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Research Article

CYTOCHROME P450 1A ISOFORM AND ITS GENE EXPRESSION IN LIVERS OF PATIENTS WITH DISORDER LIVER DISEASES

ESSAM F. AL-JUMAILY[1] ASEEL R. ABOUD[2] AND MAAN AI-KHALISI[2]

[1]Department of Biotechnology, Institute of Genetic Engineering and Biotechnology, Baghdad University.
[2] Department of Anatomy, Baghdad College of Medicine, Baghdad University.
*Corresponding Author

Abstract

BACKGROUND: Cytochrome P450 (CYP450) enzymes play a critical role in the oxidative metabolism of a variety of endogenous and exogenous compounds, including most drugs. The expression of CYP genes is affected by physiological, genetic and environmental factors. RT-PCR provides the simultaneous measurement of gene expression in many different samples for a limited number of genes, and is especially suitable when only a small number of cells are available. **AIM**: To determine whether parenchyma cells or hepatic cytochrome P450 protein was changed in disorder liver diseases, and to compare the difference of CYP1A enzyme and its gene expression between patients with chronic viral hepatitis and cirrhosis, and to investigate the pharmacologic significance behind this difference. **METHODS:** Liver samples were obtained from patients undergoing hepatic surgery with any of the chronic viral hepatitis (n=4), cirrhosis (n=7), hepatocellular carcinoma (n=6) and healthy people (controls, n=6). Total hepatic RNA was extracted and CYP1A PCR determined CYP 1A activity and protein. **RESULTS:** Compared to healthy people, the CYP1A activity and protein in liver tissue among patients with disorder liver diseases were evidently reduced. RT-PCR showed the same change in its mRNA levels. **CONCLUSION:** Hepatic levels of CYP450s and its CYP1A isoform activity were selectively changed in different disorder liver diseases. CYP1A isoenzyme and its activity declined among patients with chronic viral hepatitis, cirrhosis, and hepatocellular carcinoma as expression of *CYP1A* gene was significantly reduced. Liver's ability to eliminate many clinical therapeutic drug substrates would decline consequently; these findings may have practical implications for the use of drugs in patients with disorder liver diseases and emphasize the need to understand the metabolic fate of therapeutic compounds. Elucidation of the reasons for these different changes in hepatic CYP1A may provide insight into more fundamental aspects and mechanisms of impaired liver function.

Keywords: hepatic cytochrome P450 , liver diseases, CYP1A isoenzyme, hepatocellular carcinoma.

Introduction

Chronic liver diseases is an inflammation and necrosis of liver tissue continue for at least six months represents a series of liver disorders of many causes, the most conmen type is viral hepatitis because its one of primary risk factor for the development of cirrhosis and hepatocellular carcinoma (Ghebranious).

Hepatic cytochrome P450 enzyme constitutes a superfamily of hemoproteins which play a major role in the metabolism of endogenous compounds and in the detoxification of xenobiotic molecules. About 200 CYP450s have been found in the past 20 years, and many factors including age, gender, nutrition, hormone and general or local pathologic reaction affect CYP450s, and the biotransformation of many clinical therapeutic drugs would be changed.

Cytochrome P450 is hemoproteins have an average mass of approximately 50 KDa. The cytochrome P450 enzymes located in the membranes of the smooth

endoplasmic reticulum with the exception of several bacterial forms. Like mitochondrial cytochrome oxidase, cytochrome P450 can react with O2 and binds carbon monoxide, but it can be differentiated from cytochrome oxidase because its carbon monoxide complex reduced form absorbing light strongly at 450 nm. Cytochrome P450 has two functions, removal of carcinogens or activation of procarcinogens (Watkins *et al*., 1994).

The liver is the major site of drug biotransformation. Oxidative phase I enzymatic reactions like hydroxylation terminate the biological activity of a drug in one or more steps. Phase II conjugation reactions such as glucuronidation sequentially complete the process of transforming metabolites into more water-soluble compounds that can be more easily eliminated by the kidney (Goshman *et al*., 1999; Rooney *et al*., 2004). The cytochrome P450 enzyme system is a key pathway for drug metabolism. Many lipophilic drugs must undergo biotransformation to more hydrophilic compounds to be excreted from the body. The majority of drugs undergo phase I metabolism (e.g., oxidation, reduction) by cytochrome P450 enzymes (Beaird, 2000). Cytochrome P450-catalyzed reactions increase substrate hydrophilicity by introducing a reactive center into the substrate molecule, which can then be further conjugated by reaction with phase II enzymes such as the glutathione *S*-transferasel4s (GSTs) or *N*-acetyl transferases (NATs) (Gibson and Skett, 1994).

Several CYP enzymes metabolically activate procarcinogens to genotoxic intermediates. Phenotyping analyses revealed an association between CYP enzyme activity and the risk to develop several forms of cancer. Research carried out in the last decade demonstrated that several CYP enzymes are polymorphic due to single nucleotide polymorphism, gene duplications and deletions. As genotyping procedures became available for most human CYP, an impressive number of association studies on CYP polymorphism and cancer risk were conducted (Agundez, 2004).

CYP1A2 represents about 15% of total CYP enzymes in the human liver (Shimada *et al.,* 1994). There is some variability between individuals in the CYP1A2 enzyme levels in the human liver (Shimada *et al.,* 1994). Because of the large interindividual variation in CYP1A2 activities, the *in vivo* testing of this enzyme has been quite difficult to evaluate (Kunze and Trager, 1993). CYP1A2 substrates among the currently used drugs include theophylline, caffeine, olanzapine, ondansentron, paracetamol, phenacetin and propranolol. Conversely, smoking cessation can increase theophylline level. Caffeine and theophylline have been considered *in vivo* diagnostic probes. The usually employed *in vitro* substrate is ethoxyresorufin, which seems to be quite specific to CYP1A2 (Burke *et al.,* 1985). The substrates and their enzyme kinetic parameters have recently been reviewed by Pelkonen *et al.* (1998). The aims of the study is to determine the alterations of CYP 1A enzyme and its gene expression in patients with those disorder liver diseases, and to investigate the pharmacologic and clinical significance behind these alterations-

Materials and Methods

Source of human liver tissues and patient characters

Human biopsy liver samples were taken from patients undergoing hepatic surgery. Patients had not received medication of CYP450s inducer and inhibitor before the surgery. None of the patients were habitual consumers of alcohol or other drugs. A total of 18 liver samples from 15 men and 3 women were used. They were all cases admitted from 2004 to 2005 in Baghdad Teaching Hospital in Baghdad, Iraq. Informed content was obtained from all patients for subsequent use of their specimen tissues. These specimens were immediately dissected into small pieces under aseptic condition within half an hour. Patients' characters and liver function.

Preparation of microsomes

The preparation of hepatic and liver microsomes was done based on a method described by Tyndale and coworkers, 1990; with some modifications [31]. The mice liver cut into small pieces at 0°C and homogenized in 5 vol. of homogenizing buffer (Mixed 50 mM Tris pH 7.0 and 15 Potassium chloride KCI and store at 4 °C). After the initial centrifugation at 13000 rpm for 20 min, the supernatant was saved and the pellet was re-suspended in 3 vol. of homogenizing buffer and re-centrifuged at 13000 rpm for 20 min. The final microsomal pellet was then re-suspended in 1 vol. of storage buffer, divided into small aliquots and stored at 4°C. For spectrophotometric studies, the microsomal pellet was re-suspended in 2 vol. of homogenizing buffer, re-sedimented by centrifugation to reduce haemoglobin contamination and the final pellet was re-suspended in 1 vol. of storage buffer (Add 50 mM Tris to 20% glycerol pH 7.4).

High Performance Liquid Chromatography (HPLC)

Samples were assayed by reversed phase HPLC using a 20 ul injection onto a Waters HPLC system containing two Model 501 pumps, a Model U6K injector with sample loop, a model 484 UV detector set at 195 nm detection, and a NovalPak 3.9 mm x 15 cm C18

column. Mobile phase consisted of water as phase A and acetonitrile as phase B flowing at a rate of 2.0 ml/min, with gradient elution profile of 3.0-35.0%.

Total RNA isolation and purification

Frozen livers were thawed on ice, and total RNA was isolated using used the SV total RNA isolation system (Promega corporation. 2800 Woods Hollow Road. Madison, Wl 53711-5399 USA). The yield of total RNA obtained by determined spectrophotometrically at 260 and 280nm, where 1 absorbance unit (A260 / A280) equals 40ug of single-stranded RNA/ml. Pure RNA will exhibit an A260 / A280 ratio of 2.0. However, it should be noted that, due to the variations between mice starting materials and in performing the procedure, the expected range of A260 / A280 ratio for RNA will be 1.6-1.9.

cDNA Library Construction and Screening

Three Uni-ZAP XR cDNA libraries were constructed using the ZAP cDNA synthesis kit from Stratagene. Three libraries were constructed from hepatic RNA from patients with disorder liver diseases. Poly (A^+) RNA was prepared using oligo (dT)-cellulose columns (GIBCO-BRL) and reverse-transcribed using oligo (dT) as a primer.

Design of primers

The cDNA sequences of CYP1A. PCR primers sequences were designed by Promega Corporation. The primer used to screen the cDNA libraries was a 299-bp hepatic human cDNA PCR product based on the sequence data of human CYP1A cDNA [32]. The forward and reverse primers were 5'-TGGTCATATTGGC CTCCCTGAG-3' and 5'-GAGAAGTTTCCGATACCGCACC-3[1], respectively.

The PCR reaction involved an initial denaturing step of 95°C for 30 sec, denaturation at 95°C for 15 sec, annealing at 71 °C for 30 sec, and then at 72 °C for 30 sec and extension at 72°C for 7 min for 26 cycles. The amplified 299-bp fragment was purified on a 2% agarose gel and extracted. The specificity of *CYP* primers was confirmed through alignment within the *CYP* subfamily and by sequencing each RT-PCR amplicon.

Results

RT-PCR assay

Standard curve and reproducibility

Figure (1) shows a representative amplification plot for CYP1A that was established by plotting the delta Rn versus PCR cycle number. The resulting standard curve of Ct against log nanograms of total RNA input was highly linear, as shown by a correlation coefficient (R^2) value of >0.99 over a wide range of total RNA input(0.3-100ng).

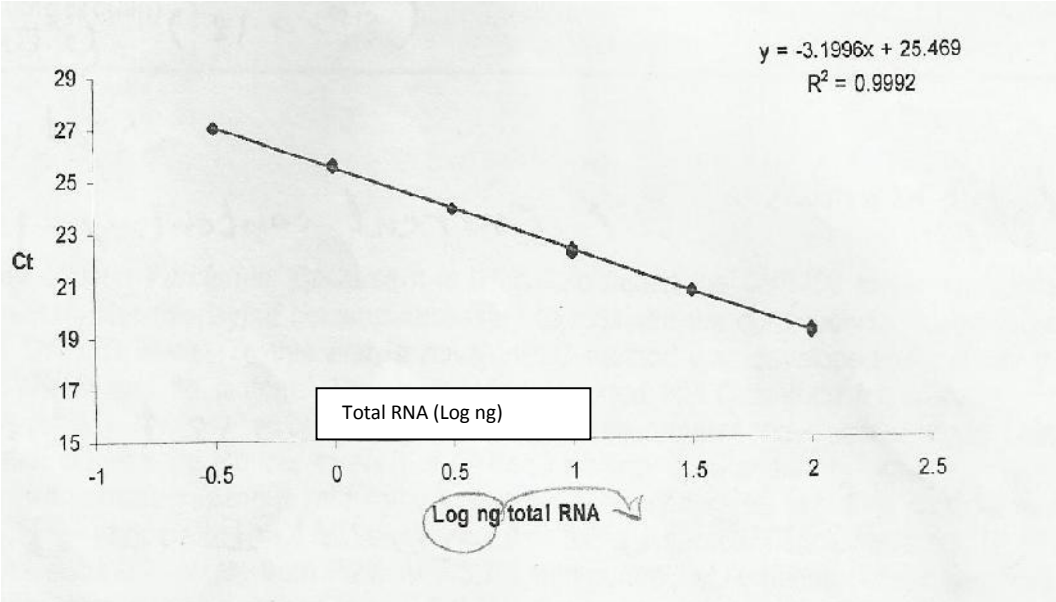


Figure 1: Amplification plot of CYP1A mRNA, the standard curve of mouse CYP1A mRNA was derived from amplification by plotting Ct verses log nanograms of total input, which shows the linearity of a large dynamic range of the starting template amount. The Ct9thermal cycle) values decrease with increasing total input.

Table 1 Microsomal protein, total P450 and CYP1A content and its activity among disorder liver diseases groups:

	Hepatocellular carcinoma	**Cirrhosis**	**Chronic viral hepatitis**	**Healthy people (control)**
Microsomal protein (g/L)	5.32 ± 0.98	5.76 ± 0.788	4.35 ± 0.8	10.45 ±2.69
CYP450 content (nmol/mg protein)	0.34 ±0.16	0.77 ±0.15	0.64 ±0.1 2	0.98 ±0.1 7
CYP1 A activity (nmol/min/mg protein)	0.12 ±0.01	0.45 ±0.1 2	0.23 ±0.10	2.94 ± 0.58

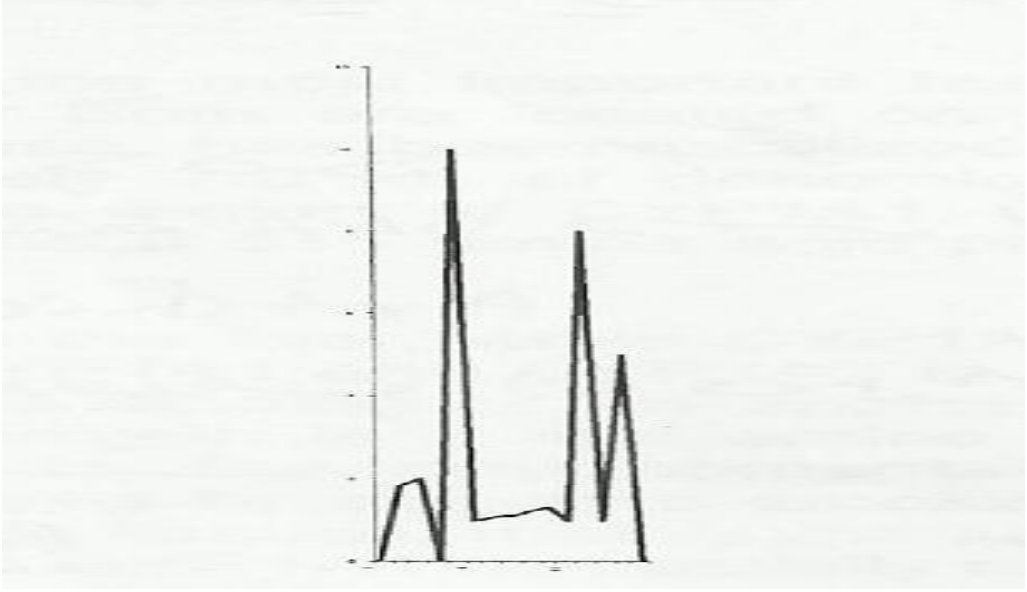
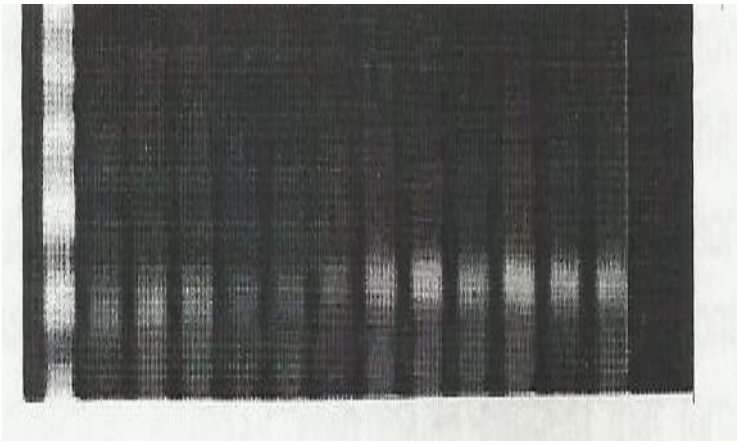


Figure (2): HPLC Chromatographs of microsome imcubation samples.



HPLC Assay Method Validation

Because it is difficult to detect the CYP450 levels from small pieces of human liver (biopsy), it became necessary to measure the corresponding decrease or increase in CYP450 levels. To this end, a novel HPLC method was developed to quantify the levels of CYP450 and its isoform. This is the first reported HPLC method for analysis of a CYP450, especially same concentration ranged from 0.6-6.3%. Cirrhosis CYP1A, as all published study measurements have been made using incubation method with the inducer of each of CYP450 isoform [33]. Standard peaks are provided in figure 1 from healthy people (control). A smaller peak apparently resulting from a less prominent CYP1A appears near 4.5 min elution but was too small to consistently quantify. CYP1A measurement accuracy ranged from 5.2% to + 3.7% with control, while hepatocellular carcinoma over the ranged from 0.7%-6.3% over the measured

concentration range of 3.5-153 nmol/ml, with the exception of a 13.7% chronic viral hepatitis at the lowest measured concentration.

Discussion

CYP1A appears to be one of the most important human CYP1A subfamily consists of two members, CYP1A1 and CYP1A2. Both are located on human chromosome 15. CYP1A1, the human aryl hydrocarbon hydroxylase, has been localized to human chromosome 15q22-24. The *CYP1A2* gene spans approximately 7.8 kb of genomic DMA and shares approximately 70% nucleotide identity with the coding region of CYP1A1. The corresponding mRNA contains an initial non-coding exon and encodes a 515 amino acid protein with a predicted molecular weight of 58 294 (Smith *et al*., 1998).

CYP1A2 is mainly expressed in the liver, while CYP1A1 is primarily expressed in extrahepatic tissues. The expression of both CYP1A1 and CYP1A2 is regulated at the level of transcription by a process which is initiated by the binding of an appropriate inducing agent to cytosolic Ah or dioxin receptor (the Ah receptor protein is a 484 amino acid helix-loop-helix transcription factor with partial sequence homology to the developmental gene *sim* and *per* from *Drosophila melanogaster)*(smith *et al*., 1998) . CYP1A1 is induced by cigarette smoking and polycyclic aromatic hydrocarbon (PAH) -type inducers in extra hepatic tissues, mainly the lung and placenta (Hirvonen, 1999) . In the liver, CYP1A1 is expressed at a very low level (Pelkonen *et al.,* 1998). CYP1A1 has activity towards some PAHs (Guengerich, 1991).

In our study, total CYP450 contents of 18 Iraqis patients were obviously lower than results those reported about Caucasian and Chinese and the activity of CYP1A isoform was also lower (Shu et al.,2002) . Although CYP1A drug metabolizing activity varied widely among individuals, it had a unimodal population distribution and did not appear to be subject to genetic polymorphism as seen with other CYP isoforms (2D6, 2C9, 2C19 and 3A4)(Tenneze *et al*., 2002;Bertilsson, 1995). The wide inter races variability was likely, in part, to be caused by ethnic or cultural differences, which might be related to an interaction between habit and diet. Therefore we could not draw any conclusion about the normal distribution character of CYP450s in Iraqis because of the limited sample number and experimental conditions. More detailed and complete studies should be performed for analyzing the distribution of CYP450s in Iraqis in the near future.

Most information on drug metabolism impairment at pathologic status has been obtained in rodent *in vivo* or *in vitro* models, and most of these studies have focused on the effects of IFNs and the major inflammatory cytokines, namely, IL-6, IL1 and TNFa(Renton, 2001; Shoda *et al*., 2000), but relatively few studies have examined the effect of liver disease on human CYP450 expression. Hepatic chronic viral hepatitis and cirrhosis are most common chronic hepatic disease in Iraqis, the change of CYP450s with chronic viral hepatitis and cirrhosis can provide us basic knowledge about the effect of pathological factors on CYP450s. The present study demonstrated that, in patients with chronic viral hepatitis and cirrhosis, CYP1A-mediated caffeine N-demethylation activity and 1A protein were significantly less than in controls, but the total CYP450 content and hepatic microsome protein still remained unchanged. These results suggest that family ingredients of CYP450s have changed in the chronic viral hepatitis and cirrhosis. That is, CYP family 2 may enhance following with CYP1A reduced, since CYP1 and CYP2 families play a major role in biotransformation of most carcinogens, but few studies described whether high morbidity of hepatic cancer in cirrhosis is correlated with these changes of drug metabolic enzymes (Tanaka, 1999) . Although many factors including age, gender, nutrition, hormone and general or local pathologic reaction affect drug elimination, the enzymatic activity as well as content of CYP450s is still a basic reason for change of drug metabolism, and the biotransformation of many clinical therapeutic drugs either enhanced or reduced (Morgan *et al*., 1998; Vuyk, 2001). This study is for the first time to examine simultaneously in patients with liver diseases the hepatic CYP450 protein level, isoform activity as well as its mRNA expression. Significant correlations with CYP1A protein level, isoform activity.

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