)
and 68 ± 10% (P < .05) of respective control (four per group).

A series of correlations were derived for the linear relations-
ships between individual steroid hydroxylation pathways
and hepatic contents of individual lipids (Table 2). Correla-
tion coefficients were in the range of 0.57 to 0.82, which
increased the expression of these proteins to 54 ± 4 (P < .001)
and 68 ± 10% (P < .05) of respective control (four per group).

Discussion

Microvesicular steatosis was evident in hepatic sections
from rats that received an experimental diet that was supple-
mented with 1% OA and sucrose for 21 days. This form of
fatty liver injury is closely associated with hepatotoxicity
produced by drugs such as valproic acid, tetracycline, and
some nonsteroidal anti-inflammatory agents (Breen et al.,
1975; Froment et al., 1990a). Mitochondrial injury, result-
ing in impaired β-oxidation of fatty acids, is considered cen-
tral to hepatotoxicity (Berson et al., 1998; Burt et al., 1998),
but the detailed effects of lipid accumulation on hepatic gene
expression are unclear. Indeed, the consequences of microve-
sicular steatosis for hepatocellular function are of interest in

TABLE 2

Correlation matrix between CYP-mediated androstenedione
hydroxylation pathways and hepatic lipid accumulation in rats fed the
SP/control or SP/OA diets for 5, 10, or 21 days

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Hydroxandrostenedione Metabolite Formation</th>
<th>6β-</th>
<th>7α-</th>
<th>16α-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation of group mean values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>−0.74</td>
<td>−0.81*</td>
<td>−0.73</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>−0.84*</td>
<td>−0.85*</td>
<td>−0.87*</td>
<td></td>
</tr>
<tr>
<td>Phospholipid</td>
<td>−0.89*</td>
<td>−0.87*</td>
<td>−0.90*</td>
<td></td>
</tr>
<tr>
<td>Correlation of individual values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>−0.58c</td>
<td>−0.62b</td>
<td>−0.60c</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.75c</td>
<td>−0.72c</td>
<td>−0.78c</td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td>−0.67c</td>
<td>−0.61b</td>
<td>−0.71c</td>
<td></td>
</tr>
</tbody>
</table>

aP < .05, bP < .01, cP < .001. Correlation coefficient significant.

light of the association between lipid deposition and disease.
In this regard, the OA-feeding model may offer some advan-
tages over established models of drug- and chemical-medi-
ated injury because potential effects of these agents on target
genes are avoided. Thus, the present study establishes that
the expression of major constitutive drug-metabolizing CYPs
is impaired in OA-induced steatosis.

An association between lipid deposition and CYP function
was suggested from previous work undertaken in a rat model
of choline deficiency (Murray et al., 1992). Similar findings
emerged from the study of Leclercq et al. (1998). In the latter
study, short-term feeding of carbohydrate-rich diets to adult
ducks (for up to 13 days) and male Wistar rats (for 2 days) led
to pronounced increases in liver weight and hepatic lipid
content. A decline in the activities of several xenobiotic oxida-
tions catalyzed by CYPs (especially aminopyrine N-de-
methylation) was observed and was attributed to lipid accu-
accumulation during nutritional manipulation. However, the
veracity of the apparent relationship was not tested further
at intermediate stages of lipid deposition in the rat. We have
explored this possibility in the SP/OA-fed rat, because the
short time frame for the development of steatosis offered
considerable flexibility: intake of the diet for periods of only a
few days enabled the assessment of CYP function during early
lipid deposition. Thus, triglycerides were increased to
about 3-fold of corresponding control after 5 days of dietary
manipulation, but cholesterol and phospholipid were un-
changed. Lipid deposition was more established at 10 days,
as reflected by the 5-fold increase in triglycerides as well as
the significant increase in both cholesterol and phospholip-
ids. After only 5 days of feeding of the SP/OA diet, CYP3A
function was markedly impaired to only 50% of control; 10
and 21 days of feeding led to further decreases in CYP3A
activity. CYP2C11 activity was also decreased after 5 days of
intake of the SP/OA diet but not to the same extent as CYP3A
activity. The loss of CYP2C11 activity was also more pro-
nounced after 10 and 21 days on the SP/OA diet. In contrast,
decreases in CYP2A function were not apparent until at least
10 days of intake of the OA-containing diet. The data were
subjected to regression analysis, and significant correlations
were detected between the extent of hepatic lipid deposition
and the extent of CYP suppression. The data were analyzed
separately (arising from individual rat livers) and as grouped
data from each treatment. Group correlations of CYP activ-
ities with cholesterol levels were generally poorer than with
triglyceride and phospholipid levels. Thus, it appears un-
likely that cholesterol ester accumulation during OA feeding
plays a role in CYP down-regulation. Accumulation of tri-
glycerides and phospholipids was more closely associated
with CYP dysregulation.
Administration of the RC/OA diet to rats afforded an opportunity to eliminate direct effects of OA on hepatic CYP function. The RC/OA diet did not promote extensive hepatic lipid deposition, which is most likely because sucrose (in the SP diet) is necessary for the stimulation of fatty acid biosynthesis. The RC/OA diet exerted only minimal effects on CYP activities, except for that of CYP3A (steroid 6β-hydroxylation). Although not statistically significant, there was a trend toward a decrease in CYP3A immunoreactive protein in microsomes from RC/OA rat liver. Such a decrease may well be responsible for the apparent decrease in steroid 6β-hydroxylation activity. Alternately, the documented sensitivity of CYP3A activity (but not necessarily CYP3A protein) to changes in the lipid environment (Imaoka et al., 1992; Gillam et al., 1993) may be the operative reason for the decline in CYP3A-mediated steroid oxidation. It is possible that the lipid membrane is especially important for catalysis by CYPs 3A because optimal association with cytochrome b5 and the reductases is essential for these CYPs. The present findings suggest that CYP3A activity is highly responsive to relatively small changes in hepatic lipids produced by dietary manipulation.

The mechanism by which OA decreases microsomal CYP expression was explored. Observed changes in CYP2C11, CYP3A, and CYP2A catalytic function were complemented by the findings that the expression of CYP apoproteins was impaired. Northern analysis demonstrated that the mRNA corresponding to CYP2C11, quantitatively the major CYP in male rat liver, was decreased in rat liver by intake of the SPO/A diet. This is consistent with regulatory impairment at a pretranslational level. Furthermore, like several other CYPs in rat liver, is known to be transcriptionally regulated by hormonal factors. Recently, it has emerged that the pattern of pituitary growth hormone secretion, which is a major determinant of CYP2C11 expression (Morgan et al., 1985; Waxman et al., 1985), signals in hepatocytes through the Janus kinase-signal transducers and activators of transcription (Jak-STAT) system (Waxman et al., 1995; Ram et al., 1996). Although the possibility was not evaluated directly in this study, the finding that CYP2C11 mRNA expression was decreased after OA feeding suggests that this signaling pathway may be perturbed in microvesicular steatosis. Other regulatory perturbations may also underlie the impairments in CYP2C11 expression. Thus, Corton et al. (1998) demonstrated that chemicals such as gemfibrozil, WY-14,643, and other ligands of the peroxisome proliferator-activated receptor also down-regulate the expression of CYP2C11 in male rat liver. Because the accumulation of lipids in liver during intake of the SP/OA diet may similarly activate peroxisome proliferator-activated receptor-α, it is conceivable that this may be the mechanism by which CYP2C11 is down-regulated. An additional possibility is that free radicals and/or cytokines released from Kupffer cells, the resident macrophages of the liver, may contribute to CYP down-regulation in the SP/OA rat. Zhong et al. (1995) demonstrated that Kupffer cells mediated reperfusion injury in rats with fatty liver produced by ethanol intake. An evaluation of possibilities such as these is now required to establish the mechanism by which CYP down-regulation occurs in OA-induced microvesicular steatosis.

Other models of steatosis have been used to assess the effect of lipid deposition on CYP expression and the development of liver disease. In earlier studies, it was demonstrated that down-regulation of CYP2C11 was a relatively early event in choline-deficient rat liver that preceded the development of chronic disease (Murray et al., 1992a). The same model has been shown to progress to hepatoma, possibly associated with methyl-group deficiency that leads to gene hypomethylation and tumorigenesis (Lombardi and Smith, 1994). Scholz et al. (1991) summarized similar findings relating to enhanced tumor development after prolonged feeding of OA.

Abundant fat in liver has been implicated in poor outcomes after orthotopic liver transplantation (Nakano et al., 1997) and, consistent with the assertions of Zhong et al. (1995), is reportedly associated with increased rates of generation of reactive oxygen species. It is possible that free radicals formed during ischemia/reperfusion resulting from lipid infiltration may promote intrahepatic cytokine production because of the activation of transcription factors such as activator protein-1 and nuclear factor-κB (Schutze et al., 1992).

Certainly, cytokines such as tumor necrosis factor-α and interleukin-6 may participate in the inflammatory response to hepatocellular injury, leading to fibrosis and cirrhosis or carcinogenesis (Thiele, 1989). In addition to the long-term impact of steatosis on liver function, the present study also suggests the need for close monitoring of recipients of livers containing significant lipid. Indeed, the hypoaclivity of CYP enzymes may seriously complicate clinical management because of the likelihood that treatment would include the administration of multiple therapeutic agents.

Acknowledgments

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