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PRE-TRANSLATIONAL REGULATION OF CYTOCHROME P450 GENES IS RESPONSIBLE FOR DISEASE-SPECIFIC CHANGES OF INDIVIDUAL P450 ENZYMES AMONG PATIENTS WITH CIRRHOSIS

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Abstract—We have recently reported that disease-specific differential alterations in the hepatic expression of xenobiotic-metabolizing cytochrome P450 (CYP P450) enzymes occur in patients with advanced liver disease. In order to determine whether the observed changes in CYP proteins are modulated at pre- or post-translational levels, we have now examined the hepatic levels of mRNA for CYPs 1A2, 2C9, 2E1 and 3A4 by solution hybridization in the same livers of 20 controls (surgical waste from histologically normal livers), 32 cases of hepatocellular and 18 of cholestatic severe chronic liver disease. CYP1A2 mRNA and CYP1A immunoreactive protein were both reduced in livers with hepatocellular and cholestatic types of cirrhosis. In contrast, CYP3A4 mRNA and protein were reduced only in livers from patients with hepatocellular diseases. For 1A2 and 3A4 there were significant correlations between mRNA species and the respective protein contents ($r_{S1A2} = 0.74$, $r_{S3A4} = 0.64$, $P<0.0001$). CYP2C9 mRNA was reduced in patients with both cholestatic and hepatocellular types of liver disease, but 2C protein was reduced only in patients with cholestatic dysfunction. The correlation between CYP2C9 mRNA and protein, was also significant ($r_S = 0.36$, $P<0.005$) but mRNA levels accounted for only 13% of the variability in protein rankings. This is probably a consequence of other CYP2C proteins apart from 2C9 being detected by the anti-2C antibody. CYP2E1 mRNA and protein were reduced in patients with cholestatic liver disease, but in hepatocellular disease the expression of only CYP2E1 mRNA was decreased. CYP2E1 mRNA was significantly correlated with CYP2E1 protein but accounted for only 18% of the variability in protein rankings ($r_S = 0.43$, $P<0.0005$). Taken collectively these data indicate that the disease-specific alterations of xenobiotic-metabolizing CYP enzymes among patients with cirrhosis is due, at least in part, to pre-translational mechanisms. The lack of a strong correlation between CYP2E1 mRNA and protein suggests that this gene, like its rat orthologue, may be subject to pre-translational as well as translational and/or post-translational regulation.

Key words: cytochrome P450; CYP; CYP regulation; CYP1A2; CYP2E1; CYP2C9; CYP3A4; human cirrhosis; liver disease

The cytochrome P450s comprise a superfamily of enzymes that play a major role in the detoxification of foreign compounds (xenobiotics) and in the metabolism of endogenous lipids. To date, 221 CYP† genes and 12 putative pseudogenes have been described in 31 eukaryote and 11 prokaryote species. Twelve gene families and 22 subfamilies have been described in man; 17 have been mapped to the genome [1]. The recommended nomenclature has been adopted in the present work; in some cases the CYP term has been dropped, e.g. 2E1 instead of CYP2E1 [1].

In man, members of the CYP1, 2 and 3 families are predominantly involved in the hepatic metabolism of xenobiotics including drugs and environmental pollutants. Induction by these substances at the gene, mRNA or protein level may alter CYP expression and catalytic function. The expression of CYP enzymes is subject to large inter-individual variation; in man, genetically determined polymorphisms in drug oxidation, and developmental and tissue-specific regulation of some CYP isoforms have been described [2–4]. CYP expression may also be modulated by hormones, cytokines and disease states.

The regulation of CYP expression is complex involving transcriptional, post-transcriptional and post-translational events. Neonatal imprinting, sex-specific and sex-independent developmental expression, and tissue-specific regulation have been described in animals [5]. Although liver disease appears to decrease pathways of hepatic drug metabolism, the expression and regulation of individual CYPs is uncertain. *In vivo* studies have shown that drug metabolism is significantly altered in severe liver disease [6–9]. However, the contribution of confounding variables such as hepatic blood flow, hepatocyte number and interference by co-administered drugs cannot be estimated or eliminated by this approach. We have recently shown

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† Abbreviations: cDNA, complementary DNA; cRNA, complementary RNA; CYP P450, cytochrome P450; IgG, immunoglobulin G; tNA, total nucleic acid; UTP, uridine triphosphate.

in vitro, using isoform-specific catalytic activities and protein immunoquantitation, that 1A2, 2C, 2E1 and 3A proteins are differentially altered in patients with different types of end stage chronic liver disease [10]. In order to determine whether the observed changes in CYP protein content could be due to impaired gene-specific protein synthesis, we now report the results of a systematic determination of hepatic mRNA levels for key protein representatives of the above CYP subfamilies.

MATERIALS AND METHODS

Materials. All chemicals were of analytical grade or similar purity and unless otherwise stated were from either Sigma (Sydney, Australia), Boehringer Mannheim (Sydney, Australia), or Merck (Darmstadt, Germany).

Human tissue. The protocols for experiments on human tissue were approved by the Human Ethics Committees of the Western Sydney Area Health Service and the Royal Prince Alfred Hospital, Sydney. Informed consent was obtained from the subjects.

The study population comprised 70 patients whose clinical characteristics have been reported elsewhere [10]. Briefly, morphologically normal liver was obtained from 20 patients undergoing hepatobiliary surgery for the resection of benign (N = 4) or malignant tumors (N = 14), or other conditions (N = 2). Cirrhotic liver was obtained at the time of orthotopic liver transplantation from 50 patients with advanced hepatic disease. Diagnoses were according to conventional criteria as recognized by the International Hepatology Informatics Group [11]. Tissue from the superior surface of the right lobe of the liver was obtained from patients with cirrhosis, and where possible (subjects undergoing right hepatic lobectomy) from this same region in controls.

Within 15 min of surgical removal, blocks of liver tissue (weight 30–50 g) from control and diseased patients were snap frozen in liquid nitrogen and stored at −70°. Aliquots of liver (weight 250–500 mg) were stored separately under the same conditions for the extraction of nucleic acids.

Data obtained from perusal of patient records included: age, sex, diagnosis, liver histology, presence and nature of non-hepatic disease, previous surgery (including portosystemic shunts), concomitant infection, a semiquantitative assessment of nutritional status, and immediate (previous week) and past history of cigarette smoking or alcohol consumption. The results of liver tests, the serum albumin concentration, prothrombin time, and investigations performed to confirm the clinical diagnosis were noted. Using the criteria of Child, as modified by Pugh, a functional assessment of the severity of liver disease was obtained [12].

All medications consumed in the 2 weeks prior to surgery were recorded. Drugs were classified according to their ability to induce or inhibit CYPs; this information for individual drugs was ascertained by searching the Medline database.

Preparation and storage of microsomes. Microsomes were prepared from liver tissue within 3 weeks of collection, using differential ultracentrifugation as previously described [13]. The resulting microsomal pellets were resuspended in 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol. Microsomes were snap frozen in liquid nitrogen and stored at −70°. CYP proteins were immunoquantitated within 8 weeks. Storage for this duration did not alter the protein content of microsomes.

Immunoquantitation of microsomal proteins. Reagents for electrophoresis and immunoblotting were from BioRad (Richmond, CA, U.S.A.). The microsomal content of CYP1A2, 2C, 2E1 and 3A was quantitated by immunoblotting [15] after electrophoresis on 7.5% polyacrylamide gels in SDS and transfer to nitrocellulose membranes [16], as described previously [10]. Immunoblotting reactions were performed following incubation with primary antibody, and then incubation with a combination of ^{125}I-labelled and peroxidase-labelled anti-sheep (1A2) or anti-rabbit IgG (2C, 2E1, 3A). Immunoreactive proteins were visualized with 4-chloro-1-naphthol/hydrogen peroxide, excised and radioactivity determined by gamma counting. Because authentic standards of the human CYPs of interest were not available, a single microsomal preparation was selected as a "control" with which to compare other samples. Patient samples were electrophoresed in duplicate and results expressed as a percentage of that in the "control".

The following polyclonal anti-CYP IgGs were used in immunochemical studies. Anti-rabbit CYP1A2 IgG raised in sheep was a gift from Dr P. Maurel (INSERM U 128, Montpellier, France). This antibody apparently recognized two proteins in rat microsomes (1A1 and 1A2), but only a single protein in human liver tissue (1A2). Anti-human CYP2C IgG generated in rabbit was a gift from Dr P. H. Beaune (Chu Necker, INSERM U 75, Paris, France). The extent of sequence similarity between members of the 2C gene subfamily in man suggests that this antibody would detect other 2C proteins in liver. Anti-human CYP2E1 IgG raised in rabbit was a gift from Dr M. Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden) and recognized a single protein in human liver. Rabbit anti-human CYP3A4 IgG was a gift from Dr F. P. Guengerich (Vanderbilt University, Nashville, Tennessee, U.S.A.). Given the amino acid sequence similarity between the various human 3A subfamily proteins, this antibody probably also recognizes 3A3, 3A5 and 3A7.

Isolation of total nucleic acid. Total nucleic acids (tNA) were isolated from liver tissue by proteinase K digestion followed by phenol–chloroform extraction as previously described [17]. tNA content was determined spectrophotometrically from the absorbance at 260 nm. The concentration of DNA was quantified using the method of Labarca and Paigen [18].

Solution hybridization analysis of CYP mRNAs. The relative abundances of the mRNAs for CYP1A2, 2C9, 2E1 and 3A4 were determined by the method of Melton *et al.* [19], using [^{35}S]UTP (1000 Ci/mmol, Amersham, Sydney, Australia) labelled cRNA probes transcribed *in vitro* using a commercial kit

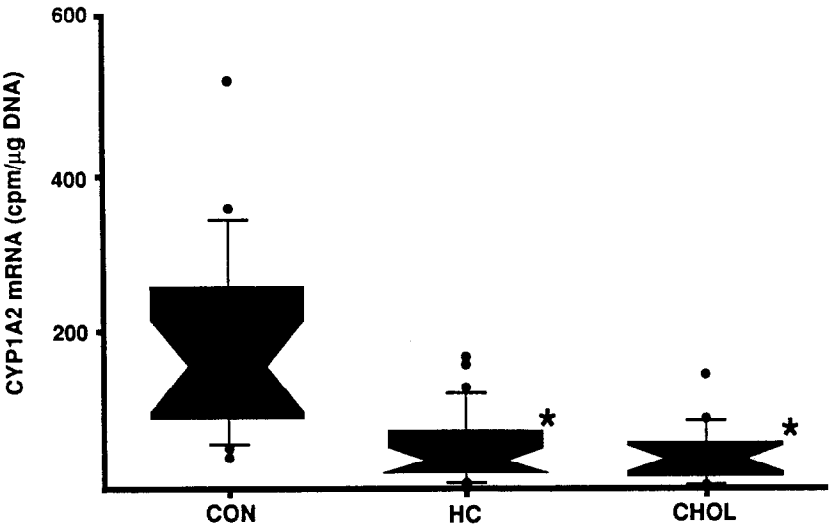

Fig. 1. CYP1A2 mRNA levels in control (CON) patients (N = 20) and in those with hepatocellular (HC, N = 32) and cholestatic (CHOL, N = 18) liver disease. Data are presented as box plots; the median values are indicated by the bar within the shaded areas, which in turn comprise the 25th to 75th percentiles. Error bars denote 10th and 90th percentiles. *P<0.0005 relative to controls.

(Riboprobe, Promega Inc, Sydney, Australia). Extensive computer assisted sequence comparisons for all known human cDNAs, particularly human CYP sequences, were carried out in order to minimize cross-hybridization with other genes. Probe sequences were as follows: CYP1A2, bases 686–735 of the published sequence [20]; CYP2C9, bases 1543–1625 [21]; CYP2E1, bases 525–574 [22]; and CYP3A4, bases 1716–1765 [23]. Probe sequences were obtained by synthesis of both DNA strands followed by annealing and ligation as previously described [24]. The 2C9 sequence was obtained by reverse transcriptase polymerase chain reaction using RNA extracted from human liver and primers whose sequences were derived from the published cDNA sequence. All probe sequences were ligated into the polylinker region of the pGEM 3Z plasmid vector. Maximum sequence similarities of the probes to CYPs within the same family or subfamily were: CYP1A2, <50%; CYP2C9, 80%; CYP2E1, <50%; CYP3A4, 82%. The greatest similarity between the CYP3A4 probe and other CYP3A sequences was with the fetal form CYP3A7, which is expressed in <50% of adults and accounts for <5% of total CYP content in adult liver microsomes [4]. It has been demonstrated previously that, under conditions of high stringency, this degree of similarity does not result in cross-reactivity between probes [24].

Optimal assay conditions with regard to temperature were determined for each probe, being set at 5–10° below the melting point of the labelled RNA–RNA hybrid. The following hybridization temperatures were used: CYP1A2, 75°; CYP2C9, 65°; CYP2E1, 75° and CYP3A4, 70°. Hybridization of aliquots of tNA were performed in triplicate. The hybridization mix (40 μL), consisted of tNA sample (5, 7.5 and 10 μL), 0.6 M NaCl, 22 mM Tris HCl (pH 7.5), 5 mM EDTA, 0.1% SDS, 1 mM dithiothreitol, 20% deionized formamide, transfer RNA (1.23 μg), and approximately 30,000 cpm of probe. After overnight incubation at the apposite temperature, samples were exposed to RNase in a 1 mL reaction mixture containing 0.3 M NaCl, 10 mM Tris HCl (pH 7.5), 2 mM EDTA, salmon sperm DNA (0.1 mg), RNase A (40 μg) and RNase T1 (500 U) for 45 min at 37°. Undigested hybrids were precipitated by the addition of 100 μL of 6 M trichloroacetic acid, and collected by vacuum filtration onto glass-fiber filters. Specific activity was determined by liquid scintillation spectrometry.

Standard curves were constructed for each assay using tNA extracted from a control liver. This confirmed linearity of probe hybridization and allowed for comparisons of results between assays. mRNA determinations from patient samples were within the linear region of the standard curve. Samples run without the addition of nucleic acid were used to determine background radioactivity. The adequacy of probe input was checked using probe solution that was not exposed to RNase. Results were expressed in cpm/μg DNA.

Statistical analyses. Results were analysed by non-parametric methods because examination of the data for frequency distributions of mRNA and protein quantitation revealed that these were not normally distributed. The Mann–Whitney U test was used for two group comparisons and the Kruskal–Wallis test if more than two groups. The Bonferroni correction was used to adjust for multiple comparisons. Correlations between mRNA and protein content were expressed as Spearman rank correlations (r_S). Data were analysed on a digital computer (Macintosh SE, Apple Computer, Cupertino, CA, U.S.A.) and a commercial statistical package (Statview 512+, Brain Power Inc, Calabasas, CA, U.S.A.). The significance level for all tests was P<0.05.

RESULTS

Characteristics of patients studied

The clinical and other characteristics of the patients have been described elsewhere [10]; there were no important differences between groups in relation to age, gender or the severity of liver disease. The 20 control subjects had normal biochemical and hematological indices, and histology of hepatic tissue used for subsequent analysis was normal. Patients with liver disease had cirrhosis confirmed on biopsy.

Five control subjects consumed alcohol (≥10 g/day) and 5 patients (3 controls, 2 cirrhotics) were current smokers (>5 cigarettes/day). Because alcohol induces 2E1 [22], and cigarette smoking increases levels of 1A2 [25], these patients were excluded from statistical analysis of the 2E1 and 1A2 data, respectively. Eight controls were currently consuming prescribed drugs, but none were taking compounds known to induce or inhibit the CYPs of interest. Patients with liver disease were usually taking multiple medications. The majority of drugs were either without effect (based on literature information) or were reversible inhibitors of CYP mediated metabolism (including the fluoroquinolones, acetaminophen, cimetidine and antihypertensives). The latter compounds are likely to be removed in the wash steps performed during the preparation of microsomes. None of the patients received drugs that have been associated with mechanism-based inhibition of CYP enzymes [26]. Compounds known to induce CYPs included rifampicin (received by 3 patients), omeprazole (2 patients) and the glucocorticoids prednisone, hydrocortisone or dexamethasone (13 patients with hepatocellular and 1 patient with cholestatic liver disease).

Microsomal CYP protein content in control and diseased liver

These results have been reported previously [10], but are summarized here in order to permit comparison with the mRNA data. Microsomal 3A protein was decreased only in hepatocellular disease, while 1A2 protein was reduced in microsomes from patients with hepatocellular and cholestatic types of hepatic dysfunction. In contrast, immunoreactive levels of 2E1 were maintained in hepatocellular disease but were decreased in livers from patients with cholestatic forms of cirrhosis. 2C protein was decreased in patients with cholestatic disease. There was a trend that suggested decreased 2C protein in hepatic microsomes from patients with hepatocellular disease, but this did not attain statistical significance.

CYP-specific mRNA levels in control and diseased liver

Large inter-individual variations (up to three orders of magnitude) were noted in the CYP-specific mRNA content of hepatic tissue from controls and patients with advanced hepatic disease. This variability in mRNA expression was greatest for CYP3A4. Thus CYP1A2, CYP2C9, CYP2E1 and CYP3A4 mRNA levels varied 14-, 9-, 11- and 136-fold in control subjects. In cirrhotic liver, this variability was even more striking being 56-, 17-, 44- and 1200-fold with regard to CYP1A2, CYP2C9, CYP2E1 and CYP3A4, respectively (Figs 1–4).

CYP1A2 mRNA levels were decreased in patients with both hepatocellular ($P<0.0005$) and cholestatic ($P<0.0005$) forms of cirrhosis (Fig. 1). In contrast, CYP3A4 mRNA was reduced in patients with hepatocellular disease ($P<0.001$) but was maintained in those with chronic cholestatic conditions (Fig. 4). CYP2C9 and CYP2E1 mRNA concentrations were significantly lower in patients that had either type of liver disease compared to controls ($P<0.05$) (Figs 2 and 3).

Correlations between CYP mRNA and content of corresponding CYP proteins

Significant correlations were noted between mRNA levels and protein content for CYP1A2 ($r_S = 0.74$, $P<0.0001$) and CYP3A4 ($r_S = 0.64$, $P<0.0001$) (Table 1, Fig. 5). For these two proteins, mRNA levels accounted for 55% (CYP1A2) and 41% (CYP3A4) of the data variance in protein rankings. The relationship between CYP2C9 and CYP2E1 mRNA concentrations and their corresponding protein concentrations, were statistically significant ($r_{S2C9} = 0.36$, $P<0.005$, $r_{S2E1} = 0.43$, $P<0.0005$), but mRNA levels accounted for a much smaller proportion of the variability in protein rankings (13% and 18%, respectively) (Table 1).

DISCUSSION

To our knowledge, no previous studies have examined the influence of advanced hepatic disease on CYP mRNA expression. Studies on normal tissue attest to the difficulty of interpreting such data. Using RNAse protection assays, Palmer *et al.* [27], noted large inter-individual variations in the expression of four CYP mRNA species in normal liver tissue. CYP1A2 and CYP2C mRNA concentrations displayed a 30-fold inter-subject variation, while 10- and 1000-fold differences were noted for the mRNAs encoding CYP2B and CYP2A, respectively. Using cDNA probes and Northern hybridization, Forrester *et al.* [28], examined the expression of CYP1A1, CYP2A6, CYP2B6, CYP2C8, CYP2D6, CYP2E1 and CYP3A3 in 12 livers from renal transplant donors. Again extremely large variations were noted in mRNA levels from samples. Moreover correlations between mRNA values and the content of the respective CYP proteins were poor. This discrepancy did not appear to be related to the integrity of the mRNA because, in samples where the mRNA levels encoding a particular CYP were low, other CYP isoform-specific mRNAs were highly expressed. Ratanasavanh *et al.* [29], correlated the levels of CYP2C and CYP3A mRNA in nine histologically normal livers (two fetal, two new borns, three children and two adults) with immunochemically determined 2C and 3A protein content. The cDNAs used recognized all mRNA species from the corresponding subfamily. CYP3A mRNA levels roughly paralleled those of 3A protein ($r = 0.71$, $P<0.05$). While there was a correlation between 2C mRNA and protein ($r = 0.66$), this was not significant ($P = 0.07$) perhaps as a consequence of the small sample size. Thus, the few studies exploring

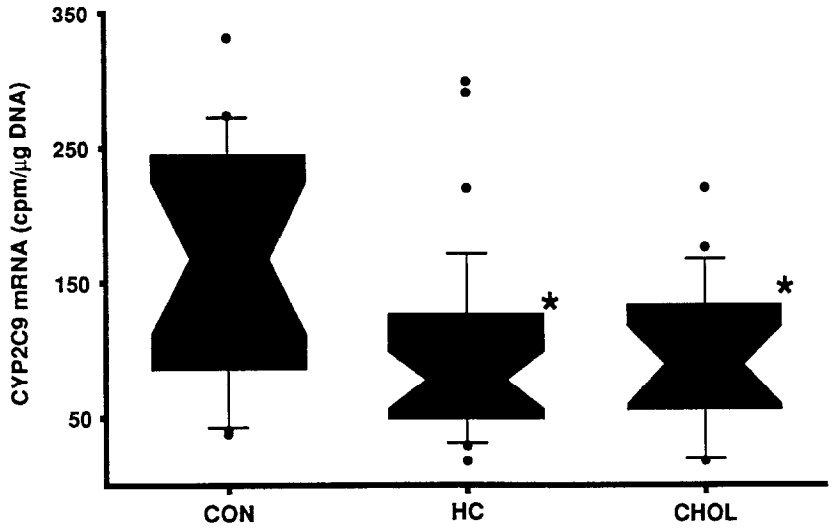

Fig. 2. CYP2C9 mRNA levels in control (CON) patients (N = 20) and in those with hepatocellular (HC, N = 32) and cholestatic (CHOL, N = 18) liver disease. Method of data presentation is the same as for Fig. 1. *P<0.05 relative to controls.

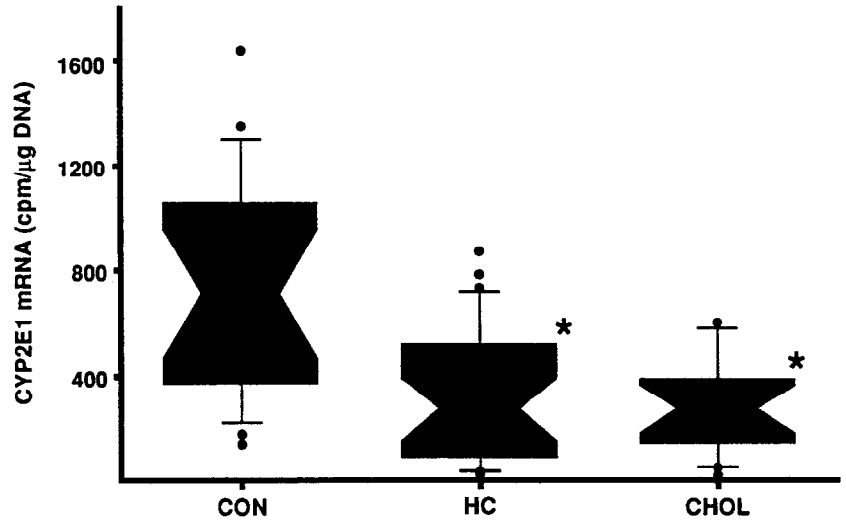

Fig. 3. CYP2E1 mRNA levels in control (CON) patients (N = 20) and in those with hepatocellular (HC, N = 32) and cholestatic (CHOL, N = 18) liver disease. Method of data presentation is the same as for Fig. 1. *P<0.005 relative to controls.

relationships between CYP mRNA and the immunoreactive protein products in healthy liver tissue have failed to find striking correlations.

It has been reported, by *in situ* hybridization, that the apparent concentrations of CYP2A and CYP2B mRNAs were increased in two cirrhotic patients compared to controls. Signals were particularly intense in isolated hepatocytes at the junctions between fibrous septa and hepatic nodules. Hybridization using CYP3A and CYP2C probes gave weak signals compared to controls [30]. Using immunohistochemical techniques, Ratanasavanh *et al.* [29] examined the intralobular distribution of 1A2, 2C and 3A proteins in four patients with cirrhosis. Striking variability in immunoreactivity for all three proteins was noted between hepatocyte nodules in cirrhotic liver.

We have shown previously that there are disease-specific alterations in CYP protein expression and catalytic activities in patients with advanced liver disease [10]. The current study extends this work, demonstrating disease-specific alterations in CYP1A2 and CYP3A4 mRNA. CYP1A2 mRNA, in tandem with changes in 1A2 protein, was reduced

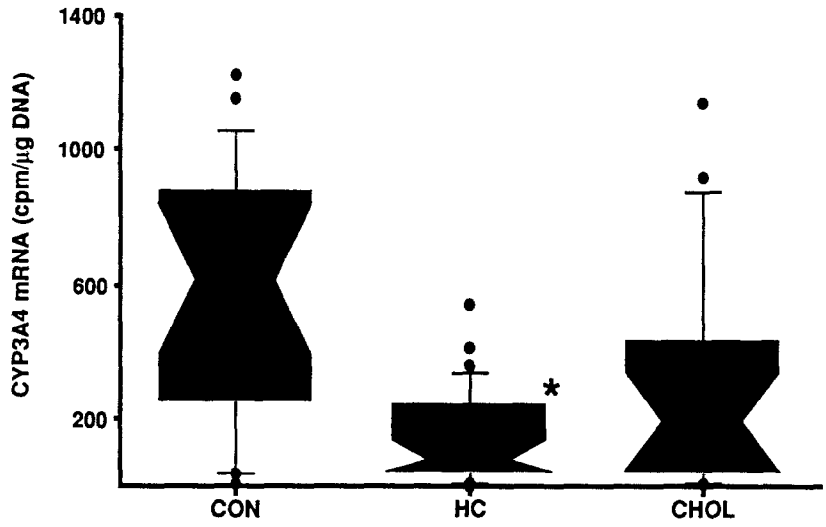

Fig. 4. CYP3A4 mRNA levels in control (CON) patients (N = 20) and in those with hepatocellular (HC, N = 32) and cholestatic (CHOL, N = 18) liver disease. Method of data presentation is the same as for Fig. 1. A single extreme value (2438 cpm/μg DNA) has not been drawn in the CHOL group to facilitate preparation of the figure. *P<0.001 relative to controls.

Table 1. Correlations between hepatic content of CYP-specific mRNA and related microsomal protein

CYP	r_S	P value
1A2	0.74	$P < 0.0001$
2C	0.36	$P < 0.005$
2E1	0.43	$P < 0.0005$
3A	0.64	$P < 0.0001$

r_S is the Spearman rank correlation. The data were derived from 20 control and 50 cirrhotic livers (see Materials and Methods).

in patients with either hepatocellular or cholestatic types of liver disease. In contrast, CYP3A4 mRNA (and 3A protein) was reduced in patients with hepatocellular disorders but appeared to be maintained in those with chronic cholestatic disease. Close correlations were evident between mRNA and protein levels for these two isoforms. These data indicate that the disease-specific alterations observed in 3A and 1A2 protein can be partly accounted for by altered pre-translational regulation of the CYP genes for these isoforms. Further, such pre-translational regulation accounts for at least 40–55% of the variability of these protein rankings. It is acknowledged that there are several reasons why levels of mRNA may be reduced, such as decreased CYP transcription and/or enhanced mRNA turnover. Which of these are operative in chronic liver disease requires further study.

CYP2C9 and CYP2E1 mRNA species were diminished in all cirrhotic livers, including those with hepatocellular and cholestatic disease. This observation is consistent with the suggestion that the mRNAs corresponding to these enzymes are at least partly subject to regulation at the pre-translational level. In contrast, protein quantitation revealed that 2C and 2E1 proteins were significantly reduced only in patients with cholestatic liver disorders, the median values compared to controls being reduced by 66% for 2C and 51% for 2E. There was a tendency towards a reduction in the median values of 2C (43%) but not of 2E1 (19%) protein in hepatocellular disease.

The polyclonal anti-human 2C9 antibody used in our studies recognized one protein in immunoblotting analyses of human microsomes. Given the high degree of sequence similarity between the various proteins in this subfamily, this signal most likely represents a summation of the changes in the levels of all members of the CYP2C subfamily. Immunoreactive 2C protein in the present study was decreased in microsomes from patients with cholestatic disease. There was also a trend suggesting a decrease in microsomes from patients with hepatocellular disease, a finding broadly reflecting those obtained by CYP2C9 mRNA quantitation. According to the selected sequences for the solution hybridization assay, it is likely that the CYP2C9 mRNA probe used recognizes CYP2C9 but not CYP2C8, CYP2C10, CYP2C17, CYP2C18 or CYP2C19. The correlation between 2C protein and mRNA levels, while significant ($P<0.005$), was not as close ($r_S = 0.36$) as that observed for CYP1A2 and CYP3A4. The complexity of the genes within the CYP2C subfamily and the lack of specificity of the polyclonal antibody used in the present study are factors that probably account for this finding.

While the regulation of CYP2E1 in man has not been elucidated, its expression in rat liver appears to be modulated at several points. Transcriptional

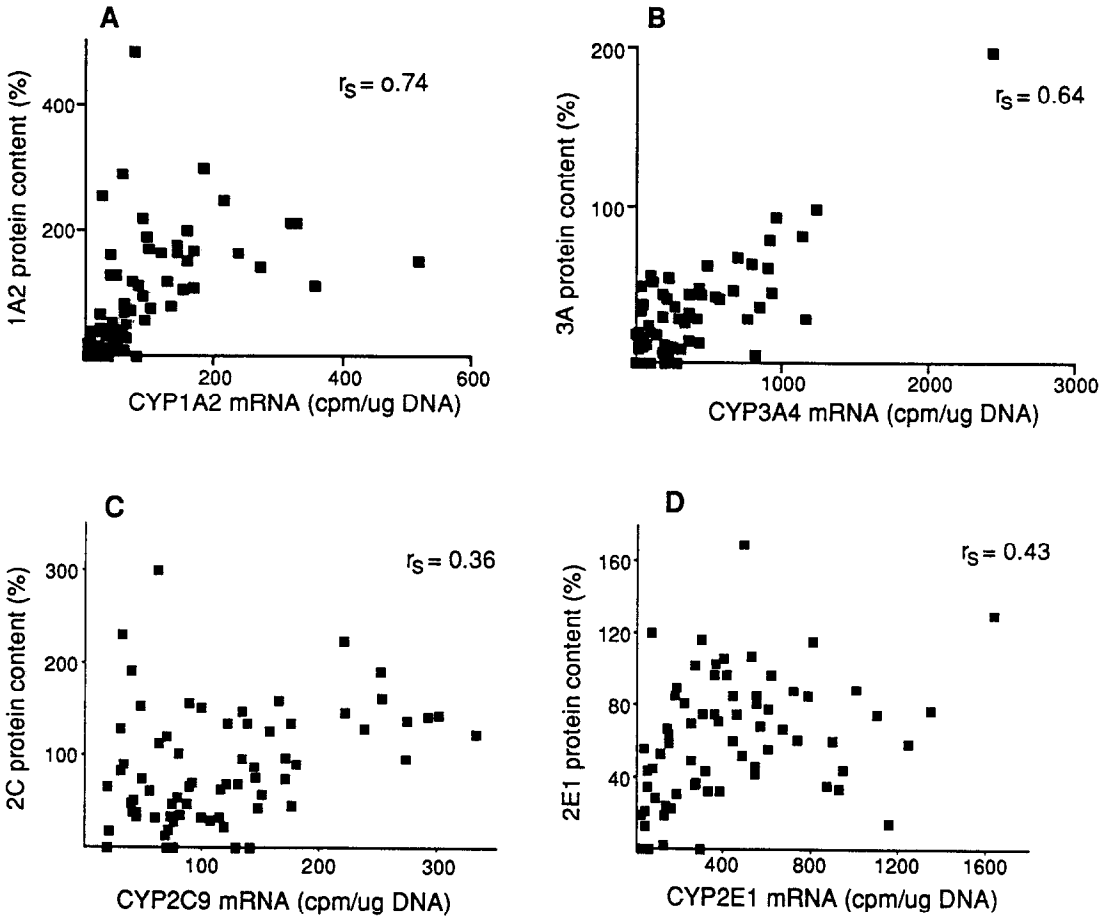

Fig. 5. Scatter plots of CYP mRNA content versus relative microsomal protein in 70 patients for CYP1A2 (A), CYP3A4 (B), CYP2C9 (C) and CYP2E1 (D). Values are Spearman rank correlation coefficients (r_S). Correlations were highly significant ($P_{1A2 \text{ and } 3A4}<0.0001$, $P_{2C9}<0.005$, $P_{2E1}<0.0005$).

activation during development [22] and pre-translational activation with increases in mRNA levels have been observed in experimental diabetes, fasting, ethanol feeding and during ketosis induced by a high fat diet [31–34]. Translational regulation and post-translational modulation by protein stabilization without elevation of CYP2E1 mRNA occur after exposure to substrates such as acetone, ethanol and pyridine [35–37]. Growth hormone and carbon tetrachloride may decrease the expression of this protein [38, 39]. In man, enhanced expression of 2E1 protein has been noted in alcoholics and in patients treated with the inducing agent isoniazid [40, 41]. Pre-translational control may be important in such regulation [42], though other studies have not confirmed this [43].

While we have demonstrated decreased levels of hepatic CYP2E1 mRNA in patients with liver disease, the lack of close correlation ($r_S = 0.43$) between protein and mRNA would suggest that differential protein stabilization or post-transcriptional regulation may also occur in man. In a study of nine healthy human liver samples, Wrighton *et al.* [43] found a similar lack of correlation between the amount of CYP2E1 mRNA and 2E1 protein. There are several factors that could account for the findings on 2E1 protein expression which have been noted in the present study as well as by others [44]. Increased stability of 2E1 protein and/or increased translation of 2E1 mRNA could occur in patients with hepatocellular disease; it is possible that opposite influences may occur in those with chronic cholestasis. Whether post-transcriptional changes contribute to the observed differential expression of 2E1 proteins in patients with advanced hepatocellular and cholestatic disease has not been addressed by the present studies. Patients with chronic cholestasis have high circulating levels of bile acids and cholesterol. These factors, as well as hormones and cytokines, are putative agents that may alter the expression of 2E1 protein by post-transcriptional processes and such potential effects warrant further study.

In summary, the present results clearly demonstrate that disease-specific changes in the expression of three xenobiotic-metabolizing CYP enzymes (1A2, 2C and 3A) can be attributed, in part, to pre-translational regulation of the respective CYP genes. In contrast, pre- and post-translational (or translational) regulation may account for the

observed differential effect of hepatocellular and cholestatic diseases on 2E1 protein expression. It is now important to identify the factors which modulate these changes in the diseased liver. *In vitro* studies using cultured human hepatocytes may be helpful in identifying potential humoral regulators and the molecular mechanisms involved.

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These results have been presented in part at the American Association for the Study of Liver Diseases Meeting, Chicago, November 1993, and have been published in abstract form in *Hepatology* **18**: 129A, 315A, 1993.

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