

Title:

Cytochrome P-450 mRNA expression in human liver and its relationship with enzyme activity.

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Relationship between CYP mRNA and activity.

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ABSTRACT

CYP activity and protein contents have been measured in human liver using different techniques. In contrast, CYP mRNA data are scarce and the relationships between CYP mRNA contents and activities have not been established. These studies deserve further attention because mRNA determinations by RT-PCR require a very small amount of material (e.g. liver needle biopsy) and could provide important data regarding CYP expression regulation. In this study we measured in 12 human liver samples the mRNA contents of ten CYPs by quantitative RT-PCR and the metabolic activities using specific substrates. mRNA contents and activities showed high correlation coefficients for CYP1A1, CYP1A2, CYP3A4, CYP2D6, and CYP2B6: 0.96, 0.94, 0.69, 0.61, and 0.52, respectively, but no significant correlations were found for CYP2C9, CYP2A6, and CYP2E1. The results suggest that the regulation of CYP1A1, CYP1A2, CYP3A4, CYP2D6, and CYP2B6 expression is essentially pre-translational and that their mRNA levels could allow a good estimate of their activity.

KEY WORDS. CYP mRNA; quantitative RT-PCR; CYP activities; specific substrates; human liver; *CYP* regulation.

Abbreviations: BROD, 7-benzyoxyresorufin O-debenzylation; COH, coumarin 7-hydroxylation; CYP, cytochrome P-450; CZOH, chlorzoxazone 6-hydroxylation; D4'OH, diclofenac 4' hydroxylation , DXOD, dextromethorphan O-demethylation; EDTA, ethylenediamine tetra-acetic acid; EROD, 7-ethoxyresorufin O-deethylation; MROD, 7-methoxyresorufin O-demethylation; NSAIDs, nonsteroidal anti-inflammatory drugs; 6 β OHT, testosterone 6 β -hydroxylation; RT-PCR, reverse transcription polymerase chain reaction and TCA, trichloro acetic acid.

INTRODUCTION

The cytochrome P-450 (CYP) is a superfamily of enzymes that catalyzes the oxidations of many endogenous and exogenous compounds (1, 2). Drugs are predominantly catalyzed in the human liver by CYPs 1A2, 2A6, 2B6, 2C8, 2D6, 2E1, and 3A4 (3, 4). As genetic, environmental, pathological, hormonal, and dietary factors all influence the expression of the CYP isoenzymes, their interindividual variability is large, and the susceptibility of humans to the pharmacological and toxicological actions of drugs and chemicals also varies widely (5-7). Because of the clinical relevance of this variability different analytical methods have been developed to measure the expression of CYP isoforms in biological samples. The most common methods are determination of the catalytic activities of individual CYPs using model substrates (8) and western immunoblot analysis using specific CYP antibodies (9). Although these techniques are reliable, the overlapping substrate specificity of CYPs makes it extremely difficult to select a compound metabolized exclusively by a single CYP, and in many cases specificity has only a relative meaning. Regarding western analysis, the quantification of CYP apoprotein levels depends on the affinity and specificity of the antibody, making the technique only semi-quantitative. Moreover, both catalytic and western-blot analyses require a large amount of biological sample, which is a serious drawback for human studies, where the available tissue is limited.

RT-PCR techniques are rapid, sensitive, and can measure precisely the amount of a specific mRNA in very small samples (10-12). mRNAs are the source of protein content and therefore enzymatic activity. However, enzymatic activity is not only the result of a transcriptional regulation, and it does not necessarily correlate with the mRNA content. This relationship, that has to be established experimentally, provides regulation information and if the correlation is high, activity levels can be estimated by measuring mRNA content. The relationships between CYP mRNA and activity have not yet been thoroughly studied, mainly because of a lack of quantitative RT-PCR data (13, 14). We recently described a quantitative

RT-PCR technique able to measure the ten most relevant drug metabolizing CYPs in human samples (10). We used this technique to quantify CYP mRNA content in 12 human livers and measure the corresponding enzyme activities in microsomes prepared from the same livers. Then we compared the results obtained by these two techniques to ascertain in which CYPs the mRNA data can be used instead of activity measurements to predict CYP-mediated drugs metabolism.

MATERIALS AND METHODS

Chemicals and reagents. Oligonucleotide primers were custom-synthesized by Life Technologies (Paisley, UK). Ribogreen was from Molecular Probes (Leiden, The Netherlands). The thermocycler used was the LightCycler Instrument from Roche (Rotkreuz, Switzerland), a “real-time” thermocycler. The PCR reactions were carried out using the LightCycler-DNA Master SYBR Green I, Roche.

Human liver tissues. A total of 12 morphologically normal liver samples were obtained from liver resections or organ donors (Table I). All liver samples were from donors who were not suspected of harboring any infectious disease and tested negative for human immunodeficiency virus and hepatitis. Liver samples were dissected into small pieces, frozen in liquid nitrogen, and stored at -80°C until used. Samples acquisition conformed to the rules of the Hospital’s Ethics Committee.

Isolation of total RNA from human liver tissues. Total RNA was extracted from approximately 50 mg of human liver using TRIzol RNA extraction kit (Life Technologies). The amount of purified RNA was estimated by ribogreen fluorescence and its purity was assessed by the absorbance ratio 260/ 280 nm. RNA integrity was examined by electrophoresis in a 1% agarose gel upon ethidium bromide staining.

Measurement of human CYPs by RT-PCR. The quantitative RT-PCR technique and the criteria used for primers design are described in detail elsewhere (10). The sequence of both the forward and reverse primers, the expected sizes of the PCR-amplified DNA, and the calculated melting temperatures are listed in Table II. The only modification introduced into the described technique consisted of adapting the PCR step to the LightCycler Instrument. The liver cDNA was diluted 10 times and the concentration of the PCR standards was

adjusted to ensure that PCR amplified products could be interpolated in the standard curve. 3µl of the diluted samples were introduced by centrifugation in the LightCycler Capillaries. The PCR reaction was carried out using the LightCycler-DNA Master SYBR Green I as recommended by the manufacturer. The final magnesium concentration for the PCR reaction was 4 mM for all amplifications except for CYP2A6, which was 6 mM. The annealing temperature was 60° C except in the case of CYPs 1A2, 2A6, 2B6, 2D6, and β-actin, where it was 62° C. All calculations to determine the concentration of specific cDNAs were carried out in the exponential phase of the PCR reaction.

Measurement of CYP activities in human liver microsomes. Liver microsomes were obtained as previously described (15). Protein content was determined by the method of Lowry (16). CYP activities were assayed by incubating 100 µg of microsome protein at 37° C for 15 min in 300 µl of 100 mM phosphate buffer pH 7.4 containing NADPH-regenerating system (5 mM Cl₂Mg, 1 mM NADP⁺, 10 mM glucose-6-phosphate, and 0.3 U/ml glucose-6-phosphate dehydrogenase). Substrate concentrations for the specific CYP assays were as follows: 10 µM ethoxyresorufin (CYP1A1/2 (17)), 10 µM methoxyresorufin (CYP1A2 (18)), 10 µM benzoxyresorufin (CYP2B6 (19)), 100 µM coumarin (CYP2A6 (20)), 200 µM diclofenac (CYP2C9 (21)), 50 µM dextromethorphan (CYP2D6, (22)), 250 µM chlorzoxazone (CYP2E1 (23)), and 200 µM testosterone (CYP3A4 (19)). 7-Ethoxyresorufin O-deethylase (EROD), 7-methoxyresorufin O-demethylase (MROD), 7-benzoxyresorufin O-debenzylase (BROD), and coumarin 7-hydroxylase (COH) assays were fluorimetrically determined as described (15). The diclofenac 4'-hydroxylase (D4'OH) and chlorzoxazone 6-hydroxylase (CZOH) assays were stopped by adding 300 µl of acetonitrile, and the metabolites formed were analyzed by HPLC as previously described (21). The dextromethorphan O-demethylase (DXOD) assay was stopped by adding 20 µl of 25% TCA, and dextromethorphan formed

was determined by HPLC analysis as described (24). The testosterone 6 β -hydroxylase (6 β OHT) assay was stopped with 500 μ l of ethylacetate, and the hydroxylated metabolite was extracted and analyzed by HPLC as described elsewhere (25).

Statistical analysis. Data are expressed as mean \pm standard deviation. Statistical analysis was performed using Pearson's product moment correlation.

RESULTS

Quantification of Human CYP mRNAs

RNA samples were extracted from 12 human livers (Table I), and CYP mRNAs were quantified by RT-PCR, as described in Methods, using a set of forward and reverse primers that ensured a specific CYP cDNA amplification. In order to improve the accuracy and precision of the correlation studies the β -actin mRNA content was also calculated and the abundance of CYP mRNAs was normalized against that value. Table III summarizes the CYP mRNA data, expressed as the mean of at least three different determinations \pm the standard deviation. A second RNA extraction was carried out obtaining similar results (data not shown).

The most abundant CYP mRNAs were those of CYP2E1 and CYP3A4, and the former more abundant than the latter in eight of the twelve livers. Similar data have been previously reported for these CYPs (10, 11, 26). Other CYP mRNAs ranked according to abundance were: CYP 2C9 > 3A5 > 2B6 > 1A2 > 2C19 > 2D6 > 2A6 > 1A1. CYP mRNA variation among livers was small (< 6 fold variation) for CYP2E1 and CYP2D6, larger for CYPs 1A1, 3A5, and 2C9 (< 60 fold variation), and CYPs 3A4, 2B6, 2A6, 2C19, and 1A2 had a variability higher than 100 fold variation. Calculated fold variation of CYP mRNA contents is given in Table III.

Enzymatic Activities of Eight Human CYP Isoenzymes

To measure the activity of individual CYP isoenzymes we used specific substrates that are catalyzed primarily by a single CYP isoenzyme to produce a given metabolite. Table IV shows the results of eight different CYP isoenzyme activities evaluated in microsomes from 12 human livers. Variability in CYP activities among the donors was small for CYPs 2A6, 2C9, and 3A4 (< 5 fold variation), larger for CYPs 1A2, 2E1, and 2B6 (between 8 and 12 fold

variation), CYP1A1 had a 21 fold variation, and CYP2D6 showed the highest activity variation with some livers with no detectable DXOD activity. Calculated fold variation of CYP activity is given in Table IV.

Correlation between mRNA Level and Metabolic Activity

The correlation between the CYP mRNA levels and activity was examined for the different CYPs. Plots showing the relationships between mRNA levels and activity for each CYP isoenzyme are represented in Fig. 1. CYP1A1 and CYP1A2 mRNAs correlated nicely with EROD and MROD enzyme activities ($r = 0.96$, $p < 0.0001$, $r = 0.94$, $p < 0.0001$, respectively). High correlations were also obtained for CYPs 3A4, 2D6, and 2B6 ($r = 0.69$, $p < 0.02$; $r = 0.61$, $p < 0.04$; and $r = 0.52$, $p < 0.08$, respectively), whereas no correlation between mRNA and their activities was found for CYPs 2A6, 2C9, and 2E1 ($r = 0.02$, $r = -0.07$, and $r = -0.003$, respectively. Data not shown).

DISCUSSION

The mRNA levels of the ten most relevant drug metabolizing human CYPs were precisely determined by a quantitative RT-PCR technique in 12 human livers. At least three different PCR determinations of the CYP mRNA levels were carried out obtaining very similar abundance profiles. The most abundant mRNAs were those of CYPs 3A4, 2E1, and 2C9 (Table III). CYP2E1 and CYP3A4 mRNAs represented altogether more than the 70% of the total CYP mRNA measured. Quantitative CYP mRNA abundance in human liver have been scarcely investigated. However, the results obtained in this study are similar to those found in the article describing this RT-PCR technique, where three human livers were used (10), and to other reports analyzing the most abundant CYPs (26-28).

Human variability of CYP expression is a well known fact with important clinical implications. Variation in CYP expression is determined by hormonal status, diet, smoking, age, genotype, and drug exposure. The CYP mRNA contents and enzymatic activities from the different livers showed a wide variation, as they did in studies previously reported (10, 11, 26, 29, 30). Inter-individual variability in CYP mRNA levels ranged from 2.0 to 582 fold variation for CYP2E1 and CYP1A2, respectively (Table III). The variations found in our study are similar to those described in 8 human livers by Andersen et al. (26), except for CYP2D6 (we found 5.9-fold and Andersen et al. a 220-fold variation) and CYP1A2 (we found 582-fold and Andersen et al. a 25-fold variation). In the case of CYP2D6, Andersen et al. reported that the 220-fold variability was mainly due to a CYP2D6*4 polymorphic donor, and its exclusion resulted in a 26-fold variation, which is closer to our results. Concerning CYP1A2 we found a 582-fold variation, which is partially attributable to the low levels of CYP1A2 mRNA in donor 9 (0.006 molecules/ molecules β -actin). This mRNA content is also lower than those described by Schweikl et al. in 19 human livers (27) (from 0.052 to 1.2 molecules/ molecules β -actin). Excluding donor 9 resulted in 70-fold variation in CYP1A2 mRNA between the remaining livers. This low CYP1A2 mRNA content could be attributed to

the fact that liver 9 corresponded to the oldest donor (71-years old woman) who was previously treated with several drugs (NSAIDs and antihypertensives) (Table I). The livers with the highest CYP1A mRNA levels did not correspond to smoker donors and there is no information of exposure to any CYP1A inductive agent. Variations in CYP activities (Table IV) were not as high as the variations in mRNA. The most variable CYP activity corresponded to CYP2D6. The high variability in CYP2D6 activity might be caused by the well known polymorphism to which this CYP is subjected (31, 32). CYP2A6 had the least variable CYP activity, with a calculated 2.0 fold variation.

mRNA determination by quantitative RT-PCR provides accurate information on gene expression, but post-transcriptional mechanisms controlling the protein translation rate, the half-lives of protein, and polymorphisms can alter a parallel mRNA/ enzyme activity relationship. Therefore, mRNA contents can not be used instead of enzymatic activity until the relationship between mRNA and activity has been established.

In our study with 12 human livers CYP1A1 and CYP1A2 mRNAs showed the best correlation with their respective enzymatic activities ($r = 0.96$, and 0.94 , respectively) (Fig. 1A and 1B). In a previous study the correlation for CYP1A2 between mRNA and protein content was investigated obtaining a correlation coefficient of $r = 0.74$ (33). In our study the mRNA quantification method is able to measure an absolute amount of mRNA which is then normalized with a internal standard (β -actin). This probably increased the precision and accuracy of the measurements and led to a higher coefficient than the one previously described. The data presented here suggest that the expression of both *CYP1A1* and *CYP1A2* is mainly regulated at a pre-translational level.

CYP3A4 is the major CYP expressed in liver and plays a significant role in the metabolism of approximately half the drugs in use today (34). We observed a significant correlation ($r = 0.69$) between the CYP3A4 mRNA level and the 6 β OHT activity (Fig. 1C). Interestingly liver 5 escaped the general pattern of distribution (and the regression increases to $r = 0.88$, $p <$

0.0004, if it is excluded). CYP3A4 protein data obtained from microsomes correlated with the measured enzyme activity (data not shown). Three allelic variants of CYP3A4 have been described: CYP3A4 *1, *2, and *3. CYP3A4*1 corresponds to the wild type allele and CYP3A4*2 encodes a protein with altered enzyme activity (35). The CYP3A4 gene of liver 5 did not correspond to this allele. There are previous studies reporting correlation coefficients of $r = 0.94$ (11), $r = 0.64$ (33), and $r = 0.71$ (36). All together, these data support that CYP3A4 mRNA can be used to estimate activity.

CYP2D6 also showed a rather high correlation value ($r = 0.61$). Polymorphisms producing less active proteins have been described (32, 37) and could account for the fact that some liver samples exhibit a high mRNA level but low activity (Fig. 1D). The correlation coefficient obtained suggests that CYP2D6 mRNA content could be used to estimate activity. However, the frequency of polymorphisms resulting in inactive enzymes is relevant and makes necessary a preliminar genotyping study to discard polymorphisms, then, mRNA could be used to estimate activity.

No polymorphic forms of CYP2B6 have been described to date, but there is a still unclear splicing mechanism that from the same gene produces different splicing forms at different levels (38, 39). It has been suggested that the activity of these alternative splicing forms could be lower than the normal splicing form. The pair of primers for CYP2B6 mRNA evaluation was designed to avoid detecting most of these splicing variants, although this mechanism could account for the lower coefficient of correlation found in this CYP ($r = 0.52$) (Fig. 1E) compared to the ones found for the CYPs 1A1, 1A2, 3A4, and 2D6.

In our study we found no significant correlation between the mRNA levels of CYPs 2A6, 2C9, and 2E1 and their activities. Data have been published indicating that in *CYP2E1* expression pre-transcriptional, pre-translational, translational and post-translational regulation take place (40-43). This would be consistent with the fact that CYP2E1 protein levels are relatively low in normal human liver (44), regard of having the highest mRNA levels. The

combined regulation mechanisms taking place in CYP2E1 would account for the lack of correlation between mRNA and activity that we have found.

CYP2C9 is an enzyme with three polymorphic variants: 2C9*1, *2, and *3 (45); *1 is the wild type and *2 (0.107 frequency) and *3 (0.074 frequency) are the two allelic variants which encode proteins with a lower activity. The polymorphic variants of CYP2C9 could account for part of the lack of correlation found in our study between mRNA levels and CYP activity. The relationship between mRNA and protein expression (which should eliminate the differences in activity caused by protein with lower activity) was previously studied. Ratanasavanh et al. (36) found a correlation between CYP2C9 mRNA and protein of $r = 0.66$, but not significant ($p = 0.07$) and George et al. (33) described a correlation coefficient of $r = 0.36$. These data suggest that in the regulation of *CYP2C9* expression there are several mechanisms involved.

In our study we did not find any correlation between CYP2A6 mRNA content and activity. CYP2A6 is a polymorphic enzyme with CYP2A6*2, *4A, *4B, *4D, and *5 allelic variants producing inactive enzymes (46-50). However, the frequencies of these variant alleles are low, which suggests that the lack of correlation found for *CYP2A6* might be determined by regulation mechanisms that are not just pre-translational.

In summary, our results indicate that the expression of CYPs 1A1, 1A2, 3A4, 2D6, and 2B6 is determined by regulatory mechanisms acting mainly at a pre-translational level, and that estimating CYP mRNA instead of CYP protein or activity levels seems to be appropriate, although, in the case of CYP2D6, genotyping analysis should be carried out first. Further studies regarding the regulatory mechanisms implicated in CYPs 2C9, and 2A6 expression may lead to better estimation of these CYPs activities by RT-PCR analysis. CYP2E1 expression is regulated at more than just the transcriptional level and, therefore, mRNA data do not seem to give a good representation of protein or activity levels.

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TABLE I

Characteristics of liver donors

| Liver n°. | Physical description (sex, age) | Smoking/ alcohol intake | Known drug intake |
|-----------|------------------------------------|---------------------------------|-----------------------------|
| 1 | Male, 38 | Smoker No alcohol intake | None |
| 2 | Male, 33 | Smoker No alcohol intake | Unknown |
| 3 | Female, 70 | Non smoker No alcohol intake | Antihypertensives |
| 4 | Male, 52 | Ex-smoker Moderate alcohol | Unknown |
| 5 | Female, 28 | Non smoker No alcohol intake | None |
| 6 | Female, 33 | Smoker No alcohol intake | None |
| 7 | Female, 46 | Non smoker No alcohol intake | Unknown |
| 8 | Female, 25 | Non smoker No alcohol intake | None |
| 9 | Female, 71 | Non smoker No alcohol intake | NSAIDs Antihypertensives |
| 10 | Female, 60 | Non smoker No alcohol intake | Unknown |
| 11 | Male, 44 | Smoker Alcohol intake | Phenytoin |
| 12 | Male, 39 | Ex-smoker No alcohol intake | None |

TABLE IIOligonucleotide PCR primers for human CYP enzymes and β -Actin and luciferase.

| Oligo ^a name | Oligonucleotide sequences 5' to 3' | Fragment size (bp) | Melting temp. (°C) |
|-------------------------|---|--------------------|--------------------|
| CYP 1A1 FP | TCC AGA GAC AAC AGG TAA AAC A | 371 | 55.7 |
| CYP 1A1 RP | AGG AAG GGC AGA GGA ATG TGA T | | |
| CYP 1A2 FP | AAC AAG GGA CAC AAC GCT GAA T | 453 | 58.6 |
| CYP 1A2 RP | GGA AGA GAA ACA AGG GCT GAG T | | |
| CYP 2A6 FP | AGG CTA TGG CGT GGT ATT CA | 521 | 58.3 |
| CYP 2A6 RP | ACT CCG TGT TGG GGT TCT TC | | |
| CYP 2B6 FP | ATG GGG CAC TGA AAA AGA CTG A | 283 | 57.8 |
| CYP 2B6 RP | AGA GGC GGG GAC ACT GAA TGA C | | |
| CYP 2C9 FP | TTC AGT CCT TTC TCA GCA GG | 383 | 53.1 |
| CYP 2C9 RP | TTG CAC AGT GAA ACA TAG GA | | |
| CYP 2C19 FP | TTC ATG CCT TTC TCA GCA GG | 277 | 55.1 |
| CYP 2C19 RP | ACA GAT AGT GAA ATT TGG AC | | |
| CYP 2D6 FP | CTA AGG GAA CGA CAC TCA TCA C | 289 | 58.2 |
| CYP 2D6 RP | CTC ACC AGG AAA GCA AAG ACA C | | |
| CYP 2E1 FP | ACA GAG ACC ACC AGC ACA ACT | 580 | 55.0 |
| CYP 2E1 RP | ATG AGC GGG GAA TGA CAC AGA | | |
| CYP 3A4 FP | CCT TAC ACA TAC ACA CCC TTT GGA AGT | 382 | 55.3 |
| CYP 3A4 RP | AGC TCA ATG CAT GTA CAG AAT CCC CGG TTA | | |
| CYP 3A5 FP | GAA GAA AAGTCG CCT CAA C | 679 | 52.8 |
| CYP 3A5 RP | AAG AAG TCC TTG CGT GTC TA | | |
| β -Actin FP | CGT ACC ACT GGC ATC GTG AT | 452 | 58.7 |
| β -Actin RP | GTG TTG GCG TAC AGG TCT TTG | | |
| Luciferase FP | TAC ACC CGA GGG GGA TGA TAA AC | 439 | 56.1 |
| Luciferase RP | CTC TTT TTC CGT CAT CGT CTT TC | | |

^a Oligo= oligonucleotide, FP= forward primer, RP= reverse primer.

TABLE IIICYPs mRNA content in 12 human livers (molecules/ molecules of β -actin)

| Liver n° | 1A1 | 1A2 | 2A6 | 2B6 | 2C9 | 2C19 | 2D6 | 2E1 | 3A4 | 3A5 |
|-----------------------------|-------------|-------------|--------------|------------|----------|--------------|-------------|-----------|------------|-----------|
| 1 | 0.040±0.012 | 0.801±0.40 | 0.154±0.05 | 1.04±0.173 | 19.5±5.7 | 0.840±0.038 | 0.051±0.020 | 21.5±5.1 | 103.4±16.7 | 1.66±0.37 |
| 2 | 0.016±0.008 | 0.094±0.06 | 0.055±0.016 | 0.33±0.067 | 12.0±2.8 | 0.062±0.0002 | 0.234±0.111 | 27.9±5.2 | 1.9±0.7 | 2.17±0.55 |
| 3 | 0.009±0.004 | 0.080±0.05 | 0.070±0.018 | 1.55±0.202 | 19.5±6.1 | 0.377±0.113 | 0.053±0.023 | 17.9±3.0 | 14.3±5.0 | 1.27±0.27 |
| 4 | 0.014±0.006 | 0.139±0.11 | 0.042±0.014 | 2.48±0.244 | 20.6±4.2 | 0.024±0.0001 | 0.106±0.038 | 32.8±3.6 | 26.2±9.5 | 7.88±0.36 |
| 5 | 0.066±0.044 | 1.273±0.57 | 0.258±0.082 | 1.67±0.267 | 27.2±7.9 | 1.195±0.004 | 0.078±0.031 | 29.1±6.6 | 93.1±26.0 | 1.59±0.16 |
| 6 | 0.017±0.005 | 0.112±0.05 | 0.133±0.034 | 0.52±0.025 | 14.9±4.3 | 0.049±0.011 | 0.215±0.100 | 30.8±11.2 | 2.2±0.9 | 1.45±0.06 |
| 7 | 0.132±0.021 | 3.496±1.03 | 0.172±0.063 | 1.92±0.098 | 16.3±3.0 | 1.513±0.658 | 0.153±0.066 | 42.0±4.6 | 55.8±22.4 | 4.07±2.48 |
| 8 | 0.036±0.017 | 0.643±0.32 | 0.200±0.051 | 0.70±0.191 | 20.6±7.6 | 0.154±0.061 | 0.131±0.068 | 19.7±2.3 | 32.3±12.0 | 2.37±0.35 |
| 9 | 0.003±0.002 | 0.006±0.005 | 0.0015±0.001 | 0.02±0.002 | 0.5±0.4 | 0.017±0.006 | 0.053±0.025 | 18.4±0.7 | 2.7±0.9 | 0.48±0.15 |
| 10 | 0.022±0.006 | 0.377±0.14 | 0.021±0.005 | 0.54±0.107 | 5.0±2.5 | 0.044±0.001 | 0.351±0.140 | 13.8±4.1 | 8.9±2.9 | 1.10±0.06 |
| 11 | 0.011±0.008 | 0.076±0.05 | 0.065±0.025 | 3.19±0.588 | 24.5±5.6 | 0.044±0.006 | 0.101±0.047 | 30.7±2.1 | 19.9±5.0 | 6.87±0.93 |
| 12 | 0.025±0.014 | 0.049±0.02 | 0.007±0.004 | 0.02±0.001 | 0.8±0.4 | 0.007±0.003 | 0.098±0.043 | 24.0±2.3 | 0.8±0.4 | 0.32±0.04 |
| Mean | 0.032 | 0.596 | 0.098 | 1.16 | 15.1 | 0.36 | 0.135 | 25.7 | 30.1 | 2.60 |
| S.D. | 0.036 | 0.993 | 0.083 | 1.01 | 8.9 | 0.53 | 0.091 | 8.0 | 35.7 | 2.44 |
| Fold variation ^a | 43 | 582 | 171 | 158 | 53 | 215 | 5.9 | 2.0 | 128 | 24 |

^a (Highest value-lowest value)/lowest value.

TABLE IV

CYPs activities in 12 microsomes derived from 12 human livers (pmol/min/mg protein)

| CYP Activity | 1A1 EROD | 1A2 MROD | 2A6 COH | 2B6 BROD | 2C9 D4'OH | 2D6 DXOD | 2E1 CZOH | 3A4 6βOHT |
|--------------------------------|-------------|-------------|------------|-------------|--------------|-------------|-------------|--------------|
| 1 | 16.7±1.7 | 20.7±0.9 | 815±13 | 16.3±0.6 | 1673±247 | 0 | 17234±5931 | 4518±584 |
| 2 | 2.7±0.9 | 4.8±2.0 | 583±65 | 6.5±1.4 | 2155±576 | 402±103 | 24952±4776 | 1452±451 |
| 3 | 5.8±2.1 | 9.7±2.1 | 637±74 | 13.8±3.4 | 1718±210 | 0 | 24942±12579 | 1448±231 |
| 4 | 4.6±1.1 | 6.7±1.0 | 616±180 | 35.3±2.3 | 1658±485 | 28±5 | 62747±18142 | 3136±613 |
| 5 | 26.7±5.2 | 25.7±1.0 | 313±21 | 3.8±1.5 | 1532±404 | 212±41 | 6715±1445 | 2182±545 |
| 6 | 10.6±2.1 | 10.5±3.0 | 530±114 | 5.8±0.1 | 2310±323 | 212±42 | 7521±2611 | 1440±175 |
| 7 | 60.2±11.5 | 40.8±3.4 | 426±25 | 10.0±3.2 | 1296±344 | 2±4 | 12163±4252 | 3639±344 |
| 8 | 12.5±2.9 | 15.4±0.5 | 679±139 | 6.1±0.3 | 2489±599 | 166±52 | 11173±491 | 3683±293 |
| 9 | 9.0±1.3 | 9.6±0.4 | 331±18 | 4.6±1.2 | 1393±82 | 93±48 | 39682±7693 | 1973±376 |
| 10 | 9.7±1.7 | 10.4±2.3 | 271±42 | 3.0±0.5 | 3847±661 | 210±31 | 38079±17377 | 2147±286 |
| 11 | 13.7±2.8 | 14.4±1.0 | 766±72 | 8.5±0.3 | 1644±208 | 141±42 | 68977±35434 | 2472±540 |
| 12 | 4.1±1.1 | 4.2±0.8 | 601±23 | 4.9±0.4 | 848±332 | 24±7 | 16338±1933 | 776±274 |
| Mean | 14.7 | 14.4 | 547 | 9.9 | 1880 | 124 | 27543 | 2406 |
| S.D. | 16 | 10 | 178 | 9.0 | 765 | 124 | 20887 | 1123 |
| Fold variation ^a | 21 | 8.7 | 2.0 | 11 | 3.5 | - | 9.3 | 4.8 |

^a (Highest value-lowest value)/lowest value

Figure Legend

FIG. 1. Relationship between CYP mRNAs and their respective metabolic activities in 12 human liver samples. mRNA was determined by quantitative RT-PCR and enzyme activities were determined using model substrates in microsomes derived from the 12 livers. A) CYP1A1 mRNA and EROD, B) CYP1A2 mRNA and MROD, C) CYP3A4 mRNA and 6 β OHT, D) CYP2D6 mRNA and , DXOD, and E) CYP2B6 mRNA and BROD.

